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NF 38

THE NATIONAL FORMULARY

Volume 2

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

Official from May 1, 2020

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

THE UNITED STATES PHARMACOPEIAL CONVENTION
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SIX-MONTH IMPLEMENTATION GUIDELINE

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

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

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USP 43–NF 38	November 1, 2019	May 1, 2020	May 1, 2021 (except as superseded by supplements, *IRAs,* and *Revision Bulletins*)
First Supplement to the USP 43–NF 38	February 1, 2020	August 1, 2020	May 1, 2021 (except as superseded by *Second Supplement, IRAs,* and *Revision Bulletins*)
Second Supplement to the USP 43–NF 38	June 1, 2020	December 1, 2020	May 1, 2021 (except as superseded by *IRAs* and *Revision Bulletins*)
USP-NF 2021 Issue 1	November 1, 2020	May 1, 2021	May 1, 2022 (except as superseded by supplements, *IRAs,* and *Revision Bulletins*)

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

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

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

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Contents

VOLUME 1

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

USP 43

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

VOLUME 2

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

VOLUME 3

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

NF 38

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

VOLUME 4

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

www.webofpharma.com

VOLUME 5

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

www.webofpharma.com

GENERAL NOTICES AND REQUIREMENTS

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

1. TITLE AND REVISION 2485

2. OFFICIAL STATUS AND LEGAL RECOGNITION 2485
2.10. Official Text 2485
2.20. Official Articles 2485
2.30. Legal Recognition 2485

3. CONFORMANCE TO STANDARDS 2486
3.10. Applicability of Standards 2486
3.20. Indicating Conformance 2487

4. MONOGRAPHS AND GENERAL CHAPTERS 2487
4.10. Monographs 2487
4.20. General Chapters 2487

5. MONOGRAPH COMPONENTS 2488
5.10. Molecular Formula 2488
5.20. Added Substances 2488
5.30. Description and Solubility 2488
5.40. Identification 2488
5.50. Assay 2488
5.60. Impurities and Foreign Substances 2489
5.70. Performance Tests 2489
5.80. USP Reference Standards 2489

6. TESTING PRACTICES AND PROCEDURES 2489
6.10. Safe Laboratory Practices 2489
6.20. Automated Procedures 2489
6.30. Alternative and Harmonized Methods and Procedures 2489
6.40. Dried, Anhydrous, Ignited, or Solvent-Free Basis 2490
6.50. Preparation of Solutions 2490
6.60. Units Necessary to Complete a Test 2490
6.70. Reagents 2490
6.80. Equipment 2491

7. TEST RESULTS 2491
7.10. Interpretation of Requirements 2491
7.20. Rounding Rules 2491

8. TERMS AND DEFINITIONS 2491
8.10. Abbreviations 2491
8.20. About 2491
8.30. Alcohol Content 2492
8.40. Atomic Weights 2492
8.50. Blank Determinations 2492
8.60. Concomitantly 2492
8.70. Desiccator 2492
8.80. Logarithms 2492
8.90. Microbial Strain 2492
8.100. Negligible 2492
8.110. NLT/NMT 2492
8.120. Odor 2492
8.130. Percent 2492
8.140. Percentage Concentrations 2492
8.150. Pressure 2492
8.160. Reaction Time 2492
8.170. Specific Gravity 2492
8.180. Temperatures 2492
8.190. Time 2492
8.200. Transfer 2493
8.210. Vacuum 2493
8.220. Vacuum Desiccator 2493
8.230. Water 2493
8.240. Weights and Measures 2493

9. PRESCRIBING AND DISPENSING 2494
9.10. Use of Metric Units 2494

10. PRESERVATION, PACKAGING, STORAGE, AND LABELING 2494
10.10. Packaging and Storage 2494
10.20. Labeling 2494

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GENERAL NOTICES AND REQUIREMENTS

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

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

1. TITLE AND REVISION

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

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

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

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

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

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

2. OFFICIAL STATUS AND LEGAL RECOGNITION

2.10. Official Text

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

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

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

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

2.20. Official Articles

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

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

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

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

2.30. Legal Recognition

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

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

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

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

3. CONFORMANCE TO STANDARDS

3.10. Applicability of Standards

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

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

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

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

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

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

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

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

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

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

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

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

3.10.30. Applicability of Standards to the Practice of Compounding

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

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

3.20. Indicating Conformance

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

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

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

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

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

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

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

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

4. MONOGRAPHS AND GENERAL CHAPTERS

4.10. Monographs

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

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

4.10.10. Applicability of Test Procedures

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

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

4.10.20. Acceptance Criteria

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

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

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

4.20. General Chapters

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

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

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

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

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5. MONOGRAPH COMPONENTS

5.10. Molecular Formula

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

5.20. Added Substances

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

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

5.20.10. Added Substances in Official Substances

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

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

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

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

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

5.20.20.1. In Compounded Preparations

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

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

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

5.20.20.2. In Dietary Supplements

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

5.30. Description and Solubility

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

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

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

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

5.40. Identification

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

5.50. Assay

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

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5.50.10. Units of Potency (Biological)

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

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

5.60. Impurities and Foreign Substances

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

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

5.60.10. Other Impurities in USP and NF Articles

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

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

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

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

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

5.60.20. Residual Solvents in USP and NF Articles

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

5.60.30. Elemental Impurities in USP Drug Products and Dietary Supplements

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

5.70. Performance Tests

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

5.80. USP Reference Standards

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

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

6. TESTING PRACTICES AND PROCEDURES

6.10. Safe Laboratory Practices

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

6.20. Automated Procedures

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

6.30. Alternative and Harmonized Methods and Procedures

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

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

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

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

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

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

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

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

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

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

6.40.10. Ignite to Constant Weight

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

6.40.20. Dried to Constant Weight

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

6.50. Preparation of Solutions

6.50.10. Filtration

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

6.50.20. Solutions

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

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

6.50.20.1. Adjustments to Solutions

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

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

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

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

6.50.20.2. Test Solutions

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

6.50.20.3. Indicator Solutions

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

6.60. Units Necessary to Complete a Test

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

6.60.10. Tablets

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

6.60.20. Capsules

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

6.70. Reagents

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

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

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

6.80. Equipment

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

6.80.10. Apparatus for Measurement

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

6.80.10.1. Pipet/Pipette

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

6.80.10.2. Light Protection

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

6.80.20. Instrumental Apparatus

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

6.80.20.1. Chromatographic Tubes and Columns

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

6.80.20.2. Tubing

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

6.80.20.3. Steam Bath

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

6.80.20.4. Water Bath

A water bath requires vigorously boiling water unless otherwise specified.

6.80.30. Temperature Reading Devices

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

7. TEST RESULTS

7.10. Interpretation of Requirements

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

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

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

7.10.5. Nominal Concentrations in Equations

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

7.10.10. Equivalence Statements in Titrimetric Procedures

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

7.20. Rounding Rules

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

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

8. TERMS AND DEFINITIONS

8.10. Abbreviations

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

8.20. About

"About" indicates a quantity within 10%.

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

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

8.30. Alcohol Content

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

8.40. Atomic Weights

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

8.50. Blank Determinations

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

8.60. Concomitantly

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

8.70. Desiccator

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

8.80. Logarithms

Logarithms are to the base 10.

8.90. Microbial Strain

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

8.100. Negligible

"Negligible" indicates a quantity not exceeding 0.50 mg.

8.110. NLT/NMT

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

8.120. Odor

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

8.130. Percent

"Percent" used without qualification means:

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

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

8.140. Percentage Concentrations

Percentage concentrations are expressed as follows:

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

8.150. Pressure

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

8.160. Reaction Time

Reaction time is 5 minutes unless otherwise specified.

8.170. Specific Gravity

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

8.180. Temperatures

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

8.190. Time

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

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8.200. Transfer

"Transfer" indicates a quantitative manipulation.

8.210. Vacuum

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

8.220. Vacuum Desiccator

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

8.230. Water

8.230.10. Water as an Ingredient in an Official Product

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

8.230.20. Water in the Manufacture of Official Substances

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

8.230.30. Water in a Compendial Procedure

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

8.240. Weights and Measures

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

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

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

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

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

Chart of Symbols and Prefixes commonly employed for SI metric units and other units:

	Units	Symbol	Notes
Length			
	meter	m	
	centimeter	cm	
	millimeter	mm	
	micrometer	µm	Previously referred to as a micron
	nanometer	nm	Previously the symbol mµ (for millimicron) was used
	Ångström	Å	Equal to 0.1 nm
Mass			
	kilogram	kg	
	gram	g	
	milligram	mg	
	microgram	µg	The symbol µg is used in the *USP* and *NF* to represent micrograms, but micrograms may be represented as "mcg" for labeling and prescribing purposes. The term "gamma," symbolized by γ, frequently is used to represent micrograms in biochemical literature.
	nanogram	ng	
	picogram	pg	
	dalton	Da	Also referred to as the unified atomic mass unit and is equal to 1/12 times the mass of the free carbon 12 atom.
	kilodalton	kDa	
Time			
	second	s	
	minute	min	
	hour	h	
Volume			
	liter	L	1 L is equal to 1000 cm^3 (cubic centimeters)
	deciliter	dL	
	milliliter	mL	1 mL is equal to 1 cm^3, sometimes referred to as cc
	microliter	µL	
Temperature			
	Celsius	°C	
Amount of Substance			
	mole	mol	Historically referred to as gram-molecular weight or gram-atomic weight
	millimole	mmol	
	micromole	µmol	
	femtomole	fmol	
	equivalent	Eq	Also referred to as gram-equivalent weight. It is used in the calculation of substance concentration in units of normality. This unit is no longer preferred for use in analytical chemistry or metrology.
	milli equivalent	mEq	
	osmole	Osmol	Osmotic pressure of a solution, related to substance concentration.
	milliosmole	mOsmol	
Pressure			
	pascal	Pa	
	kilopascal	kPa	

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	Units	Symbol	Notes
	pounds per square inch	psi	
	millimeter of mercury	mmHg	Equal to 133.322 Pa
Electrical units			
	ampere	A	
	volt	V	
	millivolt	mV	
	hertz	Hz	Unit of frequency
	kilohertz	kHz	
	megahertz	MHz	
	electron volt	eV	
	kilo-electron volt	keV	
	mega-electron volt	MeV	
Radiation			
	becquerel	Bq	SI unit of activity for radionuclides
	kilobecquerel	kBq	
	megabecquerel	MBq	
	gigabecquerel	GBq	
	curie	Ci	Non-SI unit of activity for radionuclides
	millicurie	mCi	
	microcurie	μCi	
	nanocurie	nCi	
Other			
	acceleration due to gravity	g	Used to express rate of centrifugation
	revolutions per minute	rpm	Used to express rate of centrifugation

Selected SI Prefixes

Name	Symbol	Factor
giga	G	10^9
mega	M	10^6
kilo	k	10^3
deci	d	10^{-1}
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}
femto	f	10^{-15}

9. PRESCRIBING AND DISPENSING

9.10. Use of Metric Units

Prescriptions for compendial articles shall be written to state the quantity and/or strength desired in metric units unless otherwise indicated in the individual monograph [see also section *5.50.10 Units of Potency (Biological)* above]. If an amount is prescribed by any other system of measurement, only an amount that is the metric equivalent of the prescribed amount shall be dispensed. Abbreviations for the terms "Units" or "International Units" shall not be used for labeling or prescribing purposes. Apothecary unit designations on labels and labeling shall not be used.

9.20. Changes in Volume

In the dispensing of prescription medications, slight changes in volume owing to variations in room temperatures may be disregarded.

10. PRESERVATION, PACKAGING, STORAGE, AND LABELING

10.10. Packaging and Storage

All articles in *USP* or *NF* are subject to the packaging and storage requirements specified in *Packaging and Storage Requirements* ⟨659⟩, unless different requirements are provided in an individual monograph.

10.20. Labeling

All articles in *USP* or *NF* are subject to the labeling requirements specified in *Labeling* ⟨7⟩, unless different requirements are provided in an individual monograph.

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Guide to General Chapters

(For complete alphabetical list of all general chapters in this Pharmacopeia, see under "General chapters" in the index.)

GENERAL TESTS AND ASSAYS

General Requirements for Tests and Assays

⟨1⟩ Injections and Implanted Drug Products (Parenterals)—Product Quality Tests 6405
⟨2⟩ Oral Drug Products—Product Quality Tests 6410
⟨3⟩ Topical and Transdermal Drug Products—Product Quality Tests 6415
⟨4⟩ Mucosal Drug Products—Product Quality Tests 6423
⟨5⟩ Inhalation and Nasal Drug Products—General Information and Product Quality Tests 6427
⟨7⟩ Labeling 6435
⟨11⟩ USP Reference Standards 6442

Apparatus for Tests and Assays

⟨17⟩ Prescription Container Labeling 6445
⟨31⟩ Volumetric Apparatus 6447
⟨41⟩ Balances 6448

Microbiological Tests

⟨51⟩ Antimicrobial Effectiveness Testing 6449
⟨55⟩ Biological Indicators—Resistance Performance Tests 6452
⟨60⟩ Microbiological Examination of Nonsterile Products—Tests For Burkholderia Cepacia Complex 6454
⟨61⟩ Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests 6457
⟨62⟩ Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms 6463
⟨63⟩ Mycoplasma Tests 6471
⟨64⟩ Probiotic Tests 6477
⟨71⟩ Sterility Tests 6480

Biological Tests and Assays

⟨81⟩ Antibiotics—Microbial Assays 6488
⟨85⟩ Bacterial Endotoxins Test 6508
⟨87⟩ Biological Reactivity Tests, in Vitro 6514
⟨88⟩ Biological Reactivity Tests, in Vivo 6516
⟨89⟩ Enzymes Used as Ancillary Materials in Pharmaceutical Manufacturing 6521
⟨89.1⟩ Collagenase I 6524
⟨89.2⟩ Collagenase II 6528
⟨90⟩ Fetal Bovine Serum—Quality Attributes and Functionality Tests 6533
⟨91⟩ Calcium Pantothenate Assay 6536

Biological Tests and Assays

⟨92⟩ Growth Factors and Cytokines Used in Cell Therapy Manufacturing 6539
⟨111⟩ Design and Analysis of Biological Assays 6543
⟨115⟩ Dexpanthenol Assay 6547
⟨121⟩ Insulin Assays 6547
⟨121.1⟩ Physicochemical Analytical Procedures for Insulins 6550
⟨123⟩ Glucagon Bioidentity Tests 6552
⟨124⟩ Erythropoietin Bioassays 6557
⟨126⟩ Somatropin Bioidentity Tests 6558
⟨127⟩ Flow Cytometric Enumeration of Cd34+ Cells 6560
⟨129⟩ Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies 6565
⟨130⟩ Protein A Quality Attributes 6570
⟨151⟩ Pyrogen Test 6577
⟨161⟩ Medical Devices—Bacterial Endotoxin and Pyrogen Tests 6579
⟨162⟩ Diphtheria Antitoxin Potency Testing for Human Immune Globulins 6582
⟨165⟩ Prekallikrein Activator 6583
⟨171⟩ Vitamin B12 Activity Assay 6584

Chemical Tests and Assays

IDENTIFICATION TESTS

⟨181⟩ Identification—Organic Nitrogenous Bases 6587
⟨191⟩ Identification Tests—General 6587
⟨193⟩ Identification—Tetracyclines 6592
⟨197⟩ Spectroscopic Identification Tests 6593
⟨198⟩ Nuclear Magnetic Resonance Spectroscopy Identity Testing of Bacterial Polysaccharides Used in Vaccine Manufacture 6596
⟨201⟩ Thin-Layer Chromatographic Identification Test 6600
⟨202⟩ Identification of Fixed Oils by Thin-Layer Chromatography 6601
⟨203⟩ High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin 6602

LIMIT TESTS

⟨206⟩ Aluminum 6605
⟨207⟩ Test for 1,6-Anhydro Derivative for Enoxaparin Sodium 6605
⟨208⟩ Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins 6611
⟨209⟩ Low Molecular Weight Heparin Molecular Weight Determinations 6614
⟨210⟩ Monosaccharide Analysis 6616
⟨211⟩ Arsenic 6621
⟨212⟩ Oligosaccharide Analysis 6623
⟨221⟩ Chloride and Sulfate 6636

www.webofpharma.com

Chemical Tests and Assays

⟨223⟩ Dimethylaniline 6636
⟨226⟩ 4-Epianhydrotetracycline 6636
⟨227⟩ 4-Aminophenol in Acetaminophen-Containing Drug Products 6637
⟨228⟩ Ethylene Oxide and Dioxane 6639
⟨232⟩ Elemental Impurities—Limits 6641
⟨233⟩ Elemental Impurities—Procedures 6645
⟨241⟩ Iron 6648
⟨251⟩ Lead 6649
⟨261⟩ Mercury 6650
⟨267⟩ Porosimetry by Mercury Intrusion 6653
⟨268⟩ Porosity by Nitrogen Adsorption—Desorption 6655
⟨271⟩ Readily Carbonizable Substances Test 6660
⟨281⟩ Residue on Ignition 6660
⟨291⟩ Selenium 6661

OTHER TESTS AND ASSAYS

⟨301⟩ Acid-Neutralizing Capacity 6661
⟨311⟩ Alginates Assay 6662
⟨341⟩ Antimicrobial Agents — Content 6664
⟨345⟩ Assay for Citric Acid/Citrate and Phosphate 6668
⟨351⟩ Assay for Steroids 6669
⟨381⟩ Elastomeric Closures for Injections 6669
⟨391⟩ Epinephrine Assay 6676
⟨401⟩ Fats and Fixed Oils 6676
⟨411⟩ Folic Acid Assay 6689
⟨413⟩ Impurities Testing in Medical Gases 6692
⟨415⟩ Medical Gases Assay 6693
⟨425⟩ Iodometric Assay - Antibiotics 6696
⟨429⟩ Light Diffraction Measurement of Particle Size 6697
⟨431⟩ Methoxy Determination 6702
⟨441⟩ Niacin or Niacinamide Assay 6704
⟨451⟩ Nitrite Titration 6709
⟨461⟩ Nitrogen Determination 6709
⟨466⟩ Ordinary Impurities 6710
⟨467⟩ Residual Solvents 6712
⟨469⟩ Ethylene Glycol, Diethylene Glycol, and Triethylene Glycol in Ethoxylated Substances 6733
⟨471⟩ Oxygen Flask Combustion 6735
⟨481⟩ Riboflavin Assay 6735
⟨501⟩ Salts of Organic Nitrogenous Bases 6741
⟨503⟩ Acetic Acid in Peptides 6741
⟨503.1⟩ Trifluoroacetic Acid (TFA) in Peptides 6743
⟨507⟩ Protein Determination Procedures 6744
⟨509⟩ Residual DNA Testing 6748
⟨511⟩ Single-Steroid Assay 6750
⟨525⟩ Sulfur Dioxide 6751
⟨531⟩ Thiamine Assay 6756
⟨541⟩ Titrimetry 6764

Chemical Tests and Assays

⟨551⟩ Vitamin E Assay 6767
⟨561⟩ Articles of Botanical Origin 6774
⟨563⟩ Identification of Articles of Botanical Origin 6788
⟨565⟩ Botanical Extracts 6798
⟨571⟩ Vitamin A Assay 6801
⟨580⟩ Vitamin C Assay 6805
⟨581⟩ Vitamin D Assay 6808
⟨591⟩ Zinc Determination 6817

Physical Tests and Determinations

⟨601⟩ Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests 6819
⟨602⟩ Propellants 6844
⟨603⟩ Topical Aerosols 6845
⟨604⟩ Leak Rate 6846
⟨610⟩ Alternative Microbiological Sampling Methods for Nonsterile Inhaled and Nasal Products 6846
⟨611⟩ Alcohol Determination 6848
⟨616⟩ Bulk Density and Tapped Density of Powders 6850
⟨621⟩ Chromatography 6853
⟨630⟩ Visual Comparison 6865
⟨631⟩ Color and Achromicity 6865
⟨641⟩ Completeness of Solution 6866
⟨643⟩ Total Organic Carbon 6867
⟨644⟩ Conductivity of Solutions 6868
⟨645⟩ Water Conductivity 6871
⟨651⟩ Congealing Temperature 6874
⟨659⟩ Packaging and Storage Requirements 6876
⟨660⟩ Containers—Glass 6881
⟨661⟩ Plastic Packaging Systems and Their Materials of Construction 6887
⟨661.1⟩ Plastic Materials of Construction 6893
⟨661.2⟩ Plastic Packaging Systems for Pharmaceutical Use 6912
⟨670⟩ Auxiliary Packaging Components 6916
⟨671⟩ Containers—Performance Testing 6922
⟨691⟩ Cotton 6929
⟨695⟩ Crystallinity 6931
⟨696⟩ Characterization of Crystalline Solids By Microcalorimetry and Solution Calorimetry 6931
⟨697⟩ Container Content for Injections 6934
⟨698⟩ Deliverable Volume 6935
⟨699⟩ Density of Solids 6938
⟨701⟩ Disintegration 6940
⟨705⟩ Quality Attributes of Tablets Labeled as Having a Functional Score 6944
⟨711⟩ Dissolution 6945
⟨721⟩ Distilling Range 6955
⟨724⟩ Drug Release 6957
⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions 6963
⟨730⟩ Plasma Spectrochemistry 6967

www.webofpharma.com

Physical Tests and Determinations

⟨731⟩ Loss on Drying 6970
⟨733⟩ Loss on Ignition 6970
⟨735⟩ X-Ray Fluorescence Spectrometry 6971
⟨736⟩ Mass Spectrometry 6975
⟨741⟩ Melting Range or Temperature 6980
⟨755⟩ Minimum Fill 6982
⟨761⟩ Nuclear Magnetic Resonance Spectroscopy 6984
⟨771⟩ Ophthalmic Products-Quality Tests 6993
⟨776⟩ Optical Microscopy 6998
⟨781⟩ Optical Rotation 7001
⟨782⟩ Vibrational Circular Dichroism Spectroscopy 7002
⟨785⟩ Osmolality and Osmolarity 7008
⟨786⟩ Particle Size Distribution Estimation by Analytical Sieving 7010
⟨787⟩ Subvisible Particulate Matter in Therapeutic Protein Injections 7014
⟨788⟩ Particulate Matter in Injections 7017
⟨789⟩ Particulate Matter in Ophthalmic Solutions 7020
⟨790⟩ Visible Particulates in Injections 7021
⟨791⟩ pH 7022
⟨795⟩ Pharmaceutical Compounding—Nonsterile Preparations 7025
⟨797⟩ Pharmaceutical Compounding—Sterile Preparations 7037
⟨800⟩ Hazardous Drugs—Handling in Healthcare Settings 7071
⟨801⟩ Polarography 7089
⟨811⟩ Powder Fineness 7093
⟨821⟩ Radioactivity 7094
⟨823⟩ Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses 7100
⟨825⟩ Radiopharmaceuticals—Preparation, Compounding, Dispensing, and Repackaging 7109
⟨831⟩ Refractive Index 7137
⟨841⟩ Specific Gravity 7137
⟨846⟩ Specific Surface Area 7139
⟨852⟩ Atomic Absorption Spectroscopy 7142
⟨853⟩ Fluorescence Spectroscopy 7146
⟨854⟩ Mid-Infrared Spectroscopy 7151
⟨855⟩ Nephelometry and Turbidimetry 7155
⟨856⟩ Near-Infrared Spectroscopy 7161
⟨857⟩ Ultraviolet-Visible Spectroscopy 7166
⟨861⟩ Sutures—Diameter 7175
⟨871⟩ Sutures—Needle Attachment 7178
⟨881⟩ Tensile Strength 7179
⟨891⟩ Thermal Analysis 7180
⟨905⟩ Uniformity of Dosage Units 7183
⟨911⟩ Viscosity—Capillary Methods 7187
⟨912⟩ Viscosity—Rotational Methods 7189
⟨913⟩ Viscosity—Rolling Ball Method 7193
⟨914⟩ Viscosity—Pressure Driven Methods 7195
⟨921⟩ Water Determination 7196

Physical Tests and Determinations

⟨941⟩ Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction (XRPD) 7201

GENERAL INFORMATION

⟨1004⟩ Mucosal Drug Products—Performance Tests 7227
⟨1005⟩ Acoustic Emission 7230
⟨1010⟩ Analytical Data—Interpretation and Treatment 7233
⟨1024⟩ Bovine Serum 7260
⟨1025⟩ Pancreatin 7272
⟨1027⟩ Flow Cytometry 7281
⟨1029⟩ Good Documentation Guidelines 7296
⟨1030⟩ Biological Assay Chapters—Overview and Glossary 7300
⟨1031⟩ The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants 7313
⟨1032⟩ Design and Development of Biological Assays 7320
⟨1033⟩ Biological Assay Validation 7337
⟨1034⟩ Analysis of Biological Assays 7351
⟨1039⟩ Chemometrics 7363
⟨1041⟩ Biologics 7379
⟨1043⟩ Ancillary Materials for Cell, Gene, and Tissue-Engineered Products 7381
⟨1044⟩ Cryopreservation of Cells 7389
⟨1046⟩ Cell-based Advanced Therapies and Tissue-based Products 7400
⟨1047⟩ Gene Therapy Products 7426
⟨1048⟩ Quality of Biotechnological Products: Analysis of The Expression Construct in Cells Used for Production of R-DNA Derived Protein Products 7452
⟨1049⟩ Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products 7454
⟨1050⟩ Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin 7459
⟨1050.1⟩ Design, Evaluation, and Characterization Of Viral Clearance Procedures 7472
⟨1051⟩ Cleaning Glass Apparatus 7481
⟨1052⟩ Biotechnology-Derived Articles—Amino Acid Analysis 7481
⟨1053⟩ Capillary Electrophoresis 7492
⟨1054⟩ Biotechnology-Derived Articles—Isoelectric Focusing 7499
⟨1055⟩ Biotechnology-Derived Articles—Peptide Mapping 7501
⟨1056⟩ Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis 7508
⟨1057⟩ Biotechnology-Derived Articles—Total Protein Assay 7515
⟨1058⟩ Analytical Instrument Qualification 7522
⟨1059⟩ Excipient Performance 7528
⟨1061⟩ Color—Instrumental Measurement 7553
⟨1062⟩ Tablet Compression Characterization 7556
⟨1063⟩ Shear Cell Methodology for Powder Flow Testing 7566
⟨1064⟩ Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure 7578
⟨1065⟩ Ion Chromatography 7588
⟨1066⟩ Physical Environments That Promote Safe Medication Use 7590
⟨1071⟩ Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach 7602
⟨1072⟩ Disinfectants and Antiseptics 7608

www.webofpharma.com

GENERAL INFORMATION *(continued)*

⟨1074⟩ Excipient Biological Safety Evaluation Guidelines..... 7613
⟨1078⟩ Good Manufacturing Practices for Bulk Pharmaceutical Excipients..... 7617
⟨1079⟩ Good Storage and Distribution Practices for Drug Products..... 7635
⟨1079.1⟩ Storage and Transportation of Investigational Drug Products..... 7644
⟨1080⟩ Bulk Pharmaceutical Excipients—Certificate of Analysis.... 7647
⟨1084⟩ Glycoprotein and Glycan Analysis—General Considerations..... 7655
⟨1085⟩ Guidelines on the Endotoxins Test..... 7665
⟨1086⟩ Impurities in Drug Substances and Drug Products..... 7678
⟨1087⟩ Apparent Intrinsic Dissolution—Dissolution Testing Procedures for Rotating Disk and Stationary Disk..... 7681
⟨1088⟩ In Vitro and in Vivo Evaluation of Dosage Forms..... 7684
⟨1090⟩ Assessment of Solid Oral Drug Product Performance and Interchangeability, Bioavailability, Bioequivalence, and Dissolution..... 7695
⟨1091⟩ Labeling of Inactive Ingredients..... 7706
⟨1092⟩ The Dissolution Procedure: Development and Validation. 7707
⟨1094⟩ Capsules—Dissolution Testing And Related Quality Attributes..... 7725
⟨1097⟩ Bulk Powder Sampling Procedures..... 7732
⟨1099⟩ Limit on Number of Large Deviations When Assessing Content Uniformity in Large Samples..... 7744
⟨1102⟩ Immunological Test Methods—General Considerations... 7746
⟨1103⟩ Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (Elisa)..... 7753
⟨1104⟩ Immunological Test Methods—Immunoblot Analysis..... 7763
⟨1105⟩ Immunological Test Methods—Surface Plasmon Resonance..... 7773
⟨1106⟩ Immunogenicity Assays—Design And Validation of Immunoassays to Detect Anti-Drug Antibodies..... 7788
⟨1106.1⟩ Immunogenicity Assays—Design And Validation of Assays to Detect Anti-Drug Neutralizing Antibody..... 7802
⟨1111⟩ Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use..... 7819
⟨1112⟩ Application of Water Activity Determination to Nonsterile Pharmaceutical Products..... 7820
⟨1113⟩ Microbial Characterization, Identification, and Strain Typing..... 7823
⟨1115⟩ Bioburden Control of Nonsterile Drug Substances and Products..... 7827
⟨1116⟩ Microbiological Control and Monitoring of Aseptic Processing Environments..... 7833
⟨1117⟩ Microbiological Best Laboratory Practices..... 7845
⟨1118⟩ Monitoring Devices—Time, Temperature, and Humidity.. 7850
⟨1120⟩ Raman Spectroscopy..... 7856
⟨1121⟩ Nomenclature..... 7862
⟨1125⟩ Nucleic Acid-Based Techniques—General..... 7865
⟨1126⟩ Nucleic Acid-Based Techniques—Extraction, Detection and Sequencing..... 7870
⟨1127⟩ Nucleic Acid-Based Techniques—Amplification..... 7879
⟨1128⟩ Nucleic Acid-Based Techniques—Microarray..... 7887
⟨1129⟩ Nucleic Acid-Based Techniques—Genotyping..... 7893

GENERAL INFORMATION *(continued)*

⟨1130⟩ Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)..... 7897
⟨1132⟩ Residual Host Cell Protein Measurement in Biopharmaceuticals..... 7900
⟨1136⟩ Packaging and Repackaging—Single-Unit Containers..... 7919
⟨1151⟩ Pharmaceutical Dosage Forms..... 7929
⟨1152⟩ Animal Drugs for Use in Animal Feeds..... 7952
⟨1160⟩ Pharmaceutical Calculations in Pharmacy Practice..... 7953
⟨1163⟩ Quality Assurance in Pharmaceutical Compounding 7979
⟨1168⟩ Compounding for Phase I Investigational Studies..... 7985
⟨1174⟩ Powder Flow..... 7993
⟨1176⟩ Prescription Balances and Volumetric Apparatus Used in Compounding..... 7997
⟨1177⟩ Good Packaging Practices..... 8003
⟨1178⟩ Good Repackaging Practices..... 8006
⟨1180⟩ Human Plasma 8008
⟨1181⟩ Scanning Electron Microscopy..... 8025
⟨1184⟩ Sensitization Testing..... 8035
⟨1191⟩ Stability Considerations in Dispensing Practice..... 8044
⟨1195⟩ Significant Change Guide for Bulk Pharmaceutical Excipients..... 8049
⟨1197⟩ Good Distribution Practices for Bulk Pharmaceutical Excipients..... 8060
⟨1207⟩ Package Integrity Evaluation—Sterile Products 8079
⟨1207.1⟩ Package Integrity Testing in the Product Life Cycle-Test Method Selection and Validation..... 8086
⟨1207.2⟩ Package Integrity Leak Test Technologies..... 8097
⟨1207.3⟩ Package Seal Quality Test Technologies..... 8111
⟨1208⟩ Sterility Testing—Validation of Isolator Systems..... 8113
⟨1210⟩ Statistical Tools for Procedure Validation..... 8117
⟨1211⟩ Sterility Assurance..... 8129
⟨1216⟩ Tablet Friability..... 8137
⟨1217⟩ Tablet Breaking Force..... 8138
⟨1222⟩ Terminally Sterilized Pharmaceutical Products—Parametric Release..... 8142
⟨1223⟩ Validation of Alternative Microbiological Methods..... 8144
⟨1223.1⟩ Validation of Alternative Methods to Antibiotic Microbial Assays..... 8157
⟨1224⟩ Transfer of Analytical Procedures..... 8164
⟨1225⟩ Validation of Compendial Procedures..... 8166
⟨1226⟩ Verification of Compendial Procedures..... 8171
⟨1227⟩ Validation of Microbial Recovery From Pharmacopeial Articles..... 8172
⟨1228⟩ Depyrogenation..... 8176
⟨1228.1⟩ Dry Heat Depyrogenation..... 8181
⟨1228.3⟩ Depyrogenation by Filtration..... 8184
⟨1228.4⟩ Depyrogenation by Rinsing..... 8187
⟨1228.5⟩ Endotoxin Indicators for Depyrogenation..... 8190
⟨1229⟩ Sterilization of Compendial Articles..... 8194
⟨1229.1⟩ Steam Sterilization by Direct Contact..... 8199
⟨1229.2⟩ Moist Heat Sterilization of Aqueous Liquids..... 8202
⟨1229.3⟩ Monitoring of Bioburden..... 8206

www.webofpharma.com

GENERAL INFORMATION *(continued)*

⟨1229.4⟩ Sterilizing Filtration of Liquids 8209
⟨1229.5⟩ Biological Indicators for Sterilization 8216
⟨1229.6⟩ Liquid-Phase Sterilization 8219
⟨1229.7⟩ Gaseous Sterilization 8222
⟨1229.8⟩ Dry Heat Sterilization 8225
⟨1229.9⟩ Physicochemical Integrators and Indicators for Sterilization 8227
⟨1229.10⟩ Radiation Sterilization 8228
⟨1229.11⟩ Vapor Phase Sterilization 8231
⟨1229.12⟩ New Sterilization Methods 8233
⟨1229.13⟩ Sterilization-In-Place 8233
⟨1229.14⟩ Sterilization Cycle Development 8235
⟨1229.15⟩ Sterilizing Filtration of Gases 8238
⟨1229.16⟩ Prion Inactivation 8239
⟨1230⟩ Water for Hemodialysis Applications 8240
⟨1231⟩ Water for Pharmaceutical Purposes 8242
⟨1234⟩ Vaccines for Human Use-Polysaccharide and Glycoconjugate Vaccines 8276
⟨1235⟩ Vaccines for Human Use—General Considerations 8291
⟨1236⟩ Solubility Measurements 8306
⟨1237⟩ Virology Test Methods 8319
⟨1238⟩ Vaccines for Human Use—Bacterial VaccineS 8338
⟨1240⟩ Virus Testing of Human Plasma for Further Manufacture 8350
⟨1241⟩ Water-Solid Interactions in Pharmaceutical Systems 8359
⟨1251⟩ Weighing on an Analytical Balance 8363
⟨1265⟩ Written Prescription Drug Information—Guidelines 8368
⟨1285⟩ Preparation of Biological Specimens for Histologic and Immunohistochemical Analysis 8370
⟨1285.1⟩ Hematoxylin and Eosin Staining Of Sectioned Tissue for Microscopic Examination 8374
⟨1430⟩ Analytical Methodologies Based on Scattering Phenomena —General 8376
⟨1430.1⟩ Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering 8379
⟨1430.2⟩ Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size 8386
⟨1403.4⟩ Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential) 8389
⟨1430.5⟩ Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering 8392
⟨1467⟩ Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures 8404
⟨1601⟩ Products for Nebulization—Characterization Tests 8407
⟨1602⟩ Spacers and Valved Holding Chambers Used With Inhalation Aerosols—Characterization Tests 8411
⟨1644⟩ Theory and Practice of Electrical Conductivity Measurements of Solutions 8423
⟨1660⟩ Evaluation of The Inner Surface Durability Of Glass Containers 8429
⟨1661⟩ Evaluation of Plastic Packaging Systems and Their Materials of Construction With Respect to Their User Safety Impact 8434
⟨1663⟩ Assessment of Extractables Associated With Pharmaceutical Packaging/Delivery Systems 8442

GENERAL INFORMATION *(continued)*

⟨1664⟩ Assessment of Drug Product Leachables Associated With Pharmaceutical Packaging/Delivery Systems 8455
⟨1664.1⟩ Orally Inhaled and Nasal Drug Products 8467
⟨1724⟩ Semi-Solid Drug Products—Performance Tests 8473
⟨1730⟩ Plasma Spectrochemistry—Theory and Practice 8484
⟨1735⟩ X-Ray Fluorescence Spectrometry—Theory and Practice 8490
⟨1736⟩ Applications of Mass Spectrometry 8508
⟨1761⟩ Applications of Nuclear Magnetic Resonance Spectroscopy 8529
⟨1771⟩ Ophthalmic Products—Performance Tests 8549
⟨1782⟩ Vibrational Circular Dichroism Spectroscopy—Theory and Practice 8549
⟨1787⟩ Measurement of Subvisible Particulate Matter In Therapeutic Protein Injections 8562
⟨1788⟩ Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions 8575
⟨1790⟩ Visual Inspection of Injections 8587
⟨1821⟩ Radioactivity—Theory and Practice 8604
⟨1823⟩ Positron Emission Tomography Drugs-Information 8617
⟨1850⟩ Evaluation of Screening Technologies for Assessing Medicine Quality 8626
⟨1852⟩ Atomic Absorption Spectroscopy—Theory and Practice 8632
⟨1853⟩ Fluorescence Spectroscopy—Theory and Practice 8641
⟨1854⟩ Mid-Infrared Spectroscopy—Theory and Practice 8650
⟨1856⟩ Near-Infrared Spectroscopy—Theory and Practice 8658
⟨1857⟩ Ultraviolet-Visible Spectroscopy—Theory and Practice 8670
⟨1911⟩ Rheometry 8679

DIETARY SUPPLEMENTS

⟨2021⟩ Microbial Enumeration Tests—Nutritional and Dietary Supplements 8687
⟨2022⟩ Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements 8692
⟨2023⟩ Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements 8698
⟨2030⟩ Supplemental Information for Articles of Botanical Origin 8702
⟨2040⟩ Disintegration and Dissolution of Dietary Supplements 8711
⟨2091⟩ Weight Variation of Dietary Supplements 8718
⟨2232⟩ Elemental Contaminants in Dietary Supplements 8719
⟨2250⟩ Detection of Irradiated Dietary Supplements 8722
⟨2251⟩ Screening for Undeclared Drugs and Drug Analogues 8725
⟨2750⟩ Manufacturing Practices for Dietary Supplements 8742

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Official Monographs for USP 43

Juniper Tar

DEFINITION

Juniper Tar is the empyreumatic volatile oil obtained from the woody portions of *Juniperus oxycedrus* L. (Fam. Pinaceae).

IDENTIFICATION

• **A.**

Sample solution: Shake 1 volume of Juniper Tar with 20 volumes of warm water, filter, and use the filtrate.

Analysis: To 5 mL of the cold *Sample solution* add a few drops of silver–ammonium nitrate TS.

Acceptance criteria: A black color is produced.

• **B.**

Sample solution: Shake 1 volume of Juniper Tar with 20 volumes of warm water, filter, and use the filtrate.

Analysis: To 5 mL of the *Sample solution* add a few drops of alkaline cupric tartrate TS, and heat the solution to boiling.

Acceptance criteria: A red precipitate is formed.

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩**:** 0.950–1.055

• **REACTION:** The filtrate prepared for *Identification* test *A* is acid to litmus.

• **ROSIN OR ROSIN OILS**

Sample solution: Triturate 1 mL of Juniper Tar with 15 mL of solvent hexane, and filter.

Analysis: Mix equal volumes of *Sample solution* and cupric acetate solution (10 mg/mL), shake vigorously, and allow the liquids to separate. Decant the solvent hexane layer into a test tube, and add an equal volume of ether.

Acceptance criteria: The liquid does not become dark green or blackish.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

Kanamycin Injection

DEFINITION

Kanamycin Injection contains an amount of kanamycin sulfate equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$). It contains suitable buffers and preservatives.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: 1 mg/mL of kanamycin from Injection in water

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, heated at 110° for 1 h and cooled immediately before use

Application volume: 10 µL

Developing solvent system: 150 mg/mL of monobasic potassium phosphate in water

Spray reagent: 10 mg/mL of ninhydrin in butyl alcohol

Analysis: Proceed as directed in the chapter. Allow the spots to dry, and develop in a chamber previously equilibrated for 18 h with the *Developing solvent system*. Remove the plate from the chamber, and air-dry. Spray the plate with *Spray reagent*, and dry at 110° for 10 min.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the kanamycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: 0.115 N sodium hydroxide solution

System suitability solution: 20 µg/mL of USP Amikacin RS and 8 µg/mL of USP Kanamycin Sulfate RS in water

Standard solution: 8 µg/mL of USP Kanamycin Sulfate RS in water

Sample solution: Nominally 6 µg/mL of kanamycin from Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Electrochemical

Mode: Integrated amperometric

Range: 300 nC

Output: 1 V full-scale

Electrodes

Indicator: Gold

Reference: pH silver–silver chloride

Waveform: See *Table 1*.

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.04	—
0.30	+0.04	Begin
0.50	+0.04	End
0.51	+0.80	—
0.70	+0.80	—
0.71	−0.80	—
0.90	−0.80	—

Columns

Guard: Packing L47

Analytical: 4-mm × 25-cm; packing L47

Flow rate: 0.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

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[NOTE—The relative retention times for kanamycin and amikacin are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Kanamycin Sulfate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of kanamycin in the *Sample solution* (µg/mL)

P = potency of kanamycin in USP Kanamycin Sulfate RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.5–5.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.67 USP Endotoxin Unit/mg of kanamycin
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

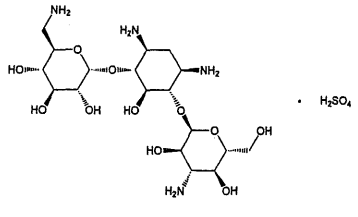
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Amikacin RS

USP Kanamycin Sulfate RS

Kanamycin Sulfate



$C_{18}H_{36}N_4O_{11} \cdot H_2SO_4$ 582.58

D-Streptamine, *O*-3-amino-3-deoxy-α-D-glucopyranosyl(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl(1→4)]-2-deoxy-, sulfate (1:1) (salt); Kanamycin sulfate (1:1) (salt) [25389-94-0].

DEFINITION

Kanamycin Sulfate has a potency equivalent to NLT 750 µg/mg of kanamycin ($C_{18}H_{36}N_4O_{11}$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 0.115 N sodium hydroxide solution

System suitability solution: 20 µg/mL of USP Amikacin RS and 8 µg/mL of USP Kanamycin Sulfate RS in water

Standard solution: 8 µg/mL of USP Kanamycin Sulfate RS in water

Sample solution: 8 µg/mL of Kanamycin Sulfate in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Pulsed amperometric electrochemical detector

Working electrode: Gold

Reference electrode: pH silver–silver chloride

Waveform: See *Table 1*.

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.04	—
0.30	+0.04	Begin
0.50	+0.04	End
0.51	+0.80	—
0.70	+0.80	—
0.71	−0.80	—
0.90	−0.80	—

Columns

Guard: 4-mm × 50-mm; 7.5-µm packing L47

Analytical: 4-mm × 25-cm; 7.5-µm packing L47

Flow rate: 0.5 mL/min

Injection volume: 20 µL

System suitability

[NOTE—The relative retention times for kanamycin and amikacin are about 1.0 and 1.3, respectively.]

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of kanamycin ($C_{18}H_{36}N_4O_{11}$) in the portion of Kanamycin Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

USP Monographs

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r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Kanamycin Sulfate RS in the *Standard solution* (µg/mL)
C_U = concentration of Kanamycin Sulfate in the *Sample solution* (µg/mL)
P = potency of kanamycin in USP Kanamycin Sulfate RS (µg/mg)

Acceptance criteria: NLT 750 µg/mg on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Analysis: Moisten the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.
Acceptance criteria: NMT 1.0%

• **ORGANIC IMPURITIES**
Adsorbent: 0.25-mm layer of chromatographic silica gel
Developing solvent system: 75 mg/mL of monobasic potassium phosphate in water
Spray reagent: 10 mg/mL of ninhydrin in butyl alcohol
Standard solution 1: 30 mg/mL of USP Kanamycin Sulfate RS in water
Standard solution 2: 0.90 mg/mL of USP Kanamycin Sulfate RS in water
Sample solution: 30 mg/mL of Kanamycin Sulfate in water
Application volume: 1 µL
Analysis: Heat the plate at 110° for 1 h immediately before use, and allow it to cool. Equilibrate for 90 min with the *Developing solvent system.*
Apply all three solutions to the plate separately, allow the spots to dry, and develop the chromatogram with the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, and air-dry. Spray the plate with *Spray reagent.* Dry the plate at 110° for 10 min, and examine the chromatograms.
Acceptance criteria: The chromatograms show principal spots at the same R_F value, and no secondary spot, if present from the *Sample solution,* is more intense than the principal spot of *Standard solution 2.*

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements

• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 6.5–8.5

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry 100 mg in a vacuum in a capillary-stoppered bottle at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 4.0%

• **STERILITY TESTS** ⟨71⟩**:** Where the label states that Kanamycin Sulfate is sterile, it meets the requirements when tested as directed for membrane filtration in *Test for Sterility of the Product to Be Examined.*

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Kanamycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.67 USP Endotoxin Unit/mg of Kanamycin.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amikacin RS
USP Kanamycin Sulfate RS

Kanamycin Sulfate Capsules

DEFINITION
Kanamycin Sulfate Capsules contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Sample solution: 1 mg/mL of kanamycin from Capsules in water
Chromatographic system
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, heated at 110° for 1 h and cooled immediately before use
Application volume: 10 µL
Developing solvent system: 150 mg/mL of monobasic potassium phosphate solution
Spray reagent: 10 mg/mL of ninhydrin in butyl alcohol
Analysis: Proceed as directed in the chapter. Allow the spots to dry, and develop in a suitable chamber, previously equilibrated for 18 h with the *Developing solvent system.* Remove the plate from the chamber, and air-dry. Spray the plate with *Spray reagent,* and dry at 110° for 10 min.
Acceptance criteria: Meet the requirements

• **B.** The retention time of the kanamycin peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**
Mobile phase: 0.115 N sodium hydroxide solution
System suitability solution: 20 µg/mL of USP Amikacin RS and 8 µg/mL of USP Kanamycin Sulfate RS
Standard solution: 8 µg/mL of USP Kanamycin Sulfate RS
Sample stock solution: Nominally 0.32 mg/mL of kanamycin, prepared as follows. Transfer a portion of the mixed contents of Capsules (NLT 10) to a suitable volumetric flask. Add water, using 20% of the final volume, and swirl to dissolve. Dilute with water to volume.
Sample solution: Nominally 6.4 µg/mL of kanamycin from the *Sample stock solution* in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Electrochemical
Mode: Integrated amperometric
Range: 300 nC
Output: 1 V full-scale
Electrodes
Indicator: Gold
Reference: pH silver–silver chloride
Waveform: See *Table 1.*

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.04	—
0.30	+0.04	Begin
0.50	+0.04	End

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Table 1 *(continued)*

Time (s)	Potential (V)	Integration
0.51	+0.80	—
0.70	+0.80	—
0.71	−0.80	—
0.90	−0.80	—

Columns
Guard: Packing L47
Analytical: 4-mm × 25-cm; packing L47
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for kanamycin and amikacin are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Kanamycin Sulfate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of kanamycin in the *Sample solution* (µg/mL)
P = potency of kanamycin in USP Kanamycin Sulfate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Kanamycin Sulfate RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Analysis: Determine the percentage of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$) dissolved by using the procedure described in the *Assay*, making any necessary modifications.
Tolerances: NLT 75% (*Q*) of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$) is dissolved.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.
Acceptance criteria: NMT 4.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amikacin RS
USP Kanamycin Sulfate RS

Kaolin

DEFINITION
Kaolin is a native hydrated aluminum silicate, powdered and freed from gritty particles by elutriation.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
Sample solution: Mix 1 g of Kaolin with 10 mL of water and 5 mL of sulfuric acid in a porcelain dish. Evaporate the mixture until the excess water is removed, and continue heating the residue until dense, white fumes of sulfur trioxide appear. Cool, cautiously add 20 mL of water, boil for a few min, and filter.
Acceptance criteria: A gray residue (impure silica) remains on the filter, and the filtrate meets the requirements of the test.

IMPURITIES
• **LOSS ON IGNITION** ⟨733⟩
Analysis: Ignite between 550° and 600°.
Acceptance criteria: NMT 15.0%
• **LEAD** ⟨251⟩
Test preparation: Transfer 1.0 g of Kaolin to a centrifuge tube, add 10 mL of 1 N nitric acid, and digest for 1 h in a boiling water bath. Centrifuge until the solids are completely separated, and pour the supernatant into a 100-mL volumetric flask. Add 5 mL of 1 N nitric acid, and digest for 15 min in a boiling water bath. Centrifuge, and add the supernatant to the previous extract in the volumetric flask. Dilute with water to volume.
Analysis: Proceed as directed in the chapter, using a 50-mL portion of this solution, 3 mL of *Ammonium Citrate Solution*, 500 µL of *Hydroxylamine Hydrochloride Solution*, and 1 mL of *Potassium Cyanide Solution*.
Acceptance criteria: NMT 5 µg of lead (NMT 10 ppm)
• **IRON**
Sample: 2.0 g
Analysis: Triturate the *Sample* in a mortar with 10 mL of water, and add 0.50 g of sodium salicylate.
Acceptance criteria: The mixture does not acquire more than a slight reddish tint.
• **ACID-SOLUBLE SUBSTANCES**
Sample: 1.0 g
Analysis: Digest the *Sample* with 20 mL of 3 N hydrochloric acid for 15 min, and filter.
Acceptance criteria: NMT 2.0%; 10 mL of the filtrate, evaporated to dryness and ignited, leaves NMT 10 mg of residue.
• **CARBONATE**
Sample: 1.0 g
Analysis: Mix the *Sample* with 10 mL of water and 5 mL of sulfuric acid.
Acceptance criteria: No effervescence occurs.

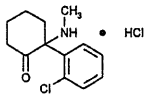
SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

USP Monographs

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Ketamine Hydrochloride



$C_{13}H_{16}ClNO \cdot HCl$ 274.19

Cyclohexanone, 2-(2-chlorophenyl)-2-(methylamino)-, hydrochloride.

(±)-2-(*o*-Chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride [1867-66-9].

» Ketamine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{16}ClNO \cdot HCl$.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Ketamine Hydrochloride RS
USP Ketamine Related Compound A RS
1-[(2-Chlorophenyl)(methylimino)methyl] cyclopentanol.
$C_{13}H_{16}NOCl$ 237.73

Clarity and color of solution—Dissolve 1 g in 5 mL of water: the solution is clear and colorless.

Identification—

Change to read:

A: ▲Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K▲ (CN 1-May-2020)—Do not dry specimens.

B: Acid solvent—The UV absorption spectrum of a solution in 0.1 N hydrochloric acid (1 in 3000) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Ketamine Hydrochloride RS, concomitantly measured, and the respective absorptivities, at the wavelengths of maximum absorbance at about 269 and 276 nm, do not differ by more than 3.0%.

Basic solvent—The UV absorption spectrum of a solution in 0.01 N sodium hydroxide (1 in 1250), in a mixture of water and methanol (1 in 20), exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Ketamine Hydrochloride RS, concomitantly measured, and the respective absorptivities, at the wavelength of maximum absorbance at about 302 nm, do not differ by more than 3.0%.

pH ⟨791⟩**:** between 3.5 and 4.1, in a solution (1 in 10).

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Related compounds—

Mobile phase—Dissolve 0.95 g of sodium 1-hexanesulfonate in 1 L of a solution consisting of a mixture of water and acetonitrile (3:1). Add 4 mL of acetic acid, and mix.

Standard solution—Dissolve accurately weighed quantities of USP Ketamine Hydrochloride RS and USP Ketamine Related Compound A RS in *Mobile phase* (sonicate if necessary) to prepare a solution containing about 0.005 mg per mL of each compound. Prepare immediately before use.

Test solution—Transfer an accurately weighed quantity of about 50.0 mg of Ketamine Hydrochloride to a 50-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, sonicating if necessary.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.0-mm × 4.0-mm guard column with a 4.0-mm × 12.5-cm analytical column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the order of elution is ketamine hydrochloride followed by ketamine related compound A; the resolution, *R,* between these two peaks is not less than 2.0; the retention time of ketamine hydrochloride is between 3.0 and 4.5 minutes (if necessary, adjust the concentration of water and acetonitrile); and the tailing factor is not greater than 1.5.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, identify the ketamine hydrochloride and ketamine related compound A peaks, and measure the areas of the major peaks. Calculate the area percentage of each impurity, relative to ketamine hydrochloride, in the portion of Ketamine Hydrochloride taken by the formula:

$$5000(C/W)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of USP Ketamine Hydrochloride RS in the *Standard solution; W* is the weight, in mg, of Ketamine Hydrochloride taken to prepare the *Test solution; r_i* is the peak area of each individual impurity peak in the *Test solution;* and r_s is the response of the ketamine hydrochloride peak obtained from the *Standard solution.* Not more than 0.1% of ketamine related compound A is found; the response of no other unknown impurity is greater than 0.3% of the ketamine peak area; and the sum of the responses of all unknown impurity peaks is not greater than 1.0% of the ketamine peak response.

Assay—

Buffer—Dissolve 5.75 g of monobasic ammonium phosphate in 1000 mL of water. Add 6 mL of triethylamine, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer* and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Transfer about 12.5 mg each, of USP Ketamine Hydrochloride RS and USP Ketamine Related Compound A RS, both accurately weighed, to a 50-mL volumetric flask, dissolve in *Mobile phase* with the aid of sonification if necessary, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of the solution so obtained to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Standard preparation—Transfer about 10 mg of USP Ketamine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add about 20 mL of *Mobile phase,* and sonicate to dissolve. Dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer about 20 mg of Ketamine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, add about 35 mL of *Mobile phase,* and sonicate to dissolve. Dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the order of elution is ketamine followed by ketamine related compound A; the resolution, *R,* between ketamine and ketamine related compound A is not less than 2.0; the column efficiency determined from the ketamine peak is not less than 9400 theoretical plates; and the tailing factor determined from the

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ketamine peak is not more than 1.6. Chromatograph the *Standard preparation,* and record the ketamine peak response as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 0.6%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{16}ClNO \cdot HCl$ in the portion of Ketamine Hydrochloride taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ketamine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the ketamine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ketamine Hydrochloride Injection

» Ketamine Hydrochloride Injection is a sterile solution of Ketamine Hydrochloride in Water for Injection. It contains an amount of ketamine hydrochloride ($C_{13}H_{16}ClNO \cdot HCl$) equivalent to not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ketamine ($C_{13}H_{16}ClNO$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light and heat.

USP Reference standards ⟨11⟩—
USP Ketamine Hydrochloride RS

Identification—

A: The UV absorption spectrum, measured in the region between 250 and 350 nm, of a dilution of Injection in 0.01 N methanolic sodium hydroxide containing ketamine hydrochloride equivalent to about 800 µg of ketamine per mL, exhibits maxima and minima at the same wavelengths as that of a similar preparation of USP Ketamine Hydrochloride RS, concomitantly measured.

B: The UV absorption spectrum of the solution employed for measurement of absorbance of the assay solution, prepared as directed in the *Assay,* exhibits maxima and minima at the same wavelengths as that of the Standard solution, prepared as directed in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.4 USP Endotoxin Unit per mg of ketamine hydrochloride.

pH ⟨791⟩: between 3.5 and 5.5.

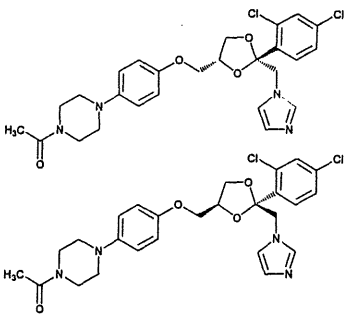
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of ketamine hydrochloride, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 20.0 mL of this solution to a 125-mL separator, add 3 mL of 0.1 N sodium hydroxide, and extract with three 15-mL portions of chloroform. Collect the chloroform extracts in a second 125-mL separator, and extract with three 30-mL portions of 0.1 N sulfuric acid, collecting the acid extracts in a 200-mL volumetric flask. Dilute with 0.1 N sulfuric acid (saturated with chloroform) to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Ketamine Hydrochloride RS in the same medium having a known concentration of about 250 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 269 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid (saturated with chloroform) as the blank. Calculate the quantity, in mg, of ketamine ($C_{13}H_{16}ClNO$) in each mL of the Injection taken by the formula:

$$(237.73/274.19)(2C/V)(A_U/A_S)$$

in which 237.73 and 274.19 are the molecular weights of ketamine and ketamine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Ketamine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Ketoconazole



$C_{26}H_{28}Cl_2N_4O_4$ 531.43

Piperazine, 1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-, *cis*-;
(±)-*cis*-1-Acetyl-4-(*p*-[[2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl)piperazine [65277-42-1].

DEFINITION

Ketoconazole contains NLT 98.0% and NMT 102.0% of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: 3.4 mg/mL of tetrabutyl ammonium hydrogen sulfate in water
Solution A: Acetonitrile and *Buffer* (5:95)
Solution B: Acetonitrile and *Buffer* (50:50)
Mobile phase: See *Table 1.*

USP Monographs

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	0	100
25	0	100
26	100	0
30	100	0

Diluent: Methanol
Standard solution: 0.1 mg/mL of USP Ketoconazole RS in *Diluent*
Sample solution: 0.1 mg/mL of Ketoconazole in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) in the portion of Ketoconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ketoconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Ketoconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩
Sample: 2 g
Acceptance criteria: NMT 0.1%
• **Organic Impurities**
Buffer, Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.01 mg/mL each of USP Ketoconazole RS and USP Terconazole RS in *Diluent*
Sample solution 10.0 mg/mL of Ketoconazole in *Diluent*
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between ketoconazole and terconazole peaks
Relative standard deviation: NMT 5.0% for the ketoconazole peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any impurities in the portion of Ketoconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurities from the *Sample solution*
r_S = peak response of Ketoconazole from the *Standard solution*
C_S = concentration of USP Ketoconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Ketoconazole in the *Sample solution* (mg/mL)

Acceptance criteria: Disregard any peak less than 0.05%.
Any individual unspecified impurity: NMT 0.10%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Sample solution: 40 mg/mL in methanol
Acceptance criteria: −1° to +1° at 20°
• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum at 80° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Ketoconazole RS
USP Terconazole RS
Piperazine, 1-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)-, *cis*-;
cis-1-(*p*-{[2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)-4-isopropylpiperazine.
$C_{26}H_{31}Cl_2N_5O_3$ 532.46

USP Monographs

Ketoconazole Compounded Oral Suspension

DEFINITION
Ketoconazole Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$).
Prepare Ketoconazole Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Ketoconazole	2.0 g
Cetylpyridinium Chloride	10 mg
Xanthan Gum	0.15 g
Purified Water	30 mL
Suspension Structured Vehicle or Sugar-Free Suspension Structured Vehicle, a sufficient quantity to make	100 mL

Place the required number of tablets in a glass mortar, and comminute to a fine powder such that they pass through a 40-mesh or 45-mesh sieve, or add *Ketoconazole* powder to the mortar. Dissolve the *Cetylpyridinium Chloride* in *Purified Water*, and dilute quantitatively, and stepwise if necessary, with *Purified Water* to obtain 10 mL of a solution containing 10 mg of *Cetylpyridinium Chloride*. Transfer this solution, in divided portions, to the mortar containing the powder, and mix to form a smooth paste. Place 20 mL of *Purified Water* in a beaker. Using moderate heat, stir to form a vortex, and slowly sprinkle the *Xanthan Gum* into the vortex

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to obtain a uniform dispersion. Add the dispersion to the wetted powder paste, and mix until smooth. Add a sufficient quantity of the *Suspension Structured Vehicle* or *Sugar-Free Suspension Structured Vehicle* to bring to final volume. Mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (25:75). Pass through a filter of 5-µm or finer pore size, and degas.

Diluent: Methanol and water (50:50)

System suitability solution: Dissolve 4 mg of USP Ketoconazole RS in 1.0 mL of a solution of potassium sorbate in water (1 in 5000). Dilute with *Diluent* to 10.0 mL.

Standard solution: 0.4 mg/mL of USP Ketoconazole RS in *Diluent*

Sample solution: [NOTE—If the Oral Suspension has settled, invert the container 10–15 times, and sonicate for 5 min, or stir on a magnetic stirrer until the Oral Suspension appears homogeneous. Examine the mixture for the presence of bubbles and unsuspended solids before sampling.] Transfer 5.0 mL of homogeneous Oral Suspension to a 250-mL volumetric flask, add 100 mL of water, and stir for 15 min to dissolve the xanthan gum. Add 135 mL of methanol, and stir for an additional 15 min. Cool, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Columns

Guard: 5-µm packing L1

Analytical: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ketoconazole and sorbate are 1.0 and 1.7, respectively.]

Suitability requirements

Resolution: NLT 2.0 between sorbate and ketoconazole, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ketoconazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketoconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.

• **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored at controlled room temperature

• **LABELING:** Label it to state that it is to be well shaken before use, and that it is to be protected from light. Label it to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ketoconazole RS

Ketoconazole Tablets

DEFINITION

Ketoconazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$).

IDENTIFICATION

• **A.**

Standard solution: 1 mg/mL of USP Ketoconazole RS in chloroform

Sample solution: Nominally 1 mg/mL of ketoconazole in chloroform prepared as follows. Transfer a quantity of finely powdered Tablets, equivalent to 50 mg of ketoconazole, to a suitable flask. Add 50 mL of chloroform, shake for 2 min, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: *n*-Hexane, ethyl acetate, methanol, glacial acetic acid, and water (42:40:15:1:2)

Analysis: Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and view under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Solution A: Diisopropylamine in methanol (1 in 500)

Solution B: 5 mg/mL of ammonium acetate in water

Mobile phase: *Solution A* and *Solution B* (7:3)

Diluent: Methanol and methylene chloride (1:1)

Internal standard solution: 5 mg/mL of USP Terconazole RS in *Diluent*

Standard solution: 0.4 mg/mL of USP Ketoconazole RS in *Diluent* prepared as follows. Transfer 20 mg of USP Ketoconazole RS to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent*.

Sample stock solution: Nominally 4 mg/mL of ketoconazole in *Diluent* prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer the nominal equivalent to 200 mg of ketoconazole to a suitable screw-capped bottle. Add 50.0 mL of *Diluent*, shake by mechanical means for 30 min, and centrifuge.

Sample solution: Nominally 0.4 mg/mL of ketoconazole in *Diluent* prepared as follows. Transfer 5.0 mL of the clear supernatant so obtained from the *Sample stock solution* to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 3 mL/min

Supplement 1

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Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ketoconazole and terconazole are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between ketoconazole and terconazole
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

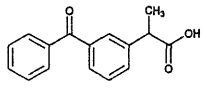
R_U = peak response ratio of ketoconazole to terconazole from the *Sample solution*
R_S = peak response ratio of ketoconazole to terconazole from the *Standard solution*
C_S = concentration of USP Ketoconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ketoconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Analytical wavelength: UV 270 nm
Standard solution: USP Ketoconazole RS in *Medium*
Sample solutions: Pass portions of the solution under test through a suitable filter of 0.45-µm pore size, and dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ketoconazole RS
USP Terconazole RS

Ketoprofen



$C_{16}H_{14}O_3$ 254.28
Benzeneacetic acid, 3-benzoyl-α-methyl-, (±)-; (±)-*m*-Benzoylhydratropic acid [22071-15-4].

DEFINITION
Ketoprofen contains NLT 98.5% and NMT 101.0% of $C_{16}H_{14}O_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelength: 258 nm
Medium: Methanol and water (3:1)
Blank: *Medium*
Sample solution: 10 µg/mL in *Medium*
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY
• **PROCEDURE**
Sample: 450 mg
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 25 mL of alcohol. Add 25 mL of water and several drops of phenol red TS. Perform a blank determination. Each mL of *Titrant* is equivalent to 25.43 mg of $C_{16}H_{14}O_3$. [NOTE—Standardize the 0.1 N sodium hydroxide by a similar titration of primary standard benzoic acid.]
Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
• **ORGANIC IMPURITIES**
[NOTE—Protect the solutions from light.]
Buffer: 68 g/L of monobasic potassium phosphate in water, and adjust with phosphoric acid to a pH of 3.5 ± 0.05
Mobile phase: Acetonitrile, water, and *Buffer* (43:55:2)
System suitability solution: 5 µg/mL of USP Ketoprofen RS and 1.5 µg/mL of USP Ketoprofen Related Compound D RS in *Mobile phase*
Standard solution: 0.002 mg/mL of USP Ketoprofen RS in *Mobile phase*
Sample solution: 1 mg/mL of Ketoprofen in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 233 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: Seven times the retention time for Ketoprofen
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for ketoprofen and ketoprofen related compound D (3-acetylbenzophenone) are about 1.0 and 1.6, respectively.]
Suitability requirements
Resolution: NLT 7.0 between ketoprofen related compound D and ketoprofen, *System suitability solution*
Column efficiency: NLT 2250 theoretical plates from ketoprofen, *System suitability solution*
Tailing factor: NMT 2.0 for ketoprofen, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Ketoprofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of impurity from the *Sample solution*
r_S = peak response of ketoprofen from the *Standard solution*
C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria
Any individual impurity: NMT 0.2%
Total impurities: NMT 1.0%

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE,** *Procedure for Class I* ⟨741⟩: 92.0°–97.0°
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL, in dehydrated alcohol
Acceptance criteria: +1° to −1°
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample in a vacuum at 60° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ketoprofen RS
USP Ketoprofen Related Compound D RS
3-Acetylbenzophenone.

Ketoprofen Capsules

DEFINITION
Ketoprofen Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
Sample solution: Shake a quantity of the contents of the Capsules containing 50 mg of ketoprofen with 5 mL of chloroform for 5 min, filter, and evaporate to dryness using a rotary evaporator.
Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
The *Standard solution* and *Sample solution* must be protected from light.
Mobile phase: Acetonitrile, glacial acetic acid, and water (90:1:110)
System suitability stock solution: 0.25 mg/mL of USP Ketoprofen RS and 0.5 mg/mL of USP Ketoprofen Related Compound A RS in *Mobile phase*
System suitability solution: 0.02 mg/mL of USP Ketoprofen RS and 0.04 mg/mL of USP Ketoprofen Related Compound A RS in *Mobile phase* from *System suitability stock solution*
Standard stock solution: 0.24 mg/mL of USP Ketoprofen RS in *Mobile phase*
Standard solution: 0.024 mg/mL of USP Ketoprofen RS in *Mobile phase* from *Standard stock solution*
Sample solution: Nominally 0.024 mg/mL of ketoprofen in *Mobile phase* prepared as follows. Remove completely the contents of NLT 20 Capsules, and transfer a quantity of the contents, equivalent to 200 mg of ketoprofen, to a 250-mL volumetric flask. Add 150 mL of *Mobile phase*, stir for 2 h, then dilute with *Mobile phase* to volume. Centrifuge a portion of the preparation. Pipet 3.0 mL of clear supernatant into a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between ketoprofen and ketoprofen related compound A, *System suitability solution*
Tailing factor: NMT 1.5 for the ketoprofen peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ketoprofen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
The *Standard solution* and *Sample solution* must be protected from light.
Medium: 0.05 M phosphate buffer, pH 7.4; 1000 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: USP Ketoprofen RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 260 nm
Cell path length: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$) dissolved:

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$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (Q) of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$) is dissolved.

IMPURITIES

• **ORGANIC IMPURITIES**

The *System suitability solution*, *Standard solution*, and *Sample solution* must be protected from light.

Buffer: 68.0 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5 ± 0.05.

Mobile phase: Acetonitrile, water, and *Buffer* (43:55:2)

Diluent: Acetonitrile and water (2:3)

System suitability solution: 5 µg/mL of USP Ketoprofen RS and 1.5 µg/mL of USP Ketoprofen Related Compound D RS in *Diluent*

Standard solution: 2 µg/mL of USP Ketoprofen RS, 2 µg/mL of USP Ketoprofen Related Compound C RS, and 3 µg/mL of USP Ketoprofen Related Compound D RS in *Diluent*

Sample solution: Nominally 1 mg/mL of ketoprofen in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 233 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 7 times the retention time of ketoprofen

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 7.0 between ketoprofen related compound D and ketoprofen

Relative standard deviation: NMT 10% for the ketoprofen peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the corresponding related compound from the *Standard solution*
C_S = concentration of the corresponding USP Ketoprofen Related Compound RS in the *Standard solution* (mg/mL); use the concentration of the USP Ketoprofen RS for unknown impurities
C_U = nominal concentration of ketoprofen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ketoprofen related compound C[a]	0.3	0.2
Ketoprofen	1.0	—
Ketoprofen related compound D[b]	1.5	0.3
Individual unspecified impurity	—	0.2
Total impurities	—	0.5

[a] 2-(3-Carboxyphenyl) propionic acid.
[b] 3-Acetylbenzophenone.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ketoprofen RS
(±)-*m*-Benzoylhydratropic acid.
$C_{16}H_{14}O_3$ 254.28

USP Ketoprofen Related Compound A RS
α-Methyl-3-(4-methylbenzoyl) benzeneacetic acid.
$C_{17}H_{16}O_3$ 268.31

USP Ketoprofen Related Compound C RS
2-(3-Carboxyphenyl) propionic acid.
$C_{10}H_{10}O_4$ 194.18

USP Ketoprofen Related Compound D RS
3-Acetylbenzophenone.
$C_{15}H_{12}O_2$ 224.25

Ketoprofen Extended-Release Capsules

DEFINITION

Ketoprofen Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩*Ultraviolet-Visible Spectroscopy*: **197U.**▲ (CN 1-May-2020)

The UV spectrum from the *Sample solution* in the *Analysis* for the *Dissolution* section corresponds to the spectrum from the *Standard solution*.

ASSAY

• **PROCEDURE**

[NOTE—Protect the *Standard solution* and *Sample solution* from light.]

Mobile phase: Acetonitrile, water, and glacial acetic acid (90:110:1)

Standard stock solution: 0.24 mg/mL of USP Ketoprofen RS in *Mobile phase*

Standard solution: 0.024 mg/mL of USP Ketoprofen RS in *Mobile phase*, from the *Standard stock solution*

System suitability solution: 0.25 mg/mL of USP Ketoprofen RS and 0.5 mg/mL of USP Ketoprofen Related Compound A RS in *Mobile phase*. Pipet 4.0 mL of this solution into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Remove completely the contents of NLT 20 Capsules, and transfer a quantity of the beads, equal to

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200 mg of ketoprofen, to a 250-mL volumetric flask. Add 150 mL of *Mobile phase* and mix; bring to volume. Centrifuge, and pipet 3.0 mL of clear supernatant that contains about 2.4 mg of ketoprofen into a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between ketoprofen and ketoprofen related compound A, *System suitability solution*
Tailing factor: NMT 1.5 for the ketoprofen peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{14}O_3$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)
C_U = concentration of ketoprofen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 2: 50 rpm
Time: 1, 4, and 8 h
Detector: UV 258 nm
Standard solution: About 0.1 mg/mL of USP Ketoprofen RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size, then pass the filtrate through a suitable filter of 0.45-µm pore size.
Capsules labeled to contain 200 mg: In a test tube, dilute 5.0 mL of filtrate with 5.0 mL of *Medium*.
Capsules labeled to contain 150 mg: In a test tube, dilute 6.0 mL of filtrate with 3.0 mL of *Medium*.
Capsules labeled to contain 100 mg: No dilution is necessary.
Capsule blank: Place 10 empty, clean Capsules of the appropriate dosage into a 1000-mL volumetric flask. Add about 800 mL of *Medium* at 37°. Stir until Capsule shells are disintegrated. After equilibration to room temperature, dilute with *Medium* to volume. Transfer 100.0 mL to a 1000-mL volumetric flask, and dilute with *Medium* to volume. Pass through a suitable filter of 10-µm pore size, then pass the filtrate through a suitable filter of 0.45-µm pore size.
Capsules labeled to contain 200 mg: In a flask, dilute 25.0 mL with 25.0 mL of *Medium*.
Capsules labeled to contain 150 mg: In a flask, dilute 30.0 mL with 15.0 mL of *Medium*.
Capsules labeled to contain 100 mg: No dilution is necessary.

Analysis
Samples: *Standard solution*, *Sample solution*, and *Capsule blank*, using *Medium* as the blank
Calculate the concentration, in mg/mL, of ketoprofen in the sample withdrawn at each time point:

$$\text{Result} = (A_U - A_{CB}) \times (C_S/A_S)$$

A_U = absorbance of the *Sample solution*
A_{CB} = absorbance of the *Capsule blank*
C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)
A_S = absorbance of the *Standard solution*

Calculate the percentage of ketoprofen dissolved at each time point:

$$\text{Result} = (D + \Sigma R) \times 100/L$$

D = [amount dissolved (mg)] = volume (mL) remaining before draw × concentration (mg/mL) of sample withdrawn at the sampling time point
R = [amount removed (mg)] = volume (mL) of sample withdrawn × concentration (mg/mL) of sample withdrawn at each time point
100 = conversion factor for percentage
L = Capsule label claim (mg)

Tolerances: The percentage of the labeled amount of ketoprofen released at the times specified conforms to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	10%–25%
4	55%–80%
8	NLT 80%

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements
Procedure for content uniformity:
[Note—Protect the *Standard solution* and *Sample solution* from light.]
Mobile phase, Standard solution, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Sample solution: Transfer the contents of 10 Capsules, 1 Capsule each, to each of 10 250-mL volumetric flasks, add about 150 mL of *Mobile phase* to each flask, and stir for 2 h. Dilute with *Mobile phase* to volume, and mix. Centrifuge, and pipet a volume of clear supernatant that contains about 2.4 mg of ketoprofen into a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between ketoprofen and ketoprofen related compound A, *System suitability solution*
Tailing factor: NMT 1.5 for the ketoprofen peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{14}O_3$ in each Capsule:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

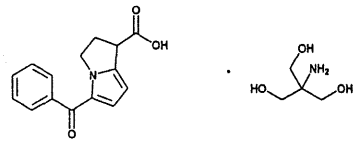
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r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)
C_U = concentration of ketoprofen in the *Sample solution* (mg/mL)

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 3.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ketoprofen RS
  USP Ketoprofen Related Compound A RS
  α-Methyl-3-(4-methylbenzoyl) benzeneacetic acid.

Ketorolac Tromethamine



$C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$ 376.40

1*H*-Pyrrolizine-1-carboxylic acid, 5-benzoyl-2,3-dihydro, (±)-, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1);
(±)-5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1) [74103-07-4].

DEFINITION
Ketorolac Tromethamine contains NLT 98.5% and NMT 101.5% of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. THIN-LAYER CHROMATOGRAPHY,** ***Tromethamine Test***

Diluent: Dichloromethane and methanol (2:1)
Standard solution: 5 mg/mL of USP Ketorolac Tromethamine RS in *Diluent*
Sample solution: 5 mg/mL of Ketorolac Tromethamine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 40 µL
Developing solvent system: Dichloromethane, acetone, and glacial acetic acid (95:5:2)
Spray reagent: Freshly prepared alcoholic solution containing 30 mg/mL of ninhydrin
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Spray the plate with *Spray reagent*, and heat the plate at about 150° for 2–5 min.
Acceptance criteria: Yellow spots with pink to purple borders develop on the plate in the areas where the *Standard solution* and the *Sample solution* were applied.

ASSAY
- **PROCEDURE**

Protect all the solutions from light.
Buffer: 5.75 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Tetrahydrofuran and *Buffer* (30:70)
Diluent: Tetrahydrofuran and water (30:70)
System suitability solution: In a 250-mL separator, mix 100 mL of water, 100 mL of dichloromethane, 30 mg of USP Ketorolac Tromethamine RS, and 1 mL of 1 N hydrochloric acid. Insert the stopper, shake, and allow the layers to separate. Transfer the lower dichloromethane layer to a stoppered borosilicate glass flask, and discard the upper layer. Expose the dichloromethane solution to direct sunlight for 10–15 min. Transfer 1.0 mL of the solution to a vial, evaporate in a current of air or in a stream of nitrogen to dryness, add 1.0 mL of *Diluent*, and swirl to dissolve. [NOTE—This solution may be stored under refrigeration and used as long as the chromatogram obtained as directed for *Analysis* is suitable for identifying the peaks due to the ketorolac 1-keto analog and ketorolac 1-hydroxy analog, and for the measurement of the resolution between the ketorolac 1-keto analog and ketorolac.]
Standard solution: 0.4 mg/mL of USP Ketorolac Tromethamine RS in *Diluent*
Sample solution: 0.4 mg/mL of Ketorolac Tromethamine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 313 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for the ketorolac 1-hydroxy analog, the ketorolac 1-keto analog, and ketorolac are about 0.63, 0.89, and 1.0, respectively. Make adjustments, if necessary, to achieve a retention time for ketorolac of about 8–12 min.]
Suitability requirements
Resolution: NLT 1.5 between ketorolac 1-keto analog and ketorolac, *System suitability solution*
Column efficiency: NLT 5500 theoretical plates, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in the portion of Ketorolac Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

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C_U = concentration of Ketorolac Tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Standard solution, and **Sample solution:** Proceed as directed in the *Assay*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 313 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: 3 times the retention time of ketorolac

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Ketorolac Tromethamine taken:

$$\text{Result} = (r_U/r_T) \times F \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity having a 0.54 relative retention time	0.54	2.2	0.5
Ketorolac 1-hydroxy analog	0.63	0.67	0.1
Impurity having a 0.66 relative retention time	0.66	0.91	0.5
Ketorolac 1-keto analog	0.89	0.52	0.1
Individual unspecified impurity	—	1.0	0.5
Ketorolac tromethamine	1.0	1.0	—
Total impurities	—	—	1.0

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 10 mg/mL

Acceptance criteria: 5.7–6.7

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 60° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ketorolac Tromethamine RS

Ketorolac Tromethamine Injection

DEFINITION

Ketorolac Tromethamine Injection is a sterile solution of Ketorolac Tromethamine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the ketorolac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Protect all solutions from light.]

Mobile phase: Methanol, water, and glacial acetic acid (55:44:1)

Diluent: Methanol and water (1:1)

Standard solution: 0.05 mg/mL of USP Ketorolac Tromethamine RS in *Diluent*

Sample solution: Nominally equivalent to 0.05 mg/mL of ketorolac tromethamine in *Diluent* from Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm. For *Identification* test *B*, use a diode array detector in the range of 200–600 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.2 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the ketorolac peak

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in each mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ketorolac from the *Sample solution*

r_S = peak response of ketorolac from the *Standard solution*

C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

[NOTE—Protect all solutions from light.]

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.10 mg/mL each of USP Ketorolac Tromethamine RS, USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in *Diluent* prepared as follows. Transfer USP Ketorolac Tromethamine RS, USP Ketorolac Related Compound A RS, USP Ketorolac Related

USP Monographs

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Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS to a suitable volumetric flask. Add 4% of the volume of the flask with methanol. Sonicate and dilute with *Diluent* to volume.

Standard solution: 0.2 µg/mL each of USP Ketorolac Tromethamine RS, USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in *Diluent* from the *Standard stock solution*

Sample solution: Prepare nominally equivalent to 0.2 mg/mL of ketorolac tromethamine in *Diluent*.

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2 between ketorolac related compound C and ketorolac

Relative standard deviation: NMT 2.8% for all the peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, and ketorolac related compound D in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, or ketorolac related compound D from the *Sample solution*

r_S = peak response of ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, or ketorolac related compound D from the *Standard solution*

C_S = concentration of the corresponding related compound in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of ketorolac from the *Standard solution*

C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ketorolac related compound A	0.4	0.20
Ketorolac related compound B	0.6	0.5
Ketorolac related compound C	0.8	0.5
Ketorolac	1.0	—
Ketorolac related compound D	2.1	0.20
Any unspecified impurity	—	0.20
Total impurities	—	1.50

SPECIFIC TESTS

- **pH** ⟨791⟩: 6.9–7.9
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 5.8 USP Endotoxin Units/mg of ketorolac tromethamine.
- **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, protected from light, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ketorolac Tromethamine RS

USP Ketorolac Related Compound A RS

5-Benzoyl-*N*-[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide.

$C_{19}H_{22}N_2O_5$ 358.39

USP Ketorolac Related Compound B RS

5-Benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-ol.

$C_{14}H_{13}NO_2$ 227.26

USP Ketorolac Related Compound C RS

5-Benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-one.

$C_{14}H_{11}NO_2$ 225.24

USP Ketorolac Related Compound D RS

5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine.

$C_{14}H_{13}NO$ 211.3

Ketorolac Tromethamine Tablets

DEFINITION

Ketorolac Tromethamine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the ketorolac peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, water, and glacial acetic acid (55:44:1)

Diluent: Methanol and water (1:1). [NOTE—Protect all volumetric solutions from light.]

USP Monographs

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Standard stock solution: 0.24 mg/mL of USP Ketorolac Tromethamine RS in methanol
Standard solution: 24 µg/mL of USP Ketorolac Tromethamine RS in *Diluent* from *Standard stock solution*
System suitability stock solution: 25 µg/mL each of USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in methanol
System suitability solution: 0.25 µg/mL each of USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in *Standard solution* from *System suitability stock solution*
Sample stock solution: 0.2 mg/mL of ketorolac tromethamine prepared as follows. Transfer 10 Tablets to a suitable volumetric flask. Add a quantity of water equivalent to about 10% of the volume of the flask, and sonicate until the Tablets are disintegrated. Add a quantity of methanol equivalent to 40% of the volume of the flask, and sonicate for 10 min to dissolve the ketorolac tromethamine. Cool to ambient temperature, dilute with methanol to volume, and mix. Centrifuge, or allow to settle.
Sample solution: 0.02 mg/mL of ketorolac tromethamine in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector
Assay: UV 254 nm
Identification test B: Diode array, UV 200–400 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 100 µL
Run time: 3.8 times the retention time of the ketorolac peak
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times are 0.8 for ketorolac related compound B and 1.0 for the ketorolac peaks.]
Suitability requirements
Resolution: NLT 1.5 each between the ketorolac and ketorolac related compound B, and ketorolac and ketorolac related compound C peaks, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of the ketorolac peak from the *Sample solution*
r_S = response of the ketorolac peak from the *Standard solution*
C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 600 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: USP Ketorolac Tromethamine RS in *Medium*
Sample solutions: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Instrumental conditions
Mode: UV absorption spectroscopy
Analytical wavelength: 322 nm
Tolerances: NLT 75% (*Q*) of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, Chromatographic system, and **Diluent:** Proceed as directed in the *Assay*.
Standard solution: Use the *System suitability solution*, prepared as directed in the *Assay*.
Sample solution: Proceed as directed for the *Sample solution* in the *Assay*.
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 each between the ketorolac and ketorolac related compound B, and ketorolac and ketorolac related compound C peaks
Relative standard deviation: NMT 5.0% for ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, and ketorolac related compound D
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each known impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each known impurity in the *Sample solution*
r_S = peak response of each known impurity in the *Standard solution*
C_S = concentration of each impurity in the *Standard solution* (mg/mL)
C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each individual impurity peak in the *Sample solution*
r_T = sum of responses for all the peaks in the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ketorolac related compound A	0.5	0.5

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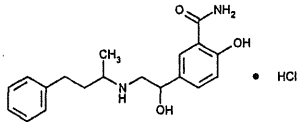
Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ketorolac related compound B	0.8	0.5
Ketorolac	1.0	—
Ketorolac related compound C	1.2	0.8
Ketorolac related compound D	2.6	0.5
Total unspecified impurity	—	0.5
Total impurities	—	1.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature, protected from light and excessive humidity.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ketorolac Tromethamine RS
  USP Ketorolac Related Compound A RS
  5-Benzoyl-*N*-[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide.
  $C_{19}H_{22}N_2O_5$ 358.39
  USP Ketorolac Related Compound B RS
  5-Benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-ol.
  $C_{14}H_{13}NO_2$ 227.26
  USP Ketorolac Related Compound C RS
  5-Benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-one.
  $C_{14}H_{11}NO_2$ 225.24
  USP Ketorolac Related Compound D RS
  5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine.
  $C_{14}H_{13}NO$ 211.26

Labetalol Hydrochloride



$C_{19}H_{24}N_2O_3 \cdot HCl$ 364.87
Benzamide, 2-hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-, monohydrochloride.
5-[1-Hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl] salicylamide monohydrochloride [32780-64-6].

» Labetalol Hydrochloride contains not less than 97.5 percent and not more than 101.0 percent of $C_{19}H_{24}N_2O_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Labetalol Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: It responds to the tests for *Chloride* ⟨191⟩.

pH ⟨791⟩**:** between 4.0 and 5.0, in a solution (1 in 100).

Loss on drying ⟨731⟩**:** Dry it in a vacuum at 105° for 4 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Chromatographic purity—

Detection reagent—Transfer 2.5 g of cadmium acetate to a 500-mL volumetric flask, add 10 mL of glacial acetic acid, dilute with alcohol to volume, and mix. Just prior to use, prepare a 0.2 in 100 solution of ninhydrin in the cadmium acetate solution for use as the *Detection reagent.*

Solvent mixture—Prepare a solution of methanol and water (4:1), and mix.

Ammonium chloride reference solution—Dissolve 60 mg of ammonium chloride in 10.0 mL of water, and mix.

Standard stock solution—Dissolve USP Labetalol Hydrochloride RS in *Solvent mixture,* and mix to obtain a solution having a known concentration of 40 mg per mL.

Standard solution 1—Quantitatively dilute a portion of the *Standard stock solution* with *Solvent mixture* to obtain a solution having a known concentration of 0.2 mg per mL.

Standard solution 2—Quantitatively dilute a portion of the *Standard solution 1* with *Solvent mixture* to obtain a solution having a known concentration of 0.1 mg per mL.

Test solution—Dissolve 200 mg of Labetalol Hydrochloride in 5.0 mL of *Solvent mixture,* and mix.

Procedure I—Apply separately 5-µL portions of the *Standard stock solution, Standard solution 1, Standard solution 2,* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of dichloromethane, methanol, and ammonium hydroxide (15:5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: the R_F value of the principal spot from the *Test solution* corresponds to that of the principal spot from the *Standard stock solution.*

Spray the plate with *Detection reagent,* heat the plate at 105° for 15 minutes, cool to room temperature, and examine the chromatogram: no individual secondary spot observed in the chromatogram of the *Test solution* is greater in size or intensity than the principal spot observed in the chromatogram of *Standard solution 1* (0.5% each). [NOTE—The spots appear as dark orange spots on a light orange to yellow background. A "negative image" spot (white) near the origin may be observed in the chromatogram of the *Test solution.* This is due to the formation of ammonium chloride during the chromatographic procedure and may be ignored.]

Procedure II—Apply separately 10-µL portions of the *Ammonium chloride reference solution,* the *Standard stock solution, Standard solution 1, Standard solution 2,* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate, isopropyl alcohol, water, and ammonium hydroxide (25:15:8:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate

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under short-wavelength UV light: no individual secondary spot (other than that due to ammonium chloride) observed in the chromatogram of the *Test solution* is greater in size or intensity than the principal spot observed in the chromatogram of *Standard solution 1* (0.5% each).

Total impurities—The sum of the intensities of all secondary spots (other than those due to ammonium chloride) observed in the chromatograms of the *Test solution* from both *Procedure I* and *Procedure II* does not exceed 1.0%.

Diastereoisomer ratio—

1-Butaneboronic acid solution—Dissolve 1-butaneboronic acid in pyridine, previously dried over a suitable molecular sieve, and mix to obtain a solution having a known concentration of 20 mg per mL.

System suitability solution—Dissolve an accurately weighed quantity of USP Labetalol Hydrochloride RS in *1-Butaneboronic acid solution,* and dilute quantitatively and stepwise with *1-Butaneboronic acid solution* to obtain a solution having a known concentration of about 1.4 mg of USP Labetalol Hydrochloride RS per mL. Allow the solution to stand at room temperature for 20 minutes before using.

Test solution—Transfer about 1 mg of Labetalol Hydrochloride to a 1-mL reaction vial, add 0.7 mL of *1-Butaneboronic acid solution,* and mix until the labetalol hydrochloride is completely dissolved. Allow the solution to stand at room temperature for 20 minutes before using.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.8-m glass column packed with 10% phase G3 on 100- to 120-mesh support S1AB. The column temperature is maintained at about 320°, and the injection port and the detector block temperatures are maintained at about 340°. Nitrogen is used as the carrier gas at the flow rate of about 30 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for the diastereoisomer B 1-butaneboronate derivative and 1.0 for the diastereoisomer A 1-butaneboronate derivative; the resolution, *R,* between the diastereoisomer A 1-butaneboronate derivative and diastereoisomer B 1-butaneboronate derivative peaks is not less than 1.5; and the relative standard deviation of the ratios of the peak areas of the diastereoisomers for replicate injections is not more than 2.0%.

Procedure—Inject about 2 µL of the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the diastereoisomer A content, in percentage, taken by the formula:

$$100r_A/(r_A + r_B)$$

in which r_A is the peak area of the diastereoisomer A 1-butaneboronate derivative peak; and r_B is the peak area of the diastereoisomer B 1-butaneboronate derivative peak. The diastereoisomer A content is not less than 45.0% and not more than 55.0%.

Assay—

Mobile phase—Prepare a suitable filtered and degassed mixture of 0.1 M monobasic sodium phosphate and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Labetalol Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer about 40 mg of Labetalol Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 20-cm column that contains packing L1 and is maintained at 60 ± 1°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 700 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$) in the portion of Labetalol Hydrochloride taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Labetalol Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Labetalol Hydrochloride Injection

» Labetalol Hydrochloride Injection is a sterile solution of Labetalol Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$).

Packaging and storage—Preserve in single-dose containers, or in multiple-dose containers not exceeding 60 mL in volume, preferably of Type I glass, at a temperature between 2° and 30°. Avoid freezing and exposure to light.

USP Reference standards ⟨11⟩—
USP Labetalol Hydrochloride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.2 USP Endotoxin Units per mg of labetalol hydrochloride.

pH ⟨791⟩: between 3.0 and 4.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a suitable filtered and degassed mixture of 0.1 M monobasic sodium phosphate and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Labetalol Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.

Resolution solution—Dissolve a quantity of methylparaben in the *Standard preparation* to obtain a solution containing about 0.08 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of labetalol hydrochloride, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

USP Monographs

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Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 20-cm column that contains packing L1 and is maintained at 60 ± 1°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 700 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for methylparaben and 1.0 for labetalol; and the resolution, *R*, between the methylparaben and labetalol is not less than 2.0.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Labetalol Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Labetalol Hydrochloride Compounded Oral Suspension

DEFINITION

Labetalol Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$).

Prepare Labetalol Hydrochloride Compounded Oral Suspension 40 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Labetalol Hydrochloride	4 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar and comminute to a fine powder, or use *Labetalol Hydrochloride* powder. Add 20 mL of the *Vehicle*, and mix to form a uniform paste. Add the *Vehicle* in small portions almost to volume. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add the *Vehicle* in portions to rinse the mortar, then add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and 0.1 M monobasic sodium phosphate (35:65). Filter, and degas.

Standard solution: 400 µg/mL of USP Labetalol Hydrochloride RS

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for labetalol hydrochloride is about 7.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.6% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Labetalol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of labetalol hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 4.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.

• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator

• **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Labetalol Hydrochloride RS

Labetalol Hydrochloride Tablets

» Labetalol Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers, at a temperature between 2° and 30°.

USP Reference standards ⟨11⟩—

USP Labetalol Hydrochloride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

USP Monographs

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Time: 45 minutes.

Procedure—Determine the amount of $C_{19}H_{24}N_2O_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 302 nm of filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Labetalol Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{19}H_{24}N_2O_3 \cdot HCl$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase , *Standard preparation,* and *Chromatographic system*—Proceed as directed in the *Assay* under *Labetalol Hydrochloride.*

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 2000 mg of labetalol hydrochloride, to a 500-mL volumetric flask, add 200 mL of water, and shake by mechanical means for 60 minutes. Dilute with water to volume, and mix. Filter the solution through a filter of 0.5 µm or finer porosity, discarding the first few mL of the filtrate. Transfer 10.0 mL of the filtrate to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$) in each Tablet taken by the formula:

$$5000(C/N)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Labetalol Hydrochloride RS in the *Standard preparation;* N is the number of Tablets taken; and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Lactase

DEFINITION

Lactase (β-D-galactoside galactohydrolase) is a hydrolytic enzyme derived from the mold *Aspergillus oryzae.* It contains NLT 30,000 USP Lactase Units/g.

[NOTE—One USP Lactase Unit is the lactase activity contained in the amount of enzyme that hydrolyzes one microequivalent of galactosidic linkage per min at a pH of 4.5 and at 37°, as directed in the *Assay* for *Lactase Activity.*]

ASSAY

- **LACTASE ACTIVITY**

Solution A: Dilute 57.5 mL of glacial acetic acid with sufficient water to make a 500-mL solution. Transfer 50 mL of the glacial acetic acid solution into a 1000-mL volumetric flask, add 11.3 mL of 4 N sodium hydroxide, and dilute with water to volume. If necessary, adjust with glacial acetic acid solution or 4 N sodium hydroxide to a pH of 4.50 ± 0.05.

Substrate solution: On the day of use, weigh 370.0 mg of *o*-nitrophenyl-β-D-galactopyranoside, and place in a 100-mL volumetric flask. Add about 50 mL of *Solution A,* swirl to dissolve, then dilute with *Solution A* to volume.

Standard solution: Transfer about 0.4 g of USP Lactase RS, accurately weighed, to a 1000-mL volumetric flask. Add about 600 mL of water, allow to stand for 15 min, swirl gently, and dilute with water to volume. Pipet 3.0 mL of this solution into a 200-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer an accurately weighed quantity of about 0.4 g of Lactase to a 1000-mL volumetric flask. Add about 600 mL of water, allow to stand for 15 min, swirl gently, and dilute with water to volume. Pipet 3.0 mL of this solution into a 200-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Analytical wavelength: 420 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Pipet 2.0 mL of *Substrate solution* into three separate test tubes labeled S, U, and B. Transfer the tubes to a thermostated water bath maintained at 37.0 ± 0.1°, and incubate for 10 min. Following the incubation, rapidly add 0.5 mL of the *Standard solution* to tube S, 0.5 mL of the *Sample solution* to tube U, and 0.5 mL of water to tube B (the reagent blank). Mix each tube on a vortex mixer for 1 s, and immediately return the tubes to the water bath, which has been maintained at 37.0 ± 0.1°. After 15 min of incubation, rapidly add 2.5 mL of a 10% sodium carbonate solution to each test tube to stop the enzyme reaction. Add 20.0 mL of water to each test tube, and mix. Concomitantly determine the absorbances of the three solutions.

Calculate the number of USP Lactase Units in the portion of Lactase taken:

$$\text{Result} = [(A_U - A_B)/(A_S - A_B)] \times P \times (W_S/W_U)$$

A_U = absorbance of the *Sample solution* (tube U)
A_B = absorbance of the reagent blank (tube B)
A_S = absorbance of the *Standard solution* (tube S)
P = potency of USP Lactase RS (USP Units/g)
W_S = weight of USP Lactase RS in the *Standard solution* (g)
W_U = weight of Lactase in the *Sample solution* (g)

Acceptance criteria: NLT 30,000 USP Lactase Units/g

IMPURITIES

- **ARSENIC** ⟨211⟩**:** NMT 3 µg/g
- **LEAD** ⟨251⟩**:** NMT 5 µg/g

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli.*
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers at room temperature.
- **LABELING:** Label it to indicate lactase activity in USP Units.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Lactase RS

Lactic Acid

Propanoic acid, 2-hydroxy-;
Lactic acid [50-21-5].

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DEFINITION

Lactic Acid is a mixture of lactic acid ($C_3H_6O_3$) and lactic acid lactate ($C_6H_{10}O_5$), equivalent to a total of NLT 88.0% and NMT 92.0%, by weight, of lactic acid ($C_3H_6O_3$). It is obtained by the lactic fermentation of sugars or is prepared synthetically. Lactic Acid obtained by fermentation of sugars is levorotatory, whereas that prepared synthetically is racemic.

[NOTE—Lactic Acid prepared by fermentation becomes dextrorotatory on dilution, which hydrolyzes L-(−)-lactic acid lactate to L-(+)-lactic acid.]

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Lactate* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Sample: 2.5 mL, accurately weighed

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Residual titration

Titrant: 1 N sodium hydroxide VS

Back-titrant: 1 N sulfuric acid VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a tared 250-mL flask, add 50.0 mL of *Titrant,* and boil the mixture for 20 min. Add phenolphthalein TS, and titrate the excess alkali in the hot solution with *Back-titrant*. Perform a blank determination. Each mL of *Titrant* is equivalent to 90.08 mg of lactic acid ($C_3H_6O_3$).

Acceptance criteria: 88.0%–92.0% (w/w)

IMPURITIES

- **CHLORIDE**

Sample solution: 1 in 100

Analysis: To 10 mL of the *Sample solution* acidified with nitric acid add a few drops of silver nitrate TS.

Acceptance criteria: No opalescence is produced immediately.

- **SULFATE**

Sample solution: 1 in 100

Analysis: To 10 mL of the *Sample solution* add 2 drops of hydrochloric acid and 1 mL of barium chloride TS.

Acceptance criteria: No turbidity is produced.

- **RESIDUE ON IGNITION** ⟨281⟩

Sample: 5 mL, accurately weighed

Acceptance criteria: NMT 3 mg (0.05%)

- **LIMIT OF CITRIC, OXALIC, PHOSPHORIC, OR TARTARIC ACID**

Sample solution: 1 in 10

Analysis: To 10 mL of the *Sample solution* add 40 mL of calcium hydroxide TS, and boil for 2 min.

Acceptance criteria: No turbidity is produced.

SPECIFIC TESTS

- **READILY CARBONIZABLE SUBSTANCES**

Sample: 5 mL

Analysis: Rinse a test tube with sulfuric acid, and allow to drain for 10 min. Add 5 mL of sulfuric acid to the test tube, carefully overlay it with the *Sample,* and maintain the tube at 15°.

Acceptance criteria: No dark color develops at the interface of the two acids within 15 min.

- **OPTICAL ROTATION,** *Angular Rotation* ⟨781A⟩: −0.05° to +0.05° for racemic Lactic Acid

- **SUGARS**

Sample: 5 drops

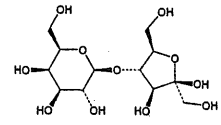
Analysis: To 10 mL of hot alkaline cupric tartrate TS add the *Sample.*

Acceptance criteria: No red precipitate is formed.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate whether it is levorotatory or racemic.

Lactulose Concentrate



$C_{12}H_{22}O_{11}$ 342.30

D-Fructose, 4-*O*-β-D-galactopyranosyl-;
4-*O*-β-D-Galactopyranosyl-D-fructofuranose [4618-18-2].

DEFINITION

Lactulose Concentrate is a solution of sugars prepared from Lactose. It consists principally of lactulose together with minor quantities of lactose and galactose, and traces of other related sugars and water. It contains NLT 95.0% and NMT 105.0% of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$). It contains no added substances.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B.**

Sample solution: Dilute a portion of Concentrate with water (1 in 20).

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A red precipitate of cuprous oxide is formed.

ASSAY

- **PROCEDURE**

Buffer: 1.15 g/L of monobasic sodium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (82:18). Ensure that the concentration of acetonitrile in the *Mobile phase* is between 78% and 85% to obtain appropriate retention times.

Standard solution: 40 mg/mL of USP Lactulose RS, 4.8 mg/mL of USP Anhydrous Lactose RS, and 3.2 mg/mL of USP Epilactose RS in a mixture of acetonitrile and water (1:1)

Sample solution: Nominally equivalent to 40 mg/mL of lactulose prepared as follows. Transfer a quantity of Concentrate containing 2.0 g of lactulose to a 50-mL volumetric flask, and dissolve in 20 mL of water. Add 25.0 mL of acetonitrile, allow the solution to reach ambient temperature, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 15-cm; 3-µm packing L8

Temperatures

Column: 40 ± 1°

Detector: 40 ± 1°

Flow rate: 1.3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times are given in *Table 1.*]

USP Monographs

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Suitability requirements
Resolution: NLT 1.5 between lactulose and lactose; NLT 0.9 between lactulose and epilactose
Relative standard deviation: NMT 2.0% for the main peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$) in the portion of Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lactulose RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lactulose in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*. To evaluate the *Suitability requirements*, use the *Standard solution* prepared as directed in the *Assay*.
Standard solution: 6.4 mg/mL of USP Galactose RS, 4.8 mg/mL of USP Anhydrous Lactose RS, 3.2 mg/mL of USP Epilactose RS, 1.2 mg/mL of USP Tagatose RS, and 0.4 mg/mL of USP Fructose RS in a mixture of acetonitrile and water (1:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of galactose, lactose, epilactose, tagatose, and fructose, if found, in the portion of Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the relevant related compound from the *Sample solution*
r_S = peak response of the relevant related compound from the *Standard solution*
C_S = concentration of the relevant USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of lactulose in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tagatose	0.30	4
Fructose	0.34	1
Galactose	0.47	16
Epilactose	0.90	8
Lactulose	1.0	—
Lactose	1.17	12

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at a temperature between 2° and 30°. Avoid subfreezing temperatures.
• **LABELING:** The label states that this article is not intended for direct administration to humans or animals.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Epilactose RS
USP Fructose RS
USP Galactose RS
USP Anhydrous Lactose RS
USP Lactulose RS
USP Tagatose RS

Lactulose Solution

DEFINITION
Lactulose Solution is a solution in water prepared from Lactulose Concentrate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.**
Sample solution: Dilute a portion of Solution with water (1 in 20).
Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A red precipitate of cuprous oxide is formed.

ASSAY
• **PROCEDURE**
Buffer: 1.15 g/L of monobasic sodium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (82:18). Ensure that the concentration of acetonitrile in the *Mobile phase* is between 78% and 85% to obtain appropriate retention times.
Standard solution: 40 mg/mL of USP Lactulose RS, 4.8 mg/mL of USP Anhydrous Lactose RS, and 3.2 mg/mL of USP Epilactose RS in a mixture of acetonitrile and water (1:1)
Sample solution: Nominally equivalent to 40 mg/mL of lactulose prepared as follows. Transfer a quantity of Solution containing 2.0 g of lactulose to a 50-mL volumetric flask, and dissolve in 20 mL of water. Add 25.0 mL of acetonitrile, allow the solution to reach ambient temperature, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 15-cm; 3-µm packing L8
Temperatures
Column: 40 ± 1°
Detector: 40 ± 1°
Flow rate: 1.3 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times are given in *Table 1*.]
Suitability requirements
Resolution: NLT 1.5 between lactulose and lactose; NLT 0.9 between lactulose and epilactose
Relative standard deviation: NMT 2.0% for the main peak
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$) in the portion of Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lactulose RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lactulose in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Oral Solution packaged in single-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*. To evaluate the *Suitability requirements*, use the *Standard solution* prepared as directed in the *Assay*.

Standard solution: 6.4 mg/mL of USP Galactose RS, 4.8 mg/mL of USP Anhydrous Lactose RS, 3.2 mg/mL of USP Epilactose RS, 1.2 mg/mL of USP Tagatose RS, and 0.4 mg/mL of USP Fructose RS in a mixture of acetonitrile and water (1:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of galactose, lactose, epilactose, tagatose, and fructose, if found, in the portion of Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the relevant related compound from the *Sample solution*
r_S = peak response of the relevant related compound from the *Standard solution*
C_S = concentration of the relevant USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of lactulose in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tagatose	0.30	4
Fructose	0.34	1
Galactose	0.47	16
Epilactose	0.90	8
Lactulose	1.0	—
Lactose	1.17	12

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total bacterial count is NMT 10^2 cfu/g of lactulose, and the tests for *Salmonella* species and *Escherichia coli* are negative.

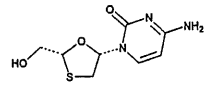
• **pH** ⟨791⟩: 2.5–6.5, after 15 min of contact with the electrodes

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at a temperature between 2° and 30°. Avoid subfreezing temperatures.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Epilactose RS
USP Fructose RS
USP Galactose RS
USP Anhydrous Lactose RS
USP Lactulose RS
USP Tagatose RS

Lamivudine



$C_8H_{11}N_3O_3S$ 229.26

$C_8H_{11}N_3O_3S \cdot 0.2\ CH_3OH$ 235.66

$C_8H_{11}N_3O_3S \cdot 0.2\ H_2O$ 232.86

2(1*H*)-Pyrimidinone, 4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-, (2*R-cis*)-;
(–)-1-[(2*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine [134678-17-4].

DEFINITION

Lamivudine contains NLT 98.0% and NMT 102.0% of lamivudine ($C_8H_{11}N_3O_3S$), calculated on the anhydrous and solvent-free basis. If labeled as a methanol solvate, it contains NLT 98.0% and NMT 102.0% of lamivudine ($C_8H_{11}N_3O_3S$), calculated on the anhydrous, methanol-free, and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Limit of Lamivudine Enantiomer*.

ASSAY

• **PROCEDURE**

Buffer: Transfer about 1.9 g of ammonium acetate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust with acetic acid to a pH of 3.8 ± 0.2, and dilute with water to volume.

Mobile phase: Methanol and *Buffer* (5:95)

System suitability solution: 0.25 mg/mL of USP Lamivudine Resolution Mixture B RS in *Mobile phase*

Standard solution: 0.25 mg/mL of USP Lamivudine RS in *Mobile phase*

Sample solution: 0.25 mg/mL of Lamivudine in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 277 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL

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System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for lamivudine diastereomer and lamivudine are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between lamivudine and lamivudine diastereomer, *System suitability solution*
Relative standard deviation: NMT 2.0% for lamivudine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lamivudine ($C_8H_{11}N_3O_3S$) in the portion of Lamivudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)
C_U = concentration of Lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis
If labeled as a methanol solvate: 98.0%–102.0% on the anhydrous, methanol-free, and solvent-free basis

OTHER COMPONENTS

• **CONTENT OF METHANOL** (if labeled as lamivudine methanol solvate)
Diluent: *N,N*-Dimethylformamide and *t*-butanol (500:1)
Standard solution: 0.625 mg/mL of methanol in diluent. Transfer 2.0 mL of this solution into a headspace vial, and immediately seal the vial.
Sample solution: Transfer 50 mg of Lamivudine to a headspace vial, add 2.0 mL of *Diluent*, and immediately seal the vial.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC headspace
Detector: Flame ionization
Column: 0.53-mm × 75-m, coated with a 3-µm film of phase G43
Temperatures
Injector: 180°
Detector: 250°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	13
40	40	240	12

Injection volume: 1.0 mL
Injection type: Split ratio, 15:1
Carrier gas: Helium
Flow rate: 6 mL/min
Headspace samplers
Oven: 95°
Loop: 175°
Transfer line: 175°
Equilibrium time: 10 min
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for methanol and *t*-butanol are 1.0 and 1.9, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for methanol
Column efficiency: NLT 25,000 for methanol
Relative standard deviation: NMT 5.0% for methanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methanol in the portion of lamivudine methanol solvate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methanol to *t*-butanol from the *Sample solution*
R_S = peak response ratio of methanol to *t*-butanol from the *Standard solution*
C_S = concentration of methanol in the *Standard solution* (mg/mL)
C_U = concentration of Lamivudine (as methanol solvate) in the *Sample solution* (mg/mL)

Acceptance criteria: 2.0%–3.0%

IMPURITIES

• **LIMIT OF LAMIVUDINE ENANTIOMER**
Buffer: 7.7 g/L of ammonium acetate in water
Mobile phase: Methanol and *Buffer* (5:95)
System suitability solution: 0.25 mg/mL of USP Lamivudine Resolution Mixture A RS in water
Sample solution: 0.25 mg/mL of Lamivudine in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; packing L45
Column temperature: 15°–30° (constant temperature)
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for lamivudine and the lamivudine enantiomer are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.5 between lamivudine and the lamivudine enantiomer
Analysis
Sample: *Sample solution*
Calculate the percentage of the lamivudine enantiomer in the portion of Lamivudine taken:

$$\text{Result} = [r_U/(r_U + r_S)] \times 100$$

r_U = peak response of the lamivudine enantiomer
r_S = peak response of lamivudine

Acceptance criteria: NMT 0.3%

• **OTHER RELATED COMPOUNDS**
Buffer, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Salicylic acid standard solution: 0.625 µg/mL of USP Salicylic Acid RS in *Mobile phase*
Sample solution: 0.25 mg/mL of Lamivudine in *Mobile phase*
Analysis
Samples: *Salicylic acid standard solution* and *Sample solution*

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Calculate the percentage of salicylic acid in the portion of Lamivudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response of USP Salicylic Acid RS from the *Salicylic acid standard solution*
C_S = concentration of salicylic acid in the *Salicylic acid standard solution* (mg/mL)
C_U = concentration of Lamivudine in the *Sample solution* (mg/mL)

Calculate the percentage of other individual impurities in the portion of Lamivudine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity other than salicylic acid from the *Sample solution*
r_T = sum of the responses of all the peaks

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamivudine-carboxylic acid[a]	0.4	0.3
Lamivudine-*trans* (lamivudine diastereomer)[b]	0.9	0.2
Lamivudine	1.0	—
Salicylic acid	2.7	0.1
Any other individual impurity	—	0.1
Total impurities	—	0.6

[a] (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.
[b] 1–[(2*RS*,5*RS*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

• **RESIDUAL SOLVENTS**
Internal standard solution: Dilute 1 mL of 2-pentanone with dimethyl sulfoxide and water (1:1) to 100.0 mL.
Standard solution: Transfer 10 mL of the *Internal standard solution* to a 100-mL volumetric flask. Add 100 µL each of the following: dehydrated alcohol, isopropyl acetate, methanol, and triethylamine. Dilute with dimethyl sulfoxide and water (1:1) to volume.
Sample solution: Transfer 5 g of Lamivudine to a 100-mL volumetric flask, add 10 mL of the *Internal standard solution*, and dilute with dimethyl sulfoxide and water (1:1) to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 50-m, coated with a 5-µm film of phase G1
Temperatures
Injector: 150°
Detector: 250°
Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	—	70	3
70	30	200	6.5

Injection volume: 0.5 µL
Injection type: Split flow rate, 320 mL/min
Carrier gas: Hydrogen (at pressure 5 psig)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each residual solvent in the portion of Lamivudine taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the respective analyte to the internal standard from the *Sample solution*
R_S = peak response ratio of the respective analyte to the internal standard from the *Standard solution*
C_S = concentration of the respective analyte in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*.

Table 4

Name	Acceptance Criteria, NMT (%)
Alcohol	0.2
Isopropyl acetate	0.2
Methanol	0.1
Triethylamine	0.1
Total residual solvents	0.3

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 2.0%
• **LIGHT ABSORPTION**
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Sample solution: 50 mg/mL in water
Analytical wavelength: 440 nm
Cell: 4 cm
Acceptance criteria: Absorptivity NMT 0.0015

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.
• **LABELING:** Where it is a methanol solvate form, the label so indicates.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lamivudine RS
USP Lamivudine Resolution Mixture A RS
USP Lamivudine Resolution Mixture B RS
USP Salicylic Acid RS

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Lamivudine Oral Solution

DEFINITION

Lamivudine Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$). It may contain a suitable preservative.

IDENTIFICATION

• **A.** The retention time of the lamivudine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

Solution A: 2.0 g/L of sodium heptanesulfonate in water. Add 1.0 mL of hydrochloric acid and 1.0 mL of triethylamine per L of the solution.

Solution B: Acetonitrile and *Solution A* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	60	40
30	10	90
33	10	90
34	100	0
45	100	0

Diluent: Acetonitrile and water (10:90)

System suitability solution: Dissolve the contents of 1 vial of USP Lamivudine Resolution Mixture C RS in 2.5 mL of *Diluent*.

Standard solution: 0.2 mg/mL of USP Lamivudine RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of lamivudine in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 277 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: ▲1 mL/min▲ (ERR 1-Jun-2018)

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between lamivudine-*S*-sulfoxide and lamivudine-*R*-sulfoxide, *System suitability solution*

Tailing factor: NMT 2.0 for the lamivudine peak, *System suitability solution*

Relative standard deviation: NMT 2% for the lamivudine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lamivudine from the *Sample solution*

r_S = peak response of lamivudine from the *Standard solution*

C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of any individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of each individual impurity

r_S = sum of the responses of all of the peaks excluding peaks due to added preservative(s) or excipients

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamivudine-uracil derivative[a]	0.34	1.2
Cytosine[b]	0.52	0.3
Lamivudine-*S*-sulfoxide[c]	0.61	0.3
Lamivudine-*R*-sulfoxide[d]	0.63	0.6
Lamivudine carboxylic acid[e,f]	0.89	—
Lamivudine *trans*[f,g]	0.94	—
Lamivudine	1.0	—
Salicylic acid[f]	1.38	—
Any other identified impurity	—	0.3
Any individual unidentified impurity	—	0.2
Total impurities	—	2.0

[a] 1-[(2*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.
[b] 4-Aminopyrimidin-2(1*H*)-one.
[c] 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[d] 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[e] (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.
[f] This impurity is controlled in the drug substance and is not to be included in the total impurities. Disregard any peak less than 0.01%.
[g] 1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 5.7–6.3

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL. The total molds and yeasts count does not exceed 10^2 cfu/mL. It

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meets the requirements of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lamivudine RS
  USP Lamivudine Resolution Mixture C RS
  [NOTE—This reference standard contains lamivudine and several related impurities.]

Lamivudine Tablets

DEFINITION

Lamivudine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
  Sample: Crush 1 Tablet and transfer it to a suitable container. Add 5 mL of methanol and shake for 15 min. Pass through a suitable filter, collecting about 2 mL of the filtrate. Evaporate the filtrate to dryness under a gentle stream of nitrogen, and use the residue.
  Standard: Dissolve a suitable amount of USP Lamivudine RS in a small amount of methanol, shaking until completely dissolved. Evaporate to dryness under a gentle stream of nitrogen, and use the residue.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 1.9 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 3.8.
  Mobile phase: Methanol and *Buffer* (50:950)
  System suitability solution: 0.2 mg/mL of USP Lamivudine Resolution Mixture B RS in *Mobile phase*
  Standard solution: 0.2 mg/mL of USP Lamivudine RS in *Mobile phase*
  Sample stock solution: Nominally about 3–4 mg/mL of lamivudine in water prepared as follows. Transfer the required number of Tablets, based on the labeled amount, to a suitable volumetric flask, and soak or shake for at least 15 min in water to disperse the sample. Dilute with water to volume, mix, and pass through a suitable filter or centrifuge.
  Sample solution: Nominally 0.2 mg/mL of lamivudine in *Mobile phase* from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 277 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 30 ± 5°
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for lamivudine diastereomer and lamivudine are 0.9 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between lamivudine and lamivudine diastereomer, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Test 1
  Procedure for products labeled as Lamivudine Tablets 100-mg or 150-mg
  Medium: Water, degassed; 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Standard solution: (*L*/900) mg/mL of USP Lamivudine RS in *Medium*, where *L* is the Tablet label claim in mg
  Sample solution: Pass a portion of the solution under test through a suitable filter to obtain a concentration similar to that of the *Standard solution*.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: 270 nm
  Cell: 1 mm
  Blank: *Medium*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) is dissolved.
Procedure for products labeled as Lamivudine Tablets 300-mg
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 15 min
Standard solution: (*L*/900) mg/mL of USP Lamivudine RS in *Medium*, where *L* is the Tablet label claim in mg
Sample solution: Pass a portion of the solution under test through a suitable filter.

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Instrumental conditions
Mode: UV
Analytical wavelength: 280 nm
Cell: 0.5 mm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Buffer: 1.93 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 3.8.
Mobile phase: Methanol and *Buffer* (40:60)
Standard solution: (L/900) mg/mL of USP Lamivudine RS in *Medium*, where L is the Tablet label claim in mg
Sample solution: Pass a portion of the solution under test through a suitable filter to obtain a concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 285 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Tailing factor: NMT 1.5
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity
r_T = sum of all the peak responses

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cytosine[a]	0.32	0.2
Lamivudine-carboxylic acid[b]	0.39	—[c]
Lamivudine-*S*-sulfoxide[d]	0.43	0.2
Lamivudine-*R*-sulfoxide[e]	0.45	0.2
Lamivudine diastereomer (Lamivudine-*trans*)[f]	0.92	—[c]
Lamivudine	1.0	—
Salicylic acid	2.2	—[c]
Any other individual impurity	—	0.2
Total impurities	—	0.6

[a] 4-Aminopyrimidin-2(1*H*)-one.
[b] (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.
[c] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.
[d] 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[e] 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[f] 1-[(2*RS*,5*RS*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Lamivudine RS
USP Lamivudine Resolution Mixture B RS
[NOTE—The resolution mixture contains lamivudine and lamivudine diastereomer. Other impurities may also be present.]

Lamivudine and Zidovudine Tablets

DEFINITION

Lamivudine and Zidovudine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) and zidovudine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION

- **A.** The retention times of the lamivudine and zidovudine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

• **PROCEDURE**

Solution A: 25 mM of ammonium acetate. Adjust with glacial acetic acid to a pH of 4.0.
Solution B: Methanol
Solution C: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	95	5	0
15.0	95	5	0
30.0	70	30	0
38.0	70	30	0
38.1	0	0	100
45.0	0	0	100
45.1	95	5	0
60.0	95	5	0

Diluent: *Solution A* and *Solution B* (19:1)
System suitability solution: 0.17 mg/mL of USP Lamivudine Resolution Mixture B RS in *Diluent*
Standard solution: 0.15 mg/mL of USP Lamivudine RS and 0.30 mg/mL of USP Zidovudine RS in *Diluent*
Sample stock solution: Transfer a counted number of Tablets, equivalent to 1500 mg of zidovudine and 750 mg of lamivudine, into a 500-mL volumetric flask. Add 250 mL of water, and disintegrate completely by shaking for a minimum of 15 min. Dilute with water to volume, and mix.
Sample solution: Pass a portion of the *Sample stock solution* through a filter of 0.45-µm pore size, discarding the first 2–3 mL. Further dilute the filtrate to obtain 0.15 mg/mL of lamivudine and 0.30 mg/mL of zidovudine in *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 3-mm × 25-cm; packing L1
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for lamivudine diastereomer and lamivudine are 0.50 and 0.52, respectively.]
Suitability requirements
Resolution: NLT 1.5 between lamivudine diastereomer and lamivudine, *System suitability solution*
Relative standard deviation: NMT 2.0% for zidovudine and lamivudine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) and zidovudine ($C_{10}H_{13}N_5O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zidovudine or lamivudine from the *Sample solution*
r_S = peak response of zidovudine or lamivudine from the *Standard solution*
C_S = concentration of USP Zidovudine RS or USP Lamivudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine or lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 2: 75 rpm
Time: 15 min
Lamivudine response factor solutions: 0.167 mg/mL of USP Lamivudine RS in *Medium*. [NOTE—Prepare in duplicate.]
Zidovudine response factor solutions: 0.333 mg/mL of USP Zidovudine RS in *Medium*. [NOTE—Prepare in duplicate.]
Sample solution: Pass a portion of the solution under test through a suitable filter (PTFE, PVDF, or equivalent) of 0.45-µm pore size.
Detector: UV 240–300 nm
Blank: *Medium*
Cell length: 0.02-cm flowcell
Analysis: The calculations of the percentages dissolved are done using a multicomponent analysis software.
Tolerances: NLT 85% (*Q*) of the labeled amount of zidovudine and lamivudine is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer solution: 7.7 g/L of ammonium acetate in water
Mobile phase: Acetonitrile and *Buffer solution* (1:9)
Standard stock solution: 1.4 mg/mL of USP Lamivudine RS and 2.8 mg/mL of USP Zidovudine RS in *Medium*. A small amount of methanol, NMT 20% of the final volume, can be used to dissolve both compounds.
Standard solution: 0.168 mg/mL of lamivudine and 0.336 mg/mL of zidovudine in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates for lamivudine and NLT 3000 theoretical plates for zidovudine
Tailing factor: NMT 2.0 for lamivudine and zidovudine
Relative standard deviation: NMT 2.0% for zidovudine and lamivudine
Calculate the percentages of lamivudine ($C_8H_{11}N_3O_3S$) and zidovudine ($C_{10}H_{13}N_5O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of lamivudine or zidovudine from the *Sample solution*
r_S = peak response of lamivudine or zidovudine from the *Standard solution*

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C_S = concentration of lamivudine or zidovudine in the *Standard solution* (mg/mL)
L = label claim for lamivudine or zidovudine (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of zidovudine and lamivudine is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for zidovudine and lamivudine

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each lamivudine related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of the peak responses of lamivudine and all lamivudine related impurities from the *Sample solution*

Calculate the percentage of each zidovudine related impurity and unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of the peak responses of zidovudine, all zidovudine related impurities, and unspecified impurities from the *Sample solution*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamivudine-(cytosine)[a]	0.11	1.0	—[b]
Lamivudine-(uracil)[c]	0.14	1.0	—[b]
Lamivudine-(carboxylic acid)[d]	0.17	1.0	0.3
Lamivudine-(*S*-sulfoxide)[e]	0.20	1.0	—[b]
Lamivudine-(*R*-sulfoxide)[f]	0.22	1.0	—[b]
Zidovudine related compound C[g]	0.27	1.7	1.5
Lamivudine diastereomer[h]	0.50	1.0	0.2
Lamivudine	0.52	—	—
Zidovudine-(thymidine)[i]	0.60	1.0	—[b]
Lamivudine-(uracil derivative)[j]	0.70	1.0	—[b]
Lamivudine-(salicylic acid)[k]	0.80	1.0	—[b]
Zidovudine	1.00	—	—
Zidovudine related compound B[l]	1.10	1.0	—[b]
Any individual unspecified impurity	—	1.0	0.1
Total lamivudine related impurities (the limit includes all lamivudine related impurities)	—	—	0.6
Total zidovudine related impurities (the limit includes individual unspecified impurities)	—	—	2.0

[a] 4-Aminopyrimidin-2(1*H*)-one.
[b] The individual impurity limit is not included because these are process/other impurities monitored individually in the drug substances.
[c] Pyrimidine-2,4(1*H*,3*H*)-dione.
[d] (2*R*,5*S*)-5-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid (2*R*,5*S*)-5-(cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.
[e] 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[f] 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[g] 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.
[h] 1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.
[i] [1-(2-Deoxy-β-D-ribofuranosyl)]thymine.
[j] (2*RS*,5*SR*)1-[(2*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.
[k] 2-Hydroxybenzoic acid.
[l] 3′-Chloro-3′-deoxythymidine.

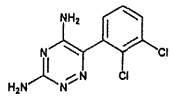
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, and store between 2° and 30°.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lamivudine RS
USP Lamivudine Resolution Mixture B RS
USP Zidovudine RS

Lamotrigine



$C_9H_7Cl_2N_5$ 256.09

1,2,4-Triazine-3,5-diamine, 6-(2,3-dichlorophenyl); 3,5-Diamino-6-(2,3-dichlorophenyl)-*as*-triazine [84057-84-1].

DEFINITION

Lamotrigine contains NLT 98.0% and NMT 102.0% of $C_9H_7Cl_2N_5$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

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• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Dilute 8.5 mL of hydrochloric acid with water to 1 L (0.1 M hydrochloric acid).
Buffer: 2.7 g/L of monobasic potassium phosphate in water
Solution A: Triethylamine and *Buffer* (1:150). Adjust with phosphoric acid to a pH of 2.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	76.5	23.5
4	76.5	23.5
14	20	80
15	76.5	23.5
19	76.5	23.5

Standard solution: 0.2 mg/mL of USP Lamotrigine RS prepared as follows. Transfer the required amount of USP Lamotrigine RS to a suitable volumetric flask, and add 5% of the final volume with methanol to facilitate dissolution. Dilute with *Diluent* to volume.
Sample solution: 0.2 mg/mL of Lamotrigine prepared as follows. Transfer the required amount of lamotrigine to a suitable volumetric flask, and add 5% of the final volume with methanol to facilitate dissolution. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lamotrigine ($C_9H_7Cl_2N_5$) in the portion of Lamotrigine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
C_U = concentration of Lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **LIMIT OF LAMOTRIGINE RELATED COMPOUND B**

Diluent, Solution A, and **Sample solution:** Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile and *Solution A* (35:65)
System suitability stock solution: 0.2 mg/mL of USP Lamotrigine RS prepared as follows. Transfer the required amount of USP Lamotrigine RS to a suitable volumetric flask, and add 5% of the final volume with methanol to facilitate dissolution. Dilute with *Diluent* to volume.
Standard stock solution: 0.01 mg/mL of USP Lamotrigine Related Compound B RS prepared as follows. Transfer the required amount of USP Lamotrigine Related Compound B RS to a volumetric flask. Add 80% of the flask volume of methanol, and acidify with 1% of the flask volume of hydrochloric acid. Allow to cool, and dilute with methanol to volume. Dilute a portion of this solution with *Diluent*.
System suitability solution: 1 µg/mL of lamotrigine related compound B from the *Standard stock solution* in *System suitability stock solution*
Standard solution: 5 µg/mL of lamotrigine related compound B from the *Standard stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: 2 times the retention time of lamotrigine related compound B
System suitability
Sample: *System suitability solution*
[NOTE—Identify the peaks in the *System suitability solution*, taking into account that lamotrigine is unretained, eluting at or near the solvent front.]
Suitability requirements
Tailing factor: NMT 2.0 for the lamotrigine related compound B peak
Relative standard deviation: NMT 5.0% for the lamotrigine related compound B peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lamotrigine related compound B in the portion of Lamotrigine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for lamotrigine related compound B from the *Sample solution*
r_S = peak response for the lamotrigine related compound B from the *Standard solution*
C_S = concentration of USP Lamotrigine Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of Lamotrigine in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.1% of lamotrigine related compound B. [NOTE—Lamotrigine related compound D, if present, will elute at a retention time of about 1.5 relative to lamotrigine related compound B. Disregard this peak as it is quantified in the test for *Organic Impurities*.]

• **ORGANIC IMPURITIES**

Diluent, Buffer, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability stock solution: 0.2 mg/mL of USP Lamotrigine RS prepared as follows. Transfer the required amount of USP Lamotrigine RS to a suitable volumetric

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flask, and add 5% of the final volume with methanol to facilitate dissolution. Dilute with *Diluent* to volume.

Impurities stock solution: 0.1 mg/mL of each of USP Lamotrigine Related Compound C RS and USP Lamotrigine Related Compound D RS prepared as follows. Transfer suitable quantities of the Reference Standards to a suitable volumetric flask. Add an amount of methanol equal to 80% of the flask volume, and acidify with 1% of the flask volume of hydrochloric acid. Allow to cool. Dilute with methanol to volume.

System suitability solution: 0.5 µg/mL each of lamotrigine related compound C and lamotrigine related compound D from *Impurities stock solution* in *System suitability stock solution*

System suitability

Sample: *System suitability solution*

[NOTE—Refer to *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between lamotrigine and lamotrigine related compound C peaks

Analysis

Samples: *Diluent* and *Sample solution*

[NOTE—Disregard any peak that may be present in the chromatogram of the *Diluent* injection. Disregard any peak due to lamotrigine related compound B, because it is quantified in the test for *Limit of Lamotrigine Related Compound B*.]

Calculate the percentage of each impurity in the portion of Lamotrigine taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for the lamotrigine peak from the *Sample solution*

F = relative response factor for each impurity from *Table 2*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamotrigine	1.0	1.0	—
Lamotrigine related compound C[a]	1.5	1.0	0.1
Lamotrigine related compound B[b,c]	3.2	—	—
Lamotrigine related compound D[d]	3.7	0.8	0.2
Any individual, unspecified impurity	—	1.0	0.1
Total impurities, excluding lamotrigine related compound B	—	—	0.2

[a] 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4*H*)-one.
[b] 2,3-Dichlorobenzoic acid.
[c] Included only for identification.
[d] *N*-[5-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Lamotrigine RS

USP Lamotrigine Related Compound B RS
2,3-Dichlorobenzoic acid.
$C_7H_4Cl_2O_2$ 191.01

USP Lamotrigine Related Compound C RS
3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4*H*)-one.
$C_9H_6Cl_2N_4O$ 257.08

USP Lamotrigine Related Compound D RS
N-[5-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide.
$C_{16}H_9Cl_4N_5O$ 429.09

Lamotrigine Tablets

DEFINITION

Lamotrigine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩**,** *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Standard solution: 0.02 mg/mL of USP Lamotrigine RS in 0.01 N hydrochloric acid

Sample solution: 0.02 mg/mL of lamotrigine from crushed powdered Tablets in 0.01 N hydrochloric acid

Acceptance criteria: The spectra of the *Standard solution* and *Sample solution* exhibit maxima and minima at the same wavelengths.

- **B.** The retention time of the lamotrigine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.8 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (60:40)

Standard solution: 0.05 mg/mL of USP Lamotrigine RS in *Mobile phase*

Sample solution: Transfer an amount equivalent to 100 mg of lamotrigine from a portion of crushed Tablets (NLT 20) to a suitable volumetric flask to obtain a nominal concentration of lamotrigine of 1.0 mg/mL. Dissolve in 70% of the flask volume of *Mobile phase* by sonicating for 20 min. Dilute with *Mobile phase* to volume. Centrifuge the solution. Quantitatively dilute a suitable volume of centrifugate with *Mobile phase* to obtain a nominal concentration of 0.05 mg/mL of lamotrigine.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for lamotrigine
Relative standard deviation: NMT 2.0% for lamotrigine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Determine the amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved by using one of the following methods.
Spectrometric method
Standard stock solution: 0.15 mg/mL of USP Lamotrigine RS in *Medium* prepared as follows. Dissolve a suitable quantity in 5% of the flask volume of methanol, then dilute with *Medium* to volume.
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 0.028 mg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium* according to *Table 1*.

Table 1

Tablet Label Claim (mg)	Volume of Sample (mL)	Volume of Volumetric Flask	Final Concentration (mg/mL)
25	—	—	0.028
100	5.0	20	0.029
150	4.0	25	0.027
200	3.0	25	0.027

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 267 nm
Blank: *Medium*
Analysis
Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Chromatographic method
Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
Standard stock solution: 0.5 mg/mL of USP Lamotrigine RS in *Medium*, prepared as follows. Dissolve a suitable quantity in 15% of the flask volume of methanol, then dilute with *Medium* to volume.
Standard solution: (L/1000) mg/mL of USP Lamotrigine RS from the *Standard stock solution* in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Column: 4.6-mm × 15-cm; 5-µm packing L1
Detector: UV 310 nm
Flow rate: 1 mL/min
Injection size: See *Table 2*.

Table 2

Label Claim (mg/Tablet)	Injection Size (µL)
25	50
100, 150, 200	10

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for lamotrigine
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of lamotrigine is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Apparatus, and **Time:** Proceed as directed for *Test 1*.
Analysis: Determine the amount of lamotrigine dissolved using either the *Spectrometric method* or *Chromatographic method* described in *Test 1*.
Tolerances: NLT 75% (Q) of the labeled amount of lamotrigine is dissolved.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Standard solution: (L/900) mg/mL of USP Lamotrigine RS in *Medium*, where L is the Tablet label claim in mg
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 270 nm

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Cell
For Tablets labeled to contain 100, 150, or 200 mg: 0.2-cm flow cell
For Tablets labeled to contain 25 mg: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of lamotrigine is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer: Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile, methanol, and *Buffer* (10:30:60)
Diluent: Methanol and *Buffer* (60:40)
System suitability solution: 1 µg/mL of Lamotrigine Related Compound B RS and 0.4 mg/mL of USP Lamotrigine RS in *Diluent*
Standard solution: 1.0 µg/mL of USP Lamotrigine RS in *Diluent*
Sample solution: Transfer an amount equivalent to 100 mg of lamotrigine from a portion of crushed Tablets (NLT 20) to a suitable volumetric flask to obtain a nominal concentration of lamotrigine of about 0.4 mg/mL. Dissolve in 70% of the flask volume of *Mobile phase* by sonicating and shaking intermittently for 30 min. Dilute with *Diluent* to volume. Pass through a membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between lamotrigine related compound B and lamotrigine, *System suitability solution*
Tailing factor: NMT 2.0 for lamotrigine, *Standard solution*
Relative standard deviation: NMT 10.0% for lamotrigine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of lamotrigine from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamotrigine related compound B[a]	0.67	0.75	0.2
Lamotrigine	1.0	—	—
Lamotrigine related compound C[b]	1.5	1.0	0.5
Any individual unspecified degradation impurity	—	1.0	0.2
Total impurities	—	—	0.75

[a] 2,3-Dichlorobenzoic acid.
[b] 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4*H*)-one.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP Reference Standards** ⟨11⟩
USP Lamotrigine RS
USP Lamotrigine Related Compound B RS
2,3-Dichlorobenzoic acid.
$C_7H_4Cl_2O_2$ 191.01

Lamotrigine Extended-Release Tablets

DEFINITION

Lamotrigine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Mobile phase: Acetonitrile, water, and trifluoroacetic acid (25: 75: 0.05)
Diluent: Acetonitrile, methanol, and water (10:20:70)
Standard solution: 0.25 mg/mL of USP Lamotrigine RS in *Diluent*. Sonication may be used to aid dissolution.
Sample stock solution: 1.0–3.0 mg/mL of lamotrigine prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask containing 10% of the flask volume of acetonitrile. Allow the Tablets to disperse. Add 20% of the flask volume of methanol. Sonicate for 10 min. Add 30% of the flask volume of 0.1 M hydrochloric acid. Sonicate for 25 min or until a fine, even dispersion is obtained. Allow to cool to room temperature. Dilute with 0.1 M hydrochloric acid to volume. Pass a portion of the solution

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through a nylon filter of 0.45-µm pore size and use the filtrate.

Sample solution: Nominally 0.2–0.3 mg/mL of lamotrigine in 0.1 M hydrochloric acid from a suitable volume of *Sample stock solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 5 µL
Run time: NLT 8 times the retention time of lamotrigine

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium 1: 0.01 M hydrochloric acid; 700 mL
Medium 2: 7.8 g of tribasic sodium phosphate, and 22.5 g of sodium dodecyl sulfate in 1 L of water. This solution has a pH of about 12.
Apparatus 2: 50 rpm with sinkers (see *Dissolution* ⟨711⟩, *Figure 2a*)
Times
For Tablets labeled to contain 25 or 50 mg: 2, 7, 15 h
For Tablets labeled to contain 100, 200, or 250 mg: 2, 5, 12 h
For Tablets labeled to contain 300 mg: 2, 6, 13 h
Procedure: Run the test with *Medium 1* for 2 h. Add 200 mL of *Medium 2*, preheated at 37°. Within 5 min of the addition of *Medium 2*, withdraw the sample for the 2-h time point. Continue the testing by drawing samples at the time points specified in *Table 1*, *Table 2*, or *Table 3*, depending on the label claim.
Diluent: *Medium 1* and *Medium 2* (70:20)
Standard solution: (*L*/900) mg/mL of USP Lamotrigine RS in *Diluent*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a suitable portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Diluent* if necessary.
Blank: *Diluent*
Instrumental conditions
Mode: UV
Analytical wavelength: 260 nm. [NOTE—Depending on the label claim, cells with suitable path lengths may be used.]

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount (Q_i) of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at each time point *i*:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor if needed
L = label claim (mg/Tablet)

Tolerances
For Tablets with 25- or 50-mg label claim: See *Table 1*.
For Tablets with 100-, 200-, or 250-mg label claim: See *Table 2*.
For Tablets with 300-mg label claim: See *Table 3*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved
1	2	NMT 10%
2	7	35%–55%
3	15	NLT 80%

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved	
		100 mg, 200 mg	250 mg
1	2	NMT 10%	NMT 10%
2	5	20%–45%	20%–40%
3	12	NLT 80%	NLT 80%

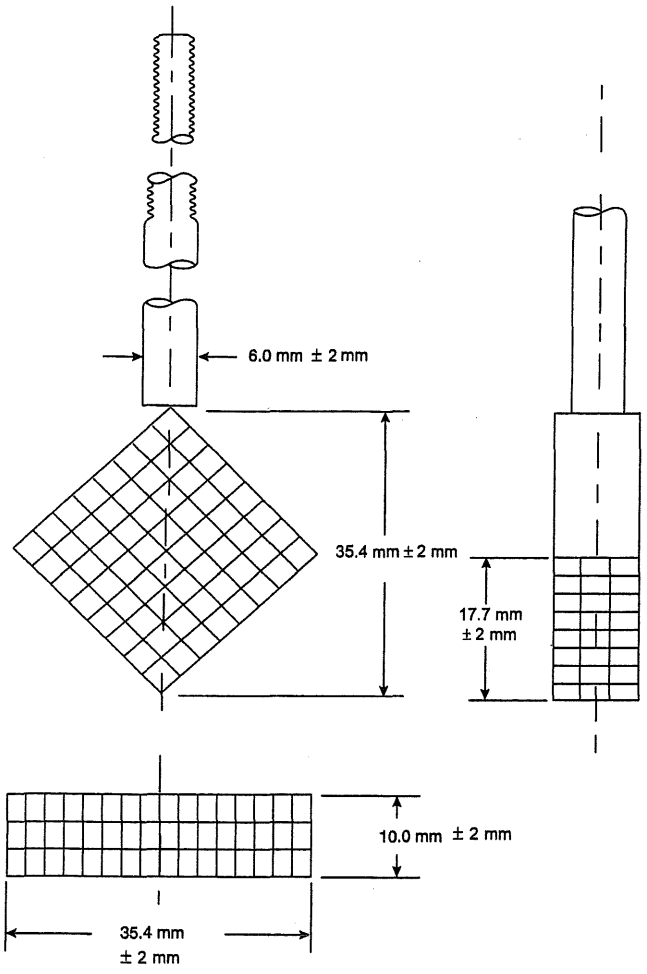
Table 3

Time Point (*i*)	Time (h)	Amount Dissolved
1	2	NMT 10%
2	6	25%–45%
3	13	NLT 80%

The percentages of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Acid stage medium: 0.01 M hydrochloric acid; 700 mL
Buffer stage stock medium: 2.83 g of sodium phosphate monobasic, 1.72 g of sodium hydroxide, and 22.5 g of sodium dodecyl sulfate in 1 L of water
Buffer stage medium: *Acid stage medium* and *Buffer stage stock medium* (70:20); 900 mL. Adjust with solution A (phosphoric acid in water prepared by diluting 1 mL of phosphoric acid with water to 50 mL) or 0.1 N sodium hydroxide, if necessary, to a pH of 6.8. Record the required volume of solution A or 0.1 N sodium hydroxide for adjustment of pH to 6.8.
Apparatus 2: 50 rpm with stationary tablet basket. See *Figures 1* and *2*.
Times
For Tablets labeled to contain 25 or 50 mg: 2 h in *Acid stage medium*; 4, 7, 9, and 15 h in *Buffer stage medium*

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NOTES

1. Rod and Basket with a Tablet cover placed in the horizontal diagonal of the basket.
2. Basket and Tablet cover material; stainless steel.
3. Basket gauze wire size: 8 mesh.

Figure 1. Stationary tablet basket. (Lamotrigine Extended-Release Tablets)

For Tablets labeled to contain 100, 200, or 300 mg: 2 h in *Acid stage medium*; 3, 5, 7, and 12 h in *Buffer stage medium*

[NOTE—The times in the *Buffer stage medium* include the time in the *Acid stage medium*.]

Procedure: Run the test with *Acid stage medium* for 2 h followed by collecting the *Acid stage medium* sample and replacing it with the same volume of *Acid stage medium*. Add 200 mL of *Buffer stage stock medium* to the above solution. If necessary, add either solution A or 0.1 N sodium hydroxide to the solution to reach a pH of 6.8. Continue the testing by drawing samples at the time points specified in *Table 4* or *Table 5*, depending on the label claim. Replace each of the volumes withdrawn with an equal volume of *Buffer stage medium*.

Buffer: Dissolve 2.76 g of sodium phosphate monobasic in 1 L of water. Add 2 mL of triethylamine and adjust with solution A to a pH of 7.0.

Mobile phase: Methanol and *Buffer* (55:45)

Standard stock solution: 1.4 mg/mL of USP Lamotrigine RS in methanol

Acid stage standard solution: (*L*/900) mg/mL of USP Lamotrigine RS from *Standard stock solution*, in *Acid stage medium*, where *L* is the label claim in mg/Tablet

Buffer stage standard solution: (*L*/900) mg/mL of USP Lamotrigine RS from *Standard stock solution*, in *Buffer stage medium*, where *L* is the label claim in mg/Tablet

Acid stage sample solution: Withdraw a 10.0-mL aliquot, and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Acid stage medium*.

Buffer stage sample solution: Withdraw a 10.0-mL aliquot, and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Buffer stage medium*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume
For 25-mg Tablets: 80 µL
For 50-mg Tablets: 40 µL
For 100-mg Tablets: 20 µL
For 200- or 300-mg Tablets: 10 µL

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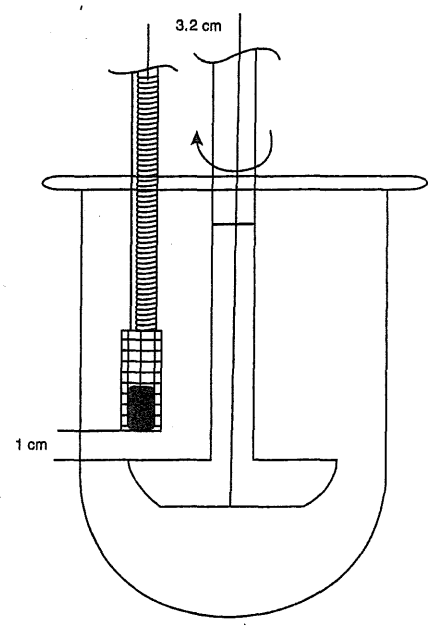


Figure 2. Drug release stationary tablet basket configuration diagram. (Lamotrigine Extended-Release Tablets)

Run time: NLT 1.8 times the retention time of lamotrigine

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage standard solution*, *Acid stage sample solution*, *Buffer stage standard solution*, and *Buffer stage sample solution*

Calculate the percentage (Q_A) of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved in the *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times V_A \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*
r_S = peak response from the *Acid stage standard solution*
C_S = concentration of USP Lamotrigine RS in the *Acid stage standard solution* (mg/mL)
V_A = volume of the *Acid stage medium*, 700 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of lamotrigine ($C_9H_7Cl_2N_5$) in the sample withdrawn from the vessel at each time point (i) during the buffer stage:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Buffer stage sample solution* at time point *i*
r_S = peak response from the *Buffer stage standard solution*
C_S = concentration of USP Lamotrigine RS in the *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at each time point (i) during the buffer stage:

$$\text{Result}_1 = [C_1 \times V_B \times (1/L) \times 100] + (Q_A \times V_S/V_A)$$

$$\text{Result}_2 = \{[(C_2 \times V_B) + (C_1 \times V_S)] \times (1/L) \times 100\} + (Q_A \times V_S/V_A)$$

$$\text{Result}_3 = (\{(C_3 \times V_B) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + (Q_A \times V_S/V_A)$$

$$\text{Result}_4 = (\{(C_4 \times V_B) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + (Q_A \times V_S/V_A)$$

C_i = concentration of lamotrigine in the *Buffer stage sample solution* withdrawn at time point *i* (mg/mL)
V_B = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
Q_A = the percentage of the labeled amount of lamotrigine dissolved in the *Acid stage medium*
V_S = volume of the sample solution withdrawn at each time point (i) during the acid stage or buffer stage (mL)
V_A = volume of the *Acid stage medium*, 700 mL

Tolerances

For Tablets labeled to contain 25 or 50 mg: See *Table 4*.

For Tablets labeled to contain 100, 200, or 300 mg: See *Table 5*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved
1	2	NMT 10%
2	4	5%–25%
3	7	30%–50%
4	9	50%–70%

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Table 4 *(continued)*

Time Point (*i*)	Time (h)	Amount Dissolved
5	15	NLT 80%

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved
1	2	NMT 10%
2	3	5%–20%
3	5	25%–50%
4	7	50%–70%
5	12	NLT 80%

The percentages of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Acid stage medium: 0.01 M hydrochloric acid; 700 mL

Buffer stage stock medium: 7.8 g of sodium phosphate tribasic and 22.5 g of sodium dodecyl sulfate in 1 L of water

Buffer stage medium: *Acid stage medium* and *Buffer stage stock medium* (70:20); 900 mL. Adjust with 2 N hydrochloric acid TS or 2 N sodium hydroxide TS, if necessary, to a pH of 6.8.

Apparatus 2: 50 rpm with stationary tablet basket. See *Figures 1* and *2* in *Test 2*.

Times

For Tablets labeled to contain 25, 50, 100, or 200 mg: 2 h in *Acid stage medium*; 4, 7, and 14 h in *Buffer stage medium*

[NOTE—The times in the *Buffer stage medium* include the time in the *Acid stage medium*.]

Procedure: Run the test with *Acid stage medium* for 2 h followed by collecting the *Acid stage medium* sample and replacing it with the same volume of *Acid stage medium*. Add 200 mL of *Buffer stage stock medium* to the above solution. Continue the testing by drawing samples at the time points specified in *Table 6*. Replace each of the volumes withdrawn with an equal volume of the *Buffer stage medium*.

Buffer: Dissolve 2.72 g of potassium phosphate monobasic in 1 L of water and adjust with dilute phosphoric acid to a pH of 3.7.

Mobile phase: Methanol, acetonitrile, and *Buffer* (50:15:35)

Standard stock solution: 0.6 mg/mL of USP Lamotrigine RS in methanol. Sonicate to dissolve as needed.

Acid stage standard solution: 0.036 mg/mL of USP Lamotrigine RS from *Standard stock solution*, in *Acid stage medium*

Buffer stage standard solution: (*L*/900) mg/mL of USP Lamotrigine RS from *Standard stock solution*, in *Buffer stage medium*, where *L* is the label claim in mg/Tablet

Acid stage sample solution: Withdraw a 10.0-mL aliquot and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 5 mL of the filtrate. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Acid stage medium*.

Buffer stage sample solution: Withdraw a 10.0-mL aliquot and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Buffer stage medium*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 1.7 times the retention time of lamotrigine

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage standard solution*, *Acid stage sample solution*, *Buffer stage standard solution*, and *Buffer stage sample solution*

Calculate the percentage (Q_A) of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved in the *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times V_A \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*

r_S = peak response from the *Acid stage standard solution*

C_S = concentration of USP Lamotrigine RS in the *Acid stage standard solution* (mg/mL)

V_A = volume of the *Acid stage medium*, 700 mL

L = label claim (mg/Tablet)

Calculate the concentration (C_i) of lamotrigine ($C_9H_7Cl_2N_5$) in the sample withdrawn from the vessel at each time point (*i*) during the buffer stage:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Buffer stage sample solution* at time point *i*

r_S = peak response from the *Buffer stage standard solution*

C_S = concentration of USP Lamotrigine RS in the *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at each time point (*i*) during the buffer stage:

$$\text{Result}_1 = [C_1 \times V_B \times (1/L) \times 100] + (Q_A \times V_S/V_A)$$

$$\text{Result}_2 = \{[(C_2 \times V_B) + (C_1 \times V_S)] \times (1/L) \times 100\} + (Q_A \times V_S/V_A)$$

$$\text{Result}_3 = (\{(C_3 \times V_B) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + (Q_A \times V_S/V_A)$$

C_i = concentration of lamotrigine in the *Buffer stage sample solution* withdrawn at time point *i* (mg/mL)

V_B = volume of the *Buffer stage medium*, 900 mL

L = label claim (mg/Tablet)

Q_A = the percentage of the labeled amount of lamotrigine dissolved in the *Acid stage medium*

V_S = volume of the sample solution withdrawn at each time point (*i*) during the acid stage or buffer stage (mL)

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V_A = volume of the *Acid stage medium*, 700 mL

Tolerances

For Tablets labeled to contain 25, 50, 100, or 200 mg: See *Table 6*.

Table 6

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 10
2	4	NMT 25
3	7	36–61
4	14	NLT 85

The percentages of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Acid stage medium: 0.01 M hydrochloric acid; 700 mL

Buffer stage stock medium: 7.8 g of sodium phosphate tribasic and 22.5 g of sodium dodecyl sulfate in 1 L of water

Buffer stage medium: *Acid stage medium* and *Buffer stage stock medium* (70:20); 900 mL

Apparatus 2: 50 rpm with sinkers

Times

For Tablets labeled to contain 25, 50, 100, 200, or 300 mg: 2 h in *Acid stage medium*; 9 and 17 h in *Buffer stage medium*

[NOTE—The times in the *Buffer stage medium* include the time in the *Acid stage medium*.]

Procedure: Run the test with *Acid stage medium* for 2 h followed by collecting the *Acid stage medium* sample and replacing it with the same volume of *Acid stage medium*. Add 200 mL of *Buffer stage stock medium* to the above solution. Continue the testing by drawing samples at the time points specified in *Table 7*. Replace each of the volumes withdrawn with an equal volume of the *Buffer stage medium*.

Buffer: Dissolve 4.1 g of potassium phosphate monobasic in 900 mL of water and adjust with dilute phosphoric acid to a pH of 2.0, and then dilute with water to 1 L. Add 1.25 g of sodium 1-hexanesulfonate to the solution and mix.

Mobile phase: Acetonitrile and *Buffer* (25:75)

Acid stage standard stock solution 1: 0.07 mg/mL of USP Lamotrigine RS prepared as follows. Transfer an appropriate amount of USP Lamotrigine RS to a suitable volumetric flask and add 20% of the flask volume of methanol. Sonicate to dissolve and dilute with *Acid stage medium* to volume. Further dilute this solution with *Acid stage medium* to obtain a final concentration of 0.07 mg/mL.

Acid stage standard stock solution 2: 0.14 mg/mL of USP Lamotrigine RS prepared as follows. Transfer an appropriate amount of USP Lamotrigine RS to a suitable volumetric flask and add 20% of the flask volume of methanol. Sonicate to dissolve and dilute with *Acid stage medium* to volume. Further dilute this solution with *Acid stage medium* to obtain a final concentration of 0.14 mg/mL.

Acid stage standard solution: 0.1 × (*L*/700) mg/mL of USP Lamotrigine RS either from *Acid stage standard stock solution 1* for Tablets labeled to contain 25, 50, 100, and 200 mg, or from *Acid stage standard stock solution 2* for Tablets labeled to contain 300 mg, in *Acid stage medium*, where *L* is the label claim in mg/Tablet. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of the filtrate.

Buffer stage standard stock solution 1: 0.55 mg/mL of USP Lamotrigine RS prepared as follows. Transfer an appropriate amount of USP Lamotrigine RS to a suitable volumetric flask and add 10% of the flask volume of methanol. Sonicate to dissolve and dilute with *Buffer stage medium* to volume.

Buffer stage standard stock solution 2: 1.1 mg/mL of USP Lamotrigine RS prepared as follows. Transfer an appropriate amount of USP Lamotrigine RS to a suitable volumetric flask and add 20% of the flask volume of methanol. Sonicate to dissolve and dilute with *Buffer stage medium* to volume.

Buffer stage standard solution: (*L*/900) mg/mL of USP Lamotrigine RS either from *Buffer stage standard stock solution 1* for Tablets labeled to contain 25, 50, 100, and 200 mg, or from *Buffer stage standard stock solution 2* for Tablets labeled to contain 300 mg, in *Buffer stage medium*, where *L* is the label claim in mg/Tablet. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of the filtrate.

Acid stage sample solution: Withdraw a 10.0-mL aliquot and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of the filtrate. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Acid stage medium*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of the filtrate.

Buffer stage sample solution: Withdraw a 10.0-mL aliquot and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Buffer stage medium*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of lamotrigine

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage standard solution*, *Acid stage sample solution*, *Buffer stage standard solution*, and *Buffer stage sample solution*

Calculate the percentage (Q_A) of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved in the *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times V_A \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*

r_S = peak response from the *Acid stage standard solution*

C_S = concentration of USP Lamotrigine RS in the *Acid stage standard solution* (mg/mL)

V_A = volume of the *Acid stage medium*, 700 mL

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L = label claim (mg/Tablet)

Calculate the concentration (C_i) of lamotrigine ($C_9H_7Cl_2N_5$) in the sample withdrawn from the vessel at each time point (i) during the buffer stage:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Buffer stage sample solution* at time point i
r_S = peak response from the *Buffer stage standard solution*
C_S = concentration of USP Lamotrigine RS in the *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at each time point (i) during the buffer stage:

$$\text{Result}_1 = [C_1 \times V_B \times (1/L) \times 100] + (Q_A \times V_S/V_A)$$
$$\text{Result}_2 = \{[(C_2 \times V_B) + (C_1 \times V_S)] \times (1/L) \times 100\} + (Q_A \times V_S/V_A)$$

C_i = concentration of lamotrigine in the *Buffer stage sample solution* withdrawn at time point i (mg/mL)
V_B = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
Q_A = the percentage of the labeled amount of lamotrigine dissolved in the *Acid stage medium*
V_S = volume of the sample solution withdrawn at each time point (i) during the acid stage or buffer stage (mL)
V_A = volume of the *Acid stage medium*, 700 mL

Tolerances

For Tablets labeled to contain 25, 50, 100, 200, or 300 mg: See *Table 7*.

Table 7

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	NMT 10
2	9	35–55
3	17	NLT 80

The percentages of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Acid stage medium: 0.01 M hydrochloric acid; 710 mL
Buffer stage stock medium: 3.36 g of anhydrous tribasic sodium phosphate and 22.5 g of sodium dodecyl sulfate in 1 L of water
Buffer stage medium: *Acid stage medium* and *Buffer stage stock medium* (70:20); 900 mL. Adjust with hydrochloric acid or 5 N sodium hydroxide TS, if necessary, to a pH of 6.8.
Apparatus 2: 50 rpm with sinkers
Times
For Tablets labeled to contain 25, 50, 100, or 200 mg: 2 h in *Acid stage medium*; 4, 7, and 17 h in *Buffer stage medium*
[NOTE—The times in the *Buffer stage medium* include the time in *Acid stage medium*.]
Procedure: Run the test with *Acid stage medium* for 2 h followed by collecting the *Acid stage medium* sample. Add 200 mL of *Buffer stage stock medium* to this solution. Continue the testing by drawing samples at the time points specified in *Table 8*.
Buffer: Dissolve 3.45 g of monobasic sodium phosphate in 1 L of water, and adjust with phosphoric acid to a pH of 3.3. To this solution, add 5.77 g of sodium dodecyl sulfate, and mix well.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Standard solution: 0.22 mg/mL of USP Lamotrigine RS in *Mobile phase*. Sonicate to dissolve as needed.
Acid stage sample solution: Withdraw a 10.0-mL aliquot, and pass a portion of the solution under test through a suitable full flow filter of 10-µm pore size.
Buffer stage sample solution: Withdraw a 2.5-mL aliquot, and pass a portion of the solution under test through a suitable full flow filter of 10-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 266 nm
Column: 4.6-mm × 5-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.6 times the retention time of lamotrigine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*
Calculate the percentage (Q_A) of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved in the *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times V_A \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
V_A = volume of the *Acid stage medium*, 710 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of lamotrigine ($C_9H_7Cl_2N_5$) in the sample withdrawn from the vessel at each time point (i) during the buffer stage:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Buffer stage sample solution* at time point i
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at each time point (i) during the buffer stage:

$$\text{Result}_1 = [C_1 \times V_B \times (1/L) \times 100] + (Q_A \times V_S/V_A)$$
$$\text{Result}_2 = \{[C_2 \times (V_B - V_S) + (C_1 \times V_S)] \times (1/L) \times 100\} + (Q_A \times V_S/V_A)$$

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$$Result_3 = (\{[C_3 \times (V_B - 2V_S)] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + (Q_A \times V_S/V_A)$$

C_i = concentration of lamotrigine in the *Buffer stage sample solution* withdrawn at time point *i* (mg/mL)
V_B = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
Q_A = the percentage of the labeled amount of lamotrigine dissolved in the *Acid stage medium*
V_S = volume of the *Sample solution* withdrawn at each time point (*i*), 10 mL for acid stage or 2.5 mL for buffer stage
V_A = volume of the *Acid stage medium*, 710 mL

Tolerances

For Tablets labeled to contain 25, 50, 100, or 200 mg: See *Table 8*.

Table 8

Time Point (*i*)	Time (h)	Amount Dissolved	
		25, 50, and 200 mg/Tablet (%)	100 mg/Tablet (%)
1	2	NMT 10	NMT 10
2	4	10–30	10–30
3	7	35–60	40–65
4	17	NLT 80	NLT 80

The percentages of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 7:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Acid stage medium: 0.01 N hydrochloric acid TS; 700 mL, degas by helium sparging before use
Buffer stage stock medium: 7.8 g of sodium phosphate tribasic in 1 L of water, add 2 mL of sodium hydroxide TS and degas by helium sparging, then add 22.5 g of sodium dodecyl sulfate and mix well. Degas by warming the solution to NMT 50° and bring back to 37° before use.
Buffer stage medium: *Acid stage medium* and *Buffer stage stock medium* (70:20), pH 6.8; 900 mL. Adjust with 1 N hydrochloric acid VS or sodium hydroxide TS, if necessary.
Apparatus 1: 75 rpm for Tablets labeled to contain 25, 50, 100, 200, or 300 mg
Apparatus 2: 50 rpm for Tablets labeled to contain 250 mg
Times: 2 h in *Acid stage medium*; see *Table 9* for times in *Buffer stage medium*. The times in the *Buffer stage medium* include the time in *Acid stage medium*.
Procedure: Run the test with *Acid stage medium* for 2 h followed by collecting the *Acid stage medium* sample and replacing it with the same volume of *Acid stage medium*. Add 200 mL of *Buffer stage stock medium* to the above solution. Continue the testing by drawing samples at the time points specified in *Table 9* without replacing the withdrawn volume.
Buffer: 3.85 g/L of ammonium acetate in water, adjust with acetic acid to a pH of 5.60 ± 0.05
Mobile phase: Acetonitrile and *Buffer* (32:68)
Standard stock solution: 2.0 mg/mL of USP Lamotrigine RS prepared as follows. Transfer an appropriate amount of USP Lamotrigine RS to a suitable volumetric flask and add 50% flask volume of methanol. Sonicate with occasional swirling for 5 min or until the Reference Standard is completely dissolved. Dilute with *Buffer stage medium* to volume.
Standard solution: 0.16 mg/mL of USP Lamotrigine RS from *Standard stock solution* in *Buffer stage medium*
Acid stage sample solution: Withdraw a suitable volume of the solution under test and replace the withdrawn aliquot with the same volume of *Acid stage medium*. Pass a portion of the withdrawn solution through a filter of suitable pore size. Discard the first few milliliters of the filtrate.
Buffer stage sample solution: Withdraw a suitable volume of the solution under test and pass a portion of the withdrawn solution through a filter of suitable pore size. Discard the first few milliliters of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 306 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.4 times the retention time of lamotrigine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*
Calculate the percentage (Q_A) of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved in the *Acid stage medium*:

$$Result = (r_U/r_S) \times C_S \times V_A \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
V_A = volume of the *Acid stage medium*, 700 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of lamotrigine ($C_9H_7Cl_2N_5$) in the sample withdrawn from the vessel at each time point (*i*) during the buffer stage:

$$Result_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Buffer stage sample solution* at time point *i*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at each time point (*i*) during the buffer stage:

$$Result_2 = [C_2 \times V_B \times (1/L) \times 100] + (Q_A \times V_S/V_A)$$
$$Result_3 = \{[C_3 \times (V_B - V_S) + (C_2 \times V_S)] \times (1/L) \times 100\} + (Q_A \times V_S/V_A)$$
$$Result_4 = (\{[C_4 \times (V_B - 2V_S)] + [(C_3 + C_2) \times V_S]\} \times (1/L) \times 100) + (Q_A \times V_S/V_A)$$

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C_i = concentration of lamotrigine in the *Buffer stage sample solution* withdrawn at time point *i* (mg/mL)
V_B = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
Q_A = percentage of the labeled amount of lamotrigine dissolved in the *Acid stage medium*
V_S = volume of the *Sample solution* withdrawn at each time point (*i*)
V_A = volume of the *Acid stage medium*, 700 mL

Tolerances: See *Table 9*.

Table 9

Time Point (*i*)	Time (h)	Amount Dissolved					
		25 mg/ Tablet (%)	50 mg/ Tablet (%)	100 mg/ Tablet (%)	200 mg/ Tablet (%)	250 mg/ Tablet (%)	300 mg/ Tablet (%)
1	2	NMT 10	NMT 10	NMT 10	NMT 10	NMT 10	NMT 10
2	4	10–30	10–30	10–30	10–30	8–25	10–28
3	7	—	—	53–73	—	—	—
	8	50–70	—	—	50–70	—	50–70
	9	—	45–65	—	—	55–75	—
4	10	—	—	NLT 80	—	—	—
	11	—	—	—	—	—	NLT 80
	12	NLT 80	—	—	NLT 80	—	—
	14	—	NLT 80	—	—	—	—
	15	—	—	—	—	NLT 85	—

The percentages of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Feb-2019)

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Diluent 1: Acetonitrile, methanol, and 0.1 M hydrochloric acid (10:20:70)
Diluent 2: Acetonitrile, methanol, and water (10:20:70)
System suitability stock solution: 0.025 mg/mL of USP Lamotrigine Related Compound C RS in *Diluent 1*
System suitability solution: 1.25 µg/mL of USP Lamotrigine Related Compound C RS and 0.25 mg/mL of USP Lamotrigine RS in *Diluent 2* prepared as follows. Transfer a suitable amount of USP Lamotrigine RS to a suitable volumetric flask. Transfer a suitable volume of *System suitability stock solution* to the flask. Dissolve and dilute with *Diluent 2* to volume.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 10 between the lamotrigine and lamotrigine related compound C peaks
Signal-to-noise ratio: NLT 100 for lamotrigine related compound C

Analysis
Sample: *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity from the *Sample solution*
r_T = sum of all of the impurity peak responses and the lamotrigine peak response from the *Sample solution*

Acceptance criteria: See ▲*Table 10*.▲ (RB 1-Feb-2019) Disregard peaks less than 0.05%.

▲Table 10▲ (RB 1-Feb-2019)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamotrigine	1.0	—
Lamotrigine related compound C	1.7	0.3
Lamotrigine dimer[a]	6.0	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	0.5

[a] This is either lamotrigine *o*-dimer [N^5,$N^{5'}$-methylenebis(6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine)] or lamotrigine *p*-dimer [N^3,$N^{3'}$-methylenebis(6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine)].

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Lamotrigine RS
USP Lamotrigine Related Compound C RS
3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4*H*)-one.
$C_9H_6Cl_2N_4O$ 257.08

Lamotrigine Tablets for Oral Suspension

DEFINITION

Lamotrigine Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$).

IDENTIFICATION

Delete the following:

- **▲A. INFRARED ABSORPTION** ⟨197K⟩

Sample: Transfer crushed powder of the Tablets for Oral Suspension, equivalent to 30 mg of lamotrigine, into an Erlenmeyer flask, and add 10 mL of chloroform. Sonicate for about 15 min. Shake the flask for another 2 min. Pass the sample through a Whatman No. 1 filter paper. Evaporate the solution to dryness. Add 250 mg of potassium bromide to the dried residue, and prepare the pellet.

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Acceptance criteria: Absorption bands at 1491 cm^{-1}, 1557 cm^{-1}, 1621 cm^{-1}, 3213 cm^{-1}, 3320 cm^{-1}, and 3451 cm^{-1} are found in the *Sample* and a similarly prepared Standard. ▲ (USP 1-Aug-2019)

Add the following:

- ▲**A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ (USP 1-Aug-2019)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 0.77 g/L of ammonium acetate ▲in water;▲ (USP 1-Aug-2019) adjusted with glacial acetic acid to a pH of 4.5
Mobile phase: Acetonitrile, methanol, and *Buffer* (30:10:60)
Diluent: Acetonitrile, methanol, and *Buffer* (30:30:40)
Standard solution: 0.05 mg/mL of USP Lamotrigine RS in *Diluent*
Sample solution: Nominally 0.05 mg/mL of lamotrigine prepared as follows. Transfer NLT 6 Tablets for Oral Suspension to a suitable volumetric flask. Sonicate in 70% of the flask volume of *Diluent* for 30 min with intermittent shaking. Dilute with *Diluent* to final volume, and pass a portion through a suitable membrane filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL, degassed
Apparatus 2: 50 rpm
Time: 15 min. [NOTE—The *Sample solution* may be analyzed using either *Chromatographic procedure 1* or *Chromatographic procedure 2*.]
Standard stock solution: 0.5 mg/mL of USP Lamotrigine RS in methanol
Standard solution: (*L*/1000) mg/mL of USP Lamotrigine RS in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Determine the amount of lamotrigine dissolved by employing one of the following chromatographic procedures.
Chromatographic procedure 1
Buffer: To 1 L of 0.77 g/L of ammonium acetate in water add 2 mL of triethylamine, and adjust with glacial acetic acid acid to a pH of 7.5.
Mobile phase: Acetonitrile, methanol, and *Buffer* (20:15:65)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 310 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Chromatographic procedure 2
Mobile phase: Acetonitrile, water, glacial acetic acid, and triethylamine (47:148:4:1). [NOTE—The *Mobile phase* is stable for 48 h at room temperature.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamotrigine dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
L = label claim of lamotrigine (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of lamotrigine is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

[NOTE—*Procedure 1* is recommended if lamotrigine related compound B is a potential organic impurity. *Procedure 2* is recommended if lamotrigine related compound C is a potential organic impurity.]

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Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 1**

Buffer, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.

Standard solution: 0.8 µg/mL of USP Lamotrigine RS in *Diluent*

Sample solution: ▲Nominally 0.25 mg/mL of lamotrigine prepared as follows.▲ (USP 1-Aug-2019) From NLT 20 Tablets for Oral Suspension ground to a fine powder, transfer an amount of powder to a suitable flask to obtain a nominal concentration of 0.25 mg/mL of lamotrigine in *Diluent*. Sonicate for 15 min to dissolve the contents. Filter a portion, and discard the first 1 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of lamotrigine from the *Standard solution*

C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamotrigine	1.0	—	—
Lamotrigine related compound B[a]	1.59	0.69	0.1
Any other individual ▲impurity▲ (USP 1-Aug-2019)	—	1.0	0.2
Total impurities	—	—	0.4

[a] 2,3-Dichlorobenzoic acid.

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 2**

Mobile phase and **Chromatographic system:** Proceed as directed in *Chromatographic procedure 2* in the *Dissolution* test.

Diluent: Methanol and water (40:60)

Standard solution: 0.2 mg/mL of USP Lamotrigine RS and 0.002 mg/mL of USP Lamotrigine Related Compound C RS prepared as follows. Transfer suitable amounts of USP Lamotrigine RS and USP Lamotrigine Related Compound C RS to a suitable volumetric flask. Add 40% of the flask volume of methanol, and sonicate until dissolved. Allow to cool to room temperature, and dilute with water to volume.

Sample solution: Nominally 0.2 mg/mL of lamotrigine. Use 10 Tablets for Oral Suspension for a label claim of 25 mg or less and 5 Tablets for Oral Suspension for a label claim of 50 mg or more prepared as follows. Transfer the appropriate number of Tablets for Oral Suspension to a suitable volumetric flask. Add 40% of the flask volume of water. Swirl until the tablets have disintegrated. Allow the effervescence to stop; and then add an additional 40% of the flask volume of methanol. Sonicate the flask for 10 min, and cool to room temperature. Dilute with water to volume. [NOTE—For Tablets for Oral Suspension with a 50 mg or higher label claim, a suitable intermediate concentration may be chosen. The final dilution to arrive at the nominal concentration is made using *Diluent*.]

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between lamotrigine and lamotrigine related compound C

Tailing factor: NMT 2.0 for lamotrigine and lamotrigine related compound C

Relative standard deviation: NMT 5.0% for lamotrigine related compound C and NMT 1.5% for lamotrigine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lamotrigine related compound C in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lamotrigine related compound C from the *Sample solution*

r_S = peak response of lamotrigine related compound C from the *Standard solution*

C_S = concentration of USP Lamotrigine Related Compound C RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified ▲impurity▲ (USP 1-Aug-2019) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other impurity from the *Sample solution*

r_S = peak response of lamotrigine from the *Standard solution*

C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

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Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamotrigine	1.0	—
Lamotrigine related compound C[a]	1.3	0.3
Any other individual unspecified ▲impurity▲ (USP 1-Aug-2019)	—	0.2
Total impurities	—	0.5

[a] 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4*H*)-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in tight, light-resistant containers, at controlled room temperature.
- **LABELING:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure the article complies. The label states that the Tablets for Oral Suspension may be swallowed whole, chewed, or dispersed in water or diluted fruit juice.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lamotrigine RS
  1,2,4-Triazine-3,5-diamine, 6-(2,3-dichlorophenyl).
  $C_9H_7Cl_2N_5$ 256.09
  USP Lamotrigine Related Compound C RS
  3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4*H*)-one.
  $C_9H_6Cl_2N_4O$ 257.08

Lamotrigine Compounded Oral Suspension

DEFINITION

Lamotrigine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$).

Prepare Lamotrigine Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Lamotrigine tablet[a] equivalent to	100 mg
Ora-Blend,[b] a sufficient quantity to make	100 mL

[a] Lamotrigine 100-mg tablet, Torrent Pharmaceuticals LTD, Kalamazoo, MI.
[b] Perrigo, Minneapolis, MN.

Place the required number of tablet(s) in a suitable mortar, and comminute to a fine powder. Add the *Ora-Blend* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Ora-Blend* to make a lamotrigine liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Ora-Blend* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water. Add 6.5 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.0.

Mobile phase: Acetonitrile and *Solution A* (20:80). Filter, and degas.

Diluent: 0.1 M hydrochloric acid

Standard solution: 0.4 mg/mL of USP Lamotrigine RS in *Diluent*

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 4 mL of Oral Suspension into a 10 mL volumetric flask, dilute with *Diluent* to volume, and mix well.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 5 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for lamotrigine is about 9.8 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lamotrigine from the *Sample solution*
r_S = peak response of lamotrigine from the *Standard solution*
C_S = concentration of lamotrigine in the *Standard solution* (mg/mL)
C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or 2°–8°.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or 2°–8°
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lamotrigine RS

Lanolin

DEFINITION

Lanolin is the purified, wax-like substance from the wool of sheep, *Ovis aries* L. (Fam. Bovidae), that has been cleaned, decolorized, and deodorized. It contains NMT 0.25% of water. It may contain NMT 0.02% of a suitable antioxidant.

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Sample solution: Boil 20 mL of alcohol with 1.0 g of Lanolin under a reflux condenser. Cool, add 1 mL of 2 N nitric acid,

USP Monographs

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and filter. To the filtrate add 5 drops of a solution of 20 mg/mL of silver nitrate in alcohol.

Blank: Boil 20 mL of alcohol under a reflux condenser. Cool, add 1 mL of 2 N nitric acid, and filter. To the filtrate add 5 drops of a solution of 20 mg/mL of silver nitrate in alcohol. Add 0.50 mL of 0.020 N hydrochloric acid.

Acceptance criteria: 0.035%; any turbidity produced by the *Sample solution* does not exceed that produced by the *Blank*.

• **FOREIGN SUBSTANCES**

Use pesticide-free grade reagents and solvents throughout this test. [NOTE—Reference materials of pesticides for use in the *Standard solution* may be obtained from any commercial source.[1]]

Standard stock solutions: Prepare stock solutions for each reference pesticide containing 100 mg/L in hexane. [NOTE—Concentrated stock solutions may be stored in glass-stoppered containers in a dark refrigerator at 2°–5° for up to 1 year. Most pesticides may be dissolved directly in hexane; however, the hexachlorocyclohexane isomers and the DDT group of pesticides may require initial dissolution in the minimum volume of acetone followed by dilution with hexane to the specified concentration.]

Standard solution: Dilute volumes of the *Standard stock solutions* quantitatively with hexane, and combine to obtain a composite *Standard solution* having the concentrations indicated in *Table 1*. Store the composite *Standard solution* in a glass-stoppered glass container in the dark at 2°–5°, and replace it every 2 months. [NOTE—Two or more separate composite *Standard solutions*, each preferably containing NMT 8 reference pesticides, may be prepared if needed. Reference pesticides should be selected for composite *Standard solutions* on the basis that relative retention times (see *Table 1*) differ sufficiently so that peaks in chromatograms will not be expected to overlap, and they should be selected and combined appropriately for the chromatographic system and detector used.]

Gel permeation chromatography cleanup system

Eluant: Methylene chloride and hexane (1:1)

Column: 25-mm × 50-cm; packed with a slurry of 35 g of styrene–divinylbenzene copolymer beads compressed to a bed length of about 20 cm

Operating pressure: 8–11 psi

Flow rate: 5 mL/min

Set up the chromatograph, adjusting to discard the fraction eluting from 0 to 12 min. Collect the fraction eluting from 12 to 32 min, and rinse for 2 min, discarding the rinse fraction.

System suitability

Elution of lanolin: Melt a suitable quantity of Lanolin, and pass through a fluted filter paper into a container. Transfer 6.0 g to a 50-mL volumetric flask. Dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the gel permeation chromatographic column, and elute with *Eluant*. Collect 100 mL of the column effluent in tared beakers in 10-mL increments. Evaporate the solvent, cool, weigh the beakers and contents, and calculate the amount of lanolin eluted in each 10-mL increment. The column is suitable if NLT 96% of the lanolin elutes in the first 60 mL.

Elution of pesticide from lanolin: Dissolve suitable quantities of diazinon, diclofenthion, bromophos ethyl, lindane, and dieldrin in hexane to obtain a *Standard solution* having concentrations of 0.4, 0.4, 1.0, 0.1, and 0.6 µg/mL, respectively. Transfer 5.0 mL of this solution to a 10-mL volumetric flask containing 1 g of USP Lanolin RS. Dilute with methylene chloride to volume. Transfer 5 mL of this solution to the gel permeation chromatographic column, and elute with 160 mL of *Eluant*. Discard the first 60-mL fraction, and collect the next 100-mL fraction (from 60 to 160 mL). Transfer this collection fraction to a concentrator fitted with a graduated collection flask, add 50 mL of hexane, and concentrate by evaporation to 5 mL. Inject this fraction into the chromatographs described in *Chromatographic system I* and *Chromatographic system II*. Record the chromatograms, and measure the heights of the peaks obtained from the five pesticides in the *Standard solution*. Calculate the recoveries of each of the five pesticides used in the fortified USP Lanolin RS solution. Prepare a test solution by mixing hexane with the *Standard solution* (1:1). Inject this into the chromatographs described in *Chromatographic system I* and *Chromatographic system II*. Record the chromatograms, and measure the peak heights of the five pesticides in the chromatogram of the *Sample solution*. Compare the peak heights from the fraction of the *Standard solution* to the peak heights of the corresponding pesticides from the *Sample solution*: NLT 85% of the added amounts of each of the five pesticides is recovered.

Sample solution: Transfer 6 g of Lanolin, previously melted to liquid form by heating on a hot water bath if necessary, to a 50-mL volumetric flask. Dissolve in 25 mL of *Eluant*, dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the column, and elute with 160 mL of *Eluant*. Discard the first 60-mL fraction, and collect the remaining fraction in a suitable evaporator. Concentrate by evaporation on a steam bath to 3 mL, add 50 mL of hexane, and evaporate again to remove all traces of methylene chloride, adjusting the volume with hexane to 3.0 mL.

Chromatographic system I

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Electron capture

Column: 0.53-mm × 30-m fused silica capillary; bonded with a 1.5-µm layer of phase G1, and a 0.53-mm × 6-m fused silica uncoated guard column connected to a modified packed column-type injector system

Column temperature: 200°. [NOTE—The initial temperature of the column may be adjusted so that the retention times of ethion and *p,p'*-DDT are 2.56 and 3.1, respectively, relative to chlorpyrifos.]

Carrier gas: Helium

Flow rate: 25 mL/min. Adjust so that the retention time of chlorpyrifos is 4 min.

Makeup gas: Nitrogen, 40 mL per minute

Injection volume: 5 µL

Chromatographic system II

Mode: GC

Detector: Flame photometric

Column: 0.53-mm × 30-m fused silica capillary; bonded with a 1.0-µm layer of phase G3, and a 0.53-mm × 6-m fused silica uncoated guard column connected to a modified packed column-type injector system

Column temperature: 200°. [NOTE—The initial temperature of the column may be adjusted so that the retention time of ethion is 3.36 relative to that of chlorpyrifos.]

Carrier gas: Helium

Flow rate: 25 mL/min. Adjust so that the retention time of chlorpyrifos is 4 min.

Makeup gas: Nitrogen, 40 mL/min

Injection volume: 5 µL

[1] Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

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Table 1 (Lanolin)

Reference Pesticide[a]	Standard Solution (Concentration in µg/mL)		Relative Retention Times (Relative to 1.0 for Chlorpyrifos)	
	Electron-Capture Detector	Flame-Photometric Detector	System I	System II
Tetrachloronitrobenzene (TCBN)	0.05	—	0.29	0.24
alpha-Hexachlorocyclohexane (alpha BHC)	0.05	—	0.40	0.35
beta-Hexachlorocyclohexane (beta BHC)	0.30	—	0.43	0.56
Hexachlorobenzene (HCB)	0.05	—	0.45	0.33
gamma-Hexachlorocyclohexane (lindane)	0.05	—	0.48	0.41
Propetamphos	—	0.30	0.48	0.42
Diazinon	—	0.20	0.52	0.40
Dichlofenthion	0.10	0.20	0.67	0.56
Ronnel	0.30	0.40	0.81	0.66
Heptachlor	0.10	—	0.83	0.60
Malathion	—	0.40	0.91	1.05
Chlorpyrifos	0.30	0.30	1.00	1.00
Aldrin	0.20	—	1.05	0.76
Pirimiphos ethyl	—	0.40	1.14	1.14
Chlorfenvinphos Z	0.40	0.40	1.17	1.40
Heptachlor epoxide	0.20	—	1.29	1.17
Chlorfenvinphos E	0.40	0.50	1.30	1.51
Bromophos ethyl	0.40	0.50	1.51	1.45
1,1′-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethene (*o,pp*-DDE)	0.30	—	1.55	1.51
1,1′-Dichloro-2-(4-chlorophenyl)-2-(4-chlorophenyl)ethene (*p,pp*-DDE)	0.30	—	1.88	1.86
Stirophos	0.60	0.80	1.58	1.97
alpha-Endosulfan	0.40	—	1.63	1.47
1,1′-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (*o,pp*-TDE)	0.40	—	1.90	2.19
Dieldrin	0.30	—	1.91	1.84
Endrin	0.40	—	2.13	2.29
beta-Endosulfan	0.40	—	2.19	2.77
1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (*p,pp*-TDE)	0.40	—	2.41	2.87
1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (*o,pp*-DDT)	0.40	—	2.55	2.70
Ethion	1.00	0.40	2.56	3.36
Carbophenothion	0.80	1.00	2.94	3.70
1,1,1-Trichloro-2,2-bix(4-chlorophenyl)ethane (*p,p*′-DDT)	0.50	—	3.13	3.50
Methoxychlor	0.60	—	4.70	7.20
Carbophenothion sulfone	5.00	—	5.10	9.20
Carbophenothion sulfoxide	5.00	—	5.40	10.00

[a] Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

Analysis

The following procedure is to be followed for *Chromatographic systems I* and *II*.

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of the individual specified residue found in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C/W) \times 30$$

r_U = peak area of each residue from the *Sample solution*

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r_S = peak area of each residue from the *Standard solution*
C = concentration of the reference pesticide in the *Standard solution* (mg/L)
W = weight of Lanolin taken (g)

Acceptance criteria
Individual specified residue: NMT 10 ppm
Total specified residue: NMT 40 ppm

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE,** *Class II* ⟨741⟩
Analysis: Determine on a sample previously cooled to 8°–10°.
Acceptance criteria: 38°–44°
- **FATS AND FIXED OILS,** *Acid Value (Free Fatty Acids)* ⟨401⟩
Sample: 10.0 g
Acceptance criteria: The free acids obtained from the *Sample* require NMT 2.0 mL of 0.10 N sodium hydroxide for neutralization.
- **FATS AND FIXED OILS,** *Iodine Value* ⟨401⟩
Sample: 780–820 mg
Acceptance criteria: 18–36
- **ALKALINITY**
Sample: 2.0 g
Analysis: Dissolve the *Sample* in 10 mL of ether, and add 2 drops of phenolphthalein TS.
Acceptance criteria: The liquid is not colored red.
- **WATER-SOLUBLE ACIDS AND ALKALIES**
Sample: 10.0 g
Analysis: Warm the *Sample* with 50 mL of water on a steam bath, constantly stirring the mixture until the Lanolin is melted.
Acceptance criteria: The fat separates completely on cooling, leaving the water layer nearly clear and neutral to litmus. Retain the water layer for the test for *Water-Soluble Oxidizable Substances* and *Ammonia*.
- **WATER-SOLUBLE OXIDIZABLE SUBSTANCES**
Sample solution: 10 mL of the solution from *Water-Soluble Acids and Alkalies*
Analysis: Add the *Sample solution* to 50 µL of 0.10 N potassium permanganate.
Acceptance criteria: The resulting solution does not completely decolorize within 10 min.
- **AMMONIA**
Sample solution: 10 mL of the solution from *Water-Soluble Acids and Alkalies*
Analysis: Add 1 mL of 1 N sodium hydroxide to the *Sample solution*, and boil.
Acceptance criteria: The vapors do not turn red litmus to blue.
- **WATER DETERMINATION,** *Method I* ⟨921⟩
Solution A: Chloroform and methanol (3:2)
Sample solution: 250 mg/mL of Lanolin in *Solution A*
Analysis: Determine the water content of a 10.0-mL portion of the *Sample solution*. Perform a blank determination on 10.0 mL of *Solution A*, and make any necessary correction.
Acceptance criteria: NMT 0.25%
- **PETROLATUM**
Sample: 3 g
Analysis: Heat the *Sample* on a steam bath, with frequent stirring, until its weight loss is NLT its water content. Boil 40 mL of dehydrated alcohol with 500 mg of the dried lanolin so obtained.
Acceptance criteria: The solution is clear or NMT opalescent.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, preferably at controlled room temperature.
- **LABELING:** The label states that it is not to be used undiluted.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Lanolin RS

Modified Lanolin

DEFINITION
Modified Lanolin is the purified wax-like substance from the wool of sheep, *Ovis aries* L. (Fam. Bovidae), that has been processed to reduce the contents of free lanolin alcohols and residues of detergent and pesticide. It contains NMT 0.25% of water. It may contain NMT 0.02% of a suitable antioxidant.

IMPURITIES
- **LIMIT OF FREE LANOLIN ALCOHOLS**
Gel permeation chromatographic cleanup system
Eluant: Methylene chloride
Column: 25-mm × 100-cm; packed with a slurry of styrene–divinylbenzene copolymer beads compressed to a bed length of approximately 77 cm
Flow rate: 4 mL/min
Set up the chromatograph, adjusting to discard the fraction eluting from 0 to 43 min. Collect the fraction eluting from 43 to 60 min, and rinse for 20 min, discarding the rinse fraction.
System suitability
Elution of lanolin alcohols: Melt a suitable quantity of USP Lanolin Alcohols RS, and pass through a fluted filter paper into a container. Transfer 1.0 g to a 10-mL volumetric flask. Dilute with *Eluant* to volume. Transfer 5 mL to the gel permeation chromatographic column, and elute with *Eluant*. Collect 172–240 mL of the column effluent in a suitable evaporator. Evaporate the solvent, cool, weigh the evaporator, and calculate the amount of lanolin alcohols eluted in the evaporator. The column is suitable if NLT 99% of the lanolin alcohols elute in the first 172–240 mL.
Standard solution: 0.5 mg/mL of USP Lanolin Alcohols RS in hexane. Store this solution in a cold, dark place for up to 4 weeks. Before using, warm just sufficiently to dissolve any precipitate if necessary.
Sample solution: Transfer 1 g of Modified Lanolin, previously melted to liquid form by heating on a hot water bath if necessary, to a 10-mL volumetric flask. Dissolve in 7 mL of *Eluant*, dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the column, and elute with 320 mL of *Eluant*. Discard the first 172-mL fraction, and collect the next 68-mL fraction (from 172 to 240 mL) in a suitable evaporator. Concentrate by evaporation on a steam bath to 3 mL. Add 50 mL of hexane, and transfer this solution to a 100-mL volumetric flask, adjusting the volume with hexane to 100 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Columns
Guard: 0.32-mm × 50-cm fused silica uncoated
Analytical: 0.33-mm × 50-m fused silica capillary; bonded with a 0.50-µm layer of phase G2
Temperatures
Detector: 290°
Column: See *Table 1*.

USP Monographs

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Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
210	3	280	—

Flow rate: 7 mL/min
Carrier gas: Nitrogen
Makeup gas: Nitrogen at 50 mL/min
Injection volume: 1 µL

Analysis

Samples: *Standard solution* and *Sample solution*
[NOTE—Allow both the *Standard solution* and the *Sample solution* to elute for NLT 40 min.]

Calculate the percentage of free lanolin alcohols in the portion of Modified Lanolin taken:

$$\text{Result} = (r_U/r_S) \times [(C \times K)/(I \times W)] \times 100$$

r_U = total peak response from the *Sample solution*
r_S = total peak response from the *Standard solution*
C = concentration of USP Lanolin Alcohols RS in the *Standard solution* (mg/mL)
I = volume injected into the gel permeation chromatography column (mL)
W = weight of Modified Lanolin taken (g)
K = corrected fraction of free lanolin alcohols in the USP Lanolin Alcohols RS in the *Standard solution* taken:

$$K = 1 + (0.0062A - 0.0119S)$$

A = acid value of USP Lanolin Alcohols RS
S = saponification value of USP Lanolin Alcohols RS

Acceptance criteria: NMT 6%

• **FOREIGN SUBSTANCES**

Use pesticide-free grade reagents and solvents throughout this test. [NOTE—Reference materials of pesticides for use in the *Standard solution* may be obtained from any commercial source.[1]]

Standard stock solutions: Prepare stock solutions for each reference pesticide containing 100 mg/L in hexane. [NOTE—Concentrated stock solutions may be stored in glass-stoppered containers in a dark refrigerator at 2°–5° for up to 1 year. Most pesticides may be dissolved directly in hexane; however, the hexachlorocyclohexane isomers and the DDT group of pesticides may require initial dissolution in the minimum volume of acetone followed by dilution with hexane to the specified concentration.]

Standard solution: Dilute volumes of the *Standard stock solutions* quantitatively with hexane, and combine to obtain a composite *Standard solution* having the concentrations indicated in *Table 2*. Store the composite *Standard solution* in a glass-stoppered glass container in the dark at 2°–5°, and replace it every 2 months. [NOTE—Two or more separate composite *Standard solutions*, each preferably containing NMT 8 reference pesticides, may be prepared if needed. Reference pesticides should be selected for composite *Standard solutions* on the basis that relative retention times (see *Table 2*) differ sufficiently so that peaks in chromatograms will not be expected to overlap, and they should be selected and combined appropriately for the chromatographic system and detector used.]

Gel permeation chromatography cleanup system

Eluant: Methylene chloride and hexane (1:1)
Column: 25-mm × 50-cm; packed with a slurry of 35 g of styrene–divinylbenzene copolymer beads compressed to a bed length of about 20 cm
Operating pressure: 8–11 psi
Flow rate: 5 mL/min. Set up the chromatograph, adjusting to discard the fraction eluting from 0 to 12 min. Collect the fraction eluting from 12 to 32 min, and rinse for 2 min, discarding the rinse fraction.

System suitability

Elution of lanolin: Melt a suitable quantity of Lanolin, and pass through a fluted filter paper into a container. Transfer 6.0 g to a 50-mL volumetric flask. Dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the gel permeation chromatographic column, and elute with *Eluant*. Collect 100 mL of the column effluent in tared beakers in 10-mL increments. Evaporate the solvent, cool, weigh the beakers and contents, and calculate the amount of lanolin eluted in each 10-mL increment. The column is suitable if NLT 96% of the lanolin elutes in the first 60 mL.

Elution of pesticide from lanolin: Dissolve suitable quantities of diazinon, diclofenthion, bromophos ethyl, lindane, and dieldrin in hexane to obtain a *Standard solution* having concentrations of 0.4, 0.4, 1.0, 0.1, and 0.6 µg/mL, respectively. Transfer 5.0 mL of this solution to a 10-mL volumetric flask containing 1 g of USP Lanolin RS. Dilute with methylene chloride to volume. Transfer 5 mL of this solution to the gel permeation chromatographic column, and elute with 160 mL of *Eluant*. Discard the first 60-mL fraction, and collect the next 100-mL fraction (from 60 to 160 mL). Transfer this collection fraction to a concentrator fitted with a graduated collection flask, add 50 mL of hexane, and concentrate by evaporation to 5 mL. Inject this fraction into the chromatographs described in *Chromatographic system I* and *Chromatographic system II*. Record the chromatograms, and measure the heights of the peaks obtained from the five pesticides in the *Standard solution*. Calculate the recoveries of each of the five pesticides used in the fortified USP Lanolin RS solution. Prepare a test solution by mixing hexane with the *Standard solution* (1:1). Inject into the chromatographs described in *Chromatographic system I* and *Chromatographic system II*. Record the chromatograms, and measure the peak heights of the five pesticides in the chromatogram of the *Sample solution*. Compare the peak heights from the fraction of the *Standard solution* to the peak heights of the corresponding pesticides from the *Sample solution*: NLT 85% of the added amounts of each of the five pesticides is recovered.

Sample solution: Transfer 6 g of Lanolin, previously melted to liquid form by heating on a hot water bath if necessary, to a 50-mL volumetric flask. Dissolve in 25 mL of *Eluant*, dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the column, and elute with 160 mL of *Eluant*. Discard the first 60-mL fraction, and collect the remaining fraction in a suitable evaporator. Concentrate by evaporation on a steam bath to 3 mL, add 50 mL of hexane, and evaporate again to remove all traces of methylene chloride, adjusting the volume with hexane to 3.0 mL.

Chromatographic system I

(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Electron capture

[1] Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

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Table 2 (Modified Lanolin)

Reference Pesticide[a]	Standard Solution (Concentration in µg/mL)		Relative Retention Times (Relative to 1.0 for Chlorpyrifos)	
	Electron-Capture Detector	Flame-Photometric Detector	System I	System II
Tetrachloronitrobenzene (TCBN)	0.05	—	0.29	0.24
alpha-Hexachlorocyclohexane (alpha BHC)	0.05	—	0.40	0.35
beta-Hexachlorocyclohexane (beta BHC)	0.30	—	0.43	0.56
Hexachlorobenzene (HCB)	0.05	—	0.45	0.33
gamma-Hexachlorocyclohexane (lindane)	0.05	—	0.48	0.41
Propetamphos	—	0.30	0.48	0.42
Diazinon	—	0.20	0.52	0.40
Dichlofenthion	0.10	0.20	0.67	0.56
Ronnel	0.30	0.40	0.81	0.66
Heptachlor	0.10	—	0.83	0.60
Malathion	—	0.40	0.91	1.05
Chlorpyrifos	0.30	0.30	1.00	1.00
Aldrin	0.20	—	1.05	0.76
Pirimiphos ethyl	—	0.40	1.14	1.14
Chlorfenvinphos Z	0.40	0.40	1.17	1.40
Heptachlor epoxide	0.20	—	1.29	1.17
Chlorfenvinphos E	0.40	0.50	1.30	1.51
Bromophos ethyl	0.40	0.50	1.51	1.45
1,1-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethene (*o,p*-DDE)	0.30	—	1.55	1.51
1,1-Dichloro-2-(4-chlorophenyl)-2-(4-chlorophenyl)ethene (*p,p*-DDE)	0.30	—	1.88	1.86
Stirophos	0.60	0.80	1.58	1.97
alpha-Endosulfan	0.40	—	1.63	1.47
1,1-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (*o,p*-TDE)	0.40	—	1.90	2.19
Dieldrin	0.30	—	1.91	1.84
Endrin	0.40	—	2.13	2.29
beta-Endosulfan	0.40	—	2.19	2.77
1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (*p,p*-TDE)	0.40	—	2.41	2.87
1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (*o,p*-DDT)	0.40	—	2.55	2.70
Ethion	1.00	0.40	2.56	3.36
Carbophenothion	0.80	1.00	2.94	3.70
1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (*p,p*-DDT)	0.50	—	3.13	3.50
Methoxychlor	0.60	—	4.70	7.20
Carbophenothion sulfone	5.00	—	5.10	9.20
Carbophenothion sulfoxide	5.00	—	5.40	10.00

[a] Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

Column: 0.53-mm × 30-m fused silica capillary; bonded with a 1.5-µm layer of phase G1, and a 0.53-mm × 6-m fused silica uncoated guard column connected to a modified packed column-type injector system

Column temperature: 200°. [NOTE—The initial temperature of the column may be adjusted so that the retention times of ethion and *p,p'*-DDT are 2.56 and 3.1, respectively, relative to chlorpyrifos.]

Carrier gas: Helium

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Flow rate: 25 mL/min. Adjust so that the retention time of chlorpyrifos is 4 min.
Makeup gas: Nitrogen, 40 mL/min
Injection volume: 5 µL
Chromatographic system II
Mode: GC
Detector: Flame photometric
Column: 0.53-mm × 30-m fused silica capillary; bonded with a 1.0-µm layer of phase G3, and a 0.53-mm × 6-m fused silica uncoated guard column connected to a modified packed column-type injector system
Column temperature: 200°. [NOTE—The initial temperature of the column may be adjusted so that the retention time of ethion is 3.36 relative to that of chlorpyrifos.]
Carrier gas: Helium
Flow rate: 25 mL/min. Adjust so that the retention time of chlorpyrifos is 4 min.
Makeup gas: Nitrogen, 40 mL/min
Injection volume: 5 µL
Analysis
The following procedure is to be followed for *Chromatographic systems I* and *II*.
Samples: *Standard solution* and *Sample solution*
Inject the appropriate composite *Standard solution* and the *Sample solution* into the gas chromatograph, record the chromatograms, and measure the areas of all the peaks observed in the chromatograms. Compare the peak areas of any of the pesticide residues in the *Sample solution* from each chromatographic system with the peak areas that correspond to the retention times in the appropriate composite *Standard solution* from each corresponding chromatographic system.
Calculate the quantity, in ppm, of the individual specified residue found in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C/W) \times 30$$

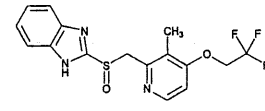
r_U = peak area of each residue from the *Sample solution*
r_S = peak area of each residue from the *Standard solution*
C = concentration of the reference pesticide in the *Standard solution* (mg/L)
W = weight of Lanolin taken (g)

Acceptance criteria
Individual specified residue: NMT 1 ppm
Total specified residues: NMT 3 ppm

SPECIFIC TESTS
• **FATS AND FIXED OILS,** *Acid Value (Free Fatty Acids)* ⟨401⟩
Sample: 12.5 g
Acceptance criteria: The free acids obtained from the *Sample* require NMT 2.0 mL of 0.10 N sodium hydroxide for neutralization.
• **ALKALINITY**
Sample: 2.5 g
Analysis: Dissolve the *Sample* in 10 mL of ether, and add 2 drops of phenolphthalein TS.
Acceptance criteria: No red color is produced.
• **WATER-SOLUBLE ACIDS AND ALKALIES**
Sample: 12.5 g
Analysis: Warm the *Sample* with 50 mL of water on a steam bath, constantly stirring the mixture until the Lanolin is melted.
Acceptance criteria: The fat separates completely on cooling, leaving the water layer nearly clear and neutral to litmus. Retain the water layer for the test for *Ammonia*.
• **AMMONIA**
Sample solution: 10 mL of the solution from *Water-Soluble Acids and Alkalies*
Analysis: Add 1 mL of 1 N sodium hydroxide to the *Sample solution*, and boil.
Acceptance criteria: The vapors do not turn red litmus to blue.
• **WATER DETERMINATION,** *Method I* ⟨921⟩
Solution A: Chloroform and methanol (3:2)
Sample solution: 250 mg/mL of Modified Lanolin in *Solution A*
Analysis: Determine the water content of a 10.0-mL portion of the *Sample solution*. Perform a blank determination on 10.0 mL of *Solution A*, and make any necessary correction.
Acceptance criteria: NMT 0.25%
• **PETROLATUM**
Sample: 3 g
Analysis: Heat the *Sample* on a steam bath, with frequent stirring, until it loses about 0.25% of its weight. Boil 40 mL of dehydrated alcohol with 500 mg of the dried lanolin so obtained.
Acceptance criteria: The solution is clear or NMT opalescent.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, preferably rust-proof containers, preferably at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lanolin RS
USP Lanolin Alcohols RS

Lansoprazole



$C_{16}H_{14}F_3N_3O_2S$ 369.36
1*H*-Benzimidazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-;
2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]-methyl] sulfinyl]benzimidazole [103577-45-3].

DEFINITION
Lansoprazole contains NLT 98.0% and NMT 102.0% of $C_{16}H_{14}F_3N_3O_2S$.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 10 µg/mL in methanol
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, water, and triethylamine (40:60:1). Adjust with phosphoric acid to a pH of 7.0.

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Diluent: Acetonitrile, water, and triethylamine (40:60:1). Adjust with phosphoric acid to a pH of 10.0.
System suitability solution: 0.1 mg/mL of USP Lansoprazole RS and 0.1 mg/mL of USP Lansoprazole Related Compound A RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Lansoprazole RS in *Diluent*
Sample solution: 0.1 mg/mL of Lansoprazole in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5 between lansoprazole and lansoprazole related compound A, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) in the portion of Lansoprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lansoprazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Lansoprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

Organic Impurities

• **Procedure**
[Note—Store and inject the lansoprazole solutions at or below 5° using a cooled autosampler. The solutions are stable for about 24 h when stored at 5°.]
Solution A: Water
Solution B: Acetonitrile, water, and triethylamine (160:40:1). Adjust with phosphoric acid to a pH of 7.0.
Diluent: Methanol and 0.1 N sodium hydroxide (1:3)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
40	20	80
50	20	80
51	90	10
60	90	10

System suitability solution: Prepare a solution containing 25 µg/mL of USP Lansoprazole RS and 25 µg/mL of USP Lansoprazole Related Compound A RS in methanol. Transfer 1 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.
Standard solution: Prepare a solution containing 25 µg/mL of USP Lansoprazole RS and 25 µg/mL of USP Lansoprazole Related Compound B RS in methanol. Transfer 1 mL of this solution into a 100-mL volumetric flask, and dilute with *Diluent* to volume.
Sample solution: 2.5 mg/mL of Lansoprazole in methanol. Transfer 1 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.
Blank: Methanol and *Diluent* (1:9)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection size: 40 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 6 between lansoprazole and lansoprazole related compound A
Relative standard deviation: NMT 3%
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Identify the lansoprazole peak and the peaks due to the impurities listed in *Table 2*. Measure the areas for the major peaks, excluding peaks obtained from the *Blank*.
Calculate the percentage of lansoprazole related compound B in the portion of Lansoprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for lansoprazole related compound B from the *Sample solution*
r_S = peak response for lansoprazole related compound B from the *Standard solution*
C_S = concentration of USP Lansoprazole Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of Lansoprazole in the *Sample solution* (µg/mL)

Calculate the percentage of lansoprazole *N*-oxide, lansoprazole sulfone, and any other individual impurity in the portion of Lansoprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for lansoprazole from the *Standard solution*
C_S = concentration of USP Lansoprazole RS in the *Standard solution* (µg/mL)
C_U = concentration of Lansoprazole in the *Sample solution* (µg/mL)
F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria
Individual impurities: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lansoprazole *N*-oxide[a]	0.8	1.3	0.1

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lansoprazole	1.0	—	—
Lansoprazole related compound A (lansoprazole sulfone)[b]	1.1	0.82	0.4
Lansoprazole related compound B (lansoprazole sulfide)[c]	1.2	—	0.1
Other individual impurity	—	1.00	0.1
Total impurities	—	—	0.6

[a] [[(1*H*-Benzimidazole-2-yl)sulfinyl]methyl]-3-methyl-4-(2,2,2-trifluoroethoxy)-pyridine 1-oxide.
[b] 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl] benzimidazole.
[c] 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl]methyl]sulfanyl]-1*H*-benzimidazole.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ia* ⟨921⟩
  Sample: 1.0 g
  [NOTE—Use 50 mL of a dehydrated mixture of pyridine and ethylene glycol (9:1 to 8:2) as the solvent.]
  Acceptance criteria: NMT 0.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature, and protect from excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lansoprazole RS
  USP Lansoprazole Related Compound A RS
  2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl] sulfonyl]benzimidazole.
  $C_{16}H_{14}F_3N_3O_3S$ 385.36
  USP Lansoprazole Related Compound B RS
  2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl] methyl]sulfanyl]-1*H*-benzimidazole.
  $C_{16}H_{14}F_3N_3OS$ 353.36

Lansoprazole Delayed-Release Capsules

DEFINITION

Lansoprazole Delayed-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  ▲[NOTE—The UV spectra of the major peaks of the *Sample solution* and the *Standard solution* as obtained in the *Assay* may also be used to meet the *Acceptance criteria*.]▲ 2S (USP41)
  Standard solution: 10 µg/mL of USP Lansoprazole RS in methanol
  Sample solution: Nominally equivalent to 10 µg/mL of lansoprazole prepared as follows. Powder a portion of Capsule contents equivalent to 5 mg of lansoprazole. Add 5 mL of methanol, shake well, and centrifuge. To 0.1 mL of the supernatant, add 10 mL of methanol.
  Acceptance criteria: The UV absorption ▲spectra exhibit a maximum between 281 and 286 nm.▲ 2S (USP41)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  Mobile phase: Acetonitrile, water, and triethylamine (40:60:1). Adjust with phosphoric acid to a pH of 7.0.
  Diluent: Acetonitrile, water, and triethylamine (40:60:1). Adjust with phosphoric acid to a pH of 10.0.
  System suitability solution: 0.1 mg/mL each of USP Lansoprazole RS and USP Lansoprazole Related Compound A RS in *Diluent*▲ 2S (USP41)
  Standard stock solution: 3.0 mg/mL of USP Lansoprazole RS in a mixture of acetonitrile and 0.1 M sodium hydroxide (2:3)
  Standard solution: ▲0.09 mg/mL of USP Lansoprazole RS in *Diluent*, from *Standard stock solution*
  Sample stock solution: Transfer the contents of NLT 10 Capsules, equivalent to 300 mg of lansoprazole, to a 100-mL volumetric flask. Add 60.0 mL of 0.1 M sodium hydroxide and sonicate until completely disintegrated. Add 20.0 mL of acetonitrile and sonicate for about 20 min, dilute with acetonitrile to volume, allow to settle, and use a clear supernatant.▲ 2S (USP41)
  Sample solution: ▲Nominally 0.09 mg/mL of lansoprazole prepared as follows. Transfer 3.0 mL of the *Sample stock solution* to a 100-mL volumetric flask and dilute with *Diluent* to volume. Pass the solution through a PVDF filter having a 0.5-µm pore size or another suitable filter, discarding the first few milliliters of the filtrate.▲ 2S (USP41)
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 285 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm.▲ 2S (USP41)
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 5 between lansoprazole and lansoprazole related compound A, *System suitability solution*
  ▲Tailing factor: NMT 1.5, *Standard solution*▲ 2S (USP41)
  Relative standard deviation: NMT 2.0%, ▲▲ 2S (USP41) *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) in the portion of Capsules taken:

$$\text{Result} = {}^{\blacktriangle}(r_U/r_S)_{\blacktriangle\ 2S\ (USP41)} \times (C_S/C_U) \times 100$$

${}^{\blacktriangle}r_{U\blacktriangle\ 2S\ (USP41)}$ = peak response ▲of lansoprazole▲ 2S (USP41) from the *Sample solution*
${}^{\blacktriangle}r_{S\blacktriangle\ 2S\ (USP41)}$ = peak response ▲of lansoprazole▲ 2S (USP41) from the *Standard solution*

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C_S = concentration of USP Lansoprazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lansoprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method A Procedure*

▲**Test 1** ▲ 2S (USP41)

Acid stage

Acid stage medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 75 rpm

Time: 60 min

Acid stage standard solution: (L × 0.08/500) mg/mL of USP Lansoprazole RS in *Acid stage medium*, where L is the label claim, in mg/Capsule

[NOTE—An amount of methanol NMT 0.5% of the total volume of the *Acid stage standard solution* may be used to dissolve USP Lansoprazole RS prior to dilution with *Acid stage medium*.]

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 306 nm

Blank: *Acid stage medium*

Analysis: Withdraw a 25-mL aliquot, leaving the remaining 475 mL in the vessel for use in the *Buffer stage*, and proceed immediately as directed for *Buffer stage sample solution*. Use a filtered portion of the aliquot as an *Acid stage sample solution*.

▲**Samples:** *Acid stage standard solution* and *Acid stage sample solution*

Calculate the percentage of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) dissolved during the *Acid stage*:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Acid stage sample solution*
A_S = absorbance of the *Acid stage standard solution*
C_S = concentration of USP Lansoprazole RS in the *Acid stage standard solution* (mg/mL)
L = label claim of lansoprazole (mg/Capsule)
V = volume of *Acid stage medium*, 500 mL ▲ 2S (USP41)

Tolerances: NMT 10% of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) is dissolved.

Buffer stage

Buffer stage medium: pH 6.8 buffer; 900 mL. Proceed as directed in *Buffer stage sample solution*.

Apparatus 2: 75 rpm

Time: 60 min

Buffer concentrate: Dissolve 65.4 g of monobasic sodium phosphate, 28.2 g of sodium hydroxide, and 12 g of sodium dodecyl sulfate in 4 L of water.

Blank solution: *Acid stage medium* and *Buffer concentrate* (19:17). Adjust, if necessary, with either phosphoric acid or sodium hydroxide solution to a pH of 6.8.

Buffer stage standard solution: (L × 0.7/900) mg/mL of USP Lansoprazole RS in the *Blank solution*, where L is the label claim in mg/Capsule

[NOTE—An amount of methanol NMT 2% of the total volume of the *Buffer stage standard solution* may be used to dissolve USP Lansoprazole RS prior to dilution with *Blank solution*.]

Buffer stage sample solution: Add 425 mL of *Buffer concentrate* to the remaining 475 mL of solution in each vessel from the *Acid stage*. Adjust, if necessary, with either phosphoric acid or sodium hydroxide solution to a pH of 6.8, and pass through a suitable filter.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: About 286 and 650 nm

Blank: *Blank solution*

Analysis

Samples: *Buffer stage standard solution* and *Buffer stage sample solution*

Determine the percentage of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) dissolved using the difference between the absorbances at the wavelengths of about 286 nm and 650 nm.

$$\text{▲Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = difference between absorbances of the *Buffer stage sample solution*
A_S = difference between absorbances of the *Buffer stage standard solution*
C_S = concentration of USP Lansoprazole RS in the *Buffer stage standard solution* (mg/mL)
L = label claim of lansoprazole (mg/Capsule)
V = volume of *Buffer stage medium*, 900 mL ▲ 2S (USP41)

Tolerances: NLT 80% (Q) of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) is dissolved.

▲**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Acid stage: Proceed as directed in *Dissolution Test 1*.

Tolerances: NMT 10% of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) is dissolved.

Buffer stage: Proceed as directed in *Dissolution Test 1*, except for the *Time*.

Time: 45 min

Tolerances: NLT 80% (Q) of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) is dissolved. ▲ 2S (USP41)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Standard solution: 12 µg/mL of USP Lansoprazole RS in a mixture of acetonitrile and 0.1 M sodium hydroxide (7:3)

Sample solution: Transfer the contents of 1 Capsule to a 100-mL volumetric flask, add 30 mL of 0.1 M sodium hydroxide, and sonicate to disintegrate. Add 65 mL of acetonitrile, cool, and dilute with acetonitrile to volume. Centrifuge a portion of the suspension and pass through a membrane filter having a 0.5-µm or finer pore size. Further dilute a portion of the filtrate with a mixture of acetonitrile and 0.1 M sodium hydroxide (7:3) to obtain a solution having a nominal concentration of about 12 µg/mL of lansoprazole.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 294 nm

Cell: 1 cm

Blank: Acetonitrile and 0.1 M sodium hydroxide (7:3)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) in the Capsule taken:

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$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Lansoprazole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of lansoprazole in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

▲• ORGANIC IMPURITIES

[NOTE—Store and inject the lansoprazole solutions at or below 5° using a cooled autosampler. The solutions are stable for about 24 h when stored at 5°.]

Solution A: Water

Solution B: Acetonitrile, water, and triethylamine (160:40:1). Adjust with phosphoric acid to a pH of 7.0.

Diluent: Methanol and 0.1 N sodium hydroxide (1:3). Adjust with phosphoric acid to a pH of 10.0.

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for at least 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
40	20	80
50	20	80

System suitability solution: 0.1 mg/mL each of USP Lansoprazole RS and USP Lansoprazole Related Compound A RS in *Diluent*

Standard solution: 2 µg/mL of USP Lansoprazole RS in *Diluent*. Use sonication to dissolve.

Sensitivity solution: 0.25 µg/mL of USP Lansoprazole RS in *Diluent*, from the *Standard solution*

Sample solution: Nominally 250 µg/mL of lansoprazole prepared as follows. Transfer a portion of Capsules contents, equivalent to about 25 mg of lansoprazole, to a 100-mL volumetric flask. Add 70 mL of *Diluent* and sonicate with occasional shaking for about 30 min, maintaining the temperature below 10°. Dilute with *Diluent* to volume, and pass the solution through a PVDF filter having a 0.45-µm pore size or another suitable filter, discarding the first few milliliters of the filtrate.

Blank: Methanol and *Diluent* (1:9)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Autosampler temperature: 5°

Flow rate: 0.8 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 6 between lansoprazole and lansoprazole related compound A, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of lansoprazole from the *Standard solution*
C_S = concentration of USP Lansoprazole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of lansoprazole in the *Sample solution* (µg/mL)
F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lansoprazole *N*-oxide[a]	0.8	1.3	0.2
Lansoprazole	1.0	—	—
Lansoprazole related compound A (lansoprazole sulfone)[b]	1.1	0.82	0.4
Lansoprazole related compound B (lansoprazole sulfide)[c]	1.2	1.0	0.2
Other individual impurity	—	1.00	0.2
Total impurities	—	—	1.5

[a] [[(1*H*-Benzimidazole-2-yl)sulfinyl]methyl]-3-methyl-4-(2,2,2-trifluoroethoxy)-pyridine 1-oxide.
[b] 2-({[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl}sulfonyl) benzimidazole.
[c] 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl]methyl]sulfanyl]-1*H*-benzimidazole. ▲ 2S (USP41)

SPECIFIC TESTS

Delete the following:

▲• LOSS ON DRYING ⟨731⟩

Sample: 1 g of Capsule contents

Analysis: Dry under vacuum over phosphorus pentoxide at a pressure not exceeding 5 mm of mercury at 60° for 5 h.

Acceptance criteria: NMT 5.0% ▲ 2S (USP41)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

Add the following:

▲• LABELING: When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. ▲ 2S (USP41)

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lansoprazole RS

USP Monographs

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USP Lansoprazole Related Compound A RS
2-({[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl} sulfonyl)benzimidazole.
$C_{16}H_{14}F_3N_3O_3S$ 385.36

Lansoprazole Compounded Oral Suspension

DEFINITION

Lansoprazole Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$). Prepare Lansoprazole Compounded Oral Suspension 3 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Lansoprazole delayed-release capsule(s)[a] equivalent to	300 mg
Vehicle: A mixture of Ora-Blend[b] and Sodium Bicarbonate Injection (8.4%) (1:1), a sufficient quantity to make	100 mL

[a] Lansoprazole 30-mg delayed-release capsules, Dr. Reddy's Laboratory Limited, Bridgewater, NJ.
[b] Perrigo Pharmaceuticals, Allegan, MI.

Empty the required number of delayed-release capsules, and pour the contents into a mortar or other suitable container. If necessary, crush the contents into a fine powder by using a pestle or other mechanical means. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add *Vehicle* to make the mortar contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.
Alternatively, a compounded 8.4% sodium bicarbonate solution may be used instead of *Sodium Bicarbonate Injection (8.4%)*. Prepare an 8.4% sodium bicarbonate solution by dissolving 8.4 g of Sodium Bicarbonate in sufficient Purified Water to make 100 mL.

ASSAY

- **PROCEDURE**

Solution A: 10 mM sodium phosphate adjusted with sodium hydroxide to a pH of 7.5. Pass through a nylon filter of 0.45-µm pore size, and degas.
Solution B: Acetonitrile and water (50:50)
Solution C: Water adjusted with 1 M sodium hydroxide to a pH of 6.5
Mobile phase: Acetonitrile and *Solution A* (45:55)
Standard stock solution: 3 mg/mL of USP Lansoprazole RS in *Solution B*. Mix well, and sonicate for 3 min. Store at 2°–8°.
Standard solution: Transfer 2.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, and dilute with *Solution C* to volume. Centrifuge an aliquot of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.
Sample solution: Shake each bottle of Oral Suspension thoroughly. Transfer 2.0 mL of Oral Suspension to a 500-mL volumetric flask, and dilute with *Solution C* to volume. Centrifuge an aliquot of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 35°
Autosampler: 5°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for lansoprazole is about 5.2 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lansoprazole from the *Sample solution*
r_S = peak response of lansoprazole from the *Standard solution*
C_S = concentration of lansoprazole in the *Standard solution* (mg/mL)
C_U = nominal concentration of lansoprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

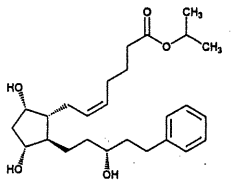
SPECIFIC TESTS

- **pH** ⟨791⟩: 8.0–8.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **LABELING:** Label Oral Suspension to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored at 2°–8° or at controlled room temperature
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Lansoprazole RS

Latanoprost



$C_{26}H_{40}O_5$ 432.59
5-Heptenoic acid, 7-[3,5-dihydroxy-2-(3-hydroxy-5-phenylpentyl)cyclopentyl]-1-methylethyl ester, [1*R*-[1α(*Z*), 2β(*R**),3α,5α]]-;
Isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate [130209-82-4].

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DEFINITION

Latanoprost contains NLT 94.0% and NMT 102.0% of latanoprost ($C_{26}H_{40}O_5$), calculated on the anhydrous and solvent-free basis.

[CAUTION—Wear protective glasses and gloves while handling the material. Avoid contact during pregnancy or while nursing.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Chromatographic hexane and dehydrated alcohol (94:6)

System suitability solution: 2.0 mg/mL of USP Latanoprost RS and 20 µg/mL of USP Latanoprost Related Compound A RS prepared as follows. Transfer USP Latanoprost RS and USP Latanoprost Related Compound A RS into a suitable volumetric flask, dissolve in dehydrated alcohol equivalent to 20% of the final volume, and dilute with chromatographic hexane to volume.

Standard solution: Transfer 2.0 mg/mL of USP Latanoprost RS into a suitable volumetric flask, dissolve in dehydrated alcohol equivalent to 20% of the final volume, and dilute with chromatographic hexane to volume.

Sample solution: Transfer 2.0 mg/mL of Latanoprost into a suitable volumetric flask, dissolve in dehydrated alcohol equivalent to 20% of the final volume, and dilute with chromatographic hexane to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 25-cm; 5-µm packing L3

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for latanoprost and latanoprost related compound A are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 2.0 between latanoprost and latanoprost related compound A, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of latanoprost ($C_{26}H_{40}O_5$) in the portion of Latanoprost taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Latanoprost RS in the *Standard solution* (mg/mL)

C_U = concentration of Latanoprost in the *Sample solution* (mg/mL)

Acceptance criteria: 94.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.50%
- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.04 mg/mL of USP Latanoprost RS in a mixture of chromatographic hexane and dehydrated alcohol (80:20) prepared as follows. Transfer USP Latanoprost RS into a suitable volumetric flask, dissolve in dehydrated alcohol equivalent to 20% of the final volume, and dilute with chromatographic hexane to volume.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between latanoprost and latanoprost related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Latanoprost taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of latanoprost from the *Standard solution*

C_S = concentration of USP Latanoprost RS in the *Standard solution* (mg/mL)

C_U = concentration of Latanoprost in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isopropyl diphenylphosphorylpentanoate[a]	0.79	2.4	0.1
Latanoprost related compound B[b]	0.89	1.0	0.5
Latanoprost	1.00	—	—
Latanoprost related compound A[c]	1.10	1.0	3.5
Any unspecified impurity	—	1.0	0.1
Total impurities[d]	—	—	0.5

[a] Isopropyl 5-(diphenylphosphoryl)pentanoate.

[b] Isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*S*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate.

[c] Isopropyl (*E*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate.

[d] Latanoprost related compound A and latanoprost related compound B are excluded.

USP Monographs

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• **LIMIT OF LATANOPROST RELATED COMPOUND E**
Solution A: Acetonitrile, phosphoric acid, and water (300:1:700)
Solution B: Acetonitrile, phosphoric acid, and water (800:1:200)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	0	100
15	0	100
16	100	0
21	100	0

Diluent: Acetonitrile and water (30:70)
Standard solution: 1.0 µg/mL of USP Latanoprost Related Compound E RS in *Diluent*
Sample solution: 1.0 mg/mL of Latanoprost in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.0-mm × 15-cm; 5-µm packing L1
Column temperature: 60°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of latanoprost related compound E in the portion of Latanoprost taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of latanoprost related compound E from the *Sample solution*
r_S = peak area of latanoprost related compound E from the *Standard solution*
C_S = concentration of USP Latanoprost Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Latanoprost in the *Sample solution* (mg/mL)

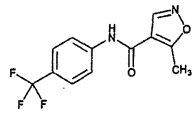
Acceptance criteria: NMT 0.2%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL of Latanoprost in acetonitrile
Acceptance criteria: +31° to +38°
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩
Sample solution: 100 mg/mL of Latanoprost in ethyl acetate. [NOTE—Alternatively, *Water Determination* ⟨921⟩, *Method Ia* may be used.]
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store in a refrigerator or a freezer.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Latanoprost RS
USP Latanoprost Related Compound A RS
Isopropyl (*E*)-7-{(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl}-5-heptenoate.
$C_{26}H_{40}O_5$ 432.59
USP Latanoprost Related Compound E RS
(*Z*)-7-{(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl}-5-heptenoic acid.
$C_{23}H_{34}O_5$ 390.51

Leflunomide



$C_{12}H_9F_3N_2O_2$ 270.21
4-Isoxazolecarboxamide, 5-methyl-*N*-[4-(trifluoromethyl) phenyl]-;
α,α,α-Trifluoro-5-methyl-4-isoxazolecarboxy-*p*-toluidide [75706-12-6].

DEFINITION
Leflunomide contains NLT 98.0% and NMT 102.0% of $C_{12}H_9F_3N_2O_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Dry the substance for 10 min at 130°.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, triethylamine, and water (70:1:130). Adjust with phosphoric acid to a pH of 4.
Standard solution: 0.5 mg/mL of USP Leflunomide RS in acetonitrile and *Mobile phase* (1:9). [NOTE—First dissolve in acetonitrile. Protect solutions from light.]
System suitability solution: 0.5 mg/mL of USP Leflunomide RS, 0.15 mg/mL of USP Leflunomide Related Compound B RS, and 0.05 mg/mL of USP Leflunomide Related Compound C RS in *Mobile phase*. [NOTE—Dissolve the Reference Standards in acetonitrile, and dilute with *Mobile phase*.]
Sample solution: 0.5 mg/mL of Leflunomide in acetonitrile and *Mobile phase* (1:9). [NOTE—First dissolve in acetonitrile. Protect solutions from light.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 12.5-cm; packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for leflunomide related compound B and leflunomide related compound C are 0.2 and 0.9, respectively.]

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Suitability requirements
Resolution: NLT 1.0 between the leflunomide and leflunomide related compound C peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{12}H_9F_3N_2O_2$ in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

Change to read:

ORGANIC IMPURITIES

• **Procedure 1: Limit of Leflunomide Related Compound A**
Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.125 mg/mL of USP Leflunomide Related Compound A RS, in acetonitrile and *Mobile phase* (1:19)
Standard solution: 0.5 µg/mL of USP Leflunomide Related Compound A RS, from the *Standard stock solution* in *Mobile phase*
Sample solution: 2.5 mg/mL of Leflunomide, in acetonitrile and *Mobile phase* (1:9)
Injection size: 20 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of leflunomide related compound A in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of leflunomide related compound A from the *Sample solution*
r_S = peak area of leflunomide related compound A from the *Standard solution*
C_S = concentration of USP Leflunomide Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.02 %
• **Procedure 2**
Mobile phase, Sample solution, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
▲**Standard stock solution:** Proceed as directed in the *Standard solution* in the *Assay*.
Standard solution: 0.5 µg/mL of USP Leflunomide RS from the *Standard stock solution* in *Mobile phase*▲ (ERR 1-Feb-2019)
Sensitivity solution: 0.25 µg/mL of Leflunomide, from the *Standard solution* in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Resolution: NLT 1.0 between leflunomide and leflunomide related compound C
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Disregard any peak with an area less than the leflunomide peak from the *Sensitivity solution*. Continue the elution for two times the retention time of the leflunomide peak.]
Calculate the percentage of each related compound and any unknown impurity (see *Impurity Table 1*) in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area for each impurity from the *Sample solution*
r_S = peak area of leflunomide from the *Standard solution*
C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)
C_U = concentration of Leflunomide in the *Sample solution* (mg/mL)

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Methylisoxazole-carboxylic acid	0.05	1.0	0.1
Leflunomide related compound B	0.22	1.0	0.3
N-(2′-Trifluoromethylphenyl)-5-methylisoxazole-4-Carboxamide	0.29	1.0	0.1
2-Cyano-acetic acid-(4′-trifluoromethyl)-anilide	0.36	1.0	0.1
Leflunomide related compound C	0.94	1.0	0.1
Any other individual impurity	—	—	0.1
Total impurities, excluding leflunomide related compound B and leflunomide related compound C	—	—	0.2
Total impurities	—	—	0.4

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 164°–168°
• **LOSS ON DRYING** ⟨731⟩: Dry a sample in a vacuum over diphosphorus pentoxide at 60° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in a well-closed container. Store at a temperature not exceeding 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Leflunomide RS
USP Leflunomide Related Compound A RS
USP Leflunomide Related Compound B RS
USP Leflunomide Related Compound C RS

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Add the following:

▲Leflunomide Compounded Oral Suspension

DEFINITION

Leflunomide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$).

Prepare Leflunomide Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Leflunomide powder	2000 mg
Vehicle: 1:1 mixture of Ora-Plus[a] and Ora-Sweet,[a] a sufficient quantity to make	100 mL

[a] Perrigo, Allegan, MI.

Place the *Leflunomide powder* in a suitable container and triturate to a fine powder. Add a small amount of *Vehicle* and mix well to form a smooth paste. Add a sufficient amount of *Vehicle* to make the contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Mix well.

[**CAUTION**—Leflunomide is a hazardous drug and must be handled accordingly.]

ASSAY

• **PROCEDURE**

Mobile phase: Add 350 mL of acetonitrile to 650 mL of water. Add 5 mL of trimethylamine and adjust with phosphoric acid to a pH of 4.0.

Standard solution: 0.5 mg/mL of USP Leflunomide RS in methanol

Sample solution: Transfer 0.625 mL of Oral Suspension into a 25-mL volumetric flask, and add methanol to volume. Pass through a filter of 0.22-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 2.1-mm × 5-cm; 1.6-µm packing L1

Temperatures

Autosampler: 10°

Column: 20°

Flow rate: 0.39 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for leflunomide is about 8.6 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of leflunomide from the *Sample solution*

r_S = peak response of leflunomide from the *Standard solution*

C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.7–4.7

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator

• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Leflunomide RS▲ 2S (USP41)

Leflunomide Tablets

DEFINITION

Leflunomide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$).

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Wavelength range: 220–360 nm

Sample solution: 0.01 mg/mL in methanol

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, triethylamine, and water (70:1:130). Adjust with phosphoric acid to a pH of 4.0.

System suitability solution A: 10 µg/mL of USP Leflunomide Related Compound A RS, 1 mg/mL of USP Leflunomide Related Compound B RS, and 100 µg/mL of USP Leflunomide Related Compound C RS in a minimum amount of acetonitrile, and diluted with *Mobile phase*

System suitability solution B: Transfer 100.0 mg of USP Leflunomide RS to a 100-mL volumetric flask. Dissolve in 2 mL of acetonitrile, add 1 mL of *System suitability solution A* and 80 mL of *Mobile phase*, and shake by mechanical means for 10 min. Dilute with *Mobile phase* to volume.

Standard solution: 1 mg/mL of USP Leflunomide RS in a minimum volume of acetonitrile, and diluted with *Mobile phase*

Sample solution: Transfer equivalent to 100 mg of leflunomide, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask. Add 20 mL of acetonitrile, dilute with *Mobile phase* to volume, and shake by mechanical means for 10 min. Pass through a membrane filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

USP Monographs

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Column: 4.0-mm × 12.5-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution B* and *Standard solution*
[NOTE—The relative retention times for leflunomide related compound B, leflunomide related compound A, leflunomide related compound C, and leflunomide are 0.2, 0.4, 0.9, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between leflunomide related compound C and leflunomide, *System suitability solution B*
Tailing factor: NMT 3.0 for leflunomide, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium
For Tablets labeled to contain 10 or 20 mg: Water, 1000 mL, deaerated
For Tablets labeled to contain 100 mg: Water containing 0.6% of polyoxyethylene (23) lauryl ether; 1000 mL, deaerated
Apparatus 2: 100 rpm
Time: 30 min
Determine the amount of leflunomide ($C_{12}H_9F_3N_2O_2$) dissolved by using one of the following methods.
Spectrometric method
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 262 nm
Standard solution: USP Leflunomide RS in *Medium*.
[NOTE—A volume of methanol not exceeding 2% of the final volume of the *Standard solution* may be used to dissolve leflunomide.]
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*, if necessary.
Chromatographic method
Mobile phase: Acetonitrile and water (1:1)
Standard solution: Transfer 22 mg of USP Leflunomide RS to a 100-mL volumetric flask. Add 40 mL of acetonitrile, and sonicate until dissolved. Add 40 mL of water, and cool to room temperature. Dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of leflunomide ($C_{12}H_9F_3N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium, Apparatus 2, Time, Spectrometric method, and **Chromatographic method:** Proceed as directed for *Test 1*.
Tolerances: NLT 75% (*Q*) of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
Procedure for content uniformity
Mobile phase, System suitability solution A, System suitability solution B, Standard solution, Chromatographic system, and **Analysis:** Proceed as directed in the *Assay*.
Sample solution: Transfer 1 Tablet to a suitable volumetric flask, and prepare a solution having a concentration of 1 mg/mL of leflunomide. Add *Mobile phase* 50% by volume, and shake to disintegrate the Tablet. After the Tablet is completely disintegrated, add acetonitrile 20% by volume, dilute with *Mobile phase* to volume, and shake again. Pass through a membrane filter.

IMPURITIES

• **PROCEDURE**
Mobile phase, System suitability solution A, System suitability solution B, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

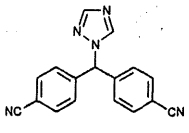
r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all the peak responses of the related compounds and leflunomide from the *Sample solution*

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Acceptance criteria
Leflunomide related compound A: NMT 0.1%
Leflunomide related compound B: NMT 3.5%
Leflunomide related compound C: NMT 0.2%
Individual impurities: NMT 0.2%
Total impurities: NMT 4.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, and humidity-resistant containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Leflunomide RS
  USP Leflunomide Related Compound A RS
  USP Leflunomide Related Compound B RS
  USP Leflunomide Related Compound C RS

Letrozole



$C_{17}H_{11}N_5$ 285.30
Benzonitrile, 4,4′-(1*H*-1,2,4-triazol-1-ylmethylene)bis-; 4,4′-(1*H*-1,2,4-Triazol-1-ylmethylene)dibenzonitrile [112809-51-5].

DEFINITION
Letrozole contains NLT 98.0% and NMT 102.0% of $C_{17}H_{11}N_5$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Diluent: Acetonitrile and water (3:7)
Standard solution: 10 µg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve USP Letrozole RS in acetonitrile, then dilute with water.]
Sample solution: 10 µg/mL of Letrozole in *Diluent*. [NOTE—Dissolve Letrozole in acetonitrile, then dilute with water.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{11}N_5$ in the portion of Letrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of letrozole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES
- **Procedure**

Solution A, Solution B, Mobile phase, Chromatographic system, and Diluent: Proceed as directed in the *Assay*.
System suitability solution: 2 µg/mL of USP Letrozole Related Compound A RS and 10 µg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve Letrozole and USP Letrozole Related Compound A RS in acetonitrile, then dilute with water.]
Standard solution: 1 µg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve USP Letrozole RS in acetonitrile, then dilute with water.]
Sample solution: Transfer 25 mg of Letrozole to a 250-mL volumetric flask. Dissolve in 75 mL of acetonitrile, and dilute with water to volume.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between letrozole related compound A and letrozole, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Letrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of letrozole from the *Standard solution*
C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)
C_U = concentration of Letrozole in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total unspecified impurities: NMT 0.3%

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Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Letrozole related compound A[a]	0.67	0.3
Letrozole	1.0	—
4,4′,4″-Methanetriyl-tribenzonitrile	2.4	0.2
Any unspecified impurity	—	0.1

[a] 4,4′-(1*H*-1,3,4-triazol-1-ylmethylene)dibenzonitrile.

[NOTE—Disregard any impurity peaks less than 0.05%.]

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.3%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Letrozole RS

USP Letrozole Related Compound A RS

4,4′-(1*H*-1,3,4-Triazol-1-ylmethylene)dibenzonitrile.

$C_{17}H_{11}N_5$ 285.31

Letrozole Tablets

DEFINITION

Letrozole Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of letrozole ($C_{17}H_{11}N_5$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: Equivalent to 2 mg/mL of letrozole from powdered Tablets in methanol. [NOTE—Shake thoroughly, sonicate for 10 min, and centrifuge.]

Application volume: 5 µL

Developing solvent system: Ethyl acetate and methanol (9:1)

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (48:52)

Diluent: Acetonitrile and water (30:70)

Standard stock solution: 0.2 mg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve letrozole in acetonitrile, and then dilute with water.]

Standard solution: 10 µg/mL of USP Letrozole RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Equivalent to 50 mg of letrozole from Tablets in a 250-mL volumetric flask. Add 20 mL of water and shake for 5 min to dissolve the Tablets. Add 75 mL of acetonitrile, shake for 30 min, and dilute with water to volume. Centrifuge a portion of the solution.

Sample solution: 10 µg/mL of letrozole in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 12.5-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of letrozole ($C_{17}H_{11}N_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Letrozole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of letrozole in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 100 rpm

Time: 30 min

Standard solution: Transfer USP Letrozole RS to a suitable volumetric flask, dissolve in acetonitrile equivalent to 10% of the final volume, and dilute with *Medium* to volume to obtain a solution of 0.05 mg/mL of letrozole. Dilute this solution with *Medium* to obtain a solution of 0.005 mg/mL of letrozole.

Sample solution: Centrifuge a portion of the solution under test at 4000 rpm for 5 min.

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*, except use an injection volume of 200 µL.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of letrozole ($C_{17}H_{11}N_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of letrozole ($C_{17}H_{11}N_5$) is dissolved.

Test 2

Medium: 0.1 N hydrochloric acid solution adjusted with 50% sodium hydroxide (NaOH) to a pH of 1.2; 900 mL, deaerated

Apparatus 2: 75 rpm

Time: 30 min

Mobile phase: Acetonitrile and water (45:55)

Standard stock solution: 0.3 mg/mL of USP Letrozole RS in *Mobile phase*

Standard solution: 3.0 µg/mL of USP Letrozole RS in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 35-µm pore size.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of letrozole ($C_{17}H_{11}N_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of letrozole ($C_{17}H_{11}N_5$) is dissolved.

Test 3
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 75 rpm
Time: 30 min
Mobile phase: Acetonitrile and water (48:52)
Standard stock solution: 0.25 mg/mL of USP Letrozole RS in *Mobile phase*
Standard solution: 0.005 mg/mL of USP Letrozole RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of letrozole ($C_{17}H_{11}N_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of letrozole ($C_{17}H_{11}N_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Diluent: Prepare as directed in the *Assay*.
System suitability solution: 10 µg/mL of USP Letrozole RS and 2 µg/mL of USP Letrozole Related Compound A RS in *Diluent*. [NOTE—Dissolve letrozole and letrozole related compound A in acetonitrile, then dilute with water.]
Standard solution: 1 µg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve letrozole in acetonitrile, then dilute with water.]
Sample solution: Nominally 0.1 mg/mL of letrozole in *Diluent*. Shake the whole Tablets (NLT 10) for about 15 min in a portion of *Diluent* to aid in dissolution. Centrifuge, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between letrozole and letrozole related compound A, *System suitability solution*
Relative standard deviation: NMT 10.0% for letrozole, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of letrozole from the *Standard solution*
C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of letrozole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Letrozole related compound A[a]	0.67	—
Letrozole	1.0	—
4,4′,4″-Methanetriyltribenzonitrile	2.4	—

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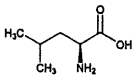
Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	0.1
Total unspecified impurities	—	0.3

[a] 4,4′-(1*H*-1,3,4-Triazol-1-ylmethylene)dibenzonitrile.
[NOTE—Letrozole related compound A and 4,4′,4″-Methanetriyltribenzonitrile are process impurities and are controlled in the drug substance monograph.]

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Letrozole RS
  USP Letrozole Related Compound A RS
  4,4′-(1*H*-1,3,4-Triazol-1-ylmethylene)dibenzonitrile.
  $C_{17}H_{11}N_5$ 285.31

Leucine



$C_6H_{13}NO_2$ 131.17
L-Leucine [61-90-5].

DEFINITION
Leucine contains NLT 98.5% and NMT 101.5% of L-leucine ($C_6H_{13}NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample: 130 mg of Leucine
  Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Endpoint detection: Potentiometric
  Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the blank determination.
  Calculate the percentage of leucine ($C_6H_{13}NO_2$) in the portion of the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N_A \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N_A = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 131.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

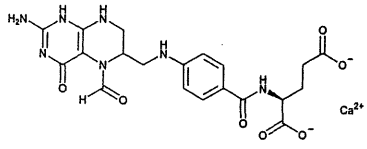
IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.4%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*
  Standard solution: 0.50 mL of 0.020 N hydrochloric acid
  Sample: 0.73 g of Leucine
  Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*
  Standard solution: 0.10 mL of 0.020 N sulfuric acid
  Sample: 0.33 g of Leucine
  Acceptance criteria: NMT 0.03%
- **IRON** ⟨241⟩: NMT 30 ppm
- **RELATED COMPOUNDS**
  System suitability solution: 0.4 mg/mL each of USP L-Leucine RS and USP L-Valine RS in 0.1 N hydrochloric acid
  Standard solution: 0.05 mg/mL of USP L-Leucine RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]
  Sample solution: 10 mg/mL of Leucine in 0.1 N hydrochloric acid
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)
  Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
  System suitability
  Sample: *System suitability solution*
  Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.
  Analysis
  Samples: *System suitability solution*, *Standard solution*, and *Sample solution*
  After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
  Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
  Individual impurities: NMT 0.5%
  Total impurities: NMT 2.0%

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 40 mg/mL in 6 N hydrochloric acid
  Acceptance criteria: +14.9° to +17.3°
- **PH** ⟨791⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: 5.5–7.0
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Leucine RS
  USP L-Valine RS

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Leucovorin Calcium



$C_{20}H_{21}CaN_7O_7$ 511.50

L-Glutamic acid, *N*-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl]amino] benzoyl]-, calcium salt (1:1);
Calcium [4-({[(*RS*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl}amino)benzoyl]-L-glutamate [1492-18-8].

DEFINITION

Leucovorin Calcium contains NLT 95.0% and NMT 105.0% of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197**▲ (CN 1-May-2020) [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.▲ (USP 1-Aug-2019)]
Do not dry specimens.
Acceptance criteria: Meets the requirements

Add the following:

▲• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Calcium*: Meets the requirements▲ (USP 1-Aug-2019)

Add the following:

▲• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**
▲▲ (USP 1-Aug-2019) Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light. Complete the *Assay* without prolonged interruption.
Solution A: 1.0 M tetrabutylammonium hydroxide in methanol
Solution B: 276 mg/mL of monobasic sodium phosphate monohydrate in water
Mobile phase: Mix 15 mL of *Solution A* with 835 mL of water. Add 125 mL of acetonitrile, adjust with *Solution B* to a pH of 7.5 ± 0.1, dilute with water to 1000 mL, and filter. Adjust the concentration of acetonitrile, if necessary.
Diluent: Mix 15 mL of *Solution A* with 900 mL of water, and adjust with *Solution B* to a pH of 7.5 ± 0.1. Dilute with water to 1000 mL.
System suitability stock solution: 0.175 mg/mL of USP Folic Acid RS in *Diluent*
Standard solution: 0.175 mg/mL of USP Leucovorin Calcium RS in *Diluent*
Sample solution: 0.2 mg/mL of Leucovorin Calcium in *Diluent*
System suitability solution: *System suitability stock solution* and *Standard solution* (1:4)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: ▲1.5 mL/min▲ (USP 1-Aug-2019)
Injection volume: 15 µL
▲Run time: 2.5 times the retention time of the leucovorin peak▲ (USP 1-Aug-2019)
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for leucovorin and folic acid are 1.0 and about 1.6, respectively.]
Suitability requirements
Resolution: NLT 3.6 between leucovorin ▲▲ (USP 1-Aug-2019) and folic acid
Relative standard deviation: NMT 2.0% ▲for the leucovorin peak▲ (USP 1-Aug-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$) in the portion of Leucovorin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)
C_U = concentration of Leucovorin Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the anhydrous basis

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**
Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light.
Buffer: Dissolve 2.6 mL of tetrabutylammonium hydroxide solution (40% in water) and 2.8 g of disodium hydrogen phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 7.8.
Mobile phase: Methanol and *Buffer* (150:850)
System suitability solution: 1.2 mg/mL of USP Leucovorin Calcium RS and 12 µg/mL each of 7,8-dihydrofolic acid and USP Folic Acid RS in water. Sonicate as necessary to dissolve.
Sensitivity solution: 0.4 µg/mL of USP Leucovorin Calcium RS in water
Standard solution: 0.12 mg/mL of USP Leucovorin Calcium RS in water. Sonicate as necessary to dissolve.
Sample solution: 1.1 mg/mL of Leucovorin Calcium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 50°

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Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 4 times the retention time of leucovorin
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between leucovorin and 7,8-dihydrofolic acid, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Leucovorin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of leucovorin from the *Standard solution*
C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)
C_U = concentration of Leucovorin Calcium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.03%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Formyl-5,6,7,8-tetrahydropteroic acid[a]	0.51	1.0	0.5
4-Aminobenzoylglutamic acid[b]	0.59	1.0	1.5
10-Formyldihydrofolic acid[c]	0.76	0.47	1.0
5,10-Diformyltetrahydrofolic acid[d]	0.85	0.49	1.5
Leucovorin	1.0	1.0	—
7,8-Dihydrofolic acid[e]	1.15	1.0	0.5
10-Formylfolic acid[f]	2.11	0.60	0.5
Folic acid	2.50[g]	1.0	1.5
Individual, unspecified impurity	—	1.0	0.3
Total impurities[h]	—	—	2.5

[a] 4-[[(2-Amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]amino]benzoic acid.
[b] *N*-(4-Aminobenzoyl)-L-glutamic acid.
[c] (4-(*N*-[(2-Amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]formamido)benzoyl)-L-glutamic acid.
[d] (4-(*N*-[(2-Amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]formamido)benzoyl)-L-glutamic acid.
[e] *N*-(4-[[(2-Amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]amino]benzoyl)-L-glutamic acid.
[f] (4-(*N*-[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]formamido)benzoyl)-L-glutamic acid.
[g] RRT may vary from 2.6 to 3.1 and may be compared with the result obtained from the *System suitability solution*.
[h] Includes all impurities in *Table 1* and 5-(γ-folinoyl)tetrahydrofolate from the test for *Limit of 5-(γ-Folinoyl)tetrahydrofolate* if the latter is possible from the manufacturing process. ▲ (USP 1-Aug-2019)

Add the following:

▲• **Limit of 5-(γ-Folinoyl)tetrahydrofolate** (if present)
The chemical name of 5-(γ-folinoyl)tetrahydrofolate is (4-[(((S)-2-amino-5-[(S)-4-(4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino)benzamido)-4-carboxybutanoyl]-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl)amino]benzoyl)-L-glutamic acid.
Solution A: Acetonitrile
Solution B: Methanol
Solution C: Dissolve 3.25 g of tetrabutylammonium hydroxide solution (40% in water) and 1.36 g of potassium dihydrogen phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 7.0.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	1.5	4.0	94.5
32	1.5	15.0	83.5
60	1.5	35.5	63.0
65	1.5	4.0	94.5
70	1.5	4.0	94.5

Standard solution: 1.0 mg/mL of USP Leucovorin Calcium RS in water
Sample solution: 1.0 mg/mL of Leucovorin Calcium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 287 nm
Column: 2.1-mm × 10-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 0.3 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for leucovorin and 5-(γ-folinoyl)tetrahydrofolate are 1.0 and about 1.7, respectively.]
Suitability requirements
Tailing factor: NMT 2.0

USP Monographs

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Relative standard deviation: NMT 2.0%

Analysis

Sample: *Sample solution*

Calculate the percentage of 5-(γ-folinoyl)tetrahydrofolate in the portion of Leucovorin Calcium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of 5-(γ-folinoyl)tetrahydrofolate from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: NMT 0.3%. It is included in the *Total impurities* in *Table 1* if it is possible from the manufacturing process. ▲ (USP 1-Aug-2019)

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 17.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

▲USP Folic Acid RS ▲ (USP 1-Aug-2019)

USP Leucovorin Calcium RS

Leucovorin Calcium Injection

DEFINITION

Leucovorin Calcium Injection is a sterile solution of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$) in Water for Injection. It contains NLT 90.0% and NMT 120.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K** ▲ (CN 1-May-2020)

Analysis: Transfer a volume of Injection, equivalent to 6 mg of leucovorin calcium, to a glass-stoppered, 50-mL centrifuge tube. Add 40 mL of acetone, mix, centrifuge for a few min, and decant and discard the liquid phase. Repeat the washing process with an additional 40 mL of acetone. Dry the precipitate so obtained with a stream of dry nitrogen.

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Use only freshly deionized water wherever water is specified throughout this *Procedure*. Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light. Complete the assay without prolonged interruption.

Solution A: 250 mg/mL of tetrabutylammonium hydroxide in methanol

Solution B: 276 mg/mL of monobasic sodium phosphate monohydrate in water

Mobile phase: Mix 15 mL of *Solution A* with 835 mL of water. Add 125 mL of acetonitrile, adjust with *Solution B* to an apparent pH of 7.5 ± 0.1, dilute with water to 1000 mL, and filter. Adjust the concentration of acetonitrile, if necessary.

Diluent: Mix 15 mL of *Solution A* with 900 mL of water, and adjust with *Solution B* to a pH of 7.5 ± 0.1. Dilute with water to 1000 mL.

Standard solution: 0.175 mg/mL of USP Leucovorin Calcium RS in *Diluent*

Sample solution: Transfer a measured volume of Injection, equivalent to 9 mg of leucovorin, to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pipet 25 mL of this solution into a 60-mL separator, add 25 mL of methylene chloride, shake the mixture, allow the layers to separate, and discard the methylene chloride extract. Repeat the extraction with two more 25-mL portions of methylene chloride, discarding the methylene chloride extracts. Filter the aqueous layer, discarding the first 5 mL of the filtrate, and collect the remaining filtrate in a glass-stoppered conical flask.

System suitability stock solution: 0.175 mg/mL of folic acid in *Diluent*

System suitability solution: *System suitability stock solution* and *Standard solution* (1:4)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1–2 mL/min

Injection volume: 15 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for leucovorin and folic acid are 1.0 and about 1.6, respectively.]

Suitability requirements

Resolution: NLT 3.6 between leucovorin calcium and folic acid

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of leucovorin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of leucovorin, 473.45

M_{r2} = molecular weight of leucovorin calcium, 511.50

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 6.5–8.5
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1.95 USP Endotoxin Units/mg of leucovorin calcium
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose, light-resistant containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Leucovorin Calcium RS

USP Monographs

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Add the following:

▲Leucovorin Calcium for Injection

DEFINITION

Leucovorin Calcium for Injection contains an amount of leucovorin calcium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Standard solution: 0.0215 mg/mL of USP Leucovorin Calcium RS in water

Sample solution: Nominally 0.02 mg/mL of leucovorin prepared by constituting Leucovorin Calcium for Injection in water

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light.

Solution A: Dissolve 2.6 mL of tetrabutylammonium hydroxide solution (40% in water) and 2.8 g of disodium hydrogen phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 7.8.

Mobile phase: Methanol and *Solution A* (150:850)

Standard solution: 0.12 mg/mL of USP Leucovorin Calcium RS in water. Sonicate as necessary to dissolve.

Sample solution: Nominally 0.1 mg/mL of leucovorin from NLT 4 vials in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 10°

Column: 50°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 2 times the retention time of the leucovorin peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in the portion of Leucovorin Calcium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of leucovorin from the *Sample solution*

r_S = peak response of leucovorin from the *Standard solution*

C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of leucovorin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of leucovorin, 473.45

M_{r2} = molecular weight of leucovorin calcium, 511.50

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light.

Solution A, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 1.2 mg/mL of USP Leucovorin Calcium RS and 12 µg/mL each of 7,8-dihydrofolic acid and USP Folic Acid RS in water. Sonicate as necessary to dissolve.

Sensitivity solution: 0.4 µg/mL of USP Leucovorin Calcium RS in water

Sample solution: Nominally 1 mg/mL of leucovorin from NLT 4 vials in water

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between leucovorin and 7,8-dihydrofolic acid, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Leucovorin Calcium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of leucovorin from the *Standard solution*

C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of leucovorin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of leucovorin, 473.45

M_{r2} = molecular weight of leucovorin calcium, 511.50

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.03%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Formyl-5,6,7,8-tetrahydropteroic acid[a]	0.51	1.0	2.0
4-Aminobenzoylglutamic acid[b]	0.59	1.0	2.0

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Formyldihydrofolic acid[c]	0.76	0.47	2.0
5,10-Diformyltetrahydrofolic acid[d]	0.85	0.49	2.0
Leucovorin	1.0	1.0	—
7,8-Dihydrofolic acid[e]	1.15	1.0	2.0
10-Formylfolic acid[f]	2.11	0.60	2.0
Folic acid	2.50[g]	1.0	2.0
Individual, unspecified impurity	—	1.0	0.5
Total impurities	—	—	2.5

[a] 4-{[(2-Amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]amino}benzoic acid.
[b] *N*-(4-Aminobenzoyl)-L-glutamic acid.
[c] (4-{*N*-[(2-Amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]formamido}benzoyl)-L-glutamic acid.
[d] (4-{*N*-[(2-Amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]formamido}benzoyl)-L-glutamic acid.
[e] *N*-(4-{[(2-Amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid.
[f] (4-{*N*-[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]formamido}benzoyl)-L-glutamic acid.
[g] Relative retention time may vary from 2.6 to 3.1 and may be compared with the result obtained from the *System suitability solution*.

SPECIFIC TESTS

- **pH** ⟨791⟩: 6.5–8.5
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Bacterial Endotoxins Test** ⟨85⟩: Meets the requirements
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose containers and protect from light. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Folic Acid RS
  USP Leucovorin Calcium RS▲ (USP 1-Aug-2019)

Leucovorin Calcium Compounded Oral Suspension

DEFINITION

Leucovorin Calcium Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

Prepare Leucovorin Calcium Compounded Oral Suspension containing 5 mg/mL of leucovorin as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Leucovorin Calcium tablets[a] equivalent to	500 mg of leucovorin
Sodium Hydroxide (1 N)	To adjust pH to 7.1–7.6
Syrup, a sufficient quantity to make	100 mL

[a] Leucovorin calcium 25-mg tablets, Teva Pharmaceuticals, Sellersville, PA.

Place the *Leucovorin Calcium tablets* into a suitable container. Wet the tablets with a small amount of *Syrup* and triturate to make a smooth paste. Add the *Syrup* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the *Syrup*. Adjust with *Sodium Hydroxide (1 N)* to a pH of 7.1–7.6. Add sufficient *Syrup* to bring to final volume. Shake to mix well. [Note—pH may decrease to 6.1 after bringing to final volume with *Syrup* without affecting the stability of the preparation.]

ASSAY

- **Procedure**

Solution A: Methanol and 5 mM tetrabutylammonium phosphate (20:80). Adjust with tetrabutylammonium hydroxide to a pH of 6.6. [Note—Tetrabutylammonium phosphate appearing wet should not be used as it may coelute with leucovorin.]

Mobile phase: See *Table 1*.

Table 1

Time (min)	Methanol (%)	Solution A (%)
0	0	100
20	10	90
20.1	0	100
30	0	100

Diluent: Methanol and water (20:80)

Standard solution: 0.05 mg/mL of leucovorin prepared from USP Leucovorin Calcium RS and *Diluent*. Vortex and sonicate until dissolved.

Sample solution: Transfer 1.0 mL of Oral Suspension to a 100-mL volumetric flask, and rinse the pipette with about 2 mL of *Diluent*. Dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 15-cm; 2.7-µm packing L7
Column temperature: 55°
Flow rate: 0.75 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[Note—The retention time for leucovorin is about 20.3 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of leucovorin from USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of leucovorin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

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SPECIFIC TESTS
- **pH** ⟨791⟩: 6.1–7.1

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant plastic containers. Store in a refrigerator or at controlled room temperature.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored in a refrigerator; NMT 30 days after the date on which it was compounded when stored at controlled room temperature
- **Labeling:** Label it to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP Reference Standards** ⟨11⟩
  USP Leucovorin Calcium RS

Leucovorin Calcium Tablets

DEFINITION
Leucovorin Calcium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

IDENTIFICATION
- **A.**
  Sample: Equivalent to 200 mg of leucovorin calcium from finely powdered Tablets
  Analysis: Transfer the *Sample* to a conical flask. Add 10 mL of water, shake vigorously, sonicate for 10 min, and filter. Transfer the filtrate to a stoppered centrifuge tube, add 125 mg of ammonium oxalate, shake vigorously, and centrifuge until a clear supernatant is obtained. Transfer the supernatant to another stoppered centrifuge tube, add 1 mL of methanol and 3 drops of hydrochloric acid, and shake vigorously. If the preparation is cloudy, add methanol until a clear solution is obtained, and filter if necessary to remove any undissolved material. Cool the preparation at 0° until a precipitate forms, and centrifuge for 1–2 min. [Note—The cooling and centrifuging steps may be repeated if necessary to increase the amount of precipitate collected.] Decant the supernatant, add 2 mL of methanol to the tube, shake vigorously to dissolve the precipitate, and transfer the contents to a beaker. Evaporate under a current of air to dryness, and dry the residue at 50° for 30 min.
  Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Leucovorin Calcium RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
  Diluent: Methanol and water (20:80)
  Mobile phase: 5 mM tetrabutylammonium phosphate in *Diluent*. Adjust with 50% (w/v) sodium hydroxide to a pH of 7.5.
  Standard solution: 0.5 mg/mL of USP Leucovorin Calcium RS and 10 µg/mL of USP 10-Formylfolic Acid RS in water
  Sample solution: Transfer finely powdered Tablets (NLT 20), equivalent to 50 mg of leucovorin, to a 100-mL volumetric flask. Add 50 mL of water, sonicate for 30 min, dilute with water to volume, mix, and filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 15-cm; packing L1
  Flow rate: 2.0 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  [Note—The relative retention times for leucovorin and 10-formylfolic acid are about 1.0 and 2.3, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between leucovorin and 10-formylfolic acid
  Relative standard deviation: NMT 2.0% for leucovorin
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U	= peak area of the *Sample solution*
r_S	= peak area of the *Standard solution*
C_S	= concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)
C_U	= nominal concentration of leucovorin in the *Sample solution* (mg/mL)
M_{r1}	= molecular weight of leucovorin, 473.45
M_{r2}	= molecular weight of leucovorin calcium, 511.50

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Detector: UV, at a maximum of about 284 nm
  Standard solution: USP Leucovorin Calcium RS in *Medium*
  Sample solution: Use filtered portion of solution under test, and dilute with water if necessary to a concentration similar to that of the *Standard solution*.
  Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

A_U	= absorbance of the *Sample solution*
A_S	= absorbance of the *Standard solution*
C_S	= concentration of the *Standard solution* (mg/mL)
V	= volume of *Medium*, 900 mL
D	= dilution factor
M_{r1}	= molecular weight of leucovorin, 473.45
M_{r2}	= molecular weight of leucovorin calcium, 511.50
L	= label claim (mg/Tablet)

  Tolerances: NLT 75% (Q) of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) is dissolved.
- **Uniformity of Dosage Units** ⟨905⟩
  Analysis for content uniformity
  Standard solution: 10 µg/mL of USP Leucovorin Calcium RS
  Sample solution: 10 µg/mL of leucovorin calcium, use individual intact Tablets.
  Blank: Water
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV
  Cell: 1 cm
  Analytical wavelength: UV, at maxima about 284 nm

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in each Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of leucovorin in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of leucovorin, 473.45
M_{r2} = molecular weight of leucovorin calcium, 511.50

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

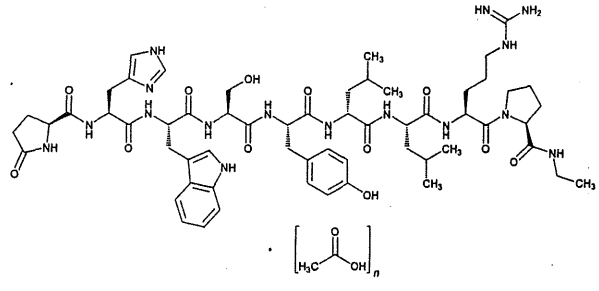
r_U = response of each impurity peak
r_T = sum of the responses of all the peaks

Acceptance criteria
Individual impurities: NMT 2.5%
Total impurities: NMT 4.0 %

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP 10-Formylfolic Acid RS
USP Leucovorin Calcium RS

Leuprolide Acetate



$C_{59}H_{84}N_{16}O_{12} \cdot (C_2H_4O_2)_n$, n = 1–2 1209.41 (as free base)
Luteinizing hormone-releasing factor, 6-D-leucine-9-(*N*-ethyl-L-prolinamide)-10-desglycinamide acetate (salt);
5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide acetate (salt);
Free base [53714-56-0].
Acetate salt [74381-53-6].

DEFINITION

Leuprolide Acetate is a synthetic nonapeptide agonist analog of luteinizing hormone-releasing factor. It contains NLT 97.0% and NMT 103.0% of leuprolide ($C_{59}H_{84}N_{16}O_{12}$), calculated on the anhydrous, acetic acid-free basis.
[NOTE—Due to the hygroscopic nature of this material, analyses are performed immediately after opening the container in a glove box under dry nitrogen purge.]
[**CAUTION**—Leuprolide Acetate is a potent hormonal manipulator. Avoid skin contact and inhalation of dusts and mists.]

IDENTIFICATION

• **A. HPLC**
Solution A, Solution B, Mobile phase, Standard solution, Degradation standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Identity sample solution: Mix equal volumes of the *Standard solution* and the *Sample solution*.
Analysis
Samples: *Standard solution, Sample solution,* and *Identity sample solution*
Examine the chromatograms of the *Standard solution*, the *Sample solution*, and the *Identity sample solution*.
Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak of the *Identity sample solution* elutes as a single peak.
• **B. AMINO ACID ANALYSIS**
[NOTE—The following method is given for informational purposes; any validated amino acid analysis method can be used.]
Standard solutions: Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. Prepare a separate, equimolar solution of L-tryptophan.
Sample solution: Transfer about 6.4 mg of Leuprolide Acetate to a suitable vacuum hydrolysis tube. Add 2.0 mL of 6 N hydrochloric acid to the tube, evacuate, and seal. Heat at 120° for 16 h. Allow to cool. Remove the solvent under vacuum. Dissolve in, and dilute to a suitable volume in, a buffer solution suitable for amino acid analysis.
Analysis: Standardize the instrument with the *Standard solutions*. Inject suitable volumes of the *Standard solutions* and the *Sample solution* into the amino acid analyzer. Record and measure the responses for each amino acid peak. Express the content of each amino acid in nmol. Calculate the mean nmol of each of the amino acids taken:

$$\text{Result} = (\text{nmol found in the } \textit{Analysis} \text{ for Glu, Pro, Tyr, His, Arg, Leu})/7$$

Divide the nmol of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria*.
Acceptance criteria
Glutamic acid, proline, tyrosine, histidine, and **arginine:** 0.85–1.1
Leucine: 1.8–2.2
Serine and tryptophan: Present

ASSAY

• **PROCEDURE**
Solution A: 15.2 mg/mL of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0.

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Solution B: Acetonitrile and *n*-propyl alcohol (3:2)
Mobile phase: *Solution A* and *Solution B* (17:3)
Standard stock solution: 1 mg/mL of USP Leuprolide Acetate RS in *Mobile phase*
Standard solution: 50 µg/mL of USP Leuprolide Acetate RS in *Mobile phase* prepared from the *Standard stock solution*
Degradation standard solution: Dilute the *Standard stock solution* with water to 0.1 mg/mL. Transfer 5 mL of the solution into a scintillation vial. Add 100 µL of 1 N sodium hydroxide solution, cap tightly, and shake vigorously. Place in an oven at 100° for 60 min. Remove, allow to cool, add 50 µL of 1 M phosphoric acid, recap, and shake vigorously to mix.
Sample solution: 50 µg/mL of Leuprolide Acetate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1–1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Mobile phase*, *Standard solution*, and *Degradation standard solution*
[NOTE—Chromatograph the *Mobile phase* and verify that no extraneous peaks are present.]
[NOTE—The relative retention times for the degradation product and leuprolide are about 0.90 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between leuprolide and the degradation product, *Degradation standard solution*
Tailing factor: 0.8–1.5, *Standard solution*
Retention time: 41–49 min for leuprolide, *Degradation standard solution*
Relative standard deviation: NMT 1.5% for leuprolide acetate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of leuprolide ($C_{59}H_{84}N_{16}O_{12}$) in the portion of Leuprolide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Leuprolide Acetate RS in the *Standard solution* (µg/mL)
C_U = concentration of Leuprolide Acetate in the *Sample solution* (µg/mL) on the anhydrous and acetic acid-free basis

Acceptance criteria: 97.0%–103.0% on the anhydrous and acetic acid-free basis

OTHER COMPONENTS

• **ACETIC ACID IN PEPTIDES** ⟨503⟩: 4.7%–9.0%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **LEUPROLIDE-RELATED IMPURITIES**
Solution A, Solution B, Mobile phase, Standard stock solution, Degradation standard solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.01 mg/mL of USP Leuprolide Acetate RS in *Mobile phase* prepared by diluting the *Standard stock solution*
Sample solution: 1 mg/mL of Leuprolide Acetate in *Mobile phase*
System suitability
Samples: *Mobile phase*, *Degradation standard solution*, and *Standard solution*
[NOTE—Chromatograph the *Mobile phase* and verify that no extraneous peaks are present.]
[NOTE—The relative retention times for the degradation product and leuprolide are about 0.90 and 1.0, respectively.]
Suitability requirements
Resolution, Tailing factor, and Retention time: Proceed as directed in the *Assay*.
Relative standard deviation: NMT 1.5% for leuprolide acetate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Record the chromatograms for 90 min.]
Calculate the percentage of each impurity in the portion of Leuprolide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of leuprolide from the *Standard solution*
C_S = concentration of USP Leuprolide Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Leuprolide Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Ser-leuprolide[a]	0.8	0.5
D-His-leuprolide[b]	0.9	0.5
Leuprolide	1.0	—
L-Leu⁶-leuprolide[c]	1.2	0.5
(*O*-Acetyl-L-ser)-leuprolide[d]	1.5	1.0
Any other individual impurity	—	0.5
Total impurities	—	2.5

[a] 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-D-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.
[b] 5-Oxo-L-prolyl-D-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.
[c] 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-L-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.
[d] 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-(*O*-acetyl)-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.

PROCESS-RELATED IMPURITIES

• **TRIFLUOROACETIC ACID (TFA) IN PEPTIDES** ⟨503.1⟩: NMT 0.25%. [NOTE—Perform this test if trifluoroacetic acid is used in the manufacturing process.]

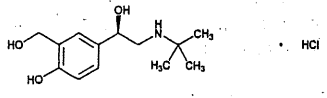
SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*, *Method Ic*: NMT 8.0%
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 166.7 USP Endotoxin Units/mg of leuprolide acetate.
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/g. The total yeast and mold count does not exceed 10^2 cfu/g.

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at a temperature not higher than 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Leuprolide Acetate RS

Levalbuterol Hydrochloride



$C_{13}H_{21}NO_3 \cdot HCl$ 275.77

(*R*)-α[1]-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene-α,α′-diol hydrochloride [50293-90-8].

DEFINITION

Levalbuterol Hydrochloride contains NLT 98.0% and NMT 102.0% of levalbuterol hydrochloride ($C_{13}H_{21}NO_3 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B.** The retention time of the major peak of the *Diluted sample solution* corresponds to that of the levalbuterol peak of the *System suitability solution*, as obtained in the test for *▲Limit of S-Albuterol.*▲ (USP 1-May-2019)

ASSAY

Change to read:

- **PROCEDURE**

Solution A: ▲Dilute 1 mL of phosphoric acid with water to 1 L.▲ (USP 1-May-2019)

Solution B: ▲Acetonitrile, methanol, and water (35:35:30). To each liter of the solution add 1 mL of phosphoric acid.▲ (USP 1-May-2019)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	91.5	8.5
15	91.5	8.5
15.01	0	100
20	0	100
20.01	91.5	8.5
30	91.5	8.5

Diluent: *Solution A*

Standard solution: 100 µg/mL of USP Levalbuterol Hydrochloride RS in *Diluent*

Sample solution: 100 µg/mL of Levalbuterol Hydrochloride in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
▲▲ (USP 1-May-2019)
Tailing factor: NMT 2.3
Relative standard deviation: ▲NMT 0.73%▲ (USP 1-May-2019)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levalbuterol hydrochloride ($C_{13}H_{21}NO_3 \cdot HCl$) in the portion of Levalbuterol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levalbuterol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

Change to read:

- **▲LIMIT OF *S*-ALBUTEROL▲** (USP 1-May-2019)

Mobile phase: ▲Acetonitrile and methanol (50:50). To each liter of the solution add 3 mL of acetic acid and 1 mL of triethylamine.▲ (USP 1-May-2019)

Diluent: *Mobile phase*

System suitability solution: 0.10 mg/mL of USP Levalbuterol Hydrochloride RS and 0.04 mg/mL of USP Albuterol RS in *Diluent*

Sample solution: 0.8 mg/mL of Levalbuterol Hydrochloride in *Diluent*

Diluted sample solution: 0.1 mg/mL from the *Sample solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L63
Flow rate: 1 mL/min
Injection volume: 10 µL
▲Run time: NLT 1.5 times the retention time of levalbuterol▲ (USP 1-May-2019)

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times of levalbuterol and (*S*)-albuterol are 1.0 and 1.16, respectively.]
Suitability requirements
Resolution: NLT 2.0 between levalbuterol and (*S*)-albuterol
Tailing factor: NMT 2.2 for levalbuterol and (*S*)-albuterol
▲▲ (USP 1-May-2019)

Analysis
Samples: *Sample solution* and *Diluted sample solution*

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Calculate the percentage of (*S*)-albuterol in the portion of Levalbuterol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of (*S*)-albuterol from the *Sample solution*

r_T = sum of the peak responses for levalbuterol and (*S*)-albuterol from the *Sample solution*

Acceptance criteria: NMT 0.2% of (*S*)-albuterol

Change to read:

- **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, and **Sample solution:** Prepare as directed in the *Assay*.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	70	30
50	28	72
50.01	0	100
55	0	100
55.01	100	0
70	100	0

System suitability solution: Prepare a solution containing the following in *Diluent*.

USP Levalbuterol Hydrochloride RS, 100 µg/mL
USP Levalbuterol Related Compound A RS, 0.05 µg/mL
USP Levalbuterol Related Compound B RS, 0.05 µg/mL
USP Levalbuterol Related Compound C RS, 0.05 µg/mL
USP Levalbuterol Related Compound D RS, 0.05 µg/mL

▲**Sensitivity solution:** 0.03 µg/mL of USP Levalbuterol Hydrochloride RS in *Diluent*▲ (USP 1-May-2019)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* ▲and *Sensitivity solution*▲ (USP 1-May-2019)

Suitability requirements

Resolution: NLT 4.9 between levalbuterol and levalbuterol related compound A; NLT 1.5 between levalbuterol related compound B and levalbuterol related compound C, ▲*System suitability solution*▲ (USP 1-May-2019)

Tailing factor: NMT 4.0 for levalbuterol, ▲*System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*▲ (USP 1-May-2019)

Analysis

Sample: *Sample solution*

▲▲ (USP 1-May-2019)

Calculate the percentage of each impurity in the portion of Levalbuterol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor for each impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. ▲The reporting threshold is 0.03%.▲ (USP 1-May-2019)

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levalbuterol	1.0	—	—
Levalbuterol related compound A	1.2	1.0	0.1
Levalbuterol related compound H▲[a]▲ (USP 1-May-2019)	1.3	1.0	0.15
Levalbuterol related compound B	1.5	1.0	0.10
Levalbuterol related compound C	1.6	1.0	0.15
Levalbuterol related compound D	1.7	3.0	0.05
Levalbuterol related compound F▲[b]▲ (USP 1-May-2019)	2.1	1.0	0.1
Levalbuterol related compound F▲[c]▲ (USP 1-May-2019)	3.5	1.2	0.10
Any individual unspecified impurity	—	—	0.10
Total impurities	—	—	0.5

[a] 4-[2-(*tert*-Butylamino)-1-methoxyethyl]-2-(hydroxymethyl)phenol acetate.
[b] α-{[(1,1-Dimethylethyl)amino]methyl}-3-(ethoxymethyl)-4-hydroxybenzenemethanol.
[c] α-{[(1,1-Dimethylethyl)amino]methyl}-4-(phenylmethoxy)-1,3-benzenedimethanol.

SPECIFIC TESTS

Delete the following:

▲• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic bacterial count is less than 10^1 cfu/g. The total combined molds and yeasts count is less than 10^1 cfu/g. It meets the requirements of the tests for the absence of *Salmonella* species, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.▲ (USP 1-May-2019)

- **pH** ⟨791⟩**:** 4.5–5.5, in a 10-mg/mL solution
- **WATER DETERMINATION** ⟨921⟩, *Method I*, *Method Ic***:** NMT 0.3%

USP Monographs

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Albuterol RS
  USP Levalbuterol Hydrochloride RS
  USP Levalbuterol Related Compound A RS
  4-(2-*tert*-Butylamino-ethyl)-2-hydroxymethyl-phenol.
  $C_{13}H_{21}NO_2$ 223.31
  USP Levalbuterol Related Compound B RS
  α-{[(1,1-Dimethylethyl)amino]methyl}-4-hydroxy-3-methyl-benzenemethanol.
  $C_{13}H_{21}NO_2$ 223.31
  USP Levalbuterol Related Compound C RS
  α-{[(1,1-Dimethylethyl)amino]methyl}-4-hydroxy-3-(methoxymethyl)-benzenemethanol.
  $C_{14}H_{23}NO_3$ 253.34
  USP Levalbuterol Related Compound D RS
  ▲5-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-hydroxybenzaldehyde sulfate (2:1) (salt);
  Also known as 5-[2-((1,1-Dimethylethyl)amino)-1-hydroxyethyl]-2-hydroxy-benzaldehyde sulfate(2:1).
  $(C_{13}H_{19}NO_3)_2 \cdot H_2SO_4$ 572.67▲ (ERR 1-May-2019)

Levalbuterol Inhalation Solution

DEFINITION

Levalbuterol Inhalation Solution is a sterile, aqueous solution of Levalbuterol Hydrochloride, prepared with Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of levalbuterol ($C_{13}H_{21}NO_3$).

IDENTIFICATION

Change to read:

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the ▲*System suitability solution*, as obtained in the test for *Limit of S-Albuterol*.▲ (USP 1-May-2019)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the major peak of the *System suitability solution*, as obtained in the test for *Limit of S-Albuterol*.▲ (USP 1-May-2019)

ASSAY

Change to read:

- **PROCEDURE**

Solution A: ▲Dilute 1 mL of phosphoric acid with water to 1 L.▲ (USP 1-May-2019)

Solution B: ▲Acetonitrile, methanol, and water (35:35:30). To each liter of the solution add 1 mL of phosphoric acid.▲ (USP 1-May-2019)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	91.5	8.5
15	91.5	8.5
15.01	0	100
20	0	100
20.01	91.5	8.5
30	91.5	8.5

Diluent: Dissolve 9.0 g of sodium chloride in 950 mL of water. Adjust with dilute sulfuric acid to a pH of 4.0, and dilute with water to 1000 mL. ▲▲ (USP 1-May-2019)

Standard solution: 0.1 mg/mL of USP Levalbuterol Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of levalbuterol hydrochloride (equivalent to 0.087 mg/mL of levalbuterol free base) in *Diluent* from an appropriately diluted volume of Inhalation Solution

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
▲▲ (USP 1-May-2019)
Tailing factor: NMT 2.3
Relative standard deviation: ▲NMT 1.0%▲ (USP 1-May-2019)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levalbuterol ($C_{13}H_{21}NO_3$) in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levalbuterol ▲▲ (USP 1-May-2019) from the *Sample solution*
r_S = peak response of levalbuterol ▲▲ (USP 1-May-2019) from the *Standard solution*
C_S = concentration of USP Levalbuterol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levalbuterol in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levalbuterol (free base), 239.31
M_{r2} = molecular weight of levalbuterol hydrochloride, 275.77

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

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IMPURITIES

Change to read:

• **▲LIMIT OF *S*-ALBUTEROL▲** (USP 1-May-2019)
Mobile phase: ▲Acetonitrile ▲and methanol (50:50).▲ (ERR 1-May-2019) To each liter of the solution add 3 mL of acetic acid and 1 mL of triethylamine.▲ (USP 1-May-2019)
Diluent: ▲Prepare as directed in the *Assay*.▲ (USP 1-May-2019)
System suitability solution: 0.10 mg/mL of USP Levalbuterol Hydrochloride RS and 0.04 mg/mL of USP Albuterol RS in *Diluent*
Sample solution: Inhalation Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm. ▲For *Identification B* use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
Column: 4.6-mm × 25-cm; 5-µm packing L63
Flow rate: 1 mL/min
Injection volume: ▲40 µL▲ (USP 1-May-2019)
Run time: ▲NLT than 1.5 times the retention time of levalbuterol▲ (USP 1-May-2019)
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times of levalbuterol and (*S*)-albuterol are 1.0 and 1.16, respectively.]
Suitability requirements
Resolution: NLT 3.0 between levalbuterol and (*S*)-albuterol
Tailing factor: NMT 1.6 for levalbuterol and NMT 2.0 for (*S*)-albuterol
▲▲ (USP 1-May-2019)
Analysis
Sample: *Sample solution*
Calculate the percentage of (*S*)-albuterol in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of (*S*)-albuterol from the *Sample solution*
r_T = sum of the peak responses of levalbuterol and (*S*)-albuterol from the *Sample solution*

Acceptance criteria: NMT 2.50% of (*S*)-albuterol in the *Sample solution*

Change to read:

• **ORGANIC IMPURITIES**
Solution A, Solution B, Diluent, and **Sample solution:** Prepare as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	70	30
50	28	72
50.01	0	100
55	0	100
55.01	100	0
70	100	0

System suitability solution: Prepare a solution containing the following in *Diluent*:
USP Levalbuterol Hydrochloride RS, 100 µg/mL
USP Levalbuterol Related Compound A RS, 0.05 µg/mL
USP Levalbuterol Related Compound B RS, 0.05 µg/mL
USP Levalbuterol Related Compound C RS, 0.05 µg/mL
USP Levalbuterol Related Compound D RS, 0.05 µg/mL
▲Sensitivity solution: 0.05 µg/mL of USP Levalbuterol Hydrochloride RS in *Diluent*▲ (USP 1-May-2019)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* ▲and *Sensitivity solution*▲ (USP 1-May-2019)
Suitability requirements
Resolution: NLT 4.9 between levalbuterol and levalbuterol related compound A; NLT 1.5 between levalbuterol related compound B and levalbuterol related compound C, ▲*System suitability solution*▲ (USP 1-May-2019)
Tailing factor: NMT 4.0 for the levalbuterol peak, ▲*System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*▲ (USP 1-May-2019)
Analysis
Sample: *Sample solution*
▲▲ (USP 1-May-2019)
Calculate the percentage of each impurity in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*
F = relative response factor for each impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. ▲The reporting threshold is 0.05%.▲ (USP 1-May-2019)

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Hydroxyalbuterol[a]	0.90	1.0	0.10
Levalbuterol	▲1.0▲ (ERR 1-May-2019)	—	—
Levalbuterol related compound A[b]	1.2	—	—
Levalbuterol related compound H[b, c]	1.3	—	—
Levalbuterol related compound B[b]	1.5	—	—

USP Monographs

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Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levalbuterol related compound C[b]	1.6	—	—
Levalbuterol related compound D	1.7	3.0	0.08
Levalbuterol related compound E[b,▲d,▲] (USP 1-May-2019)	2.1	—	—
Levalbuterol related compound F[b,▲e,▲] (USP 1-May-2019)	3.5	—	—
Any individual unspecified ▲impurity▲ (USP 1-May-2019)	—	—	0.10
Total impurities	—	—	0.70

[a] 5-[2-(*tert*-Butylamino)-1-hydroxyethyl]-3-(hydroxymethyl)benzene-1,2-diol.
[▲b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.▲ (USP 1-May-2019)
[c] 4-[2-(*tert*-Butylamino)-1-methoxyethyl]-2-(hydroxymethyl)phenol.
[d] α-{[(1,1-Dimethylethyl)amino]methyl}-3-(ethoxymethyl)-4-hydroxy-benzenemethanol.
[e] α-{[(1,1-Dimethylethyl)amino]methyl}-4-(phenylmethoxy)-1,3-benzenedimethanol.

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 3.3–4.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: See *Table 4*.

Table 4

Particle Size (µm)	Limit NMT (particles/container)
≥10	250
≥25	25
≥100	2
≥300	1

- **OSMOLALITY AND OSMOLARITY** ⟨785⟩, *Osmolality*: 280–320 mOsmol/kg

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in low-density polyethylene single-use ampuls, with a multilayer foil overwrap. Store at controlled room temperature.

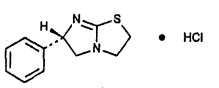
Change to read:

- **LABELING:** The outer label indicates ▲that the unit-dose container▲ (USP 1-May-2019) should be discarded if the solution is not colorless.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Albuterol RS
  USP Levalbuterol Hydrochloride RS
  USP Levalbuterol Related Compound A RS
  4-(2-*tert*-Butylamino-ethyl)-2-hydroxymethyl-phenol.
  $C_{13}H_{21}NO_2$ 223.31
  USP Levalbuterol Related Compound B RS
  α-{[(1,1-Dimethylethyl)amino]methyl}-4-hydroxy-3-methyl-benzenemethanol.
  $C_{13}H_{21}NO_2$ 223.31
  USP Levalbuterol Related Compound C RS
  α-{[(1,1-Dimethylethyl)amino]methyl}-4-hydroxy-3-(methoxymethyl)-benzenemethanol.
  $C_{14}H_{23}NO_3$ 253.34
  USP Levalbuterol Related Compound D RS
  ▲5-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-hydroxybenzaldehyde sulfate (2:1) (salt);
  Also known as 5-[2-((1,1-Dimethylethyl)amino}-1-hydroxyethyl]-2-hydroxy-benzaldehyde sulfate(2:1).
  $(C_{13}H_{19}NO_3)_2 \cdot H_2SO_4$ 572.67▲ (ERR 1-May-2019)
  ▲▲ (USP 1-May-2019)

Levamisole Hydrochloride



$C_{11}H_{12}N_2S \cdot HCl$ 240.75
Imidazo[2,1-*b*]thiazole, 2,3,5,6-tetrahydro-6-phenyl-, monohydrochloride, (*S*)-.
(–)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]thiazole monohydrochloride [16595-80-5].

» Levamisole Hydrochloride contains not less than 98.5 percent and not more than 101.0 percent of $C_{11}H_{12}N_2S \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light.
USP Reference standards ⟨11⟩—
USP Levamisole Hydrochloride RS
Completeness of solution ⟨641⟩—A test solution of 500 mg of Levamisole Hydrochloride dissolved in 10 mL of water meets the requirements.
Color of solution—The test solution prepared for the test for *Completeness of solution* is colorless or not more intensely colored than a color matching fluid prepared by mixing 2.5 mL of *Matching Fluid F* (see *Color and Achromicity* ⟨631⟩) with 97.5 mL of 0.12 N hydrochloric acid.

Identification—
A: The IR absorption spectrum of a potassium bromide dispersion of it, previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Levamisole Hydrochloride RS.
B: The color, size, and R_F value of the principal spot in the chromatogram of *Test solution B* obtained in the test for *Chromatographic purity*, when examined under short-wavelength UV light, correspond to the respective characteristics of the principal spot in the chromatogram of *Reference solution A* obtained in the test for *Chromatographic purity*.
C: A solution of it responds to the tests for *Chloride* ⟨191⟩.
Melting range ⟨741⟩: between 226° and 231°.
Light absorption—Its absorbance (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 310 nm, determined in a 0.2 N methanolic hydrochloric acid solution containing 1 mg per mL using a 1-cm cell, is not more than 0.20.
Specific rotation ⟨781S⟩: between –121.5° and –128.0°.
Test solution: 50 mg per mL, in water.

USP Monographs

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pH ⟨791⟩: between 3.0 and 4.5, in a solution (1 in 20).
Loss on drying ⟨731⟩: Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chromatographic purity—Prepare a solution of it in methanol containing 50 mg per mL (*Test solution A*). Dilute 1.0 mL of *Test solution A* to 10 mL with methanol, and mix (*Test solution B*). Prepare a solution of USP Levamisole Hydrochloride RS in methanol having a concentration of 5 mg per mL (*Reference solution A*). Dilute 1.0 mL of *Test solution B* to 20 mL with methanol, and mix (*Reference solution B*). Apply separate 10-µL portions of the four solutions on the starting line to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, acetone, and ammonium hydroxide (60:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry it at 105° for 15 minutes. Locate the spots on the plate by examination under short-wavelength UV light: any spot obtained from *Test solution A*, other than the one corresponding to levamisole, does not exceed, in size or intensity, the principal spot obtained from *Reference solution B*, corresponding to not more than 0.5% of any individual impurity. Expose the plate to iodine vapor in a closed chamber for 15 minutes, and locate the spots on the plate: any spot obtained from *Test solution A*, other than the one corresponding to levamisole, does not exceed, in size or intensity, the principal spot obtained from *Reference solution B*, corresponding to not more than 0.5% of any individual impurity, and the total of all impurities found does not exceed 1.0%.
Assay—Dissolve about 200 mg of Levamisole Hydrochloride, accurately weighed, in 30 mL of alcohol. Add 5.0 mL of 0.01 N hydrochloric acid, and titrate with 0.1 N sodium hydroxide VS, determining the two inflection points potentiometrically. Determine the volume, in mL, of 0.1 N sodium hydroxide consumed between the two inflection points. Each mL of 0.1 N sodium hydroxide consumed is equivalent to 24.08 mg of $C_{11}H_{12}N_2S \cdot HCl$.

Levamisole Hydrochloride Tablets

» Levamisole Hydrochloride Tablets contain an amount of Levamisole Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levamisole ($C_{11}H_{12}N_2S$).

Packaging and storage—Preserve in well-closed containers.
Labeling—Label it to state both the content of the active moiety and the content of the salt used in formulating the article.
USP Reference standards ⟨11⟩—
USP Levamisole Hydrochloride RS

Identification—
A: The retention time of the major peak for levamisole in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
B: The R_F value of the principal spot obtained from *Test solution B* in the *Chromatographic purity* test corresponds to that from *Standard solution A*.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of levamisole ($C_{11}H_{12}N_2S$) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 214 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Levamisole Hydrochloride RS in the same *Medium*.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{11}H_{12}N_2S$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity—
Test solution A—Transfer an amount of powdered Tablets, equivalent to 100 mg of levamisole, to a glass test tube. Add 5.0 mL of methanol, shake for 2 minutes, and filter.
Test solution B—Dilute 1.0 mL of *Test solution A* to 10 mL with methanol, and mix.
Standard solution A—Prepare a solution of USP Levamisole Hydrochloride RS in methanol having a concentration of 2.4 mg per mL (equivalent to 2.0 mg of levamisole per mL).
Standard solution B—Dilute 1.0 mL of *Standard solution A* to 20 mL with methanol, and mix.
Procedure—Apply separate 10-µL portions of *Test solutions A* and *B* and *Standard solutions A* and *B* to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, acetone, and ammonium hydroxide (60:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry the plate at 105° for 15 minutes. Locate the spots on the plate by examination under short-wavelength UV light: any spot obtained from *Test solution A*, other than that of levamisole, does not exceed, in size or intensity, the principal spot obtained from *Standard solution B*, corresponding to not more than 0.5% of any individual impurity. Expose the plate to iodine vapor in a closed chamber for 15 minutes, and locate the spots on the plate: any spot obtained from *Test solution A*, other than that of levamisole, does not exceed, in size or intensity, the principal spot obtained from *Standard solution B*, corresponding to not more than 0.5% of any individual impurity.

Assay—
Solution A—Prepare a 0.75% solution of monobasic ammonium phosphate in water, and adjust with diisopropylamine to a pH of 7.
Solution B—Use acetonitrile.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 20 mg of USP Levamisole Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add 10 mL of water, and swirl to dissolve. Dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Levamisole Hydrochloride RS per mL.
Resolution solution—Dissolve 20 mg of Levamisole Hydrochloride in 5 mL of 0.1 N sodium hydroxide, and heat

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at 100° in a closed vial for 5 hours. Allow to cool, and dilute 1 mL of the solution to 25 mL with methanol.

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 150 mg of levamisole ($C_{11}H_{12}N_2S$), to a 100-mL volumetric flask. Add 25 mL of water, and shake by mechanical means for 30 minutes. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 10-cm column that contains 3-µm packing L1. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–5	80→20	20→80	linear gradient
5–7	20	80	isocratic
7–8	20→80	80→20	linear gradient
8–12	80	20	isocratic

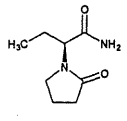
Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are 1.0 for levamisole and about 1.3 for the major degradation product; and the resolution, *R*, between levamisole and the major degradation product is not less than 6.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, is not less than 3.0; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of levamisole ($C_{11}H_{12}N_2S$) in the Tablets taken by the formula:

$$(204.29/240.75)(1000C)(r_U/r_S)$$

in which 204.29 and 240.75 are the molecular weights of levamisole and levamisole hydrochloride, respectively; *C* is the concentration, in mg per mL, of USP Levamisole Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the levamisole peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Levetiracetam



$C_8H_{14}N_2O_2$ 170.21

1-Pyrrolidineacetamide, α-ethyl-2-oxo-, (α*S*)-;
(–)-(*S*)-α-Ethyl-2-oxo-1-pyrrolidineacetamide [102767-28-2].

DEFINITION

Levetiracetam contains NLT 98.0% and NMT 102.0% of levetiracetam ($C_8H_{14}N_2O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Identification solution* corresponds to that of the levetiracetam *S*-enantiomer from the *System suitability solution*, as obtained in the test for *Limit of Levetiracetam R-Enantiomer.*

ASSAY

- **PROCEDURE**

Buffer: 2.7 g/L of monobasic potassium phosphate in water. Adjust with 2% aqueous potassium hydroxide (w/v) to a pH of 5.5.
Solution A: Acetonitrile and *Buffer* (1:19)
Solution B: Acetonitrile
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
20	71	29

System suitability solution: 0.2 mg/mL of USP Levetiracetam RS and 0.08 mg/mL of USP Levetiracetam Related Compound A RS in *Solution A.* Prepare by first dissolving the required amount of USP Levetiracetam RS in a suitable volumetric flask. Add 10% of the flask volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, and then neutralize by adding 0.1 N hydrochloric acid at 10% of the flask volume. Add the required amount of USP Levetiracetam Related Compound A RS, sonicate to dissolve, dilute with *Solution A* to volume, and mix. [NOTE—Levetiracetam related compound A is included for peak identification purposes.]
Standard solution: 0.1 mg/mL of USP Levetiracetam RS in *Solution A*
Sample solution: 0.1 mg/mL of Levetiracetam in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 0.9 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Relative standard deviation: NMT 1.0%, for the levetiracetam peak
[NOTE—If system suitability criteria cannot be met, it is recommended that the column temperature be maintained at 20° to stabilize the system.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Levetiracetam taken:

$$\text{Result} = [(r_U/r_S) \times (C_S/C_U) \times 100] - F$$

r_U = peak response of levetiracetam from the *Sample solution*

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r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)
F = percentage of levetiracetam *R*-enantiomer from the test for *Limit of Levetiracetam R-Enantiomer*

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **LIMIT OF LEVETIRACETAM *R*-ENANTIOMER**

Mobile phase: *n*-Hexane and dehydrated alcohol (80:20)
System suitability solution: 0.1 mg/mL of USP Levetiracetam Racemic Mixture RS in *Mobile phase*
Standard solution: 0.05 mg/mL of USP Levetiracetam RS in *Mobile phase*
Sample solution: 10 mg/mL of Levetiracetam in *Mobile phase*
Identification solution: 0.05 mg/mL of Levetiracetam from *Sample solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 10-µm packing L51
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Identification solution*
[NOTE—The relative retention times for levetiracetam *R*-enantiomer and levetiracetam *S*-enantiomer are 0.55 and 1.0, respectively. Use the chromatogram from the *Identification solution* for *Identification* test *B*.]
Suitability requirements
Resolution: NLT 4.0 between the *R*- and *S*-enantiomers, *System suitability solution*. [NOTE—If a loss of resolution (less than 4.0) is observed, it is recommended that the column temperature be maintained at 25° to stabilize the system.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levetiracetam *R*-enantiomer in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levetiracetam *R*-enantiomer from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.8%

• **LIMIT OF LEVETIRACETAM RELATED COMPOUND B**

[NOTE—Perform this test only if levetiracetam related compound B is a known process impurity.]
Buffer: 1.22 g of sodium 1-decanesulfonate in 1 L of water containing about 1.3 mL of phosphoric acid. Adjust with 20% (w/v) potassium hydroxide to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (3:17)
System suitability solution: 2 mg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*
Standard solution: 0.002 mg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*
Sample solution: 2.0 mg/mL of Levetiracetam in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.0 mL/min
Injection volumes
System suitability: 10 µL
Analysis: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The retention time for levetiracetam related compound B is 9 min.]
Suitability requirements
Tailing factor: NMT 3.0. [NOTE—If a significant tailing of the levetiracetam related compound B peak is observed (greater than 3.0), it is recommended that the column temperature be maintained at 27° to stabilize the system.]
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levetiracetam related compound B in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levetiracetam related compound B from the *Sample solution*
r_S = peak response of levetiracetam related compound B from the *Standard solution*
C_S = concentration of USP Levetiracetam Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levetiracetam related compound B free base, 102.1
M_{r2} = molecular weight of levetiracetam related compound B, 138.6

Acceptance criteria: NMT 0.10%
[NOTE—The amount of levetiracetam related compound B measured is to be included in the total impurities in the test for *Organic Impurities*.]

• **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.005 mg/mL of USP Levetiracetam RS in *Solution A*
Sample solution: 5 mg/mL of Levetiracetam in *Solution A*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*

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C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

[NOTE—Disregard any peak with a relative retention time of 0.19 or less.]
Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pyridin-2-ol[a]	0.37	1.0	0.025
Levetiracetam acid[b]	0.62	1.2	0.3
Levetiracetam	1.00	—	—
Levetiracetam related compound A[c]	1.25	0.35	0.05
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	0.4

[a] Not included in the total impurities limit.
[b] *(S)*-2-(2-Oxopyrrolidin-1-yl)butanoic acid. Included in the total impurities limit.
[c] *(S)*-*N*-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide. Included in the total impurities limit only if levetiracetam related compound B is a known process impurity.

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method Ia*: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Levetiracetam RS
USP Levetiracetam Racemic Mixture RS
A 1:1 mixture of:
Levetiracetam *S*-enantiomer-(2*S*)-2-(2-oxopyrrolidin-1-yl)butanamide;
Levetiracetam *R*-enantiomer (2*R*)-2-(2-oxopyrrolidin-1-yl) butanamide.
USP Levetiracetam Related Compound A RS
(*S*)-*N*-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.
$C_8H_{15}ClN_2O_2$ 206.67
USP Levetiracetam Related Compound B RS
(*S*)-2-Aminobutanamide hydrochloride.
$C_4H_{10}N_2O \cdot HCl$ 138.6

Levetiracetam Injection

DEFINITION
Levetiracetam Injection is a sterile solution of levetiracetam in Water for Injection and contains NLT 90.0% and NMT 110.0% of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$). Levetiracetam Injection may contain buffering and isotonicity agents. Levetiracetam Injection contains no antimicrobial agent.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 1.0 g/L of anhydrous dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (6:94)
Diluent: Acetonitrile and water (6:94)
System suitability solution: Solution containing levetiracetam and levetiracetam acid prepared from a solution of 0.2 mg/mL of USP Levetiracetam RS as follows. Dissolve the required amount of USP Levetiracetam RS in 10% of the final volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, then neutralize by adding 10% of the flask volume of 0.1 N hydrochloric acid. Dilute with *Diluent* to volume.
Standard solution: 100 µg/mL of USP Levetiracetam RS in *Diluent*. Sonication may be used to aid in dissolution if necessary.
Sample solution: Nominally 100 µg/mL of levetiracetam from NLT 2 mL of Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of levetiracetam
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Identify the peaks using the relative retention times given in *Table 1*.]
Suitability requirements
Tailing factor: NMT 2.0 for the levetiracetam peak, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levetiracetam from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of levetiracetam in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.1 µg/mL of USP Levetiracetam RS in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Identify the peaks using the relative retention times in *Table 1*.]
Suitability requirements
Tailing factor: NMT 2.0 for the levetiracetam peak, *System suitability solution*

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Relative standard deviation: NMT 10.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levetiracetam acid and any other unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levetiracetam acid or any individual unspecified degradation product from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of levetiracetam in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levetiracetam acid[a]	0.4	0.3
Levetiracetam	1.0	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	1.00

[a] (*S*)-2-(2-Oxopyrrolidin-1-yl)butanoic acid.

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 5.0–6.0
- **Bacterial Endotoxins Test** ⟨85⟩**:** Contains NMT 0.175 USP Endotoxin Units/mg of levetiracetam
- **Sterility Tests** ⟨71⟩**:** Meets the requirements when tested as directed for *Aqueous Solutions* under *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed Type I glass vials. Store at controlled room temperature.
- **Labeling:** Label the article to indicate that the Injection is to be diluted prior to administration.
- **USP Reference Standards** ⟨11⟩
  USP Levetiracetam RS

Levetiracetam Oral Solution

DEFINITION
Levetiracetam Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$).

IDENTIFICATION
- **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Solution A: Dilute 1 mL of phosphoric acid with water to 1 L.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	92	8
6	92	8
7	40	60
10	40	60
11	92	8
15	92	8

Standard solution: 1.0 mg/mL of USP Levetiracetam RS in *Solution A*
Sample solution: Nominally 1.0 mg/mL of levetiracetam prepared as follows. Transfer a suitable volume of the Oral Solution to a suitable volumetric flask to obtain 1.0 mg/mL final concentration of levetiracetam. Add 60% of the flask volume of *Solution A*, and sonicate at room temperature for 5 min with intermittent shaking. Allow the solution to cool, and dilute with *Solution A* to volume. Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levetiracetam from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
- **Organic Impurities**

Solution A: Dilute 2 mL of phosphoric acid with water to 1 L.
Solution B: Acetonitrile
Diluent: Acetonitrile and *Solution A* (5:95)
Mobile phase: See *Table 2*.

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Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	95	5
20	90	10
30	75	25
35	50	50
40	50	50
41	100	0
50	100	0

System suitability solution: 0.2 mg/mL of USP Levetiracetam RS and 0.1 mg/mL of USP Levetiracetam Related Compound A RS in *Diluent* prepared as follows. Dissolve the required amount of USP Levetiracetam RS in 10% of the final volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, and then neutralize by adding 0.1 N hydrochloric acid at 10% of the flask volume. Add the required amount of USP Levetiracetam Related Compound A RS, sonicate to dissolve, and dilute with *Diluent* to volume. [NOTE—This solution contains levetiracetam, levetiracetam acid, and levetiracetam related compound A.]

Standard solution: 3 µg/mL of USP Levetiracetam RS in *Solution A*

Sample solution: Nominally 2 mg/mL of levetiracetam prepared as follows. Transfer a suitable volume of the Oral Solution to a suitable volumetric flask. Add 60% of the flask volume of *Solution A*, and sonicate at room temperature for 5 min with intermittent shaking. Allow the solution to cool, and dilute with *Solution A* to volume. Pass a portion of the solution through a suitable filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between levetiracetam related compound A and levetiracetam acid, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levetiracetam	1.00	—	—
Levetiracetam related compound A[a,b]	1.38	—	—
Levetiracetam acid[c]	1.46	0.92	0.3
Any individual unspecified degradation product	—	1.0	0.10
Total impurities	—	—	1.0

[a] (*S*)-*N*-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.
[b] This is a process impurity and included for peak identification purposes only.
[c] (*S*)-2-(2-Oxopyrrolidin-1-yl)butanoic acid.

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.8–6.3
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 10^1 cfu/mL. It meets the requirement of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Levetiracetam RS
  USP Levetiracetam Related Compound A RS
  (*S*)-*N*-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.
  $C_8H_{15}ClN_2O_2$ 206.67

Levetiracetam Tablets

DEFINITION
Levetiracetam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K,197A▲** (CN 1-May-2020)

Standard solution: 1 mg/mL solution of USP Levetiracetam RS in solution prepared as follows. Transfer a suitable quantity of USP Levetiracetam RS to a suitable volumetric flask. Add 70% of the flask volume of acetone. Sonicate for 15 min. Dilute with acetone to volume.
Standard: Pass 10 mL of the *Standard solution* through a membrane filter of 0.45-µm pore size. Evaporate acetone from the filtrate completely to form crystals. Scratch the crystals. Weigh 2–4 mg of the residue and 200 mg of KBr in a mortar and pestle. Mix and grind well, and prepare the KBr pellet.
Sample solution: Transfer an amount of finely powdered Tablets (NLT 20) equivalent to 250 mg of levetiracetam to a 50-mL volumetric flask. Add 35 mL of acetone. Sonicate for 15 min. Dilute with acetone to volume.

USP Monographs

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Sample: Pass 10 mL of the *Sample solution* through a membrane filter of 0.45-µm pore size. Evaporate acetone from the filtrate completely to form crystals. Scratch the crystals. Weigh 2–4 mg of the residue and 200 mg of KBr in a mortar and pestle. Mix and grind well, and prepare the KBr pellet.
Analysis: Record the spectra of the *Standard* and *Sample* between 4000 cm^{-1} and 650 cm^{-1}.
Acceptance criteria: The spectrum of the *Sample* corresponds to that of the *Standard*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 1.4 g/L of monobasic potassium phosphate and 0.6 g/L of sodium 1-heptanesulfonate, adjusted with phosphoric acid to a pH of 2.8
Mobile phase: Acetonitrile and *Buffer* (8:92)
Diluent: Acetonitrile and water (20:80)
Standard solution: 0.35 mg/mL of USP Levetiracetam RS in *Diluent*. Sonication may be used to aid dissolution.
Sample solution: Nominally 0.4 mg/mL of levetiracetam from NLT 20 Tablets, finely crushed, in *Diluent*. Sonication may be used to aid dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 4-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: See *Table 1*.

Table 1

Tablet Strength (mg/Tablet)	Time (min)
250	15
500	15
750	15
1000	30

Buffer: 6.8 g/L of monobasic potassium phosphate, adjusted with dilute potassium hydroxide to a pH of 5.6
Mobile phase: Acetonitrile and *Buffer* (15:85)
Standard solution: (*L*/1000) mg/mL in *Medium*, where *L* is the Tablet label claim, in mg
Sample solution: Pass a portion of the solution under test though a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (*Q*) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in 15 min for Tablets labeled to contain 250, 500, or 750 mg; NLT 80% (*Q*) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in 30 min for Tablets labeled to contain 1000 mg

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Water; 900 mL, deaerate, if necessary
Apparatus 2: 50 rpm
Time: 15 min
Buffer: 1.36 g/L of monobasic potassium phosphate, adjusted with 10% potassium hydroxide to a pH of 5.0
Mobile phase: Acetonitrile and *Buffer* (10:90)
Standard solution: 54 µg/mL of USP Levetiracetam RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute an aliquot with *Medium* to obtain a concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 1*.
Tolerances: NLT 80% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) is dissolved.

▲**Test 4:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 6.8 g/L of monobasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (15:85)
Standard solution: 0.28 mg/mL of USP Levetiracetam RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 2 mL. Dilute an aliquot of the filtrate with *Medium*, if necessary, to obtain a concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of levetiracetam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 85% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) is dissolved. ▲ (RB 1-Mar-2018)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer: 6.8 g/L of monobasic potassium phosphate and 0.85 g/L of sodium 1-heptanesulfonate, adjusted with phosphoric acid to a pH of 2.8
Mobile phase: Acetonitrile and *Buffer* (5:95)
System suitability solution: 3.6 µg/mL of USP Levetiracetam RS and 3.6 µg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*
Standard solution: 3.6 µg/mL of USP Levetiracetam RS in *Mobile phase*
Sample solution: Equivalent to 1.2 mg/mL of levetiracetam from NLT 20 Tablets, finely crushed, in *Mobile phase*. [Note—Sonicate if necessary, and centrifuge the solution before passing through a suitable filter.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between levetiracetam related compound B and levetiracetam, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levetiracetam related compound B[a]	0.54	—	—
Levetiracetam	1.0	—	—

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levetiracetam related compound A[a,b]	1.7	—	—
Levetiracetam acid[c]	2.1	0.79	0.3
Any individual unspecified degradation product	—	1.0	0.1
Total impurities	—	—	0.6

[a] These impurities are listed for information only; they are process impurities, which are controlled in the drug substance.
[b] (*S*)-*N*-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.
[c] (*S*)-2-(2-Oxopyrrolidine-1-yl)butanoic acid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Levetiracetam RS
  USP Levetiracetam Related Compound B RS
  (*S*)-2-Aminobutanamide hydrochloride.
  $C_4H_{10}N_2O \cdot HCl$ 138.60

Levetiracetam Extended-Release Tablets

DEFINITION

Levetiracetam Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 1.4 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer* (10:90)
Standard stock solution: 1.0 mg/mL of USP Levetiracetam RS prepared as follows. Weigh a suitable quantity of the Reference Standard into a volumetric flask. Add *Mobile phase* to fill 60% of flask volume and tetrahydrofuran to fill 4% of flask volume. Sonicate in cool water to dissolve. Equilibrate to room temperature. Dilute with *Mobile phase* to volume.
Standard solution: 0.08 mg/mL of USP Levetiracetam RS in *Mobile phase* from *Standard stock solution*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Sample stock solution: Nominally (*L*/100) mg/mL of levetiracetam from NLT 5 Tablets prepared as follows, where *L* is the label claim in mg/Tablet. Transfer the Tablets to a volumetric flask containing tetrahydrofuran to fill about 5% of flask volume. Stir for 30 min, and allow to stand for 5 min. Sonicate for 20 min with intermittent shaking. Add *Mobile phase* to fill 80% of final volume, and sonicate in cold water for 20 min with intermittent shaking. Add methanol to fill 10% of flask volume. Dilute with *Mobile phase* to volume. Centrifuge for 15 min, and pass a portion of the solution through a suitable filter of 0.2-µm pore size. Alternatively, the *Sample stock solution*, having a nominal concentration of 3 mg/mL of levetiracetam, may be prepared as follows. Finely grind NLT 10 Tablets, and transfer an amount equivalent to 750 mg of levetiracetam to a suitable volumetric flask. Add 18% of the flask volume of acetonitrile. Sonicate for 10 min followed by shaking using a mechanical shaker for 10 min. Add 18% of the flask volume of water, and shake for 15 min using a mechanical shaker. Allow the sample to equilibrate to room temperature, and dilute with a mixture of acetonitrile and water (50:50) to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally 0.08 mg/mL of levetiracetam in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Temperatures
Column: 30°
Autosampler: 10°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 3 times the retention time of levetiracetam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levetiracetam from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1
Buffer A: Dissolve 6.8 g of potassium dihydrogen phosphate and 0.2 g of sodium hydroxide in 1 L of water. If necessary, adjust with 1 N sodium hydroxide to a pH of 6.0.
Medium: *Buffer A*; 900 mL
Apparatus 1: 100 rpm
Times: 1, 2, 4, and 8 h
Buffer B: 1.4 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer B* (10:90)

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Standard stock solution: 1.7 mg/mL of USP Levetiracetam RS in water. Sonication may be used to aid in dissolution.
Standard solution: (*L*/900) mg/mL of USP Levetiracetam RS in *Medium* from *Standard stock solution*, where *L* is the label claim in mg/Tablet. Pass a portion through a suitable filter of 0.45-µm pore size.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Temperatures
Column: 30°
Autosampler: 10°
Flow rate: 1.5 mL/min
Injection volume: 5 µL
Run time: 2 times the retention time of levetiracetam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of levetiracetam in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved	
		500 mg/Tablet (%)	750 mg/Tablet (%)
1	1	25–45	33–53
2	2	45–65	45–65
3	4	60–80	65–85
4	8	NLT 80	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 2*.
Buffer A: Dissolve 6.8 g of potassium dihydrogen phosphate and 0.2 g of sodium hydroxide in 1 L of water. If necessary, adjust with 1 N sodium hydroxide to a pH of 6.0.
Medium: *Buffer A*; 900 mL
Apparatus 1: 100 rpm
Times: 1, 2, 4, and 8 h
Buffer B: 2.82 g/L of potassium dihydrogen phosphate in water
Mobile phase: Acetonitrile and *Buffer B* (5:95). Adjust with phosphoric acid to a pH of 2.0.
Standard solution: (*L*/900) mg/mL of USP Levetiracetam RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Columns
Guard: 4.6-mm × 1-cm, 4.6-mm × 2-cm, or 4.0-mm × 2-cm; 5-µm packing L1
Analytical: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of levetiracetam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.5% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of levetiracetam in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 2*.

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Table 2

Time Point (*i*)	Time (h)	Amount Dissolved	
		500 mg/Tablet (%)	750 mg/Tablet (%)
1	1	22–42	16–36
2	2	39–59	30–50
3	4	62–82	50–70
4	8	NLT 80	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 3*.

Buffer A: Dissolve 6.8 g of potassium dihydrogen phosphate and 0.5 g of sodium hydroxide in 1 L of water. Adjust to a pH of 6.0.

Medium: *Buffer A*; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 8 h

Buffer B: 7.8 g/L of monobasic sodium phosphate dihydrate in water. Adjust with sodium hydroxide to a pH of 5.6.

Mobile phase: Acetonitrile and *Buffer B* (15:85)

Standard solution: (*L*/900) mg/mL of USP Levetiracetam RS in *Medium*, where *L* is the label claim in mg/Tablet

Sample solution: Centrifuge a portion of the solution under test.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: 2 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Relative standard deviation: NMT 2.0% for six replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i, of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of levetiracetam in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved		
		500 mg/Tablet (%)	750 mg/Tablet (%)	1000 mg/Tablet (%)
1	1	42–62	35–55	35–55
2	2	59–79	50–70	50–70
3	4	78–98	70–90	70–90
4	8	NLT 80	NLT 80	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 4*.

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 6.0.

Medium: *Buffer*; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 8 h

Standard solution: (*L*/900) mg/mL of USP Levetiracetam RS in *Medium*, where *L* is the label claim in mg/Tablet

Sample solution: Pass a suitable portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first 3 mL of the filtrate. Dilute a known volume of the remaining filtrate quantitatively with *Medium*.

Blank: *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: 210 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i, of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of levetiracetam in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL

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L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved	
		500 mg/Tablet (%)	750 mg/Tablet (%)
1	1	22–42	16–36
2	2	39–59	30–50
3	4	62–82	50–70
4	8	NLT 80	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: pH 6.0 phosphate buffer (6.8 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 6.0.); 900 mL
Apparatus 1: 100 rpm
Times
For 500- and 750-mg Tablets: 1, 4, 8, and 12 h
For 1000-mg Tablets: 1, 2, 4, and 8 h
Buffer: 2.7 g/L of monobasic potassium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (10:90)
Standard stock solution: 2.8 mg/mL of USP Levetiracetam RS in *Medium* prepared as follows. Transfer a suitable quantity of USP Levetiracetam RS to a suitable volumetric flask. Dissolve in 20% of the flask volume of methanol. Dilute with *Medium* to volume.
Standard solution: (L/900) mg/mL of USP Levetiracetam RS in *Medium* from *Standard stock solution*, where L is the label claim in mg/Tablet
Sample solution: At each time point withdraw 1 mL of the solution under test, and pass it through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L11
Flow rate: 1 mL/min
Injection volume
For 500- and 750-mg Tablets: 10 µL
For 1000-mg Tablets: 5 µL
Run time: 2 times the retention time of levetiracetam
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 4000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time for 500 and 750 mg/Tablet (h)	Time for 1000 mg/Tablet (h)	Amount Dissolved	
			500 and 750 mg/Tablet (%)	1000 mg/Tablet (%)
1	1	1	NMT 40	20–40
2	4	2	55–80	35–55
3	8	4	NLT 75	55–75
4	12	8	NLT 85	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: pH 6.0 phosphate buffer (6.9 g of monobasic potassium phosphate, and 0.23 g of sodium hydroxide in 1 L of water. Adjust with sodium hydroxide or phosphoric acid to a pH of 6.0.); 900 mL
Apparatus 1: 100 rpm
Times: 1, 2, 4, and 8 h
Mobile phase: Acetonitrile and water (10:90)
Standard solution: 0.5 mg/mL of USP Levetiracetam RS in *Medium* prepared as follows. Transfer a suitable quantity of USP Levetiracetam RS to a suitable volumetric flask. Add 4% of the flask volume of methanol and 60% of the flask volume of the *Medium*. Sonicate for NLT 5 min. Dilute with *Medium* to volume.
Sample solution: At the end of specified time interval, withdraw a known volume of the solution from the dissolution vessel. Pass a suitable portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 0.9 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of levetiracetam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

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Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of levetiracetam in *Medium* in the portion of sample withdrawn at time point i (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the solution under test (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	25–45
2	2	45–65
3	4	60–80
4	8	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 7*.
Medium: Acetate buffer, pH 4.5, prepared as follows. Dissolve 3.0 g of sodium acetate in 1 L of water and add 1.4 mL of glacial acetic acid. Adjust with 5 N sodium hydroxide or glacial acetic acid to a pH of 4.5; 230 mL.
Apparatus 3: 15 dips per min, with suitable screens
Times
For 500-mg Tablets: 1, 2, 4, and 8 h
For 750-mg Tablets: 1, 2, 4, and 10 h
Buffer: 13.6 g/L of monobasic potassium phosphate in water. Adjust with 5 N sodium hydroxide to a pH of 6.0.
Mobile phase: Methanol and *Buffer* (15:85)
Standard solution: 0.55 mg/mL of USP Levetiracetam RS in *Medium*. Sonication may be used to aid in dissolution.
Sample solution: Pass a suitable portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first 5 mL. Dilute a suitable volume of the filtrate with *Medium*, as needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of levetiracetam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i, of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point i:

$$\text{Result}_i = (r_U/r_S) \times D \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
D = dilution factor, as needed
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = C_2 \times V \times (1/L) \times 100 + \text{Result}_1$$
$$\text{Result}_3 = C_3 \times V \times (1/L) \times 100 + \text{Result}_2$$
$$\text{Result}_4 = C_4 \times V \times (1/L) \times 100 + \text{Result}_3$$

C_i = concentration of levetiracetam in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 230 mL
L = label claim (mg/Tablet)

Tolerances: See *Table 7*.

Table 7

Time Point (i)	Time (h)	Amount Dissolved	
		500 mg/Tablet (%)	750 mg/Tablet (%)
1	1	15–35	10–30
2	2	30–50	25–45
3	4	50–75	45–70
4	8	NLT 80	—
	10	—	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 8: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 8*.
Medium: Phosphate buffer, pH 6.0, prepared as follows. Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water. Adjust with 10 N sodium hydroxide solution to a pH of 6.0; 900 mL.
Apparatus 1: 100 rpm
Times: 1, 2, 4, and 12 h
Buffer: 0.26 g/L of monobasic potassium phosphate in water. Adjust with 20 g/L aqueous potassium hydroxide to a pH of 5.5.
Solution A: Acetonitrile and *Buffer* (5:95)
Mobile phase: Acetonitrile and *Solution A* (10:90)
Standard solution: (L/900) mg/mL of USP Levetiracetam RS in *Medium*, where L is the label claim in mg/Tablet. Sonicate to dissolve as needed.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 20°
Flow rate: 1 mL/min
Injection volume: 5 µL

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Run time: NLT 1.6 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.8%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i, of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of levetiracetam in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 8*.

Table 8

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	25–45
2	2	40–60
3	4	55–75
4	12	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 9:** If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 9*.

Medium: Phosphate buffer, pH 6.0, prepared as follows. Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water. Adjust with 50% (w/v) potassium hydroxide solution to a pH of 6.0; 900 mL.

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 12 h

Buffer: 5.0 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (15:85)

Standard solution: 0.56 mg/mL of USP Levetiracetam RS in *Medium*. Sonicate to dissolve as necessary.

Sample solution: Centrifuge a portion of the solution under test and use the clear supernatant. [NOTE—The use of a centrifuge speed of 2500 rpm for 10 min may be suitable.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 5 µL

Run time: NLT 2 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i, of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of levetiracetam in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 9*.

Table 9

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	10–30
2	2	25–45
3	4	45–70
4	12	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-May-2018)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Solution A: Dilute 2 mL of phosphoric acid with water to 1 L.

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Diluent: Acetonitrile and *Solution A* (5:95)
Buffer: 1.4 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer* (5:95). To each L of the mixture, add 1 g of sodium 1-hexanesulfonate monohydrate.
System suitability solution: 0.3 mg/mL of USP Levetiracetam RS in *Diluent* prepared as follows. Dissolve the required amount of USP Levetiracetam RS in 10% of the final volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, and then neutralize by adding 0.1 N hydrochloric acid at 10% of the flask volume. Dilute with *Diluent* to volume. [NOTE—This solution contains levetiracetam and levetiracetam acid.]
Standard solution: 12.5 µg/mL of USP Levetiracetam RS in water. Sonication may be used to aid in dissolution. Pass a portion of the solution through a suitable filter of 0.2-µm pore size.
Sample solution: Nominally equivalent to 2.5 mg/mL of levetiracetam in water, from a portion of crushed Tablets (NLT 20) prepared as follows. Transfer the weighed amount of crushed Tablet powder to a volumetric flask containing water to fill 80% of final volume. Sonicate in cold water for 10 min. Equilibrate to room temperature. Dilute with water to volume. Pass a portion through a suitable filter of 0.2-µm pore size.
Alternatively, the *Sample solution* having a nominal concentration of 2–3 mg/mL of levetiracetam may be prepared as follows. Finely grind NLT 10 Tablets, and transfer an amount equivalent to one Tablet to a suitable volumetric flask. Add NLT 30 mL of acetonitrile. Sonicate for 10 min, and shake using a mechanical shaker for 10 min. Add NLT 30 mL of water, and shake for 15 min using a mechanical shaker. Allow the resulting mixture to equilibrate to room temperature. Add NMT 25% of the final flask volume of acetonitrile. Dilute with water to volume. Centrifuge for 15 min, and pass a portion through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 10°
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: 5 times the retention time of levetiracetam
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between levetiracetam and levetiracetam acid peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of USP Levetiracetam RS from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: See ▲*Table 10*.

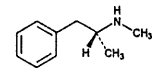
Table 10▲ (RB 1-May-2018)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levetiracetam related compound B[a, b]	0.40	—
Levetiracetam	1.0	—
Levetiracetam acid[c]	1.3	0.30
Levetiracetam related compound A[b, d]	1.9	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	1.0

[a] (*S*)-2-Aminobutanamide.
[b] Process impurities controlled in the drug substance. Included for identification purposes only. Not reported for the drug product, and not included in total impurities.
[c] (*S*)-2-(2-Oxopyrrolidin-1-yl)butanoic acid.
[d] (*S*)-*N*-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Levetiracetam RS

Levmetamfetamine



$C_{10}H_{15}N$ 149.23
Benzeneethanamine, *N*, α-dimethyl-, (*R*)-.
(–)-(*R*)-*N*, α-Dimethylphenethylamine [33817-09-3].

» Levmetamfetamine contains not less than 98.0 percent and not more than 100.5 percent of $C_{10}H_{15}N$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Levmetamfetamine RS
USP Methamphetamine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020).

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B: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *System suitability solution*, as obtained in the test for *Limit of methamphetamine*.

Specific rotation ⟨781S⟩: between −18.5° and −21.5°.

Test solution: 16 mg per mL, in 1.2 N hydrochloric acid.

Limit of methamphetamine—

Mobile phase—Prepare a filtered and degassed mixture of hexane, isopropyl alcohol, and acetonitrile (98: 1.5: 0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Mix suitable quantities of a solution of USP Methamphetamine Hydrochloride RS in chloroform and USP Levmetamfetamine RS in chloroform to obtain a solution containing about 0.025 mg per mL and 2.5 mg per mL of methamphetamine hydrochloride and levmetamfetamine, respectively. Transfer 2.0 mL of this solution to a suitable container, add 10 mg of 2-naphthyl chloroformate and 2.0 mL of chloroform, mix with a vortex mixer, and allow to stand for 5 minutes. To this solution, add 2 mL of 1 N sodium hydroxide, mix with a vortex mixer, allow to stand for 5 minutes, and discard the aqueous layer. Wash the organic layer twice with 2 mL of 1 N sodium hydroxide, discarding the aqueous layer. To the organic layer add 2 mL of 1 N hydrochloric acid, mix with a vortex mixer, and discard the aqueous layer. Wash the organic layer twice with 2 mL of 1 N hydrochloric acid, discarding the aqueous layer. To the organic layer add 2 mL of water, mix with a vortex mixer, and discard the aqueous layer. Wash the organic layer twice with 2 mL of water, discarding the aqueous layer. To the organic layer add about 1.0 g of anhydrous sodium sulfate, and mix with a vortex mixer. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter.

Test solution—Transfer about 62.5 mg of Levmetamfetamine, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with chloroform to volume, and mix. Transfer 2.0 mL of this solution to a suitable container, and proceed as directed in *Resolution solution* beginning with "add 10 mg of 2-naphthyl chloroformate and 2 mL of chloroform."

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 274-nm detector and a 4.6-mm × 25-cm column that contains packing L36. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.9 for methamphetamine and 1.0 for levmetamfetamine; and the resolution, *R*, between methamphetamine and levmetamfetamine is not less than 1.4. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 50 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the percentage of methamphetamine in the portion of Levmetamfetamine taken by the formula:

$$100r_M/(r_M + r_L)$$

in which r_M is the methamphetamine peak response obtained from the *Test solution*, and r_L is the peak response of levmetamfetamine obtained from the *Test solution:* not more than 0.1% is found.

Limit of nonvolatile residue—Heat about 1.0 g, accurately weighed, at 150° to constant weight: the limit is not more than 0.5%.

Ordinary impurities ⟨466⟩—

Test solution: chloroform.

Standard solution: chloroform.

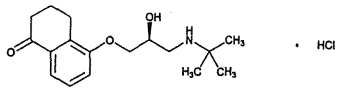
Eluant: a mixture of chloroform, cyclohexane, and diethylamine (5:4:1).

Visualization: 1.

Limits—No impurity exceeds 0.1%, and the total does not exceed 0.5%.

Assay—Transfer about 400 mg of Levmetamfetamine to a suitable container, add 50.0 mL of glacial acetic acid, and mix. Add two drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 14.92 mg of $C_{10}H_{15}N$.

Levobunolol Hydrochloride



$C_{17}H_{25}NO_3 \cdot HCl$ 327.85

1(2*H*)-Naphthalenone, 5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-, hydrochloride, (–)-(*S*); (–)-5-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2*H*)-naphthalenone hydrochloride [27912-14-7].

DEFINITION

Levobunolol Hydrochloride contains NLT 98.0% and NMT 102.0% of levobunolol hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: 5 mM sodium 1-heptanesulfonate in methanol
Solution B: 5 mM sodium 1-heptanesulfonate in water
Mobile phase: *Solution A*, 0.5 M sulfuric acid, and *Solution B* (53:1:47)
Standard solution: 100 µg/mL of USP Levobunolol Hydrochloride RS in *Mobile phase*
Sample solution: 100 µg/mL of Levobunolol Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm
Column: 3.9 mm × 25 cm; 10-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%

USP Monographs

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levobunolol hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$) in the portion of Levobunolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Levobunolol Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay*.
System suitability solution: 50 µg/mL each of USP Levobunolol Hydrochloride RS and USP Atenolol RS in *Mobile phase*
Standard solution: 5 µg/mL of USP Levobunolol Hydrochloride RS in *Mobile phase*
Sample solution: 1 mg/mL of Levobunolol Hydrochloride in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay* using *Mobile phase*.
Run time: 3 times the retention time of levobunolol
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 8 between levobunolol and atenolol peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Levobunolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of levobunolol from the *Standard solution*
C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Levobunolol Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurity: NMT 0.5%
Total impurities: NMT 1%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 30 mg/mL in methanol
Acceptance criteria: –19° to –20°
• **PH** ⟨791⟩
Sample: 50 mg/mL
Acceptance criteria: 4.5–6.5
• **LOSS ON DRYING** ⟨731⟩
Sample: 1 g
Analysis: Dry under vacuum over phosphorus pentoxide at 110° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Protect from light. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Atenolol RS
USP Levobunolol Hydrochloride RS

Levobunolol Hydrochloride Ophthalmic Solution

DEFINITION
Levobunolol Hydrochloride Ophthalmic Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of levobunolol hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

• **PROCEDURE**
Solution A: ▲1.0 g/L of▲ (USP 1-Dec-2019) sodium 1-heptanesulfonate in water
Mobile phase: Methanol, glacial acetic acid, and *Solution A* (550:5:450)
Standard solution: 0.05 mg/mL of USP Levobunolol Hydrochloride RS in *Mobile phase*
Sample solution: Nominally equivalent to 0.05 mg/mL of levobunolol hydrochloride in *Mobile phase* from Ophthalmic Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Dec-2019)
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.5 mL▲/min▲ (USP 1-Dec-2019)
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.2
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levobunolol hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

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C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levobunolol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase: Prepare as directed in the *Assay*.

Standard solution: 0.01 mg/mL each of USP Levobunolol Hydrochloride RS and USP Edetate Disodium RS in *Mobile phase*

Sample solution: Nominally equivalent to 1 mg/mL of levobunolol hydrochloride in *Mobile phase* from Ophthalmic Solution

Chromatographic system and **System suitability:** Proceed as directed in the *Assay* using wavelengths of UV 254 and 400 nm.

[NOTE—The relative retention times for levobunolol and edetate disodium are 1.0 and 0.46, respectively.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity at 254 nm in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of levobunolol from the *Standard solution*
C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of levobunolol hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any individual impurity (at 400 nm) at the retention time of levobunolol (at 254 nm) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution* at 400 nm
r_S = peak response of levobunolol from the *Standard solution* at 254 nm
C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of levobunolol hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor for the impurity, 0.2

Acceptance criteria

Individual impurity: NMT 1%

Total impurities: NMT 2.5%. Disregard any peak at 254 nm with the retention time of edetate disodium.

SPECIFIC TESTS

• **ANTIMICROBIAL EFFECTIVENESS TESTING** ⟨51⟩: Meets the requirements

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

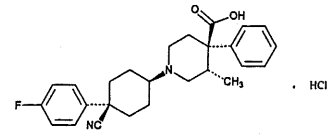
• **pH** ⟨791⟩: 5.5–7.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Edetate Disodium RS
USP Levobunolol Hydrochloride RS

Levocabastine Hydrochloride



$C_{26}H_{29}FN_2O_2 \cdot HCl$ 456.98

4-Piperidinecarboxylic acid, 1-[4-cyano-4-(4-fluorophen yl) cyclohexyl]-3-methyl-4-phenyl-, monohydrochloride, (–)-[1(*cis*),3α,4β]-;
(–)-*trans*-1-[*cis*-4-Cyano-4-(*p*-fluorophenyl)cyclohexyl]-3-methyl-4-phenylisonipecotic acid monohydrochloride [79547-78-7].

DEFINITION

Levocabastine Hydrochloride contains NLT 98.5% and NMT 101.5% of $C_{26}H_{29}FN_2O_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

• **C. OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: Meets the requirements

ASSAY

• **PROCEDURE**

Sample solution: Dissolve 175 mg of Levocabastine Hydrochloride in 50 mL of alcohol, and add 5.0 mL of 0.01 N hydrochloric acid.

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Potentiometric

Analysis
Sample: *Sample solution*
The volume of titrant required to titrate Levocabastine Hydrochloride is the difference between the first and third endpoints. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide VS is equivalent to 22.85 mg of $C_{26}H_{29}FN_2O_2 \cdot HCl$.

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%, based on a sample weight of about 1.000 g

ORGANIC IMPURITIES

• **Procedure**

[NOTE—Prepare solutions immediately before use.]

Diluent: 2 mg/mL of sodium hydroxide in water

Solution A: Dissolve 1.39 g of boric acid in water, and adjust with 1 N sodium hydroxide to a pH of 9.0. Dilute with water to 100 mL.

Run buffer: Dissolve 1.08 g of sodium dodecyl sulfate and 650 mg of hydroxypropyl-β-cyclodextrin in 5 mL of isopropyl alcohol, then dilute with *Solution A* to 50 mL.

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System suitability solution: 12.5 µg/mL of USP Levocabastine Hydrochloride RS and 12.5 µg/mL of USP Levocabastine Related Compound A RS in *Diluent*
Standard solution: Dilute 5.0 mL of the *Sample solution* with *Diluent* to 100 mL. Dilute 1.0 mL of this solution with *Diluent* to 10 mL to obtain a solution containing 12.5 µg/mL of Levocabastine Hydrochloride.
Sample solution: 2.5 mg/mL of Levocabastine Hydrochloride in *Diluent*
Capillary electrophoresis system
Detector: UV 214 nm
Column: 75-µm × 50-cm uncoated fused-silica capillary column
Column temperature: 50°
Current: See the gradient table below.

Time (min)	Current (µA)
0	0
0.17	75
15	130
40	130
60	200

[NOTE—Before performing the *System suitability*, equilibrate the capillary column with *Diluent* for 2 min, then equilibrate with *Run buffer* for at least 5 min.]
System suitability
Sample: *System suitability solution*
[NOTE—The relative migration times for levocabastine and levocabastine related compound A are approximately 1.0 and 1.07, respectively.]
Suitability requirements
Resolution: NLT 4 between levocabastine and levocabastine related compound A
[NOTE—If necessary, adjust the current gradient to achieve the required resolution.]
Analysis
Samples: *Diluent* (blank), *Standard solution*, and *Sample solution*
Separately inject equal volumes (pressure of 3450 Pa for 5 s) of the *Samples*, and record the peak responses.
[NOTE—Disregard any peak originating from the *Diluent*. Disregard any peak with an area of less than 0.1 times the major peak area of the *Standard solution* (0.05%).]
Acceptance criteria: The area for any peak in the *Sample solution*, other than the major peak, is not greater than the major peak area of the *Standard solution* (0.5%); and the sum of all peak areas in the *Sample solution*, except for the major peak, is not greater than twice the major peak area of the *Standard solution* (1.0%).

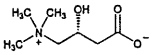
SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: −102° to −106° at 20°
Sample solution: 10 mg/mL in methanol
- **LOSS ON DRYING** ⟨731⟩: Dry about 1.000 g of the sample at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Levocabastine Hydrochloride RS
USP Levocabastine Related Compound A RS

Levocarnitine



$C_7H_{15}NO_3$ 161.20
(*R*)-3-Carboxy-2-hydroxy-*N*,*N*,*N*-trimethyl-1-propanaminium, inner salt;
(*R*)-(3-Carboxy-2-hydroxypropyl)trimethylammonium, inner salt [541-15-1].

DEFINITION

Levocarnitine contains NLT 97.0% and NMT 103.0% of levocarnitine ($C_7H_{15}NO_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Analysis: Dry the sample and USP Levocarnitine RS under vacuum at 50° for 5 h.
Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the derivatized *Sample solution* corresponds to that of the levocarnitine peak of the derivatized *System suitability solution*, as obtained in the test for *Enantiomeric Purity*.

ASSAY

- **PROCEDURE**
Sample: 100 mg of Levocarnitine
Blank: A mixture of 3 mL of formic acid and 50 mL of glacial acetic acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with the *Titrant* to an emerald green endpoint. Perform the blank determination.
Calculate the percentage of levocarnitine ($C_7H_{15}NO_3$) in the portion of Levocarnitine taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 161.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*
Standard solution: 0.50 mL of 0.020 N hydrochloric acid
Sample: 0.090 g of Levocarnitine
Acceptance criteria: NMT 0.4%

USP Monographs

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Change to read:

• **ENANTIOMERIC PURITY**

Buffer solution: Mix thoroughly 2000 mL of water with 5 mL of phosphoric acid and add accurately 13.6 mL of triethylamine dropwise while stirring.

Solution A: Mix 1500 mL of *Buffer solution* and 500 mL of acetonitrile. Adjust the solution with phosphoric acid to a pH of 2.6.

Solution B: Acetonitrile

Carbonate buffer solution: Transfer 338 mg of sodium carbonate and 152 mg of sodium bicarbonate to a 100-mL volumetric flask, and dissolve in and dilute with water to volume.

Sodium hydroxide solution: 30% solution of sodium hydroxide in water

Acetate buffer solution: Transfer 0.3 mL of glacial acetic acid to a 100-mL volumetric flask, add 90 mL of water to dissolve, adjust with *Sodium hydroxide solution* to a pH of 4.2, and dilute with water to volume.

Derivatization reagent: (+)-1-(9-Fluorenyl)ethyl chloroformate solution [(+)-FLEC]

System suitability solution: 1.25 mg/mL of USP Levocarnitine RS in water

Sample solution: 1.25 mg/mL of Levocarnitine in water

Blank: Water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Fluorescence

Excitation wavelength: 260 nm

Emission wavelength: 315 nm

Column: 4.6-mm × 7.5-cm; 2.7-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20.0 µL

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
7.4	100	0
7.5	2	98
9.1	2	98
9.3	100	0
11.0	100	0

After each sequence of samples, rinse the column with water for 10 min and then with acetonitrile and water (98:2) for another 10 min.

System suitability

[NOTE—Proceed with preparation and analysis of the derivatized *System suitability solution* as directed in *Analysis*. Relative retention times are about 0.87 and 1.0 for the (+)-FLEC derivatives of D-carnitine and L-carnitine, respectively.]

Sample: Derivatized *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between (+)-FLEC derivatives of D-carnitine and L-carnitine

Analysis: Transfer 30.0 µL of the *Blank*, *System suitability solution*, and *Sample solution* to separate 10-mL test tubes. Add 30 µL of *Carbonate buffer solution* and 80 µL of *Derivatization reagent* to each test tube and mix by vortex mixer. Allow the solutions to react for 1 h at 45° over a water bath. Cool the test tubes to room temperature, add 5.0 mL of *Acetate buffer solution* to each test tube, mix by vortex mixer, and transfer to vials for chromatographic analyses.

Separately inject equal volumes of derivatized *Blank*, derivatized *System suitability solution*, and derivatized *Sample solution* into the chromatograph and record the chromatograms. Identify the two diastereomer peaks due to (+)-FLEC derivatives of D-carnitine and L-carnitine in the chromatograms of the derivatized *System suitability solution* and derivatized *Sample solution*. Depending on the purity of the derivatizing reagent, these two peaks may contain co-eluting enantiomers of (–)-FLEC derivatives with D- and L-carnitines, which are accounted in the percentage calculations below. There should be no peaks observed at the retention times of D- and L-carnitine derivatives in the chromatogram of the derivatized *Blank*.

Calculate the percentage of L-carnitine derivative (R_L) from the derivatized *Sample solution*:

$$\text{Result} = r_L/(r_L + r_D) \times 100$$

r_L = peak response of the L-carnitine derivative from the derivatized *Sample solution*

r_D = peak response of the D-carnitine derivative from the derivatized *Sample solution*

Calculate the corrected percentage of L-carnitine ($C_7H_{15}NO_3$) (C_L) in the portion of Levocarnitine taken:

$$\text{Result} = (R_L - P_B)/(P_A - P_B) \blacktriangle \times 100 \blacktriangle$$ (ERR 1-Jun-2019)

R_L = percentage of L-carnitine derivative, calculated previously

P_B = percentage of (–)-FLEC as determined for (+)-1-(9-Fluorenyl)ethyl chloroformate solution (see Reagents, Indicators, and Solutions—Reagent Specifications)

P_A = percentage of (+)-FLEC as determined for (+)-1-(9-Fluorenyl)ethyl chloroformate solution (see Reagents, Indicators, and Solutions—Reagent Specifications)

Calculate the corrected percentage of D-carnitine in the portion of Levocarnitine taken:

$$\text{Result} = 100 - C_L$$

C_L = corrected percentage of L-carnitine, calculated previously

Acceptance criteria: NMT 0.2% of D-carnitine

• **LIMIT OF POTASSIUM**

[NOTE—The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Standard solution: 31.25 µg/mL of potassium in water, prepared from potassium chloride, previously dried at 105° for 2 h

Sample stock solution: 0.625 mg/mL of Levocarnitine in water

Sample solution A: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume. This solution contains 500 µg/mL of Levocarnitine and 0 µg/mL of added potassium from the *Standard solution*.

Sample solution B: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 2.0 mL of the *Standard solution*, and dilute with water to volume. This

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solution contains 500 µg/mL of Levocarnitine and 2.5 µg/mL of added potassium from the *Standard solution*.

Sample solution C: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 4.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 500 µg/mL of Levocarnitine and 5.0 µg/mL of added potassium from the *Standard solution*.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.7 nm

Lamp: Potassium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Sample solution A*, *Sample solution B*, *Sample solution C*, and *Blank*

Determine the absorbances of the solutions against the *Blank*. Plot the absorbances of the three *Sample solutions* versus their added potassium concentrations, in µg/mL. Draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the concentration, in µg/mL, of potassium in *Sample solution A*.

Calculate the percentage of potassium in the portion of Levocarnitine taken:

$$\text{Result} = (C_K/C_U) \times 100$$

C_K = concentration of potassium in *Sample solution A* (µg/mL), determined from the intercept of the linear regression line

C_U = concentration of Levocarnitine in *Sample solution A* (µg/mL)

Acceptance criteria: NMT 0.2%

• **LIMIT OF SODIUM**

[NOTE—The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Standard solution: 250 µg/mL of sodium in water, prepared from sodium chloride, previously dried at 105° for 2 h

Sample stock solution: 40.0 mg/mL of Levocarnitine in water

Sample solution A: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 0 µg/mL of added sodium from the *Standard solution*.

Sample solution B: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 2.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 20 µg/mL of added sodium from the *Standard solution*.

Sample solution C: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 4.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 40 µg/mL of added sodium from the *Standard solution*.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 589.0 nm

Lamp: Sodium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Sample solution A*, *Sample solution B*, *Sample solution C*, and *Blank*

Determine the absorbances of the solutions against the *Blank*. Plot the absorbances of the three *Sample solutions* versus their added sodium concentrations, in µg/mL. Draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the concentration, in µg/mL, of sodium in *Sample solution A*.

Calculate the percentage of sodium in the portion of Levocarnitine taken:

$$\text{Result} = (C_{Na}/C_U) \times 100$$

C_{Na} = concentration of sodium in *Sample solution A* (µg/mL), determined from the intercept of the linear regression line

C_U = concentration of Levocarnitine in *Sample solution A* (µg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 50 mg/mL

Acceptance criteria: 5.5–9.5

• **WATER DETERMINATION** ⟨921⟩**:** NMT 4.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers at temperatures between –15° and 35°. Protect from light.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Levocarnitine RS

Levocarnitine Injection

DEFINITION

Levocarnitine Injection is a sterile solution of Levocarnitine in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of levocarnitine ($C_7H_{15}NO_3$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. COLOR REACTION**

Analysis: Transfer 2 mL of Injection to a test tube, add 5 mL of 1 N hydrochloric acid and a few drops of ammonium reineckate TS.

Acceptance criteria: A red-violet precipitate is produced.

ASSAY

• **PROCEDURE**

Buffer: 0.05 M phosphate buffer, prepared by dissolving 6.805 g of monobasic potassium phosphate in 1 L of water

Mobile phase: Acetonitrile and *Buffer* (65:35). Adjust with phosphoric acid to a pH of 4.7, and mix.

System suitability solution: 5 mg/mL of USP Levocarnitine RS and 0.024 mg/mL of USP Levocarnitine Related Compound A RS in water

Standard solution: 10 mg/mL of USP Levocarnitine RS in water

Sample solution: Pool the contents of 10 containers, and dilute an accurately measured volume of Injection quantitatively with water to obtain a solution having a nominal concentration of about 10 mg/mL of levocarnitine.

USP Monographs

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 205 nm
Column: 3.9-mm × 30-cm; 10-µm packing L8
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between levocarnitine related compound A (crotonoylbetaine) and levocarnitine, *System suitability solution*
Relative standard deviation: NMT 2.0% for levocarnitine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of levocarnitine from the *Sample solution*
r_S = peak area of levocarnitine from the *Standard solution*
C_S = concentration of USP Levocarnitine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levocarnitine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩ **:** It contains NMT 0.1 USP Endotoxin Units/mg of levocarnitine.
- **pH** ⟨791⟩**:** 6.0–6.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩ **:** Meets the requirements for small-volume injections
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass. Store below 25°. Do not freeze.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Levocarnitine RS
USP Levocarnitine Related Compound A RS
2-Propen-1-aminium, 3-carboxy-*N*,*N*,*N*-trimethyl-, chloride.
$C_7H_{14}ClNO_2$ 179.65

Levocarnitine Oral Solution

DEFINITION
Levocarnitine Oral Solution is a solution of levocarnitine in water, and it contains suitable antimicrobial agents. It may contain a suitable flavor. It contains NLT 90.0% and NMT 110.0% of the labeled amount of levocarnitine ($C_7H_{15}NO_3$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* both relative to the internal standard, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
Buffer: 0.05 M phosphate buffer, pH 2.4, prepared by mixing 11.5 mL of phosphoric acid, 1900 mL of water, and about 100 mL of 1 N sodium hydroxide
Mobile phase: Dissolve 555 mg of sodium 1-heptanesulfonate in 980 mL of *Buffer* with stirring. Add 20 mL of methanol, and mix.
Internal standard solution: 0.02 mg/mL of *p*-aminobenzoic acid in water
Standard solution: Transfer about 10 mg of USP Levocarnitine RS to a 5-mL volumetric flask, add 1.0 mL of the *Internal standard solution,* and dilute with water to volume.
Sample stock solution: Equivalent to 10 mg/mL of levocarnitine in water from an accurately measured volume of Oral Solution
Sample solution: Wash a 10-mm × 4-cm disposable column containing 500 mg of packing L1, in order, with two column volumes of methylene chloride, two column volumes of methanol, and three column volumes of water. Pipet 5.0 mL of the *Sample stock solution* into the washed disposable column, and rinse the column twice with 6.0-mL portions of water. Collect the filtrate and washings in a 25-mL volumetric flask, add 5.0 mL of the *Internal standard solution,* and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 225 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 40 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for levocarnitine and *p*-aminobenzoic acid are 0.56 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the levocarnitine and internal standard peaks
Relative standard deviation: NMT 2.0% for levocarnitine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of levocarnitine to *p*-aminobenzoic acid from the *Sample solution*
R_S = peak area ratio of levocarnitine to *p*-aminobenzoic acid from the *Standard solution*
C_S = concentration of USP Levocarnitine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levocarnitine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 4.0–6.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Levocarnitine RS

USP Monographs

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Levocarnitine Tablets

DEFINITION
Levocarnitine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levocarnitine ($C_7H_{15}NO_3$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. COLOR REACTION**
  Analysis: Dissolve 1 Tablet in 5 mL of water, filter, and add 5 mL of 1 N hydrochloric acid. Place 2 mL of the filtrate in a test tube, and add a few drops of ammonium reineckate TS.
  Acceptance criteria: A red-violet precipitate is produced.

ASSAY
- **PROCEDURE**
  Buffer: 0.05 M phosphate buffer, pH 4.5, prepared by dissolving 6.805 g of monobasic potassium phosphate in 1 L of water
  Mobile phase: Acetonitrile and *Buffer* (65:35). Adjust with phosphoric acid to a pH of 4.7, and mix.
  System suitability solution: 1.5 mg/mL of USP Levocarnitine RS and 7 µg/mL of USP Levocarnitine Related Compound A RS in water
  Standard solution: 3 mg/mL of USP Levocarnitine RS in water
  Sample solution: Transfer 10 Tablets, accurately weighed, to a 500-mL volumetric flask, and add water to volume. Shake until the Tablets have disintegrated completely, and pass through a filter of 0.45-µm pore size. Dilute a portion of the filtrate quantitatively with water to a nominal concentration of about 3 mg/mL of levocarnitine.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 205 nm
  Column: 3.9-mm × 30-cm; 10-µm packing L8
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 1.0 between levocarnitine related compound A (crotonoylbetaine) and levocarnitine, *System suitability solution*
  Relative standard deviation: NMT 2.0% for levocarnitine, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of levocarnitine from the *Sample solution*
r_S = peak area of levocarnitine from the *Standard solution*
C_S = concentration of USP Levocarnitine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levocarnitine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 75 rpm
  Time: 30 min
  Standard solution: Known concentration of USP Levocarnitine RS in *Medium*
  Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Proceed as directed in the *Assay*, making any necessary modifications.
  Determine the percentage of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) dissolved:

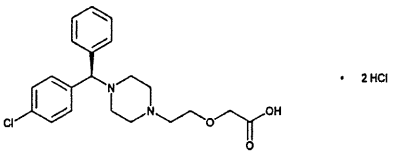
$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak area of levocarnitine in the *Sample solution*
r_S = peak area of levocarnitine in the *Standard solution*
C_S = concentration of USP Levocarnitine RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Levocarnitine RS
  USP Levocarnitine Related Compound A RS
  2-Propen-1-aminium, 3-carboxy-*N*,*N*,*N*-trimethyl-, chloride.
  $C_7H_{14}ClNO_2$ 179.65

Levocetirizine Dihydrochloride



$C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ 461.81
Acetic acid, [2-[4-[(*R*)-(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-, dihydrochloride;
(2-{4-[(*R*)-(4-Chlorophenyl)phenylmethyl]piperazin-1-yl} ethoxy)acetic acid dihydrochloride [130018-87-0].
Levocetirizine free base
$C_{21}H_{25}ClN_2O_3$ 388.89
[130018-77-8].

DEFINITION
Levocetirizine Dihydrochloride contains NLT 98.0% and NMT 102.0% of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

USP Monographs

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• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the levocetirizine peak of the *System suitability solution*, as obtained in the test for *Enantiomeric Purity*.

• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, water, and 1 M sulfuric acid TS (93: 6.6: 0.4)

Standard solution: 0.05 mg/mL of USP Levocetirizine Dihydrochloride RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Levocetirizine Dihydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L3

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) in the portion of Levocetirizine Dihydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levocetirizine from the *Sample solution*

r_S = peak response of levocetirizine from the *Standard solution*

C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Levocetirizine Dihydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**

Mobile phase: Acetonitrile, water, and 1 M sulfuric acid TS (93: 6.6: 0.4)

System suitability solution: 0.2 mg/mL of USP Levocetirizine Dihydrochloride RS and 0.2 µg/mL each of USP Levocetirizine Amide RS and USP Chlorobenzhydryl Piperazine RS in *Mobile phase*. Use the solution within 16 h.

Standard solution: 0.2 µg/mL each of USP Levocetirizine Dihydrochloride RS, USP Levocetirizine Amide RS, and USP Chlorobenzhydryl Piperazine RS in *Mobile phase*. Use the solution within 16 h.

Sample solution: 200 µg/mL of Levocetirizine Dihydrochloride in *Mobile phase*. Use the solution within 16 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L3

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of levocetirizine

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between levocetirizine and chlorobenzhydryl piperazine, *System suitability solution*

Tailing factor: NMT 2.0 for levocetirizine, *System suitability solution*

Relative standard deviation: NMT 5.0% for levocetirizine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levocetirizine amide or chlorobenzhydryl piperazine in the portion of Levocetirizine Dihydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levocetirizine amide or chlorobenzhydryl piperazine from the *Sample solution*

r_S = peak response of levocetirizine amide or chlorobenzhydryl piperazine from the *Standard solution*

C_S = concentration of USP Levocetirizine Amide RS or USP Chlorobenzhydryl Piperazine RS in the *Standard solution* (µg/mL)

C_U = concentration of Levocetirizine Dihydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Levocetirizine Dihydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of levocetirizine from the *Standard solution*

C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Levocetirizine Dihydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levocetirizine	1.0	—
Chlorobenzhydryl piperazine	1.3	0.2
Levocetirizine amide	2.5	0.2
Any individual unspecified impurity	—	0.1
Total impurities	—	0.5

• **ENANTIOMERIC PURITY**

Protect solutions containing levocetirizine from direct exposure to light.

Buffer: 1.5 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.8.

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Mobile phase: Acetonitrile and *Buffer* (30:70)
System suitability solution: 0.5 mg/mL of USP Cetirizine Hydrochloride RS in water
Sample solution: 0.5 mg/mL of Levocetirizine Dihydrochloride in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L90. [NOTE—A suitable guard column may be used.]
Column temperature: 30°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
Run time: NLT 1.8 times the retention time of levocetirizine
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for the *S*-enantiomer (of cetirizine) and levocetirizine, which is the *R*-enantiomer (of cetirizine), are about 0.83 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.4 between the *S*-enantiomer and levocetirizine
Tailing factor: NMT 2.0 for levocetirizine
Relative standard deviation: NMT 1.5% each for levocetirizine and the *S*-enantiomer
Analysis
Sample: *Sample solution*
Calculate the percentage of the *S*-enantiomer in the portion of Levocetirizine Dihydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the *S*-enantiomer from the *Sample solution*
r_T = sum of the peak responses of the *S*-enantiomer and levocetirizine from the *Sample solution*

Acceptance criteria: NMT 2.0% of the *S*-enantiomer

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 1.0%
• **pH** ⟨791⟩
Sample solution: 50 mg/mL of Levocetirizine Dihydrochloride in water
Acceptance criteria: 1.2–1.8

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cetirizine Hydrochloride RS
USP Chlorobenzhydryl Piperazine RS
(*R*)-1-[(4-Chlorophenyl)phenylmethyl]piperazine.
$C_{17}H_{19}ClN_2$ 286.80
USP Levocetirizine Amide RS
(*R*)-2-(2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetamide.
$C_{21}H_{26}ClN_3O_2$ 387.90
USP Levocetirizine Dihydrochloride RS

Levocetirizine Dihydrochloride Tablets

DEFINITION
Levocetirizine Dihydrochloride Tablets contain NLT 90% and NMT 110% of the labeled amount of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Medium: Water
Sample solution: Nominally 0.1 mg/mL of levocetirizine dihydrochloride from Tablets in water prepared as follows. Transfer 1 Tablet to a suitable volumetric flask, and add 40% of the flask volume of water. Shake for NLT 5 min to promote the disintegration of the Tablet. Dilute with water to volume. Pass a 10-mL portion of the resulting solution through a suitable filter, and discard the first mL. Use the filtrate.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 1 M sulfuric acid and water (5.7: 94.3)
Mobile phase: Acetonitrile, water, and 1 M sulfuric acid (93: 6.6: 0.4)
System suitability solution: 0.2 mg/mL of USP Levocetirizine Dihydrochloride RS and 0.2 µg/mL of USP Chlorobenzhydryl Piperazine RS in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in *Mobile phase*
Sample solution: Nominally 0.2 mg/mL of levocetirizine dihydrochloride prepared as follows. Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of *Solution A*, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Columns
Guard: 4-mm × 0.3-cm; 5-µm packing L3
Analytical: 4.6-mm × 25-cm; 5-µm packing L3
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between levocetirizine and chlorobenzhydryl piperazine
Tailing factor: NMT 1.5 for levocetirizine
Relative standard deviation: NMT 1.0% for levocetirizine
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of the labeled amount of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levocetirizine from the *Sample solution*
r_S = peak response of levocetirizine from the *Standard solution*
C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levocetirizine dihydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–110%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: (*L*/900) mg/mL of USP Levocetirizine Dihydrochloride RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 230 or 231 nm; use a suitable wavelength for background correction
Cell: 1 or 2 cm
Blank: *Medium*
Analysis
Samples: *Standard solution, Sample solution,* and *Blank*
Calculate the percentage of the labeled amount of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A, Mobile phase, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Standard solution: 0.002 mg/mL of USP Levocetirizine Dihydrochloride RS in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.
Run time: 2.3 times the retention time of levocetirizine
System suitability
Sample: *System suitability solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between levocetirizine and chlorobenzhydryl piperazine
Tailing factor: NMT 1.5 for levocetirizine
Relative standard deviation: NMT 1.0% for levocetirizine; NMT 5.0% for chlorobenzhydryl piperazine
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of levocetirizine from the *Standard solution*
C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levocetirizine dihydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levocetirizine (free base), 388.89
M_{r2} = molecular weight of levocetirizine dihydrochloride, 461.81

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.1%.

Table 1

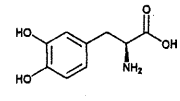
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levocetirizine	1.0	—
Chlorobenzhydryl piperazine[a]	1.4	—
Levocetirizine amide[a, b]	2.1	—
Any individual unspecified degradation product	—	0.30
Total impurities	—	1.0

[a] This is a process impurity that is included in this table for identification only. This impurity is controlled in the drug substance. This impurity is not to be reported for the drug product and is not to be included in the total impurities.
[b] (*R*)-2-(2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetamide.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Chlorobenzhydryl Piperazine RS
(*R*)-1-[(4-Chlorophenyl)phenylmethyl]piperazine.
$C_{17}H_{19}ClN_2$ 286.80
USP Levocetirizine Dihydrochloride RS

Levodopa



$C_9H_{11}NO_4$ 197.19
L-Tyrosine, 3-hydroxy-;
(–)-3-(3,4-Dihydroxyphenyl)-L-alanine [59-92-7].

USP Monographs

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DEFINITION

Levodopa contains NLT 98.0% and NMT 102.0% of levodopa ($C_9H_{11}NO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect all solutions from light, and maintain them at 10° until they are injected into the chromatograph.

Diluent: 0.1% trifluoroacetic acid in water

Mobile phase: Tetrahydrofuran and *Diluent* (3:97)

System suitability solution: 10 µg/mL each of USP Levodopa RS, USP Levodopa Related Compound B RS, and USP L-Tyrosine RS in *Diluent*

Standard solution: 0.4 mg/mL of USP Levodopa RS in *Diluent*

Sample solution: 0.4 mg/mL of Levodopa in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm L1 packing

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—Refer to *Table 1* in the test for *Organic Impurities* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between levodopa and L-tyrosine

Tailing factor: NMT 2.0 for levodopa

Relative standard deviation: NMT 2.0% for levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levodopa ($C_9H_{11}NO_4$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U = concentration of Levodopa in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%

Change to read:

- **ORGANIC IMPURITIES**

Protect all solutions from light, and maintain them at 10° until they are injected into the chromatograph.

Diluent, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Levodopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area for any impurity in the *Sample solution*

r_S = peak area for Levodopa in the *Standard solution*

C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U = concentration of Levodopa in the *Sample solution* (mg/mL)

F = relative response factor of each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A[a]	0.9	0.41	0.1
Levodopa	1.0	—	—
L-Tyrosine	1.3	0.44	0.1
Levodopa related compound B	1.6	0.83	0.5
L-Veratrylglycine[b]	2.7	0.76	0.1
Individual unknown impurity	—	1.0	0.1
Total impurities	—	—	1.1

[a] 3-(3,4,6-Trihydroxyphenyl)alanine▲; also known as 3-(2,4,5-Trihydroxyphenyl)-L-alanine.▲ (ERR 1-Nov-2018)

[b] 3-(3,4-Dimethoxyphenyl)-L-alanine.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 500 mg of Levodopa in a 25-mL volumetric flask. Add 10 mL of 1 N hydrochloric acid to dissolve the solid, add 5 g of methenamine, swirl the contents to dissolve the methenamine, and add 1 N hydrochloric acid to volume.

Analysis: Allow the *Sample solution* to stand in the dark at 25° for 3 h, and measure the rotation.

Acceptance criteria: −160° to −167°

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, store in a dry place, and prevent exposure to excessive heat.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Levodopa RS

USP Levodopa Related Compound B RS

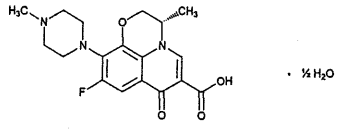
3-Methoxytyrosine.

$C_{10}H_{13}NO_4$ 211.22

USP L-Tyrosine RS

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Levofloxacin



$C_{18}H_{20}FN_3O_4 \cdot ½H_2O$ 370.38

7H-Pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-hydrate (2:1), (*S*)-;
(–)-(*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, hemihydrate [138199-71-0].
Anhydrous [100986-85-41].

DEFINITION

Levofloxacin contains NLT 98.0% and NMT 102.0% of $C_{18}H_{20}FN_3O_4$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 8.5 g/L of ammonium acetate, 1.25 g/L of cupric sulfate, pentahydrate, and 1.3 g/L of L-isoleucine in water
Mobile phase: Methanol and *Buffer* (3:7)
Standard solution: 1 mg/mL of USP Levofloxacin RS in *Mobile phase*
Sample solution: 1 mg/mL of Levofloxacin in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 360 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 0.8 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.5–1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levofloxacin from the *Sample solution*
r_S = peak response of levofloxacin from the *Standard solution*
C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)
C_U = concentration of Levofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%. Use a platinum crucible.
- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—*Procedure 1* is recommended if levofloxacin *N*-oxide is a potential organic impurity. *Procedure 2* and *Procedure 3* are recommended if levofloxacin related compound B is a potential organic impurity.]

Solution A, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 1 mg/mL of USP Levofloxacin RS in *Mobile phase*
Sensitivity solution: 0.3 µg/mL of USP Levofloxacin RS in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Relative standard deviation: NMT 1.0%, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each impurity
r_S = peak response of levofloxacin
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Desmethyl levofloxacin[a]	0.47	1.0	0.3
Diamine derivative[b]	0.52	0.9	0.3
Levofloxacin *N*-oxide[c]	0.63	1.1	0.3
9-Desfluoro levofloxacin[d]	0.73	1.0	0.3
Levofloxacin	1.0	—	—
D-Isomer[e]	1.23	1.0	0.8
Any unknown impurity	—	1.0	0.1
Total Impurities	—	—	0.5*

* Do not include the D-isomer in the calculation for total impurities.
[a] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[b] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[c] (*S*)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido-[1,2,3-*de*][1,4]benzoxazine-10-yl)-1-methyl-piperazine-1-oxide.
[d] (*S*)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[e] (*R*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

- **ORGANIC IMPURITIES, PROCEDURE 2**

[NOTE—Solutions of levofloxacin are not stable in light; use amber bottles.]

Buffer: Dissolve 3.08 g/L of ammonium acetate and 8.43 g/L of sodium perchlorate monohydrate in water. Adjust with phosphoric acid to a pH of 2.2.

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Solution A: Acetonitrile and *Buffer* (16:84)
Solution B: Acetonitrile, methanol, and *Buffer* (30:20:50)
Solution C: 0.4 mg/mL of USP Levofloxacin RS by dissolving in acetonitrile at about 8% of volume and diluting with water to volume
Solution D: 0.05 mg/mL of USP Levofloxacin Related Compound A RS in 0.2% ammonium hydroxide in methanol
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
10	82	18
15	40	60
30	40	60
30.1	100	0
38	100	0

System suitability solution: 0.1 mg/mL of USP Levofloxacin RS and 5 µg/mL of USP Levofloxacin Related Compound A RS in water from *Solution C* and *Solution D*
Levofloxacin stock solution: 0.4 mg/mL of USP Levofloxacin RS. Dissolve USP Levofloxacin RS in acetonitrile at about 8% of final volume, sonicate, and dilute with water to volume.
Levofloxacin standard solution: 0.02 mg/mL of USP Levofloxacin RS in acetonitrile and water (1:10) from *Levofloxacin stock solution*
Levofloxacin related compound B stock solution: 0.2 mg/mL USP Levofloxacin Related Compound B RS in methanol. [NOTE—Sonicate if necessary.]
Levofloxacin related compound B standard solution: 0.04 mg/mL USP Levofloxacin Related Compound B RS in methanol from *Levofloxacin related compound B stock solution*
Standard solution: 0.4 µg/mL of levofloxacin and 0.8 µg/mL of levofloxacin related compound B in acetonitrile and water (1:10) from *Levofloxacin standard solution* and *Levofloxacin related compound B standard solution*
Sample solution: 0.4 mg/mL by dissolving the sample in acetonitrile at about 8% of final volume and diluting with water to volume. [NOTE—Sonicate if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 280 nm
Column: 4.0-mm × 15-cm; 3.0-µm packing L1
Column temperature: 38°
Flow rate: 1.0 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for levofloxacin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levofloxacin related compound B in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for levofloxacin related compound B from the *Sample solution*
r_S = peak response for levofloxacin related compound B from the *Standard solution*
C_S = concentration of USP Levofloxacin Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Levofloxacin in the *Sample solution* (mg/mL)

Calculate the percentage of other impurities in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other impurity from the *Sample solution*
r_S = peak response of levofloxacin from the *Standard solution*
C_S = concentration of USP Levofloxacin RS in the *standard solution* (mg/mL)
C_U = concentration of Levofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levofloxacin related compound A (*N*-Des-methyl levofloxacin)[a]	0.9	0.20
Levofloxacin	1.0	—
Levofloxacin related compound B[b]	2.9	0.13
Any other impurity	—	0.10
Total impurities	—	0.50

[a] (*S*)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4-benzoxazine-6-carbocylic acid.
[b] (*S*)-9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4-benzoxazine-6-carbocylic acid.

• **ORGANIC IMPURITIES (ENANTIOMERIC PURITY), PROCEDURE 3**
Buffer: 1.32 g/L of D-phenylalanine and 0.75 g/L of copper(II)sulfate pentahydrate in water
Mobile phase: Methanol and *Buffer* (15:85)
System suitability solution: 0.01 mg/mL of USP Ofloxacin RS and 0.01 mg/mL of USP Levofloxacin RS in water
Sample solution: 0.08 mg/mL in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 294 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 0.7 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for D-ofloxacin and levofloxacin are 0.91 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between D-ofloxacin (D-isomer) and levofloxacin
Analysis
Sample: *Sample solution*
Calculate the percentage of D-ofloxacin in the portion of Levofloxacin taken:

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$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for D-ofloxacin
r_T = sum of responses of all peaks

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Solvent: Methanol
  Sample solution: 5 mg/mL in *Solvent*
  Acceptance criteria: –92° to –106°, at 20°
- **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** 2.0%–3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **LABELING:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Levofloxacin RS
  USP Levofloxacin Related Compound A RS
  (*S*)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
  $C_{17}H_{18}FN_3O_4$ 347.34
  USP Levofloxacin Related Compound B RS
  (*S*)-9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
  $C_{13}H_9F_2NO_4$ 281.21
  USP Ofloxacin RS

Levofloxacin Oral Solution

DEFINITION

Levofloxacin Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  [NOTE—Protect the solutions of levofloxacin from light.]
  Diluent: Acetonitrile and water (18:82)
  Mobile phase: *Diluent* that contains 1 mL of trifluoroacetic acid in each 1000 mL of solution
  Standard solution: 102.5 µg/mL of USP Levofloxacin RS in *Diluent*
  System suitability solution: 102.5 µg/mL each of USP Levofloxacin RS and USP Levofloxacin Related Compound A RS in *Diluent*
  Sample solution: 102.5 µg/mL of levofloxacin in *Diluent* based on the label claim. [NOTE—Mix the solution well after equilibrating the solution for 4 h at room temperature while protected from light.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 294 nm
  Column: 4.6-mm × 15-cm; 3.5-µm packing L11
  Column temperature: 30°
  Flow rate: 0.7 mL/min
  Run time: 2.5 times the retention time of the levofloxacin peak
  Injection size: 20 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 1.9 between levofloxacin related compound A and levofloxacin, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

- **Procedure**
  [NOTE—Protect the solutions of levofloxacin from light.]
  Diluent, Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of each individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of levofloxacin from the *Standard solution*
C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (See *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
9-Desfluoro levofloxacin[a]	0.64	1.0	—[*]
Diamine derivative[b]	0.75	1.0	—[*]
Levofloxacin related compound A[c]	0.91	0.81	0.5
Levofloxacin	1.0	—	—
Levofloxacin *N*-oxide[d]	1.55	0.93	0.5

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Impurity Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.0

* Disregard this peak because this is a process impurity controlled for the drug substance.
[a] (*S*)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[b] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[c] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carbocylic acid.
[d] (*S*)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido-[1,2,3-*de*][1,4]benzoxazine-10-yl)-1-methylpiperazine 1-oxide.

SPECIFIC TESTS
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/mL, and the total combined molds and yeast count does not exceed 10^1 cfu/mL. It also meets the requirement for absence of *Escherichia coli*.
- **Deliverable Volume** ⟨698⟩: Meets the requirements
- **pH** ⟨791⟩: 5.0–6.0

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Store at controlled room temperature, and protect from light.
- **USP Reference Standards** ⟨11⟩
  USP Levofloxacin RS
  7*H*-Pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-hydrate (2:1), (*S*)-; (–)-(*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, hemihydrate.
  Anhydrous.
  $C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$ 370.38
  USP Levofloxacin Related Compound A RS
  (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carbocylic acid.
  $C_{17}H_{18}FN_3O_4$ 347.34

Levofloxacin Tablets

DEFINITION
Levofloxacin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
  Diluent: Acetonitrile and water (20:80)
  Mobile phase: Transfer 874 mg of cupric sulfate, 918 mg of L-isoleucine, and 5.94 g of ammonium acetate to a suitable container. Add 700 mL of water, and mix until dissolved. Add 300 mL of methanol.
  Standard stock solution: 2 mg/mL of USP Levofloxacin RS in *Diluent*
  Standard solution: 0.2 mg/mL of USP Levofloxacin RS in *Mobile phase* from the *Standard stock solution*
  Sample stock solution: Nominally 5 mg/mL of levofloxacin prepared as follows. Transfer intact Tablets (NLT 5) to a volumetric flask, add 75% of the final volume of *Diluent*, and allow to stand for 15 min. Shake for 30 min, and dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discarding the first 1–2 mL of the filtrate.
  Sample solution: Nominally 0.2 mg/mL of levofloxacin in *Mobile phase* from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 360 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 45°
  Flow rate: 0.8 mL/min
  Injection volume: 25 µL
  Run time: 2 times the retention time of levofloxacin
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.8
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levofloxacin from the *Sample solution*
r_S = peak response of levofloxacin from the *Standard solution*
C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩
  Test 1
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 2: 75 rpm
  Time: 30 min
  Standard solution: 0.56 mg/mL of USP Levofloxacin RS in *Medium*
  Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV
  Analytical wavelength: 294 nm
  Cell length: 0.1 mm
  Blank: *Medium*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

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V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) is dissolved.

Test 2

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: L/900 mg/mL of USP Levofloxacin RS in *Medium*, and mix to obtain solutions with known concentrations as indicated in *Table 1*, where L is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test having a concentration similar to that of the *Standard solution* through a suitable filter of 0.45-µm pore size.

Table 1

Tablet Label Claim (mg)	Final Concentration (mg/mL)
250	0.27
500	0.55
750	0.83

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 293 nm
Cell length: 0.1 mm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) is dissolved.

Test 3

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: L/900 mg/mL of USP Levofloxacin RS in *Medium*, and mix to obtain solutions with known concentrations as indicated in *Table 1*, where L is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test having the same concentration as that of the *Standard solution* through a suitable filter of 0.45-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 326 nm
Cell length: 1 mm for a 250-mg Tablet, 0.5 mm for a 500-mg Tablet, and 0.2 mm for a 750-mg Tablet
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) is dissolved.

Test 4

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: 16 µg/mL of USP Levofloxacin RS in *Medium*
Sample solution: Pass a portion of the solution under test having the same concentration as that of the *Standard soluiton* through a suitable filter of 0.45-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 332 nm
Cell length: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Mobile phase, Standard stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.2 mg/mL of USP Levofloxacin RS from the *Standard stock solution* and 1 µg/mL of USP Levofloxacin Related Compound A RS in *Mobile phase*
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8 for the levofloxacin peak
Relative standard deviation: NMT 2.0% for the levofloxacin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levofloxacin related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

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r_U = peak response of levofloxacin related compound A from the *Sample solution*
r_S = peak response of levofloxacin related compound A from the *Standard solution*
C_S = concentration of USP Levofloxacin Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of levofloxacin from the *Standard solution*
C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

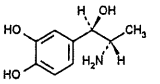
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Decarboxy levofloxacin[a]	0.38	0.60	0.3
Levofloxacin related compound A[b]	0.47	—	0.7
Diamine derivative[c]	0.52	0.83	0.3
Levofloxacin *N*-oxide[d]	0.63	0.68	0.7
9-Desfluoro levofloxacin[e]	0.73	—	—[f]
Levofloxacin	1.00	—	—
Dextrofloxacin[g]	1.23	—	—[f]
Levofloxacin 9-piperazino isomer[h]	1.69	—	—[f]
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	1

[a] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine.
[b] (*S*)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[c] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[d] (*S*)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido-[1,2,3-*de*][1,4]benzoxazine-10-yl)-1-methylpiperazine 1-oxide.
[e] (*S*)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[f] Process impurity, for information only.
[g] (*R*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
[h] (*S*)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Levofloxacin RS
  USP Levofloxacin Related Compound A RS
  (*S*)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.
  $C_{17}H_{18}FN_3O_4$ 347.34

Levonordefrin



$C_9H_{13}NO_3$ 183.20
1,2-Benzenediol, 4-(2-amino-1-hydroxypropyl)-, [*R*-(*R**,*S**)]-. (-)-α-(1-Aminoethyl)-3,4-dihydroxybenzyl alcohol [18829-78-2; 829-74-3].

» Levonordefrin, dried in vacuum at 60° for 15 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_9H_{13}NO_3$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Levonordefrin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)
B: Ultraviolet Absorption ⟨197U⟩—
Solution: 25 µg per mL.
Medium: 0.1 N hydrochloric acid.
Specific rotation ⟨781S⟩: between −28° and −31°.
Test solution: 50 mg, previously dried, per mL, in 0.3 N hydrochloric acid.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 15 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

Chromatographic purity—
Standard solutions—Dissolve an accurately weighed quantity of USP Levonordefrin RS in a mixture of methanol and glacial acetic acid (96:4) to obtain a Standard stock solution having a known concentration of 5 mg per mL. Dilute this solution quantitatively with a mixture of methanol and glacial acetic acid (96:4) to obtain *Standard solutions*, designated below by letter, having the following compositions:

Standard solution	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimen)
A	(1 in 10)	500	1.0
B	(1 in 20)	250	0.5
C	(1 in 50)	100	0.2
D	(1 in 100)	50	0.1

USP Monographs

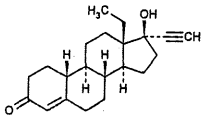
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Test solution—Dissolve an accurately weighed quantity of Levonordefrin in a mixture of methanol and glacial acetic acid (96:4) to obtain a solution containing 50 mg per mL.

Procedure— Apply separately 5 µL of the *Test solution* and 5 µL of each *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of *n*-butyl alcohol, water, and glacial acetic acid (70:20:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm, circulating air. Examine the plate under short-wavelength UV light. Expose the plate to iodine vapors, and examine again. Compare the intensities, observed by both visualizations, of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions:* the sum of the intensities of secondary spots obtained from the *Test solution* corresponds to not more than 1.0% of related compounds, with no single impurity corresponding to more than 0.5%.

Assay—Transfer about 350 mg of Levonordefrin, previously dried and accurately weighed, to a small flask, dissolve in 50 mL of glacial acetic acid, heating, if necessary, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.32 mg of $C_9H_{13}NO_3$.

Levonorgestrel



$C_{21}H_{28}O_2$ 312.45

18,19-Dinorpregn-4-en-20-yn-3-one, 13-ethyl-17-hydroxy-, (17α)-(–)-.

(–)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one [797-63-7].

» Levonorgestrel contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{28}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Levonorgestrel RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

B: Meeting the requirements of the tests for *Specific rotation* and *Melting range* provides identification distinguishing it from norgestrel.

Melting range ⟨741⟩: between 232° and 239°, but the range between beginning and end of melting does not exceed 4°.

Specific rotation ⟨781S⟩: between –30° and –35°.

Test solution: 20 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry it at 105° for 5 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.3%.

Limit of ethynyl group—Dissolve 200 mg in about 40 mL of tetrahydrofuran. Add 10 mL of silver nitrate solution (1 in 10), and titrate with 0.1 N sodium hydroxide VS, using either a glass-calomel or a silver-silver chloride electrode system with potassium nitrate filling solution. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 2.503 mg of ethynyl group (-C≡CH). Not less than 7.81% and not more than 8.18% of ethynyl group is found.

Chromatographic purity—Proceed as directed in the test for *Chromatographic purity* under *Norgestrel,* using USP Levonorgestrel RS in place of USP Norgestrel RS. The requirements of the test are met if the sum of the impurities in the *Test preparation* does not exceed 2.0% and no single impurity is greater than 0.5%.

Assay—Using USP Levonorgestrel RS, proceed as directed in the *Assay* under *Norgestrel,* except to read "Levonorgestrel" in place of "Norgestrel."

Levonorgestrel and Ethinyl Estradiol Tablets

DEFINITION

Levonorgestrel and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levonorgestrel ($C_{21}H_{28}O_2$) and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

- **A.** The retention times of the two major peaks of the *Sample solution* correspond to those of levonorgestrel and ethinyl estradiol in the *Standard solution,* as obtained in the *Assay.*
- **B.** Finely powder 20 Tablets and transfer a portion of the powder, equivalent to 4 mg of levonorgestrel, to a suitable container. Add 250 mL of a solvent mixture consisting of isooctane and chloroform (3:1). Sonicate the mixture for 3 min, and then stir it by mechanical means for 30 min. Filter the mixture and evaporate the filtrate to dryness in a rotating vacuum evaporator. Dissolve the residue in 3 mL of chloroform, and transfer with a pipet to a 60-mL separator containing 18 mL of isooctane. Rinse the evaporator flask with an additional 3-mL portion of chloroform, and add the rinsing to the separator. Add 10 mL of 1 N sodium hydroxide, shake vigorously, and allow the layers to separate. Discard the lower aqueous phase, and filter the organic phase through 3 g of anhydrous sodium sulfate on filter paper into a 50-mL beaker. Rinse the filter with several small portions of the mixture of isooctane and chloroform (3:1), adding the filtered rinsings to the filtrate, and evaporate under nitrogen on a steam bath to dryness. Dissolve the residue in 1–2 mL of hot toluene, and transfer with a pipet to a small glass vial. Reduce the volume of the solution to 0.1 mL under nitrogen with warming. [NOTE—During this step, any crystals that deposit on the vial wall should be transferred to the bottom, and allowed to redissolve.]

Store the vial containing the clear toluene solution at 4° overnight to allow crystallization to occur. Remove and discard the mother liquor with a pipet, rinse the crystals with two 0.5-mL portions of anhydrous ether, and discard

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the rinsings. Dry the vial containing the rinsed crystals in a vacuum desiccator at 60° for 4 h.

Acceptance criteria: The melting point of the dried crystals of levonorgestrel so obtained is not lower than 220°, using the procedure described under *Melting Range or Temperature* ⟨741⟩, *Class I.*

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (35:15:45)

Standard solution: 15 µg/mL of USP Levonorgestrel RS and 3 µg/mL of USP Ethinyl Estradiol RS in *Mobile phase*

Sample solution: Transfer a number of Tablets, equivalent to 3 mg of levonorgestrel, to a 200-mL volumetric flask. Dilute with *Mobile phase* to volume, sonicate to disintegrate the Tablets, then shake by mechanical means for 20 min. Centrifuge, and use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5- to 7-µm packing L7

Flow rate: 1 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and levonorgestrel are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the two major peaks

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)

C_U = nominal concentration of the corresponding analyte in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of $C_{21}H_{28}O_2$, 90.0%–110.0% of the labeled amount of $C_{20}H_{24}O_2$

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩**:** Determine the amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ dissolved by employing the following method.

Medium: Polysorbate 80 (5 µg/g) in water; 500 mL

Apparatus 2: 75 rpm

Time: 60 min

Mobile phase: Acetonitrile and water (6:4)

Standard solution: Prepare a solution of USP Levonorgestrel RS and USP Ethinyl Estradiol RS in *Medium* having known concentrations corresponding approximately to the concentrations that would be obtained by dissolving 1 Tablet in 500 mL of *Medium.* [NOTE—A volume of alcohol not exceeding 2% of the final total volume of solution may be used to aid in dissolving the Reference Standards.]

Sample solution: Withdraw 15-mL portions of liquid from each vessel, and pass through a polyvinylidene filter, discarding the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 247 nm (for levonorgestrel analysis); a spectrofluorometric detector (for ethinyl estradiol analysis), with an excitation wavelength of 285 nm, and an emission wavelength of 310 nm

Column: 4-mm × 15-cm; packing L7

Flow rate: 1 mL/min

Injection size: 100 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and levonorgestrel are about 0.7 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)

C_U = nominal concentration of the corresponding analyte in the *Sample solution* (µg/mL)

Tolerances

Uncoated Tablets: NLT 80% (Q) of the labeled amount of $C_{21}H_{28}O_2$, and 75% (Q) of the labeled amount of $C_{20}H_{24}O_2$ is dissolved.

Coated Tablets: NLT 60% (Q) of the labeled amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

Sample solution: Place 1 Tablet in a 40-mL centrifuge tube, add 10.0 mL of *Mobile phase,* and proceed as directed in the *Assay.*

ADDITIONAL REQUIREMENTS

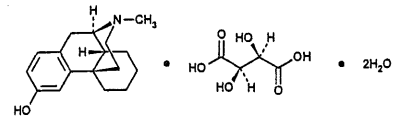
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ethinyl Estradiol RS

USP Levonorgestrel RS

Levorphanol Tartrate



$C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$ 443.49

Morphinan-3-ol, 17-methyl-, [*R*-(*R**,*R**)]-2,3-dihydroxy butanedioate (1:1) (salt), dihydrate.

USP Monographs

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17-Methylmorphinan-3-ol, tartrate (1:1) (salt) dihydrate [5985-38-6].
Anhydrous 407.47
[125-72-4].

» Levorphanol Tartrate contains not less than 99.0 percent and not more than 101.0 percent of $C_{17}H_{23}NO \cdot C_4H_6O_6$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Levorphanol Tartrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—Obtain the test specimen as follows. Dissolve 50 mg in 25 mL of water in a 125-mL separator. Add 2 mL of 6 N ammonium hydroxide, extract with 25 mL of chloroform, and filter the chloroform extract through a layer of 4 g of granular anhydrous sodium sulfate supported on glass wool into a 125-mL conical flask. Evaporate the chloroform extract on a steam bath with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1 mL of acetone, and evaporate to dryness. Dry in vacuum at 90° for 1 hour. Proceed as directed with the dried levorphanol so obtained and a similar preparation of USP Levorphanol Tartrate RS.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 130 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 279 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.
Specific rotation ⟨781S⟩: between −14.7° and −16.3°.
Test solution: 30 mg per mL, in water. Heat on a water bath or sonicate to dissolve 750 mg in 20 mL of water in a 25-mL volumetric flask, dilute with water to volume, and mix.
Water Determination, *Method I* ⟨921⟩ : between 7.0% and 9.0%.
Residue on ignition ⟨281⟩: not more than 0.1%.

Ordinary impurities ⟨466⟩—
Test solution: water.
Standard solution: water.
Eluant: a mixture of hexanes, dehydrated alcohol, and ammonium hydroxide (80:25:1).
Visualization: 17; then view immediately under short-wavelength UV light.
Assay—Dissolve about 900 mg of sample, accurately weighed, in 85 mL of glacial acetic acid, warming slightly if necessary. Titrate with 0.1 N perchloric acid VS and determine the endpoint potentiometrically. Perform a blank determination and make any nececssary corrections. Each mL of 0.1 N perchloric acid consumed by the sample is equivalent to 40.75 mg of $C_{17}H_{23}NO \cdot C_4H_6O_6$.

Levorphanol Tartrate Injection

» Levorphanol Tartrate Injection is a sterile solution of Levorphanol Tartrate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{17}H_{23}NO.C_4H_6O_6 \cdot 2H_2O$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

Identification—
A: To 1 mL of Injection add 1 drop of 3 N hydrochloric acid and 2 drops of ferric chloride TS. Heat to boiling, and add 1 mL of potassium ferricyanide solution (1 in 200): a blue-green color develops.
B: The angular rotation of the Injection is levorotatory (see *Optical Rotation* ⟨781⟩).
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 125.0 USP Endotoxin Units per mg of levorphanol tartrate.
pH ⟨791⟩: between 4.1 and 4.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 40 mg of levorphanol tartrate, to a 125-mL separator. Add 5 g of sodium chloride and sufficient sodium bicarbonate to render the solution alkaline to litmus, add an additional 100 mg of sodium bicarbonate, and extract the levorphanol with five 20-mL portions of a mixture of 3 volumes of ether and 1 volume of chloroform. Pass the combined extracts through a layer of about 10 g of granular anhydrous sodium sulfate into a 500-mL conical flask, and evaporate to a volume of about 30 mL. Add about 50 mL of chloroform and 1 drop of methanolic methyl red TS, and titrate with 0.01 N perchloric acid in dioxane VS to a red endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.01 N perchloric acid is equivalent to 4.435 mg of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$.

Levorphanol Tartrate Tablets

» Levorphanol Tartrate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of levorphanol tartrate ($C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Levorphanol Tartrate RS

Identification—
A: Powder finely a number of Tablets. To a portion of the powder, equivalent to about 1 mg of levorphanol tartrate, add 1 mL of water, 1 drop of 3 N hydrochloric acid, and 2 drops of ferric chloride TS, and heat to boiling. To the hot solution add 1 mL of potassium ferricyanide solution (1 in 200): a bluish color develops.
B: Powder a number of Tablets, equivalent to about 60 mg of levorphanol tartrate, and transfer the mixture to a small separator. Add 10 mL of water, dissolve as much of the powder as possible, add about 400 mg of sodium bicarbonate, and extract with a 50-mL portion of chloroform. Evaporate the

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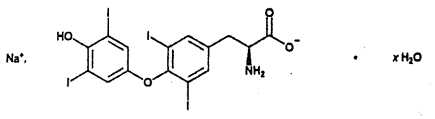
filtered chloroform extract on a steam bath to a small volume, dilute with chloroform to 10 mL, and determine the angular rotation: the solution is levorotatory (see *Optical Rotation* ⟨781⟩).

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 279 nm on filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Levorphanol Tartrate RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Transfer 1 Tablet to a glass-stoppered flask, add 25.0 mL of 0.1 N hydrochloric acid, and allow the Tablet to disintegrate. Shake well, and filter through a small filter paper, discarding the first portion of the filtrate. Dilute a portion of the filtrate quantitatively and stepwise, if necessary, to provide a solution containing about 80 µg of levorphanol tartrate per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Levorphanol Tartrate RS in the same medium having a known concentration of about 80 µg of anhydrous levorphanol tartrate per mL, in 1-cm cells at the wavelength of maximum absorbance at about 279 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$ in the Tablet taken by the formula:

$$(443.49/407.47)(TC/D)(A_U/A_S)$$

in which 443.49 and 407.47 are the molecular weights of the hydrated and anhydrous forms of levorphanol tartrate, respectively; T is the labeled quantity, in mg, of levorphanol tartrate in the Tablet; C is the concentration, in µg per mL, of USP Levorphanol Tartrate RS, on the anhydrous basis, in the Standard solution; D is the concentration, in µg per mL, of levorphanol tartrate in the solution from the Tablet, based on the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.
Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg of levorphanol tartrate, transfer to a 125-mL separator, add 20 mL of water and sufficient sodium bicarbonate to render the suspension alkaline to litmus, and proceed as directed in the *Assay* under *Levorphanol Tartrate Injection*, beginning with "add an additional 100 mg of sodium bicarbonate."

Levothyroxine Sodium



$C_{15}H_{10}I_4NNaO_4 \cdot xH_2O$ (anhydrous) 798.85
L-Tyrosine, *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-, monosodium salt, hydrate;
Monosodium L-thyroxine hydrate [25416-65-3].
Anhydrous [55-03-8].

DEFINITION
Levothyroxine Sodium is the sodium salt of L-3,3′,5,5′-tetraiodothyronine. It contains NLT 97.0% and NMT 103.0% of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*
  Sample solution: To 200 mg add 2 mL of 2 N sulfuric acid. Heat on a water bath and then carefully heat over an open flame, increasing the temperature gradually up to about 600°. [NOTE—Alternative procedures for igniting the material could also be used.] Continue the ignition until most of the particles have disappeared. Dissolve the residue in 2 mL of water.
  Acceptance criteria: The *Sample solution* meets the requirements of test *A*.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile and water (4:6) that contains 0.5 mL of phosphoric acid in each 1000 mL
  Solution A: 400 mg of sodium hydroxide in 500 mL of water. Cool and add 500 mL of methanol.
  Levothyroxine stock solution: 0.4 mg/mL of USP Levothyroxine RS in *Solution A*
  Liothyronine stock solution: 0.4 mg/mL of liothyronine from USP Liothyronine RS in *Solution A*. Make a 1:100 dilution of this solution using *Mobile phase*.
  Standard solution: 10 µg/mL of levothyroxine from *Levothyroxine stock solution* and 0.2 µg/mL of liothyronine from *Liothyronine stock solution* in *Mobile phase*
  Sample solution: 10 µg/mL of Levothyroxine Sodium in *Mobile phase*. [NOTE—A small amount of 0.01 M methanolic sodium hydroxide can be used to facilitate the dissolution of the sample.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 225 nm
  Column: 4.6-mm × 25-cm; packing L10
  Flow rate: 1.5 mL/min
  Injection volume: 100 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 5.0 between liothyronine and levothyroxine
  Relative standard deviation: NMT 2.0% for levothyroxine
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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r_U = peak response of levothyroxine from the *Sample solution*
r_S = peak response of levothyroxine from the *Standard solution*
C_S = concentration of USP Levothyroxine RS in the *Standard solution* (µg/mL)
C_U = concentration of Levothyroxine Sodium in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

[NOTE—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*. *Procedure 2* is recommended when related compounds listed in *Table 3* may be present.]

• **ORGANIC IMPURITIES, PROCEDURE 1**

Diluent: Acetonitrile and water (1:1)

Solution A: Dilute 5 mL of phosphoric acid with *Diluent* to 100.0 mL.

Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate in 200 mL of water. Add 200 mL of acetonitrile, 400 mL of methanol, and 1.0 mL of phosphoric acid. Dilute with water to 1 L.

Standard stock solution 1: Transfer 25 mg of USP Levothyroxine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard stock solution 2: Transfer 25 mg of USP Liothyronine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

System suitability solution: Transfer 5.0 mL of *Standard stock solution 1* and 5.0 mL of *Standard stock solution 2* to a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard solution: Pipet 4.0 mL of the *System suitability solution* into a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Sample solution: Transfer 25 mg of Levothyroxine Sodium to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Blank solution: Transfer 7 mL of *Solution A* to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 15 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between levothyroxine and liothyronine, *System suitability solution*
Relative standard deviation: NMT 2.0% for the levothyroxine peak, *Standard solution*

Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank solution*

[NOTE—Record the chromatograms for at least six times the retention time of the levothyroxine peak. Verify that no peaks elute in the *Blank solution* at the expected retention times for levothyroxine and related compounds.]

Calculate the area percentage of each related compound in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of levothyroxine from the *Standard solution*
C_S = concentration of USP Levothyroxine RS in the *Standard solution* (mg/mL)
C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87

[NOTE—The relative response factor for the impurities listed in *Table 1* is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]

Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Liothyronine	0.65–0.70	1.0
β-Hydroxy-T4[a]	0.71–0.76	0.15
Levothyroxine	1.0	—
T4-Hydroxyacetic acid[b]	1.13–1.28	0.15
N-Formyl-T4[c] and T4-acetamide[d]	1.47–1.53	0.15
N-Acetyl-T4[e]	1.50–1.86	0.20
T4-Acetic acid[f]	2.42–2.51	0.30
T4-Aldehyde[g]	3.17–3.45	0.15
T4-Benzoic acid[h]	3.46–3.70	0.15
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

[a] *O*-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-β-hydroxy-L-tyrosine.
[b] 2-Hydroxy-2-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl] acetic acid.
[c] *N*-Formyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
[d] 2-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetamide.
[e] *N*-Acetyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
[f] 2-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid.
[g] 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzaldehyde.
[h] 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: Dissolve 9.7 g of sulfamic acid in 2000 mL of water. Add 1.5 g of sodium hydroxide, mix to dissolve, and adjust with 2 N sodium hydroxide to a pH of 2.0.

Solution B: Acetonitrile

Diluent 1: Methanol and *Solution A* (90:10)

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Diluent 2: Acetonitrile and *Solution A* (30:70); mix with *Diluent 1* (1:1).
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	70	30
10	70	30
40	20	80
50	20	80
53	70	30
75	70	30

Identification solution: Dissolve 5.0 mg of USP Levothyroxine for Peak Identification RS in 4.5 mL of methanol. Add 0.5 mL of *Solution A*. Further dilute a portion of this solution with *Diluent 2* to obtain a solution containing about 0.2 mg/mL.
Standard stock solution: 0.1 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS in *Diluent 1*
Standard solution: 0.002 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS, prepared using the *Standard stock solution* in *Diluent 2*
Sensitivity solution: 0.0002 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS, prepared using the *Standard solution* in *Diluent 2*
Sample solution: Dissolve an amount of Levothyroxine Sodium in *Diluent 1* to obtain a solution with a known concentration of about 1.0 mg/mL. Further dilute a portion of this solution with *Diluent 2* to obtain a solution with a known concentration of about 0.2 mg/mL.
Blank solution: Use *Diluent 2*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.0-mm × 15-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 25 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 5 between levothyroxine and liothyronine, *Standard solution*
Signal-to-noise ratio: NLT 5 for each peak from the *Sensitivity solution*, calculated as follows:

$$\text{Result} = (2H)/h$$

H = measured height of the peak
h = amplitude of the average measured baseline noise

Analysis
Samples: *Blank solution*, *Standard solution*, *Identification solution*, and *Sample solution*
[NOTE—Identify the components on the basis of their relative retention times as listed in *Table 3*.]
Calculate the percentage of liothyronine sodium in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of liothyronine from the *Sample solution*
r_S = peak response of liothyronine from the *Standard solution*
C_S = concentration of USP Liothyronine RS in the *Standard solution* (mg/mL)
C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of liothyronine sodium, 672.96
M_{r2} = molecular weight of liothyronine, 650.98

Calculate the percentage of any other impurity in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of levothyroxine from the *Standard solution*
C_S = concentration of USP Levothyroxine RS in the *Standard solution* (mg/mL)
C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87

[NOTE—The relative response factor for the impurities listed in *Table 3* is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]
Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.
Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Liothyronine	0.65	1.0
Monochlorotriiodothyronine[a]	0.94	0.15
Levothyroxine *N*-methylamide[b]	0.97	0.15
Levothyroxine	1.0	—
Triiodothyroacetic acid, or T3-acetic acid[c]	1.57	0.15
O-(4-Hydroxy-3,5-diiodophenyl)thyroxine, or T6[d]	1.61	0.50
O-Methyl-tetraiodothyroethylamine, or T4-amine *O*-methyl[e]	1.76	0.30
T4-Acetic acid[f]	1.79	0.30
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

[a] (*S*)-2-Amino-3-[3-chloro-4-(4-hydroxy-3,5-diiodophenoxy)-5-iodophenyl]propanoic acid.
[b] (*S*)-2-Amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-*N*-methylpropanamide.
[c] [4-(4-Hydroxy-3-iodophenoxy)-3,5-diiodophenyl]acetic acid.
[d] (*S*)-2-Amino-3-[4-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenoxy]-3,5-diiodophenyl]propanoic acid.
[e] 2-[4-(3,5-Diiodo-4-methoxyphenoxy)-3,5-diiodophenyl]ethanamine.
[f] 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: Equivalent to 30 mg/mL of anhydrous Levothyroxine Sodium in alcohol and 1 N sodium hydroxide (2:1)
Acceptance criteria: −5° to −6°

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• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 11.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store as stated in the labeling instructions.

• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Levothyroxine RS
O-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
$C_{15}H_{11}I_4NO_4$ 776.87

USP Levothyroxine for Peak Identification RS
Levothyroxine sodium spiked with liothyronine, triiodothyroacetic acid, and tetraiodothyroacetic acid.

USP Levothyroxine Sodium RS

USP Liothyronine RS
O-(4-Hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosine.
$C_{15}H_{12}I_3NO_4$ 650.98

Levothyroxine Sodium Oral Powder

» Levothyroxine Sodium Oral Powder contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Levothyroxine RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 2.0% of its weight.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (65:35) that contains 1 mL of phosphoric acid in each 1000 mL. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

0.01 M Methanolic sodium hydroxide—Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, add 500 mL of methanol, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Levothyroxine RS in *0.01 M Methanolic sodium hydroxide,* and dilute quantitatively and stepwise with *0.01 M Methanolic sodium hydroxide* to obtain a solution having a known concentration of about 4 µg per mL.

Assay preparation—Transfer an accurately weighed portion of Oral Powder, equivalent to about 5 mg of levothyroxine sodium, to a 250-mL volumetric flask. Dilute with *0.01 M Methanolic sodium hydroxide* to volume, mix, and allow to stand for 4 hours, with occasional mixing. Pass a portion of this mixture through a filter that does not absorb levothyroxine. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, dilute with *0.01 M Methanolic sodium hydroxide* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Oral Powder taken by the formula:

$$(798.85/776.87)(1.25C)(r_U/r_S)$$

in which 798.85 and 776.87 are the molecular weights of levothyroxine sodium and levothyroxine, respectively; C is the concentration, in µg per mL, of USP Levothyroxine RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Levothyroxine Sodium Tablets

DEFINITION

Levothyroxine Sodium Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to the levothyroxine peak of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

[NOTE—Use *Sample solution 2* for Tablets labeled to meet the requirements of *Dissolution Test 3.* For all other products, use the *Sample solution.*]

Mobile phase: Acetonitrile and water (4:6) containing 0.5 mL of phosphoric acid per liter of mixture

Solution A: Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, and add 500 mL of methanol.

Diluent: Methanol and water (6:4) containing 0.5 mL of phosphoric acid per liter of mixture

Levothyroxine stock solution: 0.4 mg/mL of USP Levothyroxine RS in *Solution A*

Liothyronine stock solution: 0.4 mg/mL of USP Liothyronine RS in *Solution A.* Make a 1:100 dilution of this solution using *Mobile phase.*

Standard solution: 10 µg/mL of levothyroxine from *Levothyroxine stock solution* and 0.2 µg/mL of liothyronine from *Liothyronine stock solution,* in *Mobile phase*

Sample solution: Transfer an equivalent to about 100 µg of levothyroxine sodium, from finely powdered Tablets (NLT 20), to a centrifuge tube, add two glass beads, pipet 10 mL of *Mobile phase* into the tube, and mix on a vortex mixer for 3 min. Centrifuge to obtain a clear supernatant, filtering if necessary.

Sample solution 2 (for Tablets labeled to meet the requirements of *Dissolution Test 3*): Place the appropriate number of Tablets (see *Table 1*) into a suitable container, add 100.0 mL of *Diluent,* and shake by mechanical means for at least 30 min, or until the Tablets are fully disintegrated. Pass through a PTFE filter of 0.45-µm pore size.

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Table 1

Tablet Strength (µg/Tablet of Levothyroxine Sodium)	Number of Tablets
Less than 100	20
At least 100 but less than 200	15
200 or more	10

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between liothyronine and levothyroxine
Relative standard deviation: NMT 2.0% for the levothyroxine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Levothyroxine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of levothyroxine sodium in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
[Note—All containers that are in contact with solutions containing levothyroxine sodium are to be made of glass.]
Test 1
Medium: 0.01 N hydrochloric acid containing 0.2% sodium lauryl sulfate; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Methanol and 0.1% phosphoric acid (6:4)
Standard stock solution: 0.1 mg/mL of USP Levothyroxine RS in methanol
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a solution having a concentration similar to that expected in the *Sample solution*.
Sample solution: Pass a portion of the solution under test through a suitable filter. [Note—Before use, check the filters for absorptive loss of drug.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 800 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 4.0% for levothyroxine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) dissolved.
Tolerances: NLT 70% (*Q*) of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Apparatus 2, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed for *Test 1*.
Time: 15 min
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) is dissolved.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium, Apparatus 2, Time, Standard solution, and **Sample solution:** Proceed as directed for *Test 1*. [Note—Filter the *Standard solution* in a manner identical to that used for the *Sample solution*.]
Mobile phase: Acetonitrile and water (35:65) that contains 0.5 mL of phosphoric acid per liter of mixture
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 4.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) is dissolved.
Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
[Note—Do not use paddle stirrers with synthetic coating.]
Medium: 0.01 N hydrochloric acid; 500 mL for Tablets labeled to contain between 25 and 175 µg of levothyroxine sodium; and 900 mL for Tablets labeled to contain 200 or 300 µg of levothyroxine sodium
Apparatus 2: 75 rpm
Time: 45 min
Mobile phase: Acetonitrile, water, and phosphoric acid (500:700:2)
Standard stock solution: Transfer about 100 mg of USP Levothyroxine RS to a 100-mL volumetric flask. Add 80 mL of alcohol and 1 mL of 1 N hydrochloric acid, sonicate for 2 min, dilute with alcohol to volume, and mix.
Standard solution: Dilute the *Standard stock solution* with a mixture of alcohol and water (1:1) to obtain a

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concentration of 0.01 mg/mL of levothyroxine. Dilute the resulting solution with *Medium* to obtain a final concentration similar to that expected in the *Sample solution*.

Sample solution: Sample per *Dissolution* ⟨711⟩. Centrifuge the solution under analysis.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.0-mm × 12.5-cm; packing L7
Flow rate: 1.5 mL/min
Injection volume: 500 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 4.0% of levothyroxine

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) dissolved.

Tolerances: NLT 80% (*Q*) of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **LIMIT OF LIOTHYRONINE SODIUM**

[NOTE—Use *Sample solution 2* for Tablets labeled to meet the requirements of *Dissolution Test 3*. For all other products, use the *Sample solution*.]

Mobile phase, Liothyronine stock solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Liothyronine standard solution: 0.2 µg/mL of liothyronine from *Liothyronine stock solution*, in *Mobile phase*

Analysis
Samples: *Sample solution* and *Liothyronine standard solution*
Calculate the percentage of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

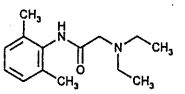
r_U = peak response of liothyronine from the *Sample solution*
r_S = peak response of liothyronine from the *Liothyronine standard solution*
C_S = concentration of USP Liothyronine RS in the *Liothyronine standard solution* (µg/mL)
C_U = nominal concentration of levothyroxine sodium in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of liothyronine sodium, 672.96
M_{r2} = molecular weight of liothyronine, 650.98

Acceptance criteria: NMT 2.0% of liothyronine sodium

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Levothyroxine RS
USP Liothyronine RS

Lidocaine



$C_{14}H_{22}N_2O$ 234.34
Acetamide, 2-(diethylamino)-*N*-(2,6-dimethylphenyl)-; 2-(Diethylamino)-2′,6′-acetoxylidide [137-58-6].

DEFINITION

Lidocaine contains NLT 97.5% and NMT 102.5% of lidocaine ($C_{14}H_{22}N_2O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020) [NOTE—Methods described under ⟨197K⟩ or ⟨197A⟩ may be used.]
Sample: Previously dried under vacuum over silica gel for 24 h
Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Water and glacial acetic acid (930:50). Adjust with 1 N sodium hydroxide to a pH of 3.4.
Mobile phase: Acetonitrile and *Solution A* (20:80)
Standard solution: 1.7 mg/mL of USP Lidocaine RS in *Mobile phase*, prepared as follows. Dissolve 85 mg of USP Lidocaine RS with 0.5 mL of ▲1 N hydrochloric acid▲ (ERR 1-Dec-2018), warming if necessary, in a 50-mL flask. Dilute with *Mobile phase* to volume.
System suitability stock solution: 220 µg/mL of methylparaben in *Mobile phase*
System suitability solution: Mix 2 mL of *System suitability stock solution* and 20 mL of *Standard solution*.
Sample solution: 1.7 mg/mL of Lidocaine in *Mobile phase*, prepared as follows. Dissolve 85 mg of Lidocaine with 0.5 mL of ▲1 N hydrochloric acid▲ (ERR 1-Dec-2018), warming if necessary, in a 50-mL flask. Dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 4-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between lidocaine and methylparaben, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Lidocaine taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lidocaine from the *Sample solution*
r_S = peak response of lidocaine from the *Standard solution*
C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
C_U = concentration of Lidocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.5%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*

Sample: 1.0 g
Analysis: Dissolve the *Sample* in a mixture of 3 mL of 2 N nitric acid and 12 mL of water, and add 1 mL of silver nitrate TS.
Acceptance criteria: The turbidity does not exceed that produced by 50 µL of 0.020 N hydrochloric acid (NMT 0.0035%).

- **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*

Sample: 100 mg
Analysis: Dissolve the *Sample* in a mixture of 1 mL of 2 N nitric acid and 10 mL of water. Filter if necessary, and add 1 mL of barium chloride TS.
Acceptance criteria: The turbidity does not exceed that produced by 0.10 mL of 0.020 N sulfuric acid (NMT 0.1%).

- **ORGANIC IMPURITIES**

Solution A: 4.85 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide solution to a pH of 8.0.
Mobile phase: Acetonitrile and *Solution A* (30:70)
Standard solution: 0.5 µg/mL of USP Ropivacaine Related Compound A RS and 5 µg/mL each of USP Lidocaine Related Compound H RS and USP Lidocaine RS in *Mobile phase*
Sample solution: 5 mg/mL of Lidocaine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between the lidocaine related compound H and 2,6-dimethylaniline (ropivacaine related compound A free base) peaks
Relative standard deviation: NMT 10.0% for 2,6-dimethylaniline
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lidocaine related compound H in the portion of Lidocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lidocaine related compound H from the *Sample solution*
r_S = peak response of lidocaine related compound H from the *Standard solution*
C_S = concentration of USP Lidocaine Related Compound H RS in the *Standard solution* (µg/mL)
C_U = concentration of Lidocaine in the *Sample solution* (µg/mL)

Calculate the percentage of 2,6-dimethylaniline (ropivacaine related compound A free base) in the portion of Lidocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of 2,6-dimethylaniline from the *Sample solution*
r_S = peak response of 2,6-dimethylaniline from the *Standard solution*.
C_S = concentration of USP Ropivacaine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Lidocaine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of 2,6-dimethylaniline, 121.18
M_{r2} = molecular weight of ropivacaine related compound A, 157.64

Calculate the percentage of any unspecified impurity in the portion of Lidocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of lidocaine from the *Standard solution*
C_S = concentration of USP Lidocaine RS in the *Standard solution* (µg/mL)
C_U = concentration of Lidocaine in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lidocaine related compound H	0.38	0.10
2,6-Dimethylaniline	0.42	0.01
Lidocaine	1.0	—
Any unspecified impurity	—	0.10
Total impurities	—	0.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Lidocaine RS
USP Lidocaine Related Compound H RS
2-Chloro-*N*-(2,6-dimethylphenyl)acetamide.
$C_{10}H_{12}ClNO$ 197.66
USP Ropivacaine Related Compound A RS
2,6-Dimethylaniline hydrochloride.
$C_8H_{11}N \cdot HCl$ 157.64

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Lidocaine Topical Aerosol

DEFINITION

Lidocaine Topical Aerosol is a solution of Lidocaine in a suitable flavored vehicle with suitable propellants in a pressurized container equipped with a metering valve. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$), and it delivers NLT 85.0% and NMT 115.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$) per actuation.

IDENTIFICATION

- **A.**

Sample solution: To 5 mL of Aerosol spray, collected in a separator, add 10 mL of water and 3 mL of dilute hydrochloric acid (1 in 2), wash with two 15-mL portions of chloroform, and discard the chloroform washings. Render the solution in the separator alkaline with 5–6 mL of ammonium hydroxide, and extract with three 20-mL portions of chloroform, filtering the chloroform extracts through a pledget of cotton previously moistened with chloroform. Evaporate the combined chloroform extracts with the aid of gentle heat to dryness, and dry the residue under vacuum over silica gel for 24 h.

Acceptance criteria: A potassium bromide dispersion of the lidocaine exhibits maxima only at the same wavelengths as that of a similar preparation of USP Lidocaine RS.

- **B.**

Analysis: To 2 mL of Aerosol spray, collected in a test tube, add 10–15 drops of cobaltous chloride TS, and shake for 2 min.

Acceptance criteria: A bright green color develops, and a fine precipitate is formed (lidocaine).

- **C.**

Analysis: To 2 mL of Aerosol spray, collected in a test tube, add 5 mL of water, 1 mL of 2 N nitric acid, and 3 mL of mercuric nitrate TS.

Acceptance criteria: A light yellow color develops (lidocaine).

ASSAY

- **PROCEDURE**

Sample solution: Weigh 1 Aerosol container and actuator. Transfer a counted number of NLT 10 doses to a 125-mL conical flask by carefully discharging the doses in such a manner as to avoid loss of material, and take precautions to protect the sample from absorption of atmospheric moisture. Weigh the container and actuator to obtain the sample weight. To the specimen, add 20 mL of chloroform, mix, and add 10 mL of dioxane and 2 drops of crystal violet TS.

Analysis: Titrate with 0.1 N perchloric acid in dioxane VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.43 mg of lidocaine ($C_{14}H_{22}N_2O$).

Acceptance criteria: It contains 90.0%–110.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$), and it delivers 85.0%–115.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$) per actuation.

PERFORMANCE TESTS

- **INHALATION AND NASAL DRUG PRODUCTS: AEROSOLS, SPRAYS, AND POWDERS—PERFORMANCE QUALITY TESTS** ⟨601⟩ and **TOPICAL AEROSOLS** ⟨603⟩

For Delivered-Dose Uniformity and Number of Discharges per Container

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in nonreactive aerosol containers equipped with metered-dose valves.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Lidocaine RS

Lidocaine Ointment

DEFINITION

Lidocaine Ointment is Lidocaine in a suitable hydrophilic ointment base. It contains NLT 95.0% and NMT 105.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Stir a quantity of Ointment, equivalent to 300 mg of lidocaine, with 20 mL of water, transfer to a separator, and extract with two 30-mL portions of solvent hexane. Wash the combined hexane extracts with 10 mL of water, evaporate with the aid of a current of warm air, and dry the residue in vacuum over silica gel for 24 h.

Acceptance criteria: The crystalline precipitate so obtained meets the requirements.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% phosphoric acid, prepared by adding 1.0 mL of 85% phosphoric acid to 1 L of water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	10	90
10.1	90	10
15	90	10

Diluent: Acetonitrile and *Solution A* (1:1)

System suitability solution: 0.1 mg/mL of USP Lidocaine RS and 0.04 mg/mL of USP Ropivacaine Related Compound A RS in *Diluent*

[NOTE—USP Ropivacaine Related Compound A RS is 2,6-dimethylaniline hydrochloride.]

Standard solution: 0.1 mg/mL of USP Lidocaine RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of lidocaine in *Diluent* from a portion of Ointment. Sonicate the solution for about 5 min.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

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Flow rate: 0.8 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for 2,6-dimethylaniline and lidocaine are about 0.93 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 1.5 for the lidocaine peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Resolution: NLT 1.8 between lidocaine and 2,6-dimethylaniline, *System suitability solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lidocaine RS
USP Ropivacaine Related Compound A RS
2,6-Dimethylaniline hydrochloride.
$C_8H_{12}ClN$ 157.64

Lidocaine Oral Topical Solution

» Lidocaine Oral Topical Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$). It contains a suitable flavor.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Lidocaine RS
Identification—Transfer a quantity of Oral Topical Solution, equivalent to about 250 mg of lidocaine, to a separator with 20 mL of water, and extract with 20 mL of chloroform. Wash the chloroform extract with 20 mL of water, and evaporate the chloroform extract with the aid of a current of warm air. Dissolve the residue in hexane, evaporate with the aid of a current of warm air, and dry the residue in vacuum over silica gel for 24 hours: the crystalline precipitate so obtained responds to *Identification* test *A* under *Lidocaine*.
Assay—Transfer an accurately measured volume of Oral Topical Solution, equivalent to about 150 mg of lidocaine, to a 125-mL conical flask, and protect from atmospheric moisture with a stopper fitted with a tube containing silica gel. Add 20 mL of glacial acetic acid and 2 drops of crystal violet TS. Titrate immediately with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.43 mg of $C_{14}H_{22}N_2O$.

Lidocaine Hydrochloride

$C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$ 288.81
Acetamide, 2-(diethylamino)-*N*-(2,6-dimethylphenyl)-, monohydrochloride, monohydrate;
2-(Diethylamino)-2′,6′-acetoxylidide monohydrochloride monohydrate [6108-05-0].
Anhydrous 270.80
[73-78-9].

DEFINITION
Lidocaine Hydrochloride contains NLT 97.5% and NMT 102.5% of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY
• **PROCEDURE**
Solution A: Water and glacial acetic acid (930:50). Adjust with 1 N sodium hydroxide to a pH of 3.40.
Mobile phase: Acetonitrile and *Solution A* (1:4)
Standard solution: 2.0 mg/mL of USP Lidocaine Hydrochloride RS in *Mobile phase*
System suitability stock solution: 220 µg/mL of methylparaben in *Mobile phase*
System suitability solution: Mix 2 mL of *System suitability stock solution* and 20 mL of *Standard solution*.
Sample solution: 2 mg/mL of Lidocaine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between lidocaine and methylparaben, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in the portion of Lidocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lidocaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Lidocaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.5% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Sample: 100 mg
  Analysis: Dissolve *Sample* in 10 mL of water, and add 1 mL of 3 N hydrochloric acid. Mix, and add 1 mL of barium chloride TS.
  Acceptance criteria: The turbidity does not exceed that produced by 0.10 mL of 0.020 N sulfuric acid (NMT 0.1%).
- **ORGANIC IMPURITIES**
  Buffer: 4.85 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide solution to a pH of 8.0.
  Mobile phase: Acetonitrile and *Buffer* (30:70)
  Standard solution: 0.5 µg/mL of USP Ropivacaine Related Compound A RS and 5 µg/mL each of USP Lidocaine Related Compound H RS and USP Lidocaine Hydrochloride RS in *Mobile phase*
  Sample solution: 5 mg/mL of Lidocaine Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 3.9-mm × 15-cm; 5-µm packing L1
  Column temperature: 30°
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  Run time: NLT 3.5 times the retention time for lidocaine
  System suitability
  Sample: *Standard solution*
  [NOTE—See *Table 1* for relative retention times.]
  Suitability requirements
  Resolution: NLT 1.5 between lidocaine related compound H and ropivacaine related compound A; NLT 2.0 between ropivacaine related compound A and lidocaine
  Relative standard deviation: NMT 10.0% for ropivacaine related compound A
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of lidocaine related compound H or ropivacaine related compound A in the portion of Lidocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for lidocaine related compound H or ropivacaine related compound A from the *Sample solution*
r_S = peak response of lidocaine related compound H or ropivacaine related compound A from the *Standard solution*
C_S = concentration of USP Lidocaine Related Compound H RS or USP Ropivacaine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Lidocaine Hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Lidocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each unspecified impurity from the *Sample solution*
r_S = peak response of lidocaine from the *Standard solution*
C_S = concentration of USP Lidocaine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Lidocaine Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lidocaine related compound H	0.38	0.10
Ropivacaine related compound A	0.42	0.01
Lidocaine	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 5.0%–7.0%
- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Lidocaine Hydrochloride is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Lidocaine Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 1.1 USP Endotoxin Units/mg of lidocaine hydrochloride.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lidocaine Hydrochloride RS
  USP Lidocaine Related Compound H RS
  2-Chloro-*N*-(2,6-dimethylphenyl)acetamide.
  $C_{10}H_{12}ClNO$ 197.66
  USP Ropivacaine Related Compound A RS
  2,6-Dimethylaniline hydrochloride.
  $C_8H_{11}N \cdot HCl$ 157.64

USP Monographs

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Lidocaine Hydrochloride Injection

» Lidocaine Hydrochloride Injection is a sterile solution of Lidocaine Hydrochloride in Water for Injection, or a sterile solution prepared from Lidocaine with the aid of Hydrochloric Acid in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Injection may be packaged in 50-mL multiple-dose containers.
Labeling—Injections that are not intended for direct injection into tissues are labeled to indicate that they are to be diluted prior to administration.
USP Reference standards ⟨11⟩—
USP Lidocaine RS
Identification—Place in a separator a volume of Injection equivalent to about 300 mg of lidocaine hydrochloride, and extract with four 15-mL portions of chloroform, discarding the chloroform extracts. Add 2 mL of 2 N sodium hydroxide to the aqueous solution remaining in the separator, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a current of warm air to dryness. Dissolve the crystals so obtained in solvent hexane, evaporate with the aid of warm air, and dry the residue in vacuum over silica gel for 24 hours: the residue so obtained responds to *Identification* test *A* under *Lidocaine*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.1 USP Endotoxin Units per mg of lidocaine hydrochloride.
pH ⟨791⟩: between 5.0 and 7.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed with Injection as directed in the *Assay for lidocaine hydrochloride* under *Lidocaine Hydrochloride and Epinephrine Injection*.

Lidocaine Hydrochloride Jelly

DEFINITION
Lidocaine Hydrochloride Jelly is Lidocaine Hydrochloride in a suitable, water-soluble, sterile, viscous base. It contains NLT 95.0% and NMT 105.0% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Standard: Prepare as directed in ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020), using USP Lidocaine RS.
  Sample: Place in a separator containing 10–15 mL of water a quantity of Jelly, equivalent to 300 mg of lidocaine hydrochloride. Mix to assure thorough dilution of the Jelly, and add 4 mL of 6 N ammonium hydroxide. Extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a current of warm air to dryness. Redissolve the crystals in solvent hexane, evaporate with the aid of warm air, and dry the residue in vacuum over silica gel for 24 h.
  Acceptance criteria: The residue so obtained meets the requirements.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 0.1% phosphoric acid, prepared by adding 1.0 mL of 85% phosphoric acid to 1 L of water
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	10	90
10.1	90	10
15	90	10

Diluent: Acetonitrile and *Solution A* (1:1)
System suitability solution: 0.1 mg/mL of USP Lidocaine RS and 0.04 mg/mL of USP Ropivacaine Related Compound A RS in *Diluent*
[NOTE—USP Ropivacaine Related Compound A RS is 2,6-dimethylaniline hydrochloride.]
Standard solution: 0.1 mg/mL of USP Lidocaine RS in *Diluent*
Sample solution: Nominally 0.12 mg/mL of lidocaine hydrochloride in *Diluent* from a portion of Jelly. Sonicate the solution for about 10 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of 2,6-dimethylaniline and lidocaine are about 0.93 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 1.5 for the lidocaine peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Resolution: NLT 1.8 between lidocaine and 2,6-dimethylaniline, *System suitability solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in the portion of Jelly taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)

USP Monographs

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C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lidocaine hydrochloride, 270.80
M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
- **PH** ⟨791⟩: 6.0–7.0
- **STERILITY TESTS** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lidocaine RS
  USP Ropivacaine Related Compound A RS
  2,6-Dimethylaniline hydrochloride.
  $C_8H_{12}ClN$ 157.64

Lidocaine Hydrochloride Oral Topical Solution

DEFINITION
Lidocaine Hydrochloride Oral Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$). It contains a suitable flavor and/or sweetening agent.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Place in a separator a volume of Oral Topical Solution, nominally equivalent to 300 mg of lidocaine hydrochloride, and extract with four 15-mL portions of chloroform, discarding the chloroform extracts. Add 2 mL of 2 N sodium hydroxide to the aqueous solution remaining in the separator, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate to dryness. Dissolve the crystals in solvent hexane, evaporate the solvent, and dry the residue under vacuum over silica gel for 24 h. [NOTE—A rotary evaporator may be used.]
  Acceptance criteria: Residue obtained from the *Sample* meets the requirements.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 4.85 g/L of monobasic potassium phosphate. Adjust with 10 N sodium hydroxide solution to a pH of 8.0.
  Mobile phase: Acetonitrile and *Solution A* (30:70)
  Standard solution: 0.85 mg/mL of USP Lidocaine RS (equivalent to 1 mg/mL of lidocaine hydrochloride) in *Mobile phase*
  Sample solution: Nominally 1 mg/mL of lidocaine hydrochloride from Oral Topical Solution in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 4.6-mm × 15-cm; 3.5-µm packing L1
  Column temperature: 45°
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in the portion of Oral Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of lidocaine from the *Sample solution*
r_S = peak response of lidocaine from the *Standard solution*
C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lidocaine hydrochloride, 270.80
M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: 95.0%–105.0%

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Standard solution: 0.0043 mg/mL of USP Lidocaine RS, 0.005 mg/mL of USP Lidocaine Related Compound H RS, and 0.00065 mg/mL of USP Ropivacaine Related Compound A RS (equivalent to 0.0005 mg/mL of 2,6-dimethylaniline) in *Mobile phase*
  Sample solution: Nominally 5 mg/mL of lidocaine hydrochloride from Oral Topical Solution in *Mobile phase*
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 1.5 between the lidocaine related compound H and 2,6-dimethylaniline (ropivacaine related compound A free base) peaks
  Relative standard deviation: NMT 2.0% for lidocaine, lidocaine related compound H, and 2,6-dimethylaniline
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of lidocaine related compound H in the portion of Oral Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lidocaine related compound H from the *Sample solution*
r_S = peak response of lidocaine related compound H from the *Standard solution*
C_S = concentration of USP Lidocaine Related Compound H RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)

USP Monographs

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Calculate the percentage of 2,6-dimethylaniline in the portion of Oral Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of 2,6-dimethylaniline from the *Sample solution*
r_S = peak response of 2,6-dimethylaniline from the *Standard solution*
C_S = concentration of USP Ropivacaine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of 2,6-dimethylaniline, 121.18
M_{r2} = molecular weight of ropivacaine related compound A, 157.64

Calculate the percentage of any unspecified degradation product in the portion of Oral Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of lidocaine from the *Standard solution*
C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lidocaine hydrochloride, 270.80
M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lidocaine related compound H	0.33	0.5
2,6-Dimethylaniline	0.37	0.5
Lidocaine	1.0	—
Any unspecified degradation product	—	0.5
Total degradation products	—	2.0

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.0–7.0

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Lidocaine RS
  USP Lidocaine Related Compound H RS
  2-Chloro-*N*-(2,6-dimethylphenyl)acetamide.
  $C_{10}H_{12}ClNO$ 197.66
  USP Ropivacaine Related Compound A RS
  2,6-Dimethylaniline hydrochloride.
  $C_8H_{11}N \cdot HCl$ 157.64

Lidocaine Hydrochloride Topical Solution

DEFINITION
Lidocaine Hydrochloride Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Place in a separator a volume of Topical Solution, nominally equivalent to 200 mg of lidocaine hydrochloride, and extract with four 15-mL portions of chloroform, discarding the chloroform extracts. Add 2 mL of 2 N sodium hydroxide to the aqueous solution remaining in the separator, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate to dryness. Dissolve the crystals in solvent hexane, evaporate the solvent, and dry the residue under vacuum over silica gel for 24 h. [Note—A rotary evaporator may be used.]
  Acceptance criteria: The residue obtained from the *Sample* meets the requirements.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
  Solution A: *water* and glacial acetic acid (930:50). Adjust with 1 N sodium hydroxide to a pH of 3.4.
  Mobile phase: *Acetonitrile* and *Solution A* (20:80)
  Standard solution: 1.7 mg/mL of USP Lidocaine RS (equivalent to 2 mg/mL of lidocaine hydrochloride) in *Mobile phase*, prepared as follows. Transfer a weighed quantity of USP Lidocaine RS to a suitable volumetric flask, and add 1 N hydrochloric acid to fill 1% of the final volume. Warm if necessary, and dilute with *Mobile phase* to volume.
  System suitability stock solution: 220 µg/mL of USP Methylparaben RS in *Mobile phase*
  System suitability solution: Mix 2 mL of *System suitability stock solution* with 20 mL of the *Standard solution*.
  Sample solution: Nominally 2 mg/mL of lidocaine hydrochloride from Topical Solution in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; 10-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 3.0 between lidocaine and methylparaben, *System suitability solution*
  Relative standard deviation: NMT 1.5%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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r_U = peak response of lidocaine from the *Sample solution*
r_S = peak response of lidocaine from the *Standard solution*
C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lidocaine hydrochloride, 270.80
M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Mobile phase, and **Sample solution:** Prepare as directed in the *Assay*.

Standard solution: 0.0017 mg/mL of USP Lidocaine RS (equivalent to 0.002 mg/mL of lidocaine hydrochloride), 0.0026 mg/mL of USP Ropivacaine Related Compound A RS (equivalent to 0.002 mg/mL of 2,6-dimethylaniline), and 0.002 mg/mL of USP Lidocaine Related Compound H RS in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between the lidocaine related compound H and 2,6-dimethylaniline (ropivacaine related compound A free base) peaks

Relative standard deviation: NMT 2.0% for lidocaine, lidocaine related compound H, and 2,6-dimethylaniline

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of lidocaine related compound H in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lidocaine related compound H from the *Sample solution*
r_S = peak response of lidocaine related compound H from the *Standard solution*
C_S = concentration of USP Lidocaine Related Compound H RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of 2,6-dimethylaniline in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of 2,6-dimethylaniline from the *Sample solution*
r_S = peak response of 2,6-dimethylaniline from the *Standard solution*
C_S = concentration of USP Ropivacaine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of 2,6-dimethylaniline, 121.18
M_{r2} = molecular weight of ropivacaine related compound A, 157.64

Calculate the percentage of any unspecified degradation product in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of lidocaine from the *Standard solution*
C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lidocaine hydrochloride, 270.80
M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lidocaine	1.0	—
2,6-Dimethylaniline	3.2	0.5
Lidocaine related compound H	3.8	0.5
Any unspecified degradation product	—	0.5
Total degradation products	—	2.0

SPECIFIC TESTS

• **pH** ⟨791⟩: 5.0–7.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Lidocaine RS
USP Lidocaine Related Compound H RS
2-Chloro-*N*-(2,6-dimethylphenyl)acetamide.
$C_{10}H_{12}ClNO$ 197.66
USP Methylparaben RS
USP Ropivacaine Related Compound A RS
2,6-Dimethylaniline hydrochloride.
$C_8H_{11}N \cdot HCl$ 157.64

Lidocaine Hydrochloride and Dextrose Injection

» Lidocaine Hydrochloride and Dextrose Injection is a sterile solution of Lidocaine Hydrochloride and Dextrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of lidocaine

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hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) and dextrose ($C_6H_{12}O_6 \cdot H_2O$).

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
USP Reference standards ⟨11⟩—
USP Lidocaine RS

Identification—
A: Place in a separator a volume of Injection equivalent to about 300 mg of lidocaine hydrochloride, add 2 mL of 2 N sodium hydroxide, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a current of warm air to dryness. Dissolve the crystals so obtained in solvent hexane, evaporate with the aid of warm air, and dry the residue in vacuum over silica gel for 24 hours: the residue so obtained responds to *Identification* test *A* under *Lidocaine*.
B: Add a few drops of a solution (1 in 20) to 5 mL of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide is formed.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 1.1 USP Endotoxin Units per mg of lidocaine hydrochloride.
pH ⟨791⟩**:** between 3.0 and 7.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay for lidocaine hydrochloride—Proceed with Injection as directed in the *Assay for lidocaine hydrochloride* under *Lidocaine and Epinephrine Injection*.
Assay for dextrose—Determine the angular rotation of Injection in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩). Calculate the percentage (g per 100 mL) of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively; *A* is 100 mm divided by the length of the polarimeter tube, in mm; and *R* is the observed rotation, in degrees.

Lidocaine Hydrochloride and Epinephrine Injection

» Lidocaine Hydrochloride and Epinephrine Injection is a sterile solution prepared from Lidocaine Hydrochloride and Epinephrine with the aid of Hydrochloric Acid in Water for Injection, or a sterile solution prepared from Lidocaine and Epinephrine with the aid of Hydrochloric Acid in Water for Injection, or a sterile solution of Lidocaine Hydrochloride and Epinephrine Bitartrate in Water for Injection. The content of epinephrine does not exceed 0.002 percent (1 in 50,000). Lidocaine Hydrochloride and Epinephrine Injection contains the equivalent of not less than 95.0 percent and not more than 105.0 percent of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) and the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

Packaging and storage—Preserve in single-dose or multiple-dose light-resistant containers, preferably of Type I glass.
Labeling—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
USP Reference standards ⟨11⟩—
USP Epinephrine Bitartrate RS
USP Lidocaine RS
Color and clarity—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.7 USP Endotoxin Unit per mg of lidocaine hydrochloride.
pH ⟨791⟩**:** between 3.3 and 5.5.
Other requirements—It responds to the *Identification* test under *Lidocaine Hydrochloride Injection*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for lidocaine hydrochloride—
Mobile phase—Mix 50 mL of glacial acetic acid and 930 mL of water, and adjust with 1 N sodium hydroxide to a pH of 3.40. Mix about 4 volumes of this solution with 1 volume of acetonitrile, so that the retention time of lidocaine is about 4 to 6 minutes. Pass through a membrane filter having a 1-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve about 85 mg of USP Lidocaine RS, accurately weighed, with warming if necessary, in 0.5 mL of 1 N hydrochloric acid in a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 1.7 mg of lidocaine per mL.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of lidocaine hydrochloride, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Resolution preparation—Prepare a solution of methylparaben in *Mobile phase* containing about 220 µg per mL. Mix 2 mL of this solution and 20 mL of the *Standard preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph about 20 µL of the *Resolution preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between lidocaine and methylparaben is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in each mL of the Injection taken by the formula:

$$(270.80/234.34)(50)(C/V)(r_U/r_S)$$

in which 270.80 and 234.34 are the molecular weights of lidocaine hydrochloride and lidocaine, respectively; *C* is the concentration, in mg per mL, of USP Lidocaine RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and r_U and r_S are the lidocaine peak responses obtained from

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the *Assay preparation* and the *Standard preparation,* respectively.

Assay for epinephrine—

Mobile phase—Mix 50 mL of glacial acetic acid and 930 mL of water, and adjust with 1 N sodium hydroxide to a pH of 3.40. Dissolve 1.1 g of sodium 1-heptanesulfonate in this solution, add 1.0 mL of 0.1 M edetate disodium, and mix. Mix about 9 volumes of this solution with 1 volume of methanol, so that the retention time of epinephrine is about 4 to 6 minutes. Pass through a membrane filter having a 1-µm or finer porosity, and degas.

Standard preparation—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in *Mobile phase* to obtain a solution having a known concentration of about 9 µg of epinephrine bitartrate per mL. Pipet 10 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 1.8 µg of epinephrine bitartrate per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 µg of epinephrine, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is fitted with a 3.9-mm × 30-cm stainless steel column that contains packing L1 and is equipped with an electrochemical detector held at a potential of +650 mV, a controller capable of regulating the background current, and a suitable recorder. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure:* the relative standard deviation of the peak responses of successive injections of the *Standard preparation* is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters so that satisfactory chromatography and peak responses are obtained. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of epinephrine ($C_9H_{13}NO_3$) in each mL of the Injection taken by the formula:

$$(183.20/333.29)(50)(C/V)(r_U/r_S)$$

in which 183.20 and 333.29 are the molecular weights of epinephrine and epinephrine bitartrate, respectively; C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Lidocaine and Prilocaine Cream

» Lidocaine and Prilocaine Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of lidocaine ($C_{14}H_{22}N_2O$) and prilocaine ($C_{13}H_{20}N_2O$).

Packaging and storage—Preserve in collapsible tubes or in tight containers. Do not store above 30°. Do not freeze.

USP Reference standards ⟨11⟩—
USP Lidocaine RS
USP Prilocaine Hydrochloride RS
USP Prilocaine Related Compound B RS
(*RS*)-*N*-(4-Methylphenyl)-2-(propylamino)propanamide.
$C_{13}H_{20}N_2O$ 220.31

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The total aerobic microbial count does not exceed 100 cfu per g, and the total combined molds and yeasts count does not exceed 50 cfu per g.

Minimum fill ⟨755⟩**:** meets the requirements.

pH ⟨791⟩**:** between 8.7 and 9.7, determined in a solution (1 in 10) or in the undiluted Cream.

Related compounds—

Solution A, Solution B, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Dissolve accurately weighed quantities of USP Lidocaine RS and USP Prilocaine Hydrochloride RS in *Solution A,* and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a solution having a known concentration of about 0.002 mg per mL of each compound. Immediately store this solution at or below 10°.

Test solution—Use the *Assay preparation,* prepared as directed in the *Assay.*

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are listed in *Table 1;* and the resolution, *R,* between prilocaine and prilocaine related compound B is not less than 1.4. Chromatograph the *Standard solution* a minimum of six times, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each related compound in the portion of the Cream taken by the formula:

$$100C(r_U/r_S)(V/W)(100/L)(1/F)(220.31/256.77)$$

in which C is the individual concentration, in mg per mL, of either USP Lidocaine RS or USP Prilocaine Hydrochloride RS in the *Standard solution;* r_U is the individual peak response of the impurities obtained from the *Test solution;* r_S is the individual peak response for either lidocaine or prilocaine obtained from the *Standard solution; V* is the volume, in mL, of the *Test solution; W* is the weight, in mg, of the Cream taken to prepare the *Test solution; L* is the individual label claim, in percent, for either lidocaine or prilocaine; *F* is the relative response factor for each related compound as listed in *Table 1;* and 220.31 and 256.77 are the molecular weights of prilocaine and prilocaine hydrochloride, respectively (these are used only for calculation involving prilocaine related compounds). The percentages of lidocaine related compounds and prilocaine related compounds are calculated using the concentration and peak response from USP Lidocaine RS and USP Prilocaine Hydrochloride RS, respectively. The designation of whether an impurity is a lidocaine related compound or prilocaine related compound is specified in *Table 1.* The percentage of any individual unknown related compound is determined using the concentration and peak response from USP Prilocaine Hydrochloride RS in the *Standard solution.*

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Table 1 (Lidocaine and Prilocaine Cream)

Related Compound	Relative Retention Time[a]	Relative Response Factor (*F*)	Limit
o-Toluidine	0.38	2.3 (P)[b]	not more than 2.0%
n-Chloroacetyl-2,6-xylidine	0.54	1.0 (L)[c]	not more than 0.1%
2,6-Dimethylaniline	0.67	3.3 (L)[c]	not more than 0.1%
Prilocaine	1.00	—	—
2-Diethylaminoaceto-2,4-xylidine	1.33	0.8 (L)[c]	not more than 0.1%
Lidocaine	2.14	—	—
n-Dichloroacetyl-2,6-xylidine	2.98	2.2 (L)[c]	not more than 0.1%
Any other individual related compounds	—	1.0 (P)[b]	not more than 0.2%
Total related compounds, excluding o-toluidine	—	—	not more than 1.0%

[a] Relative to the prilocaine peak.
[b] P designates a prilocaine related compound.
[c] L designates a lidocaine related compound.

Assay—

Solution A—Dissolve about 2.73 g of monobasic potassium phosphate in 630 mL of water, and adjust with 5 N sodium hydroxide to a pH of 7.20 ± 0.02. Dilute with acetonitrile to 1 L.

Solution B—Dissolve about 2.73 g of monobasic potassium phosphate in 900 mL of water, and adjust with 5 N sodium hydroxide to a pH of 7.20 ± 0.02. Dilute with acetonitrile to 1 L.

Mobile phase—Use variable mixtures of filtered and degassed *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Lidocaine RS and USP Prilocaine Hydrochloride RS in *Solution A*, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a solution having a known concentration of about 0.2 mg per mL of each compound. Immediately store this solution at or below 10°.

System suitability solution—Dissolve an accurately weighed quantity of USP Prilocaine Related Compound B RS in the *Standard preparation*, and dilute quantitatively, and stepwise if necessary, with the *Standard preparation*, to obtain a solution having a known concentration of about 0.08 mg per mL of prilocaine related compound B.

Assay preparation—Transfer a portion of the Cream, equivalent to about 20 mg each of lidocaine and prilocaine, accurately weighed, to a 100-mL volumetric flask. Add 5 mL of 5 N sodium hydroxide to disperse the Cream, and mix. Add 5 mL of 5 N hydrochloric acid, and dilute with *Solution A* to volume, and mix. Pass a portion through a nylon filter having a 0.2-µm or finer porosity, discarding the first 1 mL, and use the filtrate. Immediately store this solution at or below 10°.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 232-nm detector and a 4.6-mm × 10-cm column that contains 3-µm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. The samples are maintained at or below 10°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	67	33	equilibration
0–11.0	67	33	isocratic
11.0–22.0	67→100	33→0	linear gradient
22.0–32.0	100	0	isocratic

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are 1.00 for prilocaine, 1.09 for prilocaine related compound B, and 2.14 for lidocaine; and the resolution, *R*, between prilocaine and prilocaine related compound B is not less than 1.4. Chromatograph the *Standard preparation* a minimum of five times, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 5000 theoretical plates, based on the prilocaine peak; the tailing factor is not more than 1.5, based on the prilocaine peak; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the lidocaine and prilocaine peaks. Calculate the percentage of the label claim of lidocaine ($C_{14}H_{22}N_2O$) and prilocaine ($C_{13}H_{20}N_2O$) in the portion of Cream taken by the formula:

$$100C(r_U/r_S)(V/W)(100/L)(220.31/256.77)$$

in which C is the individual concentration, in mg per mL, of either USP Lidocaine RS or USP Prilocaine Hydrochloride RS in the *Standard preparation*; r_U and r_S are either the individual peak responses of lidocaine or prilocaine obtained from the *Assay preparation* and the *Standard preparation*, respectively; V is the volume, in mL, of the *Assay preparation*; W is the weight, in mg, of the Cream taken to prepare the *Assay preparation*; L is the individual label claim, in percent, for either lidocaine or prilocaine; and 220.31 and 256.77 are the molecular weights of prilocaine and prilocaine hydrochloride, respectively (these are used only for calculating the percentage of prilocaine in the Cream).

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Lime

CaO 56.08

Calcium oxide [1305-78-8].

DEFINITION

Lime, when freshly ignited to constant weight, contains NLT 95.0% of lime (CaO).

IDENTIFICATION

• **A.**

Analysis:

Moisten a suitable quantity of Lime with water: heat is generated, and a white powder is obtained (calcium hydroxide or slaked lime). Mix the powder with 3 or 4 times its weight of water.

Acceptance criteria: A smooth magma of lime forms that is alkaline to litmus.

• **B. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩

Sample solution:

Slake 1 g with 20 mL of water, and add 6 N acetic acid until the lime is dissolved.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample solution: Ignite 1 g of Lime in a muffle furnace to constant weight. Cool, weigh accurately, and dissolve in 20 mL of 3 N hydrochloric acid. Cool the solution, transfer to a 500-mL volumetric flask with the aid of water, and dilute with water to volume.

Analysis: Transfer 50.0 mL to a suitable container, add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 2.804 mg of lime (CaO).

Acceptance criteria: NLT 95.0%

IMPURITIES

• **INSOLUBLE SUBSTANCES**

Sample: 5.0 g

Analysis: Slake the *Sample*, then mix with 100 mL of water, followed by hydrochloric acid, dropwise, with agitation, until solution takes place: the resulting solution after boiling and cooling is acid. Filter the solution through a tared crucible, wash with water until free of chlorides, and dry at 105° for 1 h.

Acceptance criteria: NMT 50 mg (1.0%) of insoluble substances

• **MAGNESIUM AND ALKALI SALTS**

Sample solution: Dissolve 500 mg in 30 mL of water and 15 mL of 3 N hydrochloric acid. Neutralize the solution with 6 N ammonium hydroxide, heat to boiling, and add ammonium oxalate TS to precipitate the calcium completely. Heat the mixture on a steam bath for 1 h. Cool, dilute with water to 100 mL, mix, and filter.

Analysis: To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite in a tared platinum crucible to constant weight.

Acceptance criteria: The weight of the residue does not exceed 9 mg.

• **CARBONATE**

Sample: 1 g

Analysis:

Slake the *Sample*, mix with 50 mL of water, and decant the greater portion of the milky liquid.

Acceptance criteria: The addition of an excess of 3 N hydrochloric acid to the residue does not cause more than a slight effervescence.

SPECIFIC TESTS

• **LOSS ON IGNITION** ⟨733⟩

Analysis:

Ignite a portion to constant weight in a tared platinum crucible at 1100 ± 50°.

Acceptance criteria: NMT 10.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Lincomycin Injection

» Lincomycin Injection contains an amount of Lincomycin Hydrochloride in Water for Injection equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$). It contains benzyl alcohol as a preservative.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—

USP Lincomycin Hydrochloride RS

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 USP Endotoxin Unit per mg of lincomycin.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 3.0 and 5.5.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride.*

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 600 mg of lincomycin, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Lincomycin Hydrochloride.* Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in each mL of the Injection taken by the formula:

$$0.625(CP/V)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Lincomycin Oral Solution

» Lincomycin Oral Solution contains an amount of lincomycin hydrochloride ($C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled

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amount of lincomycin ($C_{18}H_{34}N_2O_6S$), and one or more suitable colors, flavors, preservatives, and sweeteners in water.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Lincomycin Hydrochloride RS

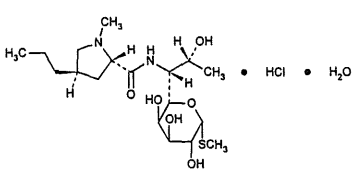
Uniformity of dosage units ⟨905⟩—
F *OR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS:* meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 3 and 5.5.

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride.*
Assay preparation—Transfer an accurately measured volume of Oral Solution, freshly mixed and free of air bubbles, equivalent to about 100 mg of lincomycin, to a suitable container. Add 0.5 mL of sodium carbonate solution (3 in 10), and swirl for 30 seconds, noting that a precipitate forms. Add 1.0 mL of 0.2 N sodium hydroxide, swirl for 30 seconds, add 10.0 mL of chloroform, and shake by mechanical means for 10 minutes. Centrifuge, and remove the upper aqueous layer by suction. Transfer 1.0 mL of the clear chloroform layer to a suitable container, and evaporate under a stream of nitrogen to dryness. Add 10.0 mL of *Mobile phase* to the residue, and dissolve by swirling, sonicating if necessary.
Procedure—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride,* recording the chromatogram over a period seven times the retention time of lincomycin. Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in each mL of the Oral Solution taken by the formula:

$$(CP/10V)(r_U/r_S)$$

in which V is the volume, in mL, of Oral Solution taken; and the other terms are as defined therein.

Lincomycin Hydrochloride



$C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$ 461.01
D-*erythro*-α-D-*galacto*-Octopyranoside, methyl 6,8-dideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-, monohydrochloride, monohydrate, (2*S*-*trans*)-.
Methyl 6,8-dideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside monohydrochloride monohydrate [7179-49-9].
Anhydrous 443.01
[859-18-7].

» Lincomycin Hydrochloride has a potency equivalent to not less than 790 µg of lincomycin ($C_{18}H_{34}N_2O_6S$) per mg.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Lincomycin Hydrochloride RS

Change to read:

Identification, ▲ ***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197M***▲ (CN 1-May-2020)
Specific rotation ⟨781S⟩: between +135° and +150°.
Test solution: 20 mg per mL, in water.
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 3.0 and 5.5, in a solution (1 in 10).
Water Determination, *Method I* ⟨921⟩: between 3.0% and 6.0%.
Limit of lincomycin B—Use the chromatogram obtained from the *Assay preparation* in the *Assay:* the area of the lincomycin B peak is not greater than 5.0% of the sum of the areas of the lincomycin B peak and the lincomycin peak.
Other requirements—Where the label states that Lincomycin Hydrochloride is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Lincomycin Injection.* Where the label states that Lincomycin Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Lincomycin Injection.*

Assay—
Mobile phase—Add 13.5 mL of phosphoric acid to 1000 mL of water, and adjust with ammonium hydroxide to a pH of 6.0. Prepare a filtered and degassed mixture of this solution, acetonitrile, and methanol (780:150:150). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Lincomycin Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 1.2 mg per mL, using sonication if necessary to effect solution.
Assay preparation—To about 12 mg of Lincomycin Hydrochloride, accurately weighed, add 10.0 mL of *Mobile phase.* Shake by mechanical means for 5 minutes, and sonicate if necessary to effect solution.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7 and is maintained at a temperature of 46°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the tailing factor for the main lincomycin peak is not more than 1.3; the column efficiency determined from the main lincomycin peak is not less than 4000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. The relative retention times are about 0.5 for lincomycin B and 1.0 for lincomycin. Calculate the quantity, in µg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in each mg of the Lincomycin Hydrochloride taken by the formula:

$$10(CP/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Lincomycin Hydrochloride RS in the *Standard preparation; P* is the designated potency, in µg of lincomycin per mg, of USP

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Lincomycin Hydrochloride RS; *W* is the weight, in mg, of the portion of Lincomycin Hydrochloride taken to prepare the *Assay preparation;* and r_U and r_S are the lincomycin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lincomycin Hydrochloride Capsules

» Lincomycin Hydrochloride Capsules contain an amount of $C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$ equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Lincomycin Hydrochloride RS

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Filter a portion of about 20 mL of the solution under test. Transfer about 5 mL of the eluant into a small test tube, and add 250 µL of 0.01 M sodium sulfate internal standard solution. Evaporate until dry using a vacuum centrifuge. Add 10.0 µL of water to the precipitate and place on a vortex mixer until all solid material is dissolved. Transfer this solution to a capillary tube, place it in a Raman spectrometer, and obtain the Raman spectrum using suitable instrumental conditions . Integrate the Raman intensity, applying baseline corrections, between 660 cm^{-1} and 720 cm^{-1}. Divide this result by the integrated intensity between 966 cm^{-1} and 994 cm^{-1}. Determine the amount of $C_{18}H_{34}N_2O_6S$ dissolved in comparison with an aqueous Standard solution having a known concentration of USP Lincomycin Hydrochloride RS.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{18}H_{34}N_2O_6S$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 7.0%.

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride.*
Assay preparation—Remove, as completely as possible, the contents of not less than 10 Capsules, taking care to prevent capsule shell fragments from being combined with the capsule contents and to remove any shell fragments from the contents. Weigh and mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of lincomycin, to a suitable container. Add 50.0 mL of *Mobile phase,* and shake by mechanical means for 5 minutes. Use the solution thus obtained as the *Assay preparation.*
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Lincomycin Hydrochloride.* Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in the portion of Capsule contents taken by the formula:

$$(CP/20)(r_U/r_S)$$

in which the terms are as defined therein.

Lincomycin Hydrochloride Soluble Powder

» Lincomycin Hydrochloride Soluble Powder contains an amount of Lincomycin Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$).

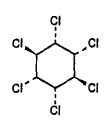
Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Lincomycin Hydrochloride RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 6.0%.

Assay—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride.*
Standard preparation—Dissolve an accurately weighed quantity of USP Lincomycin Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 1.2 mg per mL.
Assay preparation—Remove as completely as possible the contents of not fewer than 5 containers. Weigh and mix the combined contents, and transfer an accurately weighed portion of the Soluble Powder, equivalent to about 400 mg of lincomycin ($C_{18}H_{34}N_2O_6S$), to a 100-mL volumetric flask. Add about 80 mL of *Mobile phase,* and swirl to dissolve. Dilute with *Mobile phase* to volume, and mix. Transfer 25.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Procedure—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride.* Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in the portion of Soluble Powder taken by the formula:

$$0.4CP(r_U/r_S)$$

in which the terms are as defined therein.

Lindane



$C_6H_6Cl_6$ 290.83
Cyclohexane, 1,2,3,4,5,6-hexachloro-, (1α,2α,3β,4α,5α,6β)-.
γ-1,2,3,4,5,6-Hexachlorocyclohexane [58-89-9].

» Lindane is the gamma isomer of hexachlorocyclohexane. It contains not less than 99.0 percent and not more than 101.0 percent of lindane (γ-$C_6H_6Cl_6$).

USP Monographs

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Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Lindane RS

Change to read:

Identification, ▲ ***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)
Congealing temperature ⟨651⟩: not less than 112.0°.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Chloride ion—Place about 100 mg in a test tube with 10 mL of water, shake, and filter. Add 1 mL of nitric acid and 3 mL of silver nitrate TS to the filtrate: no turbidity develops.

Assay—
Internal standard solution—Dissolve *n*-octadecane in methylene chloride to obtain a solution having a concentration of about 0.5 mg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Lindane RS in *Internal standard solution* to obtain a solution having a known concentration of about 2 mg per mL.
System suitability solution—Prepare solutions of α-benzene hexachlorides (BHC) at 1000 µg per mL of methanol, β-BHC at 1000 µg per mL of acetone, and δ-BHC at 1000 µg per mL of methanol. Transfer 100 µL each of α-BHC, β-BHC and δ-BHC solutions to a 4-mL conical vial, and evaporate under a stream of nitrogen to dryness. Add to the vial a 100-µL aliquot of the *Standard preparation*. Insert the stopper, and shake vigorously to dissolve the residue.
Assay preparation—Transfer about 10 mg of Lindane, accurately weighed, to a 5-mL volumetric flask. Dissolve in and dilute with *Internal standard solution* to volume.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm×30-m fused-silica column coated with a 1-µm phase G46. The chromatograph is programmed as follows. The initial column temperature is maintained at 120° for 1 minute. Then, the temperature is increased at a rate of 20° per minute to 150°, and then ramped at a rate of 10° per minute to 280° and maintained at that temperature for 4 minutes. The injection port and detector temperatures are maintained at 300°. The injection split ratio is 50:1. Chromatograph the *Standard preparation* and the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times for *n*-octadecane and lindane are about 0.85 and 1.0, respectively. [NOTE—Typical retention times for α-BHC, β-BHC, γ-BHC, δ-BHC, and *n*-octadecane are 15.7, 17.8, 16.5, 18.8, and 13.9 minutes, respectively.] The resolution, *R,* between *n*-octadecane and α-BHC is not less than 21, between lindane (γ-BHC) and α-BHC is not less than 9, between β-BHC and lindane is not less than 14, and between δ-BHC and β-BHC is not less than 8; the tailing factors for *n*-octadecane and lindane are less than 1.5 and 1.2, respectively; and the relative standard deviation of the ratios of peak area responses of lindane to *n*-octadecane for replicate injections of *Standard preparation* is not more than 1.5%.
Procedure—Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of γ-$C_6H_6Cl_6$ in the portion of Lindane taken by the formula:

$$5C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Lindane RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak responses of lindane to *n*-octadecane, obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Lindane Cream

DEFINITION
Lindane Cream is Lindane in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lindane (γ-$C_6H_6Cl_6$).

IDENTIFICATION
• **A.**
Analysis: Wind a strip of 20-mesh copper gauze 1.5 cm wide and 5 cm long around the end of a copper wire. Heat the gauze in the nonluminous flame of a Bunsen burner until it glows without coloring the flame green. Allow the gauze to cool, and repeat the heating and cooling step several times until a thorough coating of oxide is formed. Apply a small amount of Cream to the cooled gauze, ignite, and allow to burn freely in the air. Hold the gauze in the outer edge of the burner flame at a height of 4 cm.
Acceptance criteria: A bright green color is imparted to the flame.

ASSAY
• **PROCEDURE**
Mobile phase: Mix 18 mL of anhydrous ethyl ether with 280 mL of chromatographic hexane.
Internal standard solution: 1 mg/mL of *n*-docosane in methylene chloride
Standard stock solution: 2 mg/mL of USP Lindane RS in methylene chloride
Standard solution: Transfer 5.0 mL of *Standard stock solution* to a graduated centrifuge tube, add 5.0 mL of *Internal standard solution,* and evaporate with the aid of gentle heat and a current of dry air to 3 mL. Avoid evaporating to dryness. If the mixture is inadvertently evaporated to dryness, discard it, and begin another *Standard solution.*
Solid support: 60- to 100-mesh magnesium silicate that has been heated previously at 300° for 2 h
Sample stock solution: Nominally 2 mg/mL of lindane from a quantity of Cream, equivalent to 10 mg of lindane, prepared as follows. Place a pledget of cotton on a removable porous plate at the base of a 25-mm × 200-mm chromatographic tube fitted with a polytef stopcock. Add 50 mL of *Mobile phase* and 10 g of *Solid support,* and stir the mixture to expel air bubbles. Add 1.5 g of anhydrous sodium sulfate to the column, and elute until the surface of the liquid is 4 cm above the *Solid support,* discarding the eluate. Transfer a portion of Cream to a 150-mL beaker, and add 10 g of *Solid support.* Mix with a spatula, adding chromatographic hexane as necessary to produce a homogeneous mixture, and continue stirring until a free-flowing powder is produced. Transfer this mixture to the chromatographic column with the aid of three 5-mL portions of *Mobile phase,* and elute the column with 225 mL of the *Mobile phase* at a flow rate of 2–3 mL/min, collecting the eluate in a 250-mL beaker. Remove the chromatographic column, add 5.0 mL of *Internal standard solution* to the eluate, and evaporate with the aid of gentle heat and a current of dry air to 5 mL.
Sample solution: Transfer the *Sample stock solution* to a graduated centrifuge tube with the aid of 1 mL of methylene chloride, and evaporate with the aid of gentle heat and a current of dry air to 3 mL. Avoid evaporating to

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dryness. If the mixture is inadvertently evaporated to dryness, discard it, and begin another *Sample solution.*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: GC
Detector: Flame ionization
Column: 1.8-m × 2-mm glass; packed with 3% liquid phase G3 on support S1A
Temperatures
Column: 195°
Injection port: 250°
Detector: 250°
Flow rate: 40 mL/min
Injection volume: 1 µL
Carrier gas: Dry nitrogen

System suitability
Sample: *Standard solution* (6–10 replicate injections)
Suitability requirements
Resolution: NLT 5 between lindane and *n*-docosane
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lindane (γ-$C_6H_6Cl_6$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of lindane to *n*-docosane in the *Sample solution*
R_S = peak response ratio of lindane to *n*-docosane in the *Standard solution*
C_S = concentration of USP Lindane RS in the *Standard stock solution* (mg/mL)
C_U = nominal concentration of lindane in the *Sample stock solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 1-in-5 dilution
Acceptance criteria: 8.0–9.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Lindane RS

Lindane Lotion

» Lindane Lotion is Lindane in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lindane (γ-$C_6H_6Cl_6$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Lindane RS
Identification—It responds to the *Identification* test under *Lindane Cream.*
pH ⟨791⟩**:** between 6.5 and 8.5.
Assay—Proceed as directed in the *Assay* under *Lindane Cream,* substituting "Lotion" for "Cream" throughout.

Lindane Shampoo

DEFINITION
Lindane Shampoo is Lindane in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lindane (γ-$C_6H_6Cl_6$).

IDENTIFICATION
• **A.**
Analysis: Wind a 1.5-cm × 5-cm strip of 20-mesh copper gauze around the end of a copper wire. Heat the gauze in the nonluminous flame of a Bunsen burner until it glows, without coloring the flame green. Allow the gauze to cool, and repeat the heating and cooling step several times until a thorough coating of oxide is formed. Apply a small amount of Shampoo to the cooled gauze, ignite, and allow to burn freely in the air. Hold the gauze in the outer edge of the burner flame at a height of 4 cm.
Acceptance criteria: A bright green color is imparted to the flame.

ASSAY
• **Procedure**
Mobile phase: Anhydrous ethyl ether and chromatographic solvent hexane (18:280)
Internal standard solution: 1 mg/mL of *n*-docosane in methylene chloride
Standard stock solution: 2 mg/mL of USP Lindane RS in methylene chloride
Standard solution: Transfer 5.0 mL of *Standard stock solution* to a graduated centrifuge tube, add 5.0 mL of *Internal standard solution,* and evaporate with the aid of gentle heat and a current of dry air to 3 mL. Avoid evaporating to dryness. If the mixture is inadvertently evaporated to dryness, discard it, and begin another *Standard solution.*
Solid support: Use 60- to 100-mesh magnesium silicate that has been previously heated at 300° for 2 h.
Sample stock solution: Nominally 2 mg/mL of lindane from a quantity of Shampoo, equivalent to 10 mg of lindane, prepared as follows. Place a pledget of cotton on a removable porous plate at the base of a 25-mm × 200-mm chromatographic column that is fitted with a polytef stopcock. Add 50 mL of *Mobile phase* and 10 g of *Solid support,* and stir the mixture to expel air bubbles. Add 1.5 g of anhydrous sodium sulfate to the column, and elute until the surface of the liquid is 4 cm above *Solid support,* discarding the eluate. Transfer a weighed portion of Shampoo, corresponding to 10 mg of lindane, to a 150-mL beaker, and add 10 g of *Solid support.* Mix with a spatula, adding chromatographic solvent hexane as necessary to produce a homogeneous mixture, and continue stirring until a free-flowing powder is produced. Transfer this mixture to the chromatographic column with the aid of three 5-mL portions of *Mobile phase.* Elute the column with 225 mL of *Mobile phase* at a flow rate of 2–3 mL/min, collecting the eluate in a 250-mL beaker. Remove the chromatographic column, add 5.0 mL of *Internal standard solution* to the eluate, and evaporate with the aid of gentle heat and a current of dry air to 5 mL.
Sample solution: Transfer the *Sample stock solution* to a graduated centrifuge tube with the aid of 1 mL of methylene chloride, and evaporate with the aid of gentle heat and a current of dry air to 3 mL. Avoid evaporating to dryness. If the mixture is inadvertently evaporated to dryness, discard it, and begin another *Sample solution.*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: GC
Detector: Flame ionization

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Column: 1.8-m × 2-mm glass; packed with 3% liquid phase G3 on support S1A
Temperatures
Injector: 250°
Detector: 250°
Column: 195°
Carrier gas: Dry nitrogen
Flow rate: 40 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5 between lindane and *n*-docosane
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0% for 6–10 replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lindane (γ-$C_6H_6Cl_6$) in the portion of Shampoo taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of lindane to *n*-docosane from the *Sample solution*
R_S = peak response ratio of lindane to *n*-docosane from the *Standard solution*
C_S = concentration of USP Lindane RS in the *Standard stock solution* (mg/mL)
C_U = nominal concentration of lindane in the *Sample stock solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

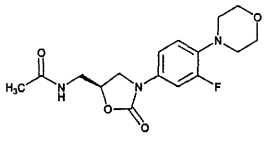
SPECIFIC TESTS

• **pH** ⟨791⟩: 6.2–7.0

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Lindane RS

Linezolid



$C_{16}H_{20}FN_3O_4$ 337.35
Acetamide, *N*-[[3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]-, (*S*)-;
N-[[(*S*)-3-(3-Fluoro-4-morpholinophenyl)-2-oxo-5-oxazolidinyl]methyl]acetamide [165800-03-3].

DEFINITION

Linezolid contains NLT 98.0% and NMT 102.0% of linezolid ($C_{16}H_{20}FN_3O_4$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)**:** If a difference appears in the IR spectra of the analyte and the Standard, dissolve equal portions of the test specimen and the USP Reference Standard in equal volumes of methanol. Evaporate the solutions to dryness and perform the test on the residues.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Buffer: 1.4 g/L of monobasic potassium phosphate
Solution A: Methanol, acetonitrile, and *Buffer* (15:5:80)
Solution B: Methanol and *Buffer* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
8	57	43
18	0	100
25	0	100

Return to original conditions, and equilibrate the system for 5 min.
Diluent: Acetonitrile and water (35:65)
System suitability solution: 50 µg/mL each of USP Linezolid RS and USP Linezolid Related Compound D RS in *Diluent*
Standard solution: 0.08 mg/mL of USP Linezolid RS in *Diluent*
Sample solution: 0.08 mg/mL of Linezolid in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 7.5-cm; 3-µm packing L1
Temperatures
Autosampler: 15°
Column: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for linezolid and linezolid related compound D are about 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 3.0 between linezolid and linezolid related compound D, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of linezolid ($C_{16}H_{20}FN_3O_4$) in the portion of Linezolid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Linezolid RS in the *Standard solution* (mg/mL)
C_U = concentration of Linezolid in the *Sample solution* (mg/mL)

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Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.0008 mg/mL of USP Linezolid RS in *Diluent*

Sample solution: 0.8 mg/mL of Linezolid in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for linezolid and linezolid related compound D are about 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 3.0 between linezolid and linezolid related compound D, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Linezolid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of linezolid from the *Standard solution*

C_S = concentration of USP Linezolid RS in the *Standard solution* (mg/mL)

C_U = concentration of Linezolid in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Linezolid *N*-oxide[a]	0.20	0.3
Linezolid related compound C (linezolid amine)[b]	0.31	0.2
Desfluoro linezolid (if present)[c, d]	0.63	0.3
Linezolid	1.0	—
Any individual unspecified impurity	—	0.1

[a] (*S*)-4-{4-[5-(Acetamidomethyl)-2-oxooxazolidin-3-yl]-2-fluorophenyl} morpholine 4-oxide.
[b] (*S*)-5-(Aminomethyl)-3-(3-fluoro-4-morpholinophenyl)oxazolidin-2-one.
[c] (*S*)-*N*-{[3-(4-Morpholinophenyl)-2-oxooxazolidin-5-yl]methyl}acetamide.
[d] If possible from the manufacturing process.

- **ENANTIOMERIC PURITY**

Mobile phase: Hexane, absolute alcohol, and trifluoroacetic acid (650:350:1)

System suitability solution: 25 µg/mL each of USP Linezolid RS and USP Linezolid R-Isomer RS in absolute alcohol

Sample solution: 0.5 mg/mL of Linezolid in absolute alcohol. Sonicate as needed to dissolve.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L51

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: NLT 2.8 times the retention time of linezolid

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for linezolid *R*-isomer and linezolid are 0.84 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between linezolid *R*-isomer and linezolid

Analysis

Sample: *Sample solution*

Calculate the percentage of linezolid *R*-isomer in the portion of Linezolid taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of linezolid *R*-isomer from the *Sample solution*

r_T = sum of the peak responses of both enantiomers from the *Sample solution*

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Linezolid RS

USP Linezolid Related Compound D RS

(*R*)-[3-(3-Fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl]methyl methanesulfonate.

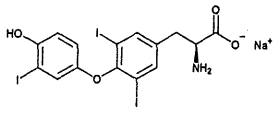
$C_{15}H_{19}FN_2O_6S$ 374.38

USP Linezolid R-Isomer RS

N-{[(*R*)-3-(3-Fluoro-4-morpholinophenyl)-2-oxo-5-oxazolidinyl]methyl}acetamide.

$C_{16}H_{20}FN_3O_4$ 337.35

Liothyronine Sodium



$C_{15}H_{11}I_3NNaO_4$ 672.96

L-Tyrosine, *O*-(4-hydroxy-3-iodophenyl)-3,5-diiodo-, monosodium salt;

Monosodium L-3-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]alanine [55-06-1].

DEFINITION

Liothyronine Sodium is the sodium salt of L-3,3′,5-triiodothyronine. It contains NLT 95.0% and NMT 101.0% of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$), calculated on the dried basis.

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IDENTIFICATION

• **A.**

Diluent: Solution of hydrochloric acid in 80% alcohol (1 in 50)

Sample solution: 0.1-mg/mL solution in *Diluent*

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima at the same wavelengths as those of a similar solution of USP Liothyronine RS, concomitantly measured; and the respective absorptivities, both calculated on the dried basis in terms of the acid at the wavelength of maximum absorbance at about 297 nm, do not differ by more than 5.0%.

• **B.**

Analysis: Heat 50 mg with a few drops of sulfuric acid in a porcelain crucible.

Acceptance criteria: Violet vapors of iodine are evolved.

• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*: The residue from the ignition of Liothyronine Sodium meets the requirements.

• **D.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Mixture of acetonitrile and water (4:6) that contains 0.5 mL of phosphoric acid in each liter of the mixture

Solution A: Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, and add 500 mL of methanol.

Levothyroxine stock solution: 0.4 mg/mL of USP Levothyroxine RS in *Solution A*. Make a 1:100 dilution of this solution using *Mobile phase*.

Liothyronine stock solution: 0.4 mg/mL of USP Liothyronine RS in *Solution A*

Standard solution: 10 µg/mL of liothyronine from *Liothyronine stock solution* and 0.5 µg/mL of levothyroxine from *Levothyroxine stock solution*, in *Mobile phase*

Sample solution: 10 µg/mL of Liothyronine Sodium in *Mobile phase*

[NOTE—A small amount of 0.01 M methanolic sodium hydroxide can be used to facilitate the dissolution of the sample.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between levothyroxine and liothyronine

Relative standard deviation: NMT 2.0% for liothyronine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) in the portion of Liothyronine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of liothyronine from the *Sample solution*

r_S = peak response of liothyronine from the *Standard solution*

C_S = concentration of USP Liothyronine RS in the *Standard solution* (µg/mL)

C_U = concentration of Liothyronine Sodium in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of liothyronine sodium, 672.96

M_{r2} = molecular weight of liothyronine, 650.97

Acceptance criteria: 95.0%–101.0% on the dried basis

IMPURITIES

• **CHLORIDE CONTENT**

Sample solution: Transfer 100 mg of Liothyronine Sodium, previously dried, into a platinum dish. Ignite over a low flame, protecting the dish from air currents during the ignition. When carbonization is complete, cool the dish, add 2 drops of water, and break up the charred mass thoroughly with a stirring rod. Add 10 mL of water and 5 mL of ammonium hydroxide, and mix. Transfer the slurry to a glass-stoppered, 50-mL flask, and wash the platinum dish and the stirring rod with water, adding the washings to the flask, until the volume of the solution is about 25 mL. Add 10 mL of silver nitrate solution (1 in 20), shake thoroughly, and filter through a retentive paper into a 50-mL color-comparison tube. Wash the flask and the filter paper with 10 mL of water, and add the washings to the tube. Acidify the combined filtrate and washings to litmus with nitric acid, and dilute with water to 50 mL.

Control solution: Mix 5 mL of ammonium hydroxide, 20 mL of water, and 10 mL of silver nitrate solution (1 in 20). Filter the mixture through a retentive paper into a 50-mL color-comparison tube, and then wash the filter paper with 10 mL of water into the tube. Acidify the contents of the tube to litmus with nitric acid, and dilute with water to 50 mL.

Sodium chloride solution: 1-mg/mL solution of sodium chloride in water

Analysis: Add the *Sodium chloride solution* in 0.1-mL increments to the *Control solution* until the turbidity of the *Control solution* matches that of the *Sample solution*.

Acceptance criteria: NMT 2.0 mL of *Sodium chloride solution* is required (1.2%).

• **LIMIT OF LEVOTHYROXINE SODIUM**

Mobile phase, Levothyroxine stock solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Levothyroxine standard solution: 0.5 µg/mL of levothyroxine from *Levothyroxine stock solution*, in *Mobile phase*

Analysis

Samples: *Levothyroxine standard solution* and *Sample solution*

Calculate the percentage of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Liothyronine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levothyroxine from the *Sample solution*

r_S = peak response of levothyroxine from the *Levothyroxinestandard solution*

C_S = concentration of USP Levothyroxine RS in the *Levothyroxinestandard solution* (µg/mL)

C_U = concentration of Liothyronine Sodium in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of levothyroxine sodium, 798.85

M_{r2} = molecular weight of levothyroxine, 776.87

Acceptance criteria: NMT 5.0% of levothyroxine sodium

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SPECIFIC TESTS

- **SODIUM CONTENT**

Analysis: Transfer 100 mg, previously dried, into a platinum dish. Add 8–10 drops of sulfuric acid, and ignite to constant weight, taking care to avoid spattering. Each mg of residue is equivalent to 0.324 mg of sodium (Na).

Correct the result for the quantity of sodium equivalent to the sodium chloride (NaCl) found in the test for *Chloride Content*.

Acceptance criteria: 2.9%–4.0%

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures,Specific Rotation*

Diluent: A mixture of alcohol and 1.2 N hydrochloric acid (4:1)

Sample solution: 20 mg/mL in *Diluent*

Acceptance criteria: +18° to +22°

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 4.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Levothyroxine RS
USP Liothyronine RS

Liothyronine Sodium Tablets

» Liothyronine Sodium Tablets contain an amount of $C_{15}H_{11}I_3NNaO_4$ equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of liothyronine ($C_{15}H_{12}I_3NO_4$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Levothyroxine RS
USP Liothyronine RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to the liothyronine peak in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Dissolution ⟨711⟩— [NOTE—All containers that are in contact with solutions containing liothyronine sodium are to be made of glass.]

Medium: pH 10.0 ± 0.05 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 250 mL.

Apparatus 3: 30 dips per minute, using 20-mesh screen on the top and 40-mesh screen on the bottom of the glass reciprocating cylinder.

Time: 45 minutes.

Determine the amount of liothyronine sodium ($C_{15}H_{12}I_3NO_4$) dissolved by employing the following method.

Ammoniated solution—Add 0.05 mL of ammonium hydroxide to 200 mL of water.

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (55:45) that contains 1 mL of phosphoric acid in each 1000 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Liothyronine RS in *Ammoniated solution*, and dilute quantitatively, and stepwise if necessary, with *Ammoniated solution* to obtain a solution having a known concentration of about 10 µg of USP Liothyronine RS per mL. Dilute a portion of this solution quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.5 µg of USP Liothyronine RS per mL.

Test solution—Transfer 20 mL of the solution under test to a centrifuge tube, and centrifuge until a clear supernatant is obtained.

Resolution solution—Prepare a solution of USP Liothyronine RS and USP Levothyroxine RS in *Ammoniated solution* having known concentrations of about 10 µg of each USP Reference Standard per mL. Dilute with water to obtain a concentration of about 0.5 µg of each USP Reference Standard per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between liothyronine and levothyroxine is not less than 3.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 200 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of $C_{15}H_{12}I_3NO_4$ dissolved.

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{15}H_{12}I_3NO_4$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Liothyronine Sodium*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 µg of liothyronine sodium, to a centrifuge tube, add 2 glass beads, pipet 10 mL of *Mobile phase* into the tube, and mix using a vortex mixer for 3 minutes. Centrifuge to obtain a clear supernatant, filtering if necessary.

Procedure—Proceed as directed in the *Assay* under *Liothyronine Sodium*. Calculate the quantity, in µg, of liothyronine ($C_{15}H_{12}I_3NO_4$) in the portion of Tablets taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Liothyronine RS in the *Standard preparation;* and r_U and r_S are the liothyronine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Liotrix Tablets

» Liotrix Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of Levothyroxine Sodium ($C_{15}H_{10}I_4NNaO_4$) and Liothyronine Sodium ($C_{15}H_{11}I_3NNaO_4$) in a ratio by weight of 4 to 1, respectively.

Packaging and storage—Preserve in tight containers.

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USP Reference standards ⟨11⟩—
USP Levothyroxine RS
USP Liothyronine RS

Identification—The retention time of the two major peaks in the chromatogram of the *Assay preparation* corresponds to the levothyroxine and liothyronine peaks in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Disintegration ⟨701⟩: 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (65:35) that contains 2 mL of trifluoroacetic acid in each 1000 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

0.01 M Methanolic sodium hydroxide—Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, add 500 mL of methanol, and mix.

Levothyroxine stock solution—Dissolve an accurately weighed quantity of USP Levothyroxine RS in *0.01 M Methanolic sodium hydroxide* to obtain a solution having a known concentration of about 0.4 mg of levothyroxine per mL.

Liothyronine stock solution—Dissolve an accurately weighed quantity of USP Liothyronine RS in *0.01 M Methanolic sodium hydroxide* to obtain a solution having a known concentration of about 0.4 mg of liothyronine per mL. Make a 1:10 dilution of this solution using *Mobile phase.*

Standard preparation—Transfer appropriate volumes of *Levothyroxine stock solution* and *Liothyronine stock solution* to a suitable container, and dilute quantitatively and stepwise, if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 10 µg of levothyroxine per mL and 2.5 µg of liothyronine per mL.

Assay preparation—Transfer 20 Tablets to a 200-mL volumetric flask, add 180 mL of *Mobile phase,* and sonicate for 15 minutes, occasionally swirling the flask to accelerate the disintegration of the Tablets. Cool to room temperature, and dilute with *Mobile phase* to volume. Transfer a portion of the solution to a centrifuge tube, and centrifuge for 10 minutes at 5000 rpm. Quantitatively dilute a portion of the clear supernatant with *Mobile phase* to obtain concentrations of about 10.0 µg of levothyroxine sodium per mL and 2.5 µg of liothyronine sodium per mL.

Chromatographic system—Proceed as directed in the *Assay* under *Levothyroxine Sodium.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Levothyroxine Sodium.* Calculate the quantity, in µg, of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Tablets taken by the formula:

$$(798.86/776.87)(10C)(r_U/r_S)$$

in which 798.86 and 776.87 are the molecular weights of levothyroxine sodium and levothyroxine, respectively; C is the concentration, in µg per mL, of USP Levothyroxine RS in the *Standard preparation;* and r_U and r_S are the levothyroxine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Calculate the quantity, in µg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) in the portion of Tablets taken by the formula:

$$(672.96/650.98)(10C)(r_U/r_S)$$

in which 672.96 and 650.98 are the molecular weights of liothyronine sodium and liothyronine, respectively; C is the concentration, in µg per mL, of USP Liothyronine RS in the *Standard preparation;* and r_U and r_S are the liothyronine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Lipid Injectable Emulsion

» Lipid Injectable Emulsion used in total parenteral nutrition is a sterile 10 (0.10 g per mL), 20 (0.20 g per mL), or 30 (0.30 g per mL) percent w/v emulsion in an aqueous vehicle. The aqueous phase contains 0.6 percent to 1.8 percent w/v parenteral Egg Phospholipids in Water for Injection and contains, if necessary, an osmotic agent, such as glycerin in amounts of 1.7 percent to 2.5 percent w/v, or a suitable stabilizer, such as a fatty acid salt. The most frequently used oil present is Soybean Oil, which provides an ample supply of the essential fatty acids: linoleic acid and linolenic acid. Other oils, such as Safflower Oil, Medium-Chain Triglycerides, Olive Oil, Fish Oil, or other suitable oils, can be mixed with Soybean Oil. Hence, Soybean Oil can be the only oil or be part of a mixture of these other oils. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of the total oil(s). It contains no antimicrobial agents. The final products are terminally sterilized.

Packaging and storage—Preserve in an appropriate container (see *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*). Use elastomeric closures that are compatible with both the oil and water phases of the Emulsion. Store at a temperature not below 4° (protect from freezing) or above 30° (protect from excessive heat).

Labeling—The label states the identity and the quantities of the specific oils in the Emulsion. The label states the total osmolar concentration (or osmolarity) in mOsm per L. The labeling provides the following information: do not use if there is evidence of excessive creaming or aggregation, if excessive free oil droplets are visible, or if there are other indications of compromised integrity, such as microbial growth, present in the product.

Fatty acid composition—Transfer a volume of the Emulsion, equivalent to about 200 mg of lipids, to a stoppered extraction vessel, add 10 mL of ether, and mix. Add 5 g of anhydrous sodium sulfate, mix, and allow the mixture to stand until separation of the layers is complete. Wet the packing of a chromatographic silica cartridge with a few mL of ether, transfer about 5 mL of the ether layer from the extraction vessel to the column reservoir, and elute at a rate of between 5 and 10 drops per minute into a suitable vessel. Evaporate the ether from the eluant, and dissolve the residue in 5.0 mL of toluene. Transfer 1.0 mL of the toluene solution to a reaction vial, and add 0.4 mL of (*m*-trifluoromethylphenyl) trimethylammonium hydroxide in methanol. Cover, mix, and allow to stand for 30 minutes. Inject about 1 µL of this solution into a gas chromatograph equipped with a 0.53-mm × 50-m wide-bore, fused-silica capillary column coated with a 2.0-µm thickness of liquid phase G16 and maintained at a temperature of 200°. The column is connected to a flame-ionization detector. Helium is used as the carrier gas at a flow rate of

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about 10 mL per minute. Measure the main peak areas of the methyl esters of the fatty acids. The relative peak areas expressed as a percentage of the main peaks are in the known ranges for the oil (e.g., Soybean Oil, USP; Safflower Oil, USP) as specified on the label. For oil mixtures, analysis of each oil should be performed to identify known peaks prior to emulsification as specified on the label.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 USP Endotoxin Unit per mL.

pH ⟨791⟩: between 6.0 and 9.0.

Globule size limits—The Injectable Emulsion meets the requirements of the limits specified in both *Method I* and *Method II* as directed under *Globule Size Distribution in Lipid Injectable Emulsions* ⟨729⟩.

Limit of oil droplet mean diameters (See *Method I—Light Scattering Method* under *Globule Size Distribution in Lipid Injectable Emulsions* ⟨729⟩)—Using the method of light scattering, determine the mean droplet diameter (MDD): the sample meets the requirements. The intensity-weighted mean droplet diameter (MDD) for the Injectable Emulsion must be ≤500 nm, or 0.5 µm, irrespective of the concentration of the dispersed lipid phase.

Limit of large globule volume-diameter (See *Method II—Light Obscuration or Extinction Method* under *Globule Size Distribution in Lipid Injectable Emulsions* ⟨729⟩)—Using the method of light obscuration, determine the size distribution of globules in the large-diameter tail of the dispersion (detection threshold ≥2.0 µm). Calculate the volume-weighted mass of lipid in the form of globules with diameters in excess of 5.0 µm per 100 mL of the Injectable Emulsion. The volume-weighted, large-diameter fat globule limits of the dispersed phase, expressed as the percentage of fat residing in globules larger than 5 µm (PFAT5) for a given Injectable Emulsion, is not to exceed 0.05%.

Limit of free fatty acid—

Solvent—Prepare a mixture of heptane, isopropanol, and water (400:400:200) in a separatory funnel. Allow the phases to separate, and discard the lower phase. Filter the upper phase (heptane solution) through 40 g of anhydrous sodium sulfate. Store in a tightly capped glass container, and use within 1 week.

Chromatographic column—Prepare a slurry of heptane and chromatographic silica gel having an average pore size of 6 nm, and activate at a temperature of 110° for not less than 1 hour prior to use. Transfer the slurry to a 2.3-cm chromatographic tube (see *Column Chromatography* under *Chromatography* ⟨621⟩), and pack to a bed height of between 5 cm and 6 cm. Wash the column with about 40 mL of heptane, and drain the heptane through the column to a level of about 0.5 cm above the silica gel bed.

Procedure —Transfer 20.0 mL of the Injectable Emulsion to a flask, freeze, and lyophilize. Dissolve the residue in 30 mL of *Solvent,* and transfer the solution to the column. Rinse the flask with three 30-mL portions of *Solvent,* and transfer the washings to the column, allowing each rinsing to drain to the top of the column bed before applying the next rinse. Collect a total of 120 mL of effluent. Add 10 drops of phenolphthalein TS to the effluent, bubble nitrogen through the solution, and titrate with 0.02 N alcoholic potassium hydroxide VS until the solution remains pale pink after mixing for 10 seconds. Titrate a blank using 120 mL of *Solvent.* Calculate the quantity, in mEq, of free fatty acids per g of oil in the Injectable Emulsion using the formula:

$$(V_U - V_B)N/20C$$

in which V_U is the volume, in mL, of 0.02 N alcoholic potassium hydroxide consumed by the eluant; V_B is the volume, in mL, of 0.02 N alcoholic potassium hydroxide consumed by the blank; N is the normality of the 0.02 N alcoholic potassium hydroxide; and C is the labeled concentration, in g per mL, of the total oil(s) in the Injectable Emulsion: not more than 0.07 mEq of free fatty acids per g of oil is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase —Prepare a filtered and degassed mixture of isopropanol, ethyl acetate, and glacial acetic acid (179:20:1).

Standard preparation —Dissolve an accurately weighed portion of Soybean Oil (or other relevant oils used in the Emulsion) in *Mobile phase* to obtain a solution having a known concentration of about 8 mg per mL.

Assay preparation —Transfer an accurately measured portion of Emulsion, equivalent to about 800 mg of oil, to a 100-mL volumetric flask with the aid of additional portions of *Mobile phase.* Dilute with *Mobile phase* to volume, and mix to obtain a solution containing about 8 mg of oil per mL.

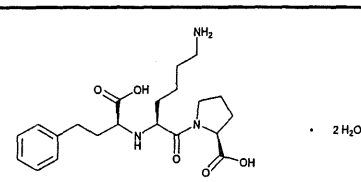
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector and a 4.1-mm × 25-cm column that contains packing L21. The flow rate is about 1 mL per minute, adjusted so that the peak due to oil elutes at about 6.5 minutes. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the capacity factor, k', is not less than 1.0; the tailing factor for the oil peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure —Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of oil in the portion of Emulsion taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of Soybean Oil or other relevant oils used in the Emulsion in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Lisinopril



$C_{21}H_{31}N_3O_5 \cdot 2H_2O$ 441.52

L-Proline, 1-[N^2-(1-carboxy-3-phenylpropyl)-L-lysyl]-, dihydrate, (*S*)-;
1-[N^2-[(*S*)-1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline dihydrate [83915-83-7].

DEFINITION

Lisinopril contains NLT 98.0% and NMT 102.0% of lisinopril ($C_{21}H_{31}N_3O_5$), calculated on the anhydrous basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 2.76 g/L of monobasic sodium phosphate in water prepared as follows. Dissolve 2.76 g of monobasic sodium phosphate in about 900 mL of water in a 1000-mL volumetric flask. Adjust with 1 N sodium hydroxide to a pH of 5.0 and dilute with water to volume.
Mobile phase: Acetonitrile and *Solution A* (4:96)
Standard solution: 0.3 mg/mL of USP Lisinopril RS in water
Sample solution: 0.3 mg/mL of Lisinopril in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.7
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lisinopril ($C_{21}H_{31}N_3O_5$) in the portion of Lisinopril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lisinopril from the *Sample solution*
r_S = peak response of lisinopril from the *Standard solution*
C_S = concentration of USP Lisinopril RS in the *Standard solution* (mg/mL)
C_U = concentration of Lisinopril in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer: 3.53 g/L of monobasic sodium phosphate dihydrate in water adjusted with phosphoric acid to a pH of 4.1
Solution A: Acetonitrile and *Buffer* (7:193)
Solution B: Acetonitrile and *Buffer* (20:80)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	60	40
55	60	40
60	100	0

Standard solution: 0.006 mg/mL of USP Lisinopril RS in *Solution A*
Sensitivity solution: 1.0 µg/mL of USP Lisinopril RS in *Solution A* from *Standard solution*
Sample solution: 2 mg/mL of Lisinopril in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 45°
Flow rate: 1.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Relative standard deviation: NMT 10.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Lisinopril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of lisinopril from the *Standard solution*
C_S = concentration of USP Lisinopril RS in the *Standard solution* (mg/mL)
C_U = concentration of Lisinopril in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Alkyl-L-lysine[a]	0.57	0.35	0.3
DL-Homophenylalanine[b]	0.72	1.08	0.30
Lisinopril	1.00	1.00	—
Lisinopril epimer[c]	1.33	0.76	0.3
Lisinopril cyclohexyl analog[d]	2.93	0.39	0.30
R,S,S-Diketopiperazine[e]	3.88	0.79	0.3
S,S,S-Diketopiperazine (lisinopril related compound A)[f]	4.04	0.76	0.3
N-Alkyl lisinopril[g]	4.60	0.86	0.15
Any individual unspecified impurity	—	1.00	0.1

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities[h]	—	—	0.5

[a] [(*S*)-1-Carboxy-3-phenylpropyl]-L-lysine.
[b] 2-Amino-4-phenylbutanoic acid.
[c] [(*R*)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline.
[d] [(*S*)-1-Carboxy-3-cyclohexylpropyl]-L-lysyl-L-proline.
[e] (*S*)-2-[(3*S*,8a*R*)-3-(4-Aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid.
[f] (*S*)-2-[(3*S*,8a*S*)-3-(4-Aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid.
[g] N^2,N^6-Bis[(*S*)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline.
[h] Total impurities does not include lisinopril epimer.

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Specific Rotation*
  Diluent: 0.25 M zinc acetate solution prepared as follows. Mix 600 mL of water with 150 mL of glacial acetic acid and 54.9 g of zinc acetate, and stir to dissolve the zinc acetate. While stirring, add 150 mL of ammonium hydroxide, cool to room temperature, and adjust with ammonium hydroxide to a pH of 6.4. Transfer the solution to a 1000-mL volumetric flask, and dilute with water to volume.
  Sample solution: 10 mg/mL of Lisinopril in *Diluent*
  Acceptance criteria: −115.3° to −122.5° (λ = 405 nm)
- **WATER DETERMINATION** ⟨921⟩, *Method I*: 8.0%–9.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lisinopril RS

Lisinopril Compounded Oral Suspension

DEFINITION
Lisinopril Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$).
Prepare Lisinopril Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Lisinopril tablets[a] equivalent to	100 mg of lisinopril
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Prinivil 10-mg tablets, Merck & Co., West Point, PA.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Lisinopril tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a lisinopril liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY
- **PROCEDURE**
  Solution A: 4.1 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0.
  Mobile phase: 1.0 g/L of sodium 1-hexanesulfonate in acetonitrile and *Solution A* (18:82). Filter and degas.
  Diluent: Methanol and water (20:80)
  Standard solution: 0.2 mg/mL of USP Lisinopril RS in *Diluent*
  Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Mix 1.0 mL of Oral Suspension with 4.0 mL of *Diluent* to obtain a solution having a nominal concentration of 0.2 mg/mL of lisinopril.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 215 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Column temperature: 40°
  Flow rate: 1.0 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The retention time for lisinopril is about 12.9 min.]
  Suitability requirements
  Column efficiency: NLT 1800 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0% for replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lisinopril RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lisinopril in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **PH** ⟨791⟩: 4.3–5.3

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lisinopril RS

Lisinopril Tablets

» Lisinopril Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{31}N_3O_5$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Lisinopril RS

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Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Determine the amount of lisinopril dissolved using the following method.
Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay*.
Determine the amount of lisinopril dissolved by one of the following procedures.
PROCEDURE FOR POOLED SAMPLE—Proceed as directed for *Procedure* in *Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms* under *Dissolution* ⟨711⟩. Combine equal volumes of the filtered solutions of the 6 or 12 individual specimens withdrawn, and use the pooled sample as the test solution. Inject a volume of the pooled sample into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of $C_{21}H_{31}N_3O_5$ dissolved in comparison with a Standard solution having a known concentration of USP Lisinopril RS in the same *Medium* and similarly chromatographed.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{21}H_{31}N_3O_5$ in the Tablets is dissolved in 30 minutes: the requirements are met if the quantities of active ingredient dissolved from the pooled sample conform to the accompanying *Acceptance Table for a Pooled Sample*. Continue testing through the three stages unless the results conform at either S_1 or S_2. The quantity, *Q*, is the amount of dissolved active ingredient specified, expressed as a percentage of the labeled content.

Acceptance Table for a Pooled Sample

Stage	Number Tested	Acceptance Criteria
S_1	6	Average amount dissolved is not less than $Q + 10\%$.
S_2	6	Average amount dissolved ($S_1 + S_2$) is equal to or greater than $Q + 5\%$.
S_3	12	Average amount dissolved ($S_1 + S_2 + S_3$) is equal to or greater than *Q*.

PROCEDURE FOR UNIT SAMPLE—Proceed as directed for *Procedure* in *Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms* under *Dissolution* ⟨711⟩. Inject a volume of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the amount of $C_{21}H_{31}N_3O_5$ dissolved in comparison with a Standard solution having a known concentration of USP Lisinopril RS in the *Medium* and similarly chromatographed.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{21}H_{31}N_3O_5$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—
Phosphate solution, Mobile phase, and Chromatographic system—Prepare as directed in the *Assay*.
Diluent—Dissolve 2.72 g of monobasic potassium phosphate in 800 mL of water, adjust with phosphoric acid to a pH of 4.0, dilute with water to 1000 mL, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Lisinopril RS in *Diluent* to obtain a solution having a known concentration of about 0.2 mg per mL.
Test preparation—Place one Tablet in a volumetric flask of appropriate size, based on the labeled quantity, in mg, of lisinopril in the Tablet, to obtain a solution containing 0.2 mg of lisinopril per mL. Fill the flask to about 50% volume with *Diluent*, sonicate for 5 minutes, and shake by mechanical means for 20 minutes. Dilute with *Diluent* to volume, mix, and filter.
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{31}N_3O_5$ in the Tablet taken by the formula:

$$(TC/D)(r_U/r_S)$$

in which *T* is the labeled quantity, in mg, of lisinopril in the Tablet; *C* is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Lisinopril RS in the *Standard preparation; D* is the concentration, in mg per mL, of lisinopril in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and r_U and r_S are the lisinopril peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.

Related compounds—
Phosphate solution, Mobile phase, Diluent, and *Chromatographic system*—Prepare as directed in the *Assay*.
Standard solution—Dilute the *Standard preparation*, prepared as directed in the *Assay*, with *Diluent* to obtain a solution having a known concentration of about 20 µg per mL.
Test solution—Use the *Assay preparation*.
Procedure—Proceed as directed in the *Assay*. Measure the responses of the lisinopril peak obtained from the *Standard solution*, and of all peaks other than that of lisinopril obtained from the *Test solution*. Calculate the percentage of related compounds in each Tablet taken by the formula:

$$100(V/10)(C/L)(r_U/r_S)$$

in which *V* is the volume, in mL, of the *Test solution; C* is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Lisinopril RS in the *Standard solution; L* is the quantity, in mg, of lisinopril in each Tablet, taken as determined in the *Assay; r_U* is the sum of all peak responses other than that of lisinopril obtained from the *Test solution;* and r_S is the lisinopril peak response obtained from the *Standard solution:* not more than 2.0%, calculated on the basis of the quantity, in mg, of lisinopril in the portion of Tablets taken, as determined in the *Assay*, is found.

Assay—
Phosphate solution—Dissolve 4.1 g of monobasic potassium phosphate in about 900 mL of water in a 1000-mL volumetric flask, and adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, and mix.
Mobile phase—Dissolve 1.0 g of sodium 1-hexanesul fonate in 820 mL of *Phosphate solution*. Add 180 mL of acetonitrile, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Prepare a mixture of water and methanol (4:1).
Standard preparation—Dissolve an accurately weighed quantity of USP Lisinopril RS in *Diluent* to obtain a solution having a known concentration of about 0.2 mg per mL.
Assay preparation—Transfer to a suitable size volumetric flask 10 Tablets, which when diluted with *Diluent* will yield a solution having a concentration of about 0.2 mg per mL. Add *Diluent*, and sonicate for 5 minutes. Shake the flask by mechanical means for 20 minutes, dilute with *Diluent* to volume, mix, and filter.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 20-cm column that contains packing

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L7 and is maintained at a temperature of 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 700 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; the capacity factor, *k'*, for the analyte peak is greater than 1.5; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{31}N_3O_5$ in each Tablet taken by the formula:

$$(L/D)C(r_U/r_S)$$

in which L is the labeled quantity, in mg, of lisinopril in each Tablet, D is the concentration, in mg per mL, of lisinopril in the *Assay preparation* based on the labeled quantity per Tablet and the extent of dilution; C is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Lisinopril RS in the *Standard preparation;* and r_U and r_S are the lisinopril peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Lisinopril and Hydrochlorothiazide Tablets

DEFINITION

Lisinopril and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

The Tablets must be assayed within 24 h of the time they are put in the solution.

Buffer: Dissolve 4.08 g of monobasic potassium phosphate in 800 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute with water to 1 L.

Mobile phase: Acetonitrile, triethylamine, water, and phosphoric acid (280:3:1480:15)

Lisinopril standard stock solution: 0.5 mg/mL of USP Lisinopril RS in *Buffer*

Hydrochlorothiazide standard stock solution: 3.12 mg/mL of USP Hydrochlorothiazide RS in methanol

Lisinopril related compound A stock solution: 0.05 mg/mL of USP Lisinopril Related Compound A RS in methanol

Benzothiadiazine related compound A stock solution: 0.016 mg/mL of USP Benzothiadiazine Related Compound A RS in methanol

Standard solution: 0.1 mg/mL of USP Lisinopril RS, 0.125 mg/mL of USP Hydrochlorothiazide RS, 2 µg/mL of USP Lisinopril Related Compound A RS, and 1.3 µg/mL of USP Benzothiadiazine Related Compound A RS in *Buffer* from *Lisinopril standard stock solution, Hydrochlorothiazide standard stock solution, Lisinopril related compound A stock solution,* and *Benzothiadiazine related compound A stock solution.* For Tablet strengths 20/12.5 of lisinopril/hydrochlorothiazide, the concentration of USP Lisinopril RS and USP Lisinopril Related Compound A RS in the *Standard solution* is 0.2 mg/mL and 4 µg/mL, respectively.

Sample stock solution: Transfer 10 Tablets to a suitable volumetric flask. Add *Buffer* (0.25 mL/mg of total lisinopril), sonicate for 5 min, and then add methanol (0.5 mL/mg of total lisinopril). Sonicate for an additional 10 min. Add more *Buffer* (0.75 mL/mg of total lisinopril), and mix by mechanical means for 20 min. Dilute with water to volume to prepare solutions as described in *Table 1.*

Table 1

Tablet Strength of Lisinopril/ Hydrochlorothiazide (mg/Tablet)	Nominal Concentration of Lisinopril/ Hydrochlorothiazide (mg/mL)
10/12.5	0.4/0.5
20/12.5	0.4/0.25
20/25	0.4/0.5

Sample solution: Dilute the *Sample stock solution* with *Buffer* to prepare solutions as described in *Table 2.* Pass a portion through a suitable filter of 0.45-µm pore size.

Table 2

Tablet Strength of Lisinopril/ Hydrochlorothiazide (mg/Tablet)	Nominal Concentration of Lisinopril/ Hydrochlorothiazide (mg/mL)
10/12.5	0.1/0.12
20/12.5	0.2/0.125
20/25	0.1/0.12

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: NLT 10 min

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between lisinopril and benzothiadiazine related compound A and NLT 4.0 between hydrochlorothiazide and benzothiadiazine related compound A

Tailing factor: NMT 2 for both the lisinopril and hydrochlorothiazide peaks

Relative standard deviation: NMT 2.0% for both the lisinopril and hydrochlorothiazide peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lisinopril or hydrochlorothiazide from the *Sample solution*

r_S = peak response of lisinopril or hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Lisinopril RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of lisinopril or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min for hydrochlorothiazide; 30 min for lisinopril

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

Lisinopril standard stock solution: 0.5 mg/mL of USP Lisinopril RS in *Buffer*

Hydrochlorothiazide standard stock solution: 0.44 mg/mL of USP Hydrochlorothiazide RS in methanol

Standard solution: Prepare solutions in *Medium* as described in *Table 3* from the *Lisinopril standard stock solution* and *Hydrochlorothiazide standard stock solution*.

Table 3

Tablet Strength of Lisinopril/ Hydrochlorothiazide (mg/Tablet)	Nominal Concentration of Lisinopril/ Hydrochlorothiazide (mg/mL)
10/12.5	0.01/0.013
20/12.5	0.02/0.013
20/25	0.02/0.026

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first few mL of the filtrate.

Chromatographic system: Proceed as directed in the *Assay*, except use an injection volume of 20 µL.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between the lisinopril and hydrochlorothiazide peaks

Tailing factor: NMT 2 for both the lisinopril and hydrochlorothiazide peaks

Column efficiency: NLT 6000 theoretical plates for the hydrochlorothiazide peak

Relative standard deviation: NMT 2.0% for both the lisinopril and hydrochlorothiazide peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of lisinopril or hydrochlorothiazide from the *Sample solution*

r_S = peak response of lisinopril or hydrochlorothiazide from the *Standard solution*

C_S = concentration of lisinopril and hydrochlorothiazide in the *Standard solution* from *Table 3* (mg/mL)

L = labeled amount of lisinopril and hydrochlorothiazide (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times: 30 min for both lisinopril and hydrochlorothiazide

Buffer: 2.76 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (6:94)

Standard stock solution 1 (for Tablets labeled to contain 10 mg/12.5 mg or 20 mg/25 mg of lisinopril/hydrochlorothiazide): 0.11 mg/mL of USP Lisinopril RS and 0.14 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Add acetonitrile to fill 1.5% of the total volume, sonicate until dissolved, and dilute with *Medium* to volume.

Standard stock solution 2 (for Tablets labeled to contain 20 mg/12.5 mg of lisinopril/hydrochlorothiazide): 0.22 mg/mL of USP Lisinopril RS and 0.14 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Add acetonitrile to fill 1.5% of the total volume, sonicate until dissolved, and dilute with *Medium* to volume.

Standard solution: Prepare solutions, in *Medium*, of lisinopril and hydrochlorothiazide as described in *Table 4* from either *Standard stock solution 1* or *Standard stock solution 2*.

Table 4

Tablet Strength of Lisinopril/ Hydrochlorothiazide (mg/Tablet)	Nominal Concentration of Lisinopril/ Hydrochlorothiazide (mg/mL)
10/12.5	0.011/0.014
20/12.5	0.022/0.014
20/25	0.022/0.028

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 20-cm; 5-µm packing L7

Column temperature: 50°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8 for the lisinopril peak and NMT 1.5 for the hydrochlorothiazide peak

Resolution: NLT 3.5 between the lisinopril and hydrochlorothiazide peaks

Relative standard deviation: NMT 2.0% for both peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of lisinopril or hydrochlorothiazide from the *Sample solution*

r_S = peak response of lisinopril or hydrochlorothiazide from the *Standard solution*

C_S = concentration of lisinopril or hydrochlorothiazide in the *Standard solution* (mg/mL)

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L = labeled amounts of lisinopril or hydrochlorothiazide (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$) and NLT 75% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Buffer, Mobile phase, Benzothiadiazine related compound A stock solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Standard solution 1: Use the *Standard solution*, prepared as directed in the *Assay*.

Standard solution 2: 1.28 µg/mL of USP Benzothiadiazine Related Compound A RS in *Buffer* from the *Benzothiadiazine related compound A stock solution*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of lisinopril related compound A and benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of lisinopril related compound A or benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of lisinopril related compound A or benzothiadiazine related compound A from *Standard solution 1* or *Standard solution 2*
C_S = concentration of USP Lisinopril Related Compound A RS in *Standard solution 1* or USP Benzothiadiazine Related Compound A RS in *Standard solution 2* (mg/mL)
C_U = nominal concentration of lisinopril or hydrochlorothiazide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lisinopril, 405.5, for calculating the percentage of lisinopril related compound A; or molecular weight of hydrochlorothiazide, 297.74, for calculating the percentage of benzothiadiazine related compound A
M_{r2} = molecular weight of lisinopril related compound A, 387.47; or benzothiadiazine related compound A, 285.73

Acceptance criteria

Individual impurities: NMT 2% of lisinopril related compound A; NMT 1% of benzothiadiazine related compound A

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.

• **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP Reference Standards** ⟨11⟩

USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73

USP Hydrochlorothiazide RS

USP Lisinopril RS

USP Lisinopril Related Compound A RS
(*S*)-2-{(3*S*,8a*S*)-3-(4-Aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazin-2(1*H*)-yl}-4-phenylbutanoic acid.
$C_{21}H_{29}N_3O_4$ 387.47

Lithium Oral Solution

DEFINITION

Lithium Oral Solution is prepared from Lithium Citrate or Lithium Hydroxide to which an excess of Citric Acid has been added. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lithium (Li).

IDENTIFICATION

• **A.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. Identification Tests—General,** *Citrate* ⟨191⟩: Meets the requirements

ASSAY

• **Procedure**

Surfactant solution: 1%–2% solution of nonionic surfactant, such as t-dodecyl mercaptan ethoxylate or polyoxyethylene (20) sorbitan monolaurate, in water

Standard stock solution: 0.3 mg/mL of USP Lithium Carbonate RS prepared as follows. Transfer the required quantity of USP Lithium Carbonate RS to a suitable volumetric flask, and add 20% of the flask volume of water and 0.5% of the flask volume of hydrochloric acid. Shake until dissolved.

Standard solution: 6 µg/mL of USP Lithium Carbonate RS from the *Standard stock solution* prepared as follows. Transfer a suitable volume of the *Standard stock solution* to a suitable volumetric flask. Add 80% of the flask volume of water and 2% of the flask volume of *Surfactant solution*, and dilute with water to volume. Determine the pH of the solution.

Sample stock solution: Nominally 0.06 mg/mL of lithium in water prepared as follows. Transfer a volume of Oral Solution equivalent to NLT 60 mg of lithium to a suitable volumetric flask. Dilute with water to volume.

Sample solution: Nominally 1.2 µg/mL of lithium from the *Sample stock solution* prepared as follows. Transfer a suitable volume of the *Sample stock solution* to a suitable volumetric flask. Add 95% of the flask volume of water, 0.2% of the flask volume of 1 N hydrochloric acid, and 2% of the flask volume of the *Surfactant solution*. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to the same pH (±0.1 pH unit) as that of the *Standard solution*, and dilute with water to volume.

Blank: *Surfactant solution*

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: About 671 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of lithium (Li) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (A_r/M_r) \times F \times 100$$

r_U = photometer reading of the *Sample solution*
r_S = photometer reading of the *Standard solution*

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C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of lithium in the *Sample solution* (µg/mL)
A_r = atomic weight of lithium, 6.94
M_r = molecular weight of lithium carbonate, 73.89
F = number of lithium ions in one mole of lithium carbonate, 2

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements for oral solution packaged in single-unit containers

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lithium Carbonate RS

Lithium Carbonate

Li_2CO_3 73.89
Carbonic acid, dilithium salt;
Dilithium carbonate [554-13-2].

DEFINITION
Lithium Carbonate contains NLT 99.0% of lithium carbonate (Li_2CO_3), calculated on the dried basis.

IDENTIFICATION
- **A.** It effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately forms a white precipitate.
- **B.** When moistened with hydrochloric acid, it imparts an intense crimson color to a nonluminous flame.

ASSAY
- **PROCEDURE**

Sample solution: Dissolve 0.5 g of Lithium Carbonate in 25.0 mL of 1 N hydrochloric acid VS.
Blank: 25.0 mL of 1 N hydrochloric acid VS
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 1 N sodium hydroxide VS
Endpoint detection: Visual
Indicator: Methyl orange TS
Analysis
Samples: *Sample solution* and *Blank*
Titrate the excess acid in the *Sample solution* with *Titrant*. Calculate the percentage of lithium carbonate (Li_2CO_3) in the portion of Lithium Carbonate taken:

$$\text{Result} = (V_B - V_S) \times N \times F \times (1/W) \times 100$$

V_B = *Titrant* volume consumed by the *Blank* (mL)
V_S = *Titrant* volume consumed by the *Sample solution* (mL)
N = normality of *Titrant* (mEq/mL)
F = equivalent weight of Lithium Carbonate, 36.95 mg/mEq
W = weight of Lithium Carbonate in the *Sample solution* (mg)

Acceptance criteria: NLT 99.0% on the dried basis

IMPURITIES
- **ALUMINUM AND IRON**

Sample solution: Dissolve 500 mg of Lithium Carbonate in 10 mL of water by the dropwise addition, with agitation, of hydrochloric acid.
Analysis: Boil the *Sample solution*, then cool it. To 5 mL of the solution add 6 N ammonium hydroxide until the reaction is alkaline.
Acceptance criteria: No turbidity or precipitate is observed.
- **CALCIUM**

Sample solution: Suspend 5.0 g of Lithium Carbonate in 50 mL of water, and add a slight excess of 3 N hydrochloric acid. Boil the clear solution to expel carbon dioxide, add 5 mL of ammonium oxalate TS, render alkaline with 6 N ammonium hydroxide, and allow to stand for 4 h. Pass through a filtering crucible, and wash with warm water until the last washing yields no turbidity with calcium chloride TS. Place the crucible in a beaker, cover the crucible with water, add 3 mL of sulfuric acid, and heat to 70°.
Analysis: Titrate the *Sample solution* with 0.10 N potassium permanganate to a pale pink color that persists for 30 s.
Acceptance criteria: NMT 3.8 mL of 0.10 N potassium permanganate is consumed (0.15%).
- **SODIUM**

Standard stock solution: 500 µg/mL of sodium prepared as follows. Dissolve 1.271 g of sodium chloride, previously dried at 130° to constant weight, in water in a 1000-mL volumetric flask. Dilute with water to volume.
Sample stock solution: 100 mg/mL of Lithium Carbonate prepared as follows. Suspend 20.0 g of Lithium Carbonate in 100 mL of water, cautiously add 50.0 mL of hydrochloric acid, transfer to a 200-mL volumetric flask, and dilute with water to volume.
Standard solution: Transfer 1 mL of *Standard stock solution* and 5 mL of *Sample stock solution* to a 100-mL volumetric flask, and dilute with water.
Sample solution: 5 mg/mL of Lithium Carbonate from *Sample stock solution* diluted with water
Instrumental conditions
Mode: Flame photometry
Analytical wavelengths: 580 and 589 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Set the flame photometer for maximum emission at 589 nm, using the *Standard solution*. Measure the emission intensities of the *Sample solution* at 580 and 589 nm.
Acceptance criteria: The difference between the intensities observed at 580 and 589 nm for the *Sample solution* does not exceed the difference between the intensities observed at 589 nm for the *Sample solution* and the *Standard solution*, respectively (0.1%).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Standard solution: Transfer 1 mL of 0.020 N sulfuric acid and 1 mL of 3 N hydrochloric acid to a suitable container. Dilute with water to 40 mL.
Sample solution: Transfer 1.0 g of Lithium Carbonate to a suitable container. Dissolve 10 mL of 3 N hydrochloric acid. Dilute with water to 40 mL.
Analysis: To the *Standard solution* and the *Sample solution*, separately, add 1 mL of barium chloride TS.
Acceptance criteria: The turbidity produced in the *Sample solution*, after 3 min, is NMT that produced in the *Standard solution* (0.1%).

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• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard solution: Transfer 0.5 mL of 0.02 N hydrochloric acid and 1.2 mL of nitric acid to a suitable container. Dilute with water to 50 mL.
Sample solution: Transfer 500 mg of Lithium Carbonate to a suitable container. Add 1.2 mL of nitric acid. Dilute with water to 50 mL.
Analysis: To the *Standard solution* and the *Sample solution,* separately, add 1 mL of silver nitrate TS.
Acceptance criteria: The turbidity produced in the *Sample solution* is NMT that produced in the *Standard solution* (0.07%).

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 200° for 4 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Lithium Carbonate Capsules

DEFINITION
Lithium Carbonate Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of lithium carbonate (Li_2CO_3).

IDENTIFICATION
• **A.** A portion of the Capsule contents effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately forms a white precipitate.
• **B.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Surfactant solution: 1%–2% (v/v) solution of nonionic surfactant such as t-dodecyl mercaptan ethoxylate or polyoxyethylene (20) sorbitan monolaurate in water
Blank: *Surfactant solution* and water (1:50)
Standard stock solution: 0.3 mg/mL of USP Lithium Carbonate RS prepared as follows. Transfer the required quantity of USP Lithium Carbonate RS to a suitable volumetric flask, and add water to 20% of the flask volume and hydrochloric acid to 0.5% of the flask volume. Shake until dissolved, and dilute with water to volume.
Standard solution: 0.006 mg/mL of USP Lithium Carbonate RS from *Standard stock solution* prepared as follows. Pipet a suitable volume of *Standard stock solution* into a suitable volumetric flask. Add water to 80% of the flask volume and *Surfactant solution* to 2% of the flask volume, and dilute with water to volume.
Sample stock solution: Nominally 0.6 mg/mL of lithium carbonate from the contents of NLT 20 Capsules prepared as follows. Transfer a portion of powder, equivalent to NLT 600 mg of lithium carbonate, into a suitable volumetric flask. Add water to 4% of the flask volume and hydrochloric acid to 0.5% of the flask volume, shake until the solid is well disintegrated, and dilute with water to volume.
Sample solution: Nominally 0.006 mg/mL of lithium carbonate from *Sample stock solution* prepared as follows. Pipet a suitable volume of *Sample stock solution* into a suitable volumetric flask. Add water to 80% of the flask volume and *Surfactant solution* to 2% of the flask volume, and dilute with water to volume.
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: About 671 nm
Analysis
Samples: *Blank, Standard solution,* and *Sample solution*
Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and *Sample solution.*
Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = emission response from the *Sample solution*
r_S = emission response from the *Standard solution*
C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Surfactant solution, Blank, and **Standard solution:** Prepare as directed in the *Assay.*
Sample solution: Pass the solution under test through a suitable filter. Transfer 20.0 mL of the filtrate to a 1000-mL volumetric flask. Add 500 mL of water, 1 drop of hydrochloric acid, and 20 mL of *Surfactant solution.* Dilute with water to volume.
Analysis
Samples: *Blank, Standard solution,* and *Sample solution*
Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and *Sample solution.*
Calculate percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

r_U = emission response from the *Sample solution*
r_S = emission response from the *Standard solution*
C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
V = volume of the *Medium,* 900 mL
D = dilution factor
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of lithium carbonate (Li_2CO_3) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lithium Carbonate RS

Lithium Carbonate Tablets

DEFINITION
Lithium Carbonate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of lithium carbonate (Li_2CO_3).

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IDENTIFICATION

A portion of the powdered Tablets meets the requirements of the following tests.

- **A.** It effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately forms a white precipitate.
- **B.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Surfactant solution: 1%–2% solution of nonionic surfactant such as t-dodecyl mercaptan ethoxylate or polyoxyethylene (20) sorbitan monolaurate in water

Blank: *Surfactant solution* and water (1:50)

Standard solution: Transfer 30 mg of USP Lithium Carbonate RS to a 100-mL volumetric flask, and add 20 mL of water and 0.5 mL of hydrochloric acid. Shake until dissolved, and dilute with water to volume. Pipet 20 mL of the resulting solution into a 1000-mL volumetric flask, add 800 mL of water and 20 mL of a suitable surfactant solution, and dilute with water to volume.

Sample solution: Powder NLT 20 Tablets. Transfer a portion of powder, nominally equivalent to 600 mg of lithium carbonate, into a 1000-mL volumetric flask. Add 40 mL of water and 5 mL of hydrochloric acid, shake until the solid is well disintegrated, and dilute with water to volume. Pipet 10 mL of the resulting solution into a 1000-mL volumetric flask, add 800 mL of water and 20 mL of the surfactant solution, and dilute with water to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelength: About 671 nm

[NOTE—Adjust the instrument with the *Surfactant solution*.]

Analysis

Samples: *Blank*, *Standard solution*, and *Sample solution*

Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of lithium carbonate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Surfactant solution, Blank, and **Standard solution:** Proceed as directed in the *Assay*.

Sample solution: Dilute 900 mL of the solution under test with *Medium* to 1000 mL. Pass through a suitable filter. Transfer 20.0 mL of the filtrate to a 1000-mL volumetric flask. Add 500 mL of water, 1 drop of hydrochloric acid, and 20 mL of *Surfactant solution*. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelength: About 671 nm

Analysis

Samples: *Blank*, *Standard solution*, and *Sample solution*

Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of lithium carbonate (Li_2CO_3) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from moisture. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Lithium Carbonate RS

Lithium Carbonate Extended-Release Tablets

DEFINITION

Lithium Carbonate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lithium carbonate (Li_2CO_3).

IDENTIFICATION

- **A.** A portion of powdered Tablets effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately forms a white precipitate.
- **B.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Dilute 5 mL of hydrochloric acid with water to 1 L.

Standard solution: 0.3 mg/mL of USP Lithium Carbonate RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Lithium Carbonate RS to an appropriate volumetric flask. Add water to 20% of the flask volume, and hydrochloric acid to 0.5% of the flask volume. Shake until dissolved, and dilute with water to volume.

Sample stock solution: Nominally 12 mg/mL of lithium carbonate from a number of Tablets, nominally equivalent to NLT 1200 mg of lithium carbonate prepared as follows. Transfer the required number of Tablets to a suitable container. Add the required amount of *Diluent*. Shake until completely dissolved. Filter, and discard the first 25 mL. Use the remaining filtrate.

Sample solution: Nominally 0.3 mg/mL from *Sample stock solution* in *Diluent*

[NOTE—The *Standard solution* and *Sample solution* may be diluted quantitatively with *Diluent*, if necessary, to yield solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: About 671 nm

Analysis

Samples: *Standard solution* and *Sample solution*

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Use the *Diluent* to zero the instrument. Measure the emission responses for the *Standard solution* and the *Sample solution.*

Calculate the percentage of labeled amount of lithium carbonate (Li_2CO_3) in the portion of Tablets:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times D \times 100$$

r_U = emission response from the *Sample solution*
r_S = emission response from the *Standard solution*
C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lithium carbonate in the *Sample solution* (mg/mL)
D = dilution factor, if needed

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Dilute hydrochloric acid (7 in 1000); 800 mL
Apparatus 1: 100 rpm
Time: 15, 45, 90, and 120 min
Standard solution: USP Lithium Carbonate RS at a known concentration in *Medium*
Sample solution: At each time point *i*, withdraw 8.0 mL of the *Sample solution,* and pass through a filter of 35-µm or finer pore size. Use the filtrate as the *Sample solution,* suitably diluted with *Medium* if necessary.
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: About 671 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Use the *Medium* to zero the instrument. Measure the emission responses for the *Standard solution* and the *Sample solution.*
Calculate the concentration, C_i, of lithium carbonate (Li_2CO_3) in *Medium* (mg/mL) at each time point *i*:

$$C_i = (r_U/r_S) \times C_S$$

r_U = emission response from the *Sample solution*
r_S = emission response from the *Standard solution*
C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount (Q_i) of lithium carbonate (Li_2CO_3) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_i = (\{C_i \times [V - (i-1) \times V_S]\} + [(C_{i-1} + C_{i-2} \ldots + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of lithium carbonate in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium,* 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 1.*

Table 1

Time Point (*i*)	Time (min)	Amount Dissolved (%)
1	15	2–16
2	45	25–45
3	90	60–85
4	120	NLT 85

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 2: If the product complies with this procedure, the labeling indicates that it meets *USP Dissolution Test 2.*
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 1, 3, and 7 h
Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1.*
Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at each time point as described in *Test 1.*
Tolerances: See *Table 2.*

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 40
2	3	45–75
3	7	NLT 70

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 3: If the product complies with this test, the labeling indicates that it meets *USP Dissolution Test 3.*
Medium: Water; 250 mL
Apparatus 3: 6 dips/min, 20-mesh top screen and 100-mesh bottom screen
Time: 1, 2, and 6 h
Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1.*
Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at each time point as described in the *Test 1.*
Tolerances: See *Table 3.*

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	10–45
2	2	25–75
3	6	NLT 70

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 4: If the product complies with this procedure, the labeling indicates that it meets *USP Dissolution Test 4.*
Medium: Dilute hydrochloric acid (7 in 1000); 800 mL
Apparatus 1: 100 rpm
Time: 15, 45, 90, and 120 min
Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1.*

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Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at each time point as described in the *Test 1*.
Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (min)	Amount Dissolved (%)
1	15	NMT 15
2	45	20–45
3	90	50–80
4	120	NLT 70

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.
Test 5: If the product complies with this procedure, the labeling indicates that it meets *USP Dissolution Test 5*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30, 90, and 150 min
Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1*.
Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at each time point as described in the *Test 1*.
Tolerances: See *Table 5*.

Table 5

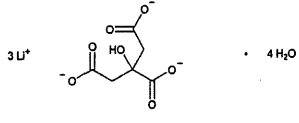
Time Point (*i*)	Time (min)	Amount Dissolved (%)
1	30	10–30
2	90	55–75
3	150	NLT 85%

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Protect from moisture. Store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lithium Carbonate RS

Lithium Citrate



$C_6H_5Li_3O_7 \cdot 4H_2O$ 281.98
$C_6H_5Li_3O_7$ 209.93

1,2,3-Propanetricarboxylic acid, 2-hydroxy-trilithium salt tetrahydrate;
Trilithium citrate tetrahydrate [6080-58-6].
Anhydrous [919-16-4].

DEFINITION

Lithium Citrate contains NLT 98.0% and NMT 102.0% of lithium citrate ($C_6H_5Li_3O_7$), calculated on the anhydrous basis.

IDENTIFICATION

• **A.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩: Meets the requirement

ASSAY

• **PROCEDURE**
Surfactant solution: 1%–2% solution of nonionic surfactant such as *t*-dodecyl mercaptan ethoxylate or polyoxyethylene (20) sorbitan monolaurate in water
Blank: *Surfactant solution* and water (1:50)
Standard stock solution: 0.3 mg/mL of USP Lithium Carbonate RS prepared as follows. Transfer the required quantity of USP Lithium Carbonate RS to a suitable volumetric flask, and add 20% of the flask volume of water and 0.5% of the flask volume of hydrochloric acid. Shake until dissolved, and dilute with water to volume.
Standard solution: 0.006 mg/mL of USP Lithium Carbonate RS from *Standard stock solution* prepared as follows. Pipet a suitable volume of *Standard stock solution* into a suitable volumetric flask. Add 80% of the flask volume of water and 2% of the flask volume of the *Surfactant solution*, and dilute with water to volume.
Sample stock solution: 0.8 mg/mL of Lithium Citrate prepared as follows. Transfer a suitable amount of Lithium Citrate to a suitable volumetric flask. Dissolve in water. Add 0.05% of the flask volume of hydrochloric acid. Dilute with water to volume.
Sample solution: Nominally 0.006 mg/mL of Lithium Citrate from *Sample stock solution* prepared as follows. Pipet a suitable volume of *Sample stock solution* into a suitable volumetric flask. Add 80% of the flask volume of water and 2% of the flask volume of the *Surfactant solution*, and dilute with water to volume.
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: 671 nm
Analysis
Samples: *Blank*, *Standard solution*, and *Sample solution*
Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and the *Sample solution*.
Calculate the percentage of lithium citrate ($C_6H_5Li_3O_7$) in the portion of Lithium Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F \times 100$$

r_U = emission response of the *Sample solution*
r_S = emission response of the *Standard solution*
C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
C_U = concentration of Lithium Citrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of anhydrous lithium citrate, 209.93
M_{r2} = molecular weight of lithium carbonate, 73.89

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F = ratio of the number of moles of lithium in 1 mol of lithium carbonate to that of the number of moles of lithium in 1 mol of lithium citrate, 0.66

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

SPECIFIC TESTS

- **CARBONATE**

Analysis: Add 0.5 g of Lithium Citrate to 5 mL of 6 N acetic acid.

Acceptance criteria: NMT a slight effervescence is produced.

- **pH** ⟨791⟩

Sample solution: 50-mg/mL solution of Lithium Citrate in water

Acceptance criteria: 7.0–10.0

- **WATER DETERMINATION,** *Method III* ⟨921⟩

Analysis: Dry a sample at 150° for 3 h.

Acceptance criteria: 24.0%–28.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Lithium Carbonate RS

Lithium Hydroxide

$LiOH \cdot H_2O$ 41.96
LiOH 23.95
Lithium hydroxide monohydrate [1310-66-3].
Anhydrous [1310-65-2].

DEFINITION

Lithium Hydroxide contains NLT 98.0% and NMT 102.0% of lithium hydroxide (LiOH), calculated on the anhydrous basis.
[**CAUTION**—Exercise great care in handling Lithium Hydroxide, as it rapidly destroys tissues.]

IDENTIFICATION

- **A.** When moistened with hydrochloric acid, it imparts an intense crimson color to a nonluminous flame.

ASSAY

- **PROCEDURE**

Sample solution: Nominally equivalent to 10 mg/mL of anhydrous lithium hydroxide from Lithium Hydroxide in carbon dioxide-free water

Analysis

Preliminary titration: Pipet 50 mL of the *Sample solution* into a 250-mL conical flask. Start the titration by adding 35 mL of 0.5 N hydrochloric acid VS with continuous vigorous stirring. Add 20 mL of 1 N barium chloride and 3 drops of phenolphthalein TS, and allow to stand for 2 min. Continue the titration with 0.5 N hydrochloric acid VS. At the discharge of the pink color of the indicator, record the volume of acid solution consumed.

Final titration: Pipet 50 mL of the *Sample solution* into a 250-mL conical flask. While pipeting and during the subsequent titrations, keep the contents of the flask blanketed with a stream of carbon dioxide-free air. Start the titration by adding with continuous vigorous swirling a volume of 0.5 N hydrochloric acid VS that is 0.50 mL less than that consumed in the preliminary titration. Add 20 mL of 1 N barium chloride and 3 drops of phenolphthalein TS, and allow to stand for 2 min. Rinse the sides of the flask with carbon dioxide-free water, and continue the titration with 0.1 N hydrochloric acid VS. At the discharge of the pink color of the indicator, record the volume of acid solution consumed. Each mL of 0.5 N hydrochloric acid VS and 0.1 N hydrochloric acid VS is equivalent to 11.975 and 2.395 mg of total alkali, respectively, calculated as lithium hydroxide (LiOH).

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample: 2.0 g

Acceptance criteria: It shows no more sulfate than corresponds to 1.0 mL of 0.020 N sulfuric acid (0.05%).

- **CALCIUM**

Sample solution: Dissolve 3.33 g in 50 mL of 3 N hydrochloric acid. Boil the clear solution to expel carbon dioxide, add 5 mL of ammonium oxalate TS, render alkaline with 6 N ammonium hydroxide, and allow to stand for 4 h. Pass through a filtering crucible, and wash with warm water until the last washing yields no turbidity with calcium chloride TS. Place the crucible in a beaker, cover it with water, add 3 mL of sulfuric acid, and heat to 70°.

Analysis: Titrate the *Sample solution* with 0.10 N potassium permanganate to a pale pink color that persists for 30 s.

Acceptance criteria: NMT 3.34 mL of 0.10 N potassium permanganate is consumed (0.20%).

- **CARBONATE**

[NOTE—While pipeting and during the subsequent titrations, keep the contents of the flasks blanketed with a stream of carbon dioxide-free air.]

Analysis: To the flask containing the completed *Final titration* obtained in the *Assay*, add 1 drop of methyl orange TS. Titrate with 0.1 N hydrochloric acid VS until a persistent orange color is produced and no undissolved barium carbonate remains. Perform a blank titration to determine the volume of 0.1 N hydrochloric acid consumed in going from the phenolphthalein endpoint to the methyl orange endpoint. To 100 mL of carbon dioxide-free water in a 250-mL conical flask, add 3 drops of the *Sample solution* from the *Assay*, 20 mL of 1 N barium chloride, and 3 drops of phenolphthalein TS. Allow to stand for 2 min. Titrate this solution with 0.1 N hydrochloric acid. At the discharge of the pink color of the indicator, add 1 drop of methyl orange TS, and titrate with 0.1 N hydrochloric acid VS until a persistent orange color is produced.

Acceptance criteria: The titration shows no more carbon dioxide than corresponds to 1.5 mL of 0.10 N hydrochloric acid (0.7%).

SPECIFIC TESTS

- **LITHIUM CONTENT**

Standard stock solution: 0.3 mg/mL of USP Lithium Carbonate RS prepared as follows. Dissolve first in water using 20% final volume and hydrochloric acid using 0.5% of final volume. Dilute with water to volume.

Standard solution: 6.0 µg/mL of USP Lithium Carbonate RS from the *Standard stock solution* prepared as follows. Pipet a volume of the *Standard stock solution* into a suitable volumetric flask, add water to fill 80% of final volume, and a suitable surfactant solution to fill 2% of final volume. Dilute with water to volume. Measure the pH.

Sample stock solution: 0.4 mg/mL of Lithium Hydroxide in water

Sample solution: Pipet 20 mL of the *Sample stock solution* into a 1000-mL volumetric flask. Add 950 mL of water, 2 mL of 1 N hydrochloric acid, and 20 mL of a surfactant solution, and mix. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to the same pH (±0.1 pH unit) as that of the *Standard solution*, and dilute with water to volume.

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Instrumental conditions
Mode: Flame photometry
Analytical wavelength: 671 nm. Adjust the instrument with the surfactant solution.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lithium (Li) in the portion of Lithium Hydroxide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (A_r/M_r) \times F \times 100$$

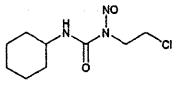
r_U = photometric reading of the *Sample solution*
r_S = photometric reading of the *Standard solution*
C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
C_U = concentration of Lithium Hydroxide in the *Sample solution* (mg/mL)
A_r = atomic weight of lithium, 6.94
M_r = molecular weight of lithium carbonate, 73.89
F = number of lithium ions per mole of lithium carbonate, 2

Acceptance criteria: 28.1%–29.9% on the anhydrous basis

• **WATER DETERMINATION,** *Method III* ⟨921⟩
Analysis: Dry at 135° at a pressure of NMT 5 mm of mercury for 1 h.
Acceptance criteria: 41.0%–43.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lithium Carbonate RS

Lomustine



$C_9H_{16}ClN_3O_2$ 233.70
Urea, *N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitroso-;
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea [13010-47-4].

DEFINITION
Lomustine contains NLT 97% and NMT 103% of lomustine ($C_9H_{16}ClN_3O_2$), calculated on the as-is basis.
[**CAUTION**—Great care should be taken to prevent inhalation of particles of lomustine and to prevent exposure to the skin.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
[NOTE—Protect the solutions from light. Use freshly prepared solutions.]
Mobile phase: Acetonitrile and water (35:65)
Standard solution: 0.2 mg/mL of USP Lomustine RS in acetonitrile
Sample solution: 0.2 mg/mL of Lomustine in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 75-mm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Tailing factor: NMT 1.3
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lomustine ($C_9H_{16}ClN_3O_2$) in the portion of Lomustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lomustine RS in the *Standard solution* (mg/mL)
C_U = concentration of Lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: 97%–103% on the as-is basis

IMPURITIES
• **ORGANIC IMPURITIES**
[NOTE—Protect the solutions from light. Use freshly prepared solutions.]
Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3	90	10
40	65	35
47	65	35
52	50	50
55	50	50
55.1	5	95
60	5	95
60.1	90	10
65	90	10

Standard solution A: 0.032 mg/mL each of USP Carmustine Related Compound A RS, USP Lomustine Related Compound B RS, and USP Lomustine Related Compound C RS, and 0.04 mg/mL of USP Lomustine Related Compound D RS, and 2 mg/mL of USP Lomustine RS in acetonitrile
Standard solution B: 8 µg/mL of USP Lomustine RS in acetonitrile
Sample solution: 8 mg/mL of Lomustine in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 and 230 nm
Column: 4.6-mm × 10-cm; 2.6-µm packing L43

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Temperatures
Column: 35°
Sample: 15°
Flow rate: 0.8 mL/min
Injection volume: 4 µL
System suitability
Samples: *Standard solution A* and *Standard solution B*
Suitability requirements
Resolution: NLT 1.2 between the lomustine and lomustine related compound D peaks determined at 230 nm, *Standard solution A*
Relative standard deviation: NMT 10% for carmustine related compound A and lomustine related compounds B and C determined at 195 nm, *Standard solution A*; NMT 10% for lomustine related compound D determined at 230 nm, *Standard solution A*; NMT 10% for lomustine determined at 230 nm, *Standard solution B*
Tailing factor: Between 0.7 and 1.3 for the lomustine peak determined at 230 nm, *Standard solution A*
Analysis
Samples: *Standard solution A, Standard solution B,* and *Sample solution*
Calculate the percentage of carmustine related compound A and lomustine related compounds B and C in the portion of Lomustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*, determined at 195 nm
r_S = peak area of the corresponding related compound from *Standard solution A*, determined at 195 nm
C_S = concentration of the corresponding USP Carmustine Related Compound A RS, USP Lomustine Related Compound B RS, or USP Lomustine Related Compound C RS in *Standard solution A* (mg/mL)
C_U = concentration of Lomustine in the *Sample solution* (mg/mL)

Detect at 230 nm, and compare the peak area of lomustine related compound D in the *Sample solution* with the peak area of lomustine related compound D in *Standard solution A*. The peak area of lomustine related compound D in the *Sample solution* is NMT the peak area of lomustine related compound D in *Standard solution A* (0.5%).
Calculate the percentage of any unspecified impurity in the portion of Lomustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of any unspecified impurity from the *Sample solution*, determined at 195 nm or 230 nm. If the impurity is detected at both wavelengths, use the higher peak area in the formula.
r_S = peak area of lomustine from *Standard solution B*, determined at 230 nm
C_S = concentration of USP Lomustine RS in *Standard solution B* (mg/mL)
C_U = concentration of Lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carmustine related compound A[a]	0.11	0.4[b]
Lomustine related compound B[c]	0.39	0.4[b]
Lomustine related compound C[d]	0.73	0.4[b]
Lomustine	1.0	—
Lomustine related compound D[e]	1.02	0.5
Any individual unspecified impurity	—	0.2
Total impurities[f]	—	1

[a] 1,3-Bis(2-chloroethyl)urea.
[b] No more than one such impurity (carmustine related compound A, lomustine related compound B, or lomustine related compound C) is greater than 0.2%.
[c] 1-(2-Chloroethyl)-3-cyclohexylurea.
[d] 1,3-Dicyclohexylurea.
[e] 3-(2-Chloroethyl)-1-cyclohexyl-1-nitrosourea.
[f] Lomustine related compound D is not included in the *Total impurities*.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store between 2° and 8°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carmustine Related Compound A RS
1,3-Bis(2-chloroethyl)urea.
$C_5H_{10}Cl_2N_2O$ 185.05
USP Lomustine RS
USP Lomustine Related Compound B RS
1-(2-Chloroethyl)-3-cyclohexylurea.
$C_9H_{17}ClN_2O$ 204.70
USP Lomustine Related Compound C RS
1,3-Dicyclohexylurea.
$C_{13}H_{24}N_2O$ 224.34
USP Lomustine Related Compound D RS
3-(2-Chloroethyl)-1-cyclohexyl-1-nitrosourea.
$C_9H_{16}ClN_3O_2$ 233.70

Lomustine Capsules

DEFINITION
Lomustine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of lomustine ($C_9H_{16}ClN_3O_2$).
[**CAUTION**—Great care should be taken to prevent inhalation of particles of lomustine and to prevent exposure to the skin.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer the contents of 2 Capsules to a stoppered Erlenmeyer flask containing 25 mL of methylene chloride. Shake vigorously, and filter. Evaporate the methylene chloride from the filtrate under a stream of dry nitrogen. Prepare a potassium bromide pellet of the residue.

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Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Protect the solutions from light. Use freshly prepared solutions.]

Mobile phase: Acetonitrile and water (7:13)

Standard solution: 0.2 mg/mL of USP Lomustine RS in acetonitrile

Sample solution: 0.2 mg/mL of lomustine in acetonitrile prepared as follows. Transfer a portion of the Capsule fill (from NLT 20 Capsules) to a suitable volumetric flask, and add acetonitrile equivalent to 75% of the volume. Shake for 15 min, and dilute with acetonitrile to volume. Pass through a suitable filter, and discard the first few mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 7.5-cm; 3-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Tailing factor: NMT 1.3

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lomustine ($C_9H_{16}ClN_3O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of lomustine in the *Standard solution* (mg/mL)

C_U = nominal concentration of lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

[NOTE—Protect the solutions from light. Use freshly prepared solutions.]

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3	90	10
40	65	35
47	65	35
52	50	50
55	50	50
55.1	5	95
60	5	95
60.1	90	10
65	90	10

Standard solution A: 0.032 mg/mL each of USP Carmustine Related Compound A RS, USP Lomustine Related Compound B RS, and USP Lomustine Related Compound C RS, and 0.04 mg/mL of USP Lomustine Related Compound D RS and 2 mg/mL of USP Lomustine RS in acetonitrile

Standard solution B: 8 µg/mL of USP Lomustine RS in acetonitrile

Sample solution: 8 mg/mL of lomustine in acetonitrile prepared as follows. Transfer a portion of the Capsule fill (from NLT 20 Capsules) to a suitable volumetric flask, and dilute with acetonitrile to volume. Sonicate for 30 min, and then stir for 30 min. Pass a portion of the solution through a suitable filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 195 and 230 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing L43

Temperatures

Column: 35°

Sample: 15°

Flow rate: 0.8 mL/min

Injection volume: 4 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.2 between the lomustine and lomustine related compound D peaks determined at 230 nm, *Standard solution A*

Relative standard deviation: NMT 10% for carmustine related compound A, lomustine related compounds B and C determined at 195 nm, *Standard solution A*; NMT 10% for lomustine determined at 230 nm, *Standard solution B*

Tailing factor: Between 0.7 and 1.3 for the lomustine peak determined at 230 nm, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of carmustine related compound A and lomustine related compounds B and C in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*, determined at 195 nm

r_S = peak area of the corresponding related compound from *Standard solution A*, determined at 195 nm

C_S = concentration of the corresponding USP Carmustine Related Compound A RS, USP Lomustine Related Compound B RS, or USP Lomustine Related Compound C RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of lomustine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Capsules taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of any unspecified impurity from the *Sample solution*, determined at 195 nm or 230 nm. If the impurity is detected at both wavelengths, use the higher peak area in the formula.
r_S = peak area of lomustine from *Standard solution B*, determined at 230 nm
C_S = concentration of USP Lomustine RS in the *Standard solution B* (mg/mL)
C_U = nominal concentration of lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carmustine related compound A[a]	0.11	0.4[b]
Lomustine related compound B[c]	0.39	0.4[b]
Lomustine related compound C[d]	0.73	0.4[b]
Lomustine	1.0	—
Lomustine related compound D[e]	1.02	—
Any individual unspecified impurity	—	0.2
Total impurities	—	2

[a] 1,3-Bis(2-chloroethyl)urea.
[b] No more than one such impurity (carmustine related compound A, lomustine related compound B, or lomustine related compound C) is greater than 0.2%.
[c] 1-(2-Chloroethyl)-3-cyclohexylurea.
[d] 1,3-Dicyclohexylurea.
[e] This process impurity is included in the table for identification only, and it is not to be reported or included in the *Total impurities*.

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩: 20 min
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Complete the analysis within 1 h after the preparation of *Sample solution*.

Standard solution: 0.02 mg/mL of USP Lomustine RS in methanol

Sample solution: Nominally equivalent to 0.02 mg/mL of lomustine in methanol prepared as follows. Transfer the content of a Capsule into a 100-mL volumetric flask, and dilute with methanol to volume. Allow the excipients to settle for at least 15 min. Transfer a measured volume of the clear supernatant to a suitable volumetric flask, and dilute to volume with methanol.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 230 nm

Cell length: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lomustine ($C_9H_{16}ClN_3O_2$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of USP Lomustine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Carmustine Related Compound A RS
1,3-Bis(2-chloroethyl)urea.
$C_5H_{10}Cl_2N_2O$ 185.05

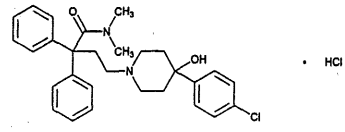
USP Lomustine RS

USP Lomustine Related Compound B RS
1-(2-Chloroethyl)-3-cyclohexylurea.
$C_9H_{17}ClN_2O$ 204.70

USP Lomustine Related Compound C RS
1,3-Dicyclohexylurea.
$C_{13}H_{24}N_2O$ 224.34

USP Lomustine Related Compound D RS
3-(2-Chloroethyl)-1-cyclohexyl-1-nitrosourea.
$C_9H_{16}ClN_3O_2$ 233.70

Loperamide Hydrochloride



$C_{29}H_{33}ClN_2O_2 \cdot HCl$ 513.50

1-Piperidinebutanamide, 4-(4-chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-, monohydrochloride;
4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide hydrochloride [34552-83-5].

DEFINITION

Loperamide Hydrochloride contains NLT 98.0% and NMT 102.0% NLT of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*, **197A** or **197K** ▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*

Sample solution: Dissolve about 30 mg of Loperamide Hydrochloride in 0.5 mL of methanol Add 1.5 mL of water and 1 mL of 6 N ammonium hydroxide. A precipitate forms. Centrifuge, decant, and acidify the supernatant with diluted nitric acid.

Acceptance criteria: Meets the requirements

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ASSAY

Change to read:

• **PROCEDURE**

▲Sample solution: Dissolve 400 mg of Loperamide Hydrochloride in 50 mL of alcohol and add 5.0 mL of 0.01 N hydrochloric acid.

Analysis: Titrate the *Sample solution* with 0.1 N sodium hydroxide VS (see *Titrimetry* ⟨541⟩), determining the endpoint potentiometrically. Read the volume of 0.1 N sodium hydroxide added between the two points of inflection. Each milliliter of 0.1 N sodium hydroxide is equivalent to 51.35 mg of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$). ▲ (USP 1-May-2020)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**

▲Solution A: 17.0 g/L of tetrabutylammonium hydrogen sulfate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	30	70
17	30	70

System suitability solution: 10 mg/mL of USP Loperamide System Suitability Mixture RS in methanol. See *Table 2* for the relative retention times of the main components of the mixture.

Standard solution: 20 µg/mL of USP Loperamide Hydrochloride RS in methanol

Sensitivity solution: 5 µg/mL of USP Loperamide Hydrochloride RS in methanol, from the *Standard solution*

Sample solution: 10 mg/mL of Loperamide Hydrochloride in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Peak-to-valley ratio: NLT 1.5 for loperamide related compounds G and H; NLT 1.5 for loperamide related compounds E and A, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Loperamide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of loperamide from the *Standard solution*

C_S = concentration of USP Loperamide Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Loperamide Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlorophenylpiperidinol	0.2	1.0	0.2
Deschloroloperamide	0.8	0.59	0.2
Loperamide	1.0	—	—
Loperamide *trans*-*N*-oxide	1.1	1.0	0.2
Loperamide *cis*-*N*-oxide	1.16	1.0	0.2
Anhydroloperamide	1.18	1.0	0.2
Loperamide piperidinolamide	1.3	1.0	0.2
Loperamide biphenyl analog	1.4	0.77	0.2
Loperamide quaternary salt	1.7	1.0	0.2
Any other individual impurity	—	1.0	0.1 0 ▲ (USP 1-May-2020)

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Loperamide Hydrochloride RS

▲USP Loperamide System Suitability Mixture RS

The mixture contains loperamide hydrochloride and the following impurities (other impurities may also be present):

Chlorophenylpiperidinol;

4-(4-Chlorophenyl)piperidin-4-ol.

$C_{11}H_{14}ClNO$ 211.69

Deschloroloperamide;

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4-(4-Hydroxy-4-phenylpiperidin-1-yl)-*N,N*-dimethyl-2,2-diphenylbutanamide.
$C_{29}H_{34}N_2O_2$ 442.59
Loperamide *trans*-*N*-oxide;
(1*r*,4*s*)-4-(4-Chlorophenyl)-1-[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidine 1-oxide.
$C_{29}H_{33}ClN_2O_3$ 493.04
Loperamide *cis*-*N*-oxide;
(1*s*,4*r*)-4-(4-Chlorophenyl)-1-[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidine 1-oxide.
$C_{29}H_{33}ClN_2O_3$ 493.04
Anhydroloperamide;
4-[4-(4-Chlorophenyl)-5,6-dihydropyridin-1(2*H*)-yl]-*N,N*-dimethyl-2,2-diphenylbutanamide.
$C_{29}H_{31}ClN_2O$ 459.02
Loperamide piperidinolamide;
1,4-Bis[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-2,2-diphenylbutan-1-one.
$C_{38}H_{40}Cl_2N_2O_3$ 643.64
Loperamide biphenyl analog;
4-[4-(4′-Chlorobiphenyl-4-yl)-4-hydroxypiperidin-1-yl]-*N,N*-dimethyl-2,2-diphenylbutanamide.
$C_{35}H_{37}ClN_2O_2$ 553.13
Loperamide quaternary salt;
4-(4-Chlorophenyl)-1,1-bis[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidin-1-ium chloride.
$C_{47}H_{53}Cl_2N_3O_3$ 778.85▲ (USP 1-May-2020)

Loperamide Hydrochloride Capsules

» Loperamide Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Loperamide Hydrochloride RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution—Transfer a quantity of the contents of the Capsules, equivalent to about 10 mg of loperamide hydrochloride, to a 37-mL stoppered vial, add 10 mL of methanol, shake for 5 minutes, and filter.
Standard solution: a solution of USP Loperamide Hydrochloride RS in methanol containing about 10 mg per mL.
Application volume: 10 µL of the *Test solution* and 1 µL of the *Standard solution*.
Developing solvent system: a mixture of chloroform, methanol, and formic acid (85:10:5).
Procedure—Proceed as directed in the chapter. Visualize the spots by exposing to iodine vapors.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: pH 4.7 acetate buffer, prepared by mixing 200 mL of 1 N acetic acid with 600 mL of water, adjusting with 1 N sodium hydroxide to a pH of 4.70 ± 0.05, diluting with water to 1000 mL, and mixing; 500 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Determine the amount of loperamide hydrochloride dissolved using the following method.
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*.
Procedure—Inject a volume (about 50 µL) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ dissolved in comparison with a Standard solution having a known concentration of USP Loperamide Hydrochloride RS in the same medium and similarly chromatographed.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase—Transfer 500 mL of acetonitrile to a 1000-mL volumetric flask. Dilute with water to volume, add 20 drops of phosphoric acid, mix, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Loperamide Hydrochloride RS in a mixture of acetonitrile and 0.5 N hydrochloric acid (1:1) to obtain a solution having a known concentration of about 0.2 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a mixture of acetonitrile and water (1:1) to volume, and mix to obtain a solution having a known concentration of about 10 µg per mL.
Assay preparation—Transfer, as completely as possible, the contents of not less than 20 Capsules to a suitable tared container, and determine the average weight per capsule. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 20 mg of loperamide hydrochloride, to a 100-mL volumetric flask. Add about 35 mL of 0.5 N hydrochloric acid and sonicate for 15 minutes. Add 35 mL of acetonitrile and sonicate for an additional 15 minutes. Dilute with a mixture of acetonitrile and 0.5 N hydrochloric acid (1:1) to volume, mix, and filter. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a mixture of acetonitrile and water (1:1) to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 25-cm column that contains 10-µm packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*:the column efficiency, N, determined from the analyte peak is not less than 1900 theoretical plates, the capacity factor, *K′*, is not less than 3.5, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ in the portion of Capsules taken by the formula:

$$2000C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Loperamide Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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Loperamide Hydrochloride Oral Solution

DEFINITION

Loperamide Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Sample: Transfer a volume of Oral Solution containing a suitable amount of loperamide hydrochloride (typically 12–24 mg) to a separator containing about 100 mL of water and 1 mL of 50% sodium hydroxide solution, and gently swirl the contents. Add 50 mL of methylene chloride, shake gently by hand, releasing pressure often, and then shake by mechanical means for 20 min. Allow the layers to separate. Transfer the lower methylene chloride layer to a separator containing 100 mL of water. Shake gently by hand, releasing pressure often, and then shake by mechanical means for 10 min. Allow the layers to separate. Transfer the lower methylene chloride layer to a 250-mL beaker, and evaporate to dryness on a steam bath with the aid of a current of air. Add 10 mL of methanol and 500 mg of potassium bromide to the beaker. Evaporate to dryness on a steam bath with the aid of a current of air, and use the residue.

Acceptance criteria: The spectrum obtained from the *Sample* shows bands at approximately 3400 cm^{-1}, 2929 cm^{-1}, 1624 cm^{-1}, and 1493 cm^{-1}, similar to the spectrum from a Standard similarly obtained.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 3.0 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (37:63), adjusted with 5% phosphoric acid to a pH of 3.0

Standard stock solution: Prepare 2 mg/mL of USP Loperamide Hydrochloride RS in methanol. Dilute this solution with water to obtain a 0.1-mg/mL solution.

Standard solution: 0.01 mg/mL of USP Loperamide Hydrochloride RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Nominally 0.01 mg/mL of loperamide hydrochloride from Oral Solution, prepared as follows. Transfer a volume of Oral Solution, equivalent to about 1.0 mg of loperamide hydrochloride, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, mix, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.0-mm × 8.0-cm; 5-µm packing L7, 4.6-mm × 7.5-cm; 3.5-µm packing L7, or 4.6-mm × 12.5-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Loperamide Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of loperamide hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩ (if present)**:** 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH)

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers

Acceptance criteria: Meets the requirements

- **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **PH** ⟨791⟩**:** 2.7–6.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store below 40°, preferably between 15° and 30°, unless otherwise specified by the manufacturer.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Loperamide Hydrochloride RS

Loperamide Hydrochloride Tablets

DEFINITION

Loperamide Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

[NOTE—This procedure is not applicable for Tablets labeled as chewable.]

Wavelength range: 250–300 nm

Standard solution: About 0.4 mg/mL of USP Loperamide Hydrochloride RS, prepared as follows. Transfer an amount of USP Loperamide Hydrochloride RS to a suitable volumetric flask, dissolve first in isopropyl alcohol, using 50% of the final volume. Add 0.1 N hydrochloric acid equivalent to 10% of the final volume, and dilute with isopropyl alcohol to volume.

Sample solution: Transfer a quantity of finely powdered Tablets equivalent to about 10 mg of loperamide hydrochloride to a test tube. Add 20.0 mL of isopropyl

USP Monographs

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alcohol, shake by mechanical means for 1 min, and allow to settle. Pipet 9.0 mL of the supernatant into a 10-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

Acceptance criteria: The spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, concomitantly measured.

THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩

[NOTE—For Tablets labeled as chewable, use the following procedure.]

Standard solution: 1.0 mg/mL of USP Loperamide Hydrochloride RS in methanol

Sample solution: Grind a number of Tablets, equivalent to 10 mg of loperamide hydrochloride, with 10 mL of methanol for about 2 min. Centrifuge the mixture, and use the supernatant.

Application volume: 10 µL

Developing solvent system: Chloroform, methanol, and formic acid (75:25:1)

Analysis: Visualize the spots by using Dragendorff's TS.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solvent mixture: Methanol and acetonitrile (3:1)

Ion pairing solution: Solution containing 2.35 g/L of sodium 1-hexanesulfonate and 2.88 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 3.2

Mobile phase: *Solvent mixture* and *Ion pairing solution* (55:45)

System suitability solution: 0.2 mg/mL of USP Loperamide Hydrochloride RS and 0.002 mg/mL of USP Loperamide Related Compound F RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Loperamide Hydrochloride RS in *Mobile phase*

Sample solution: Fill a 100-mL volumetric flask with *Mobile phase*. Immediately transfer a number of Tablets equivalent to 20 mg of loperamide hydrochloride to the flask, and cap tightly. Sonicate for 15–30 min with intermittent shaking. Allow the contents to settle, and use a clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 219 nm

Column: 3.9-mm × 15-cm; 5-µm or 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between loperamide and loperamide related compound F, *System suitability solution*

Tailing factor: NMT 2.0 for both peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Loperamide Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of loperamide hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Loperamide Hydrochloride RS at a known concentration in *Medium*. [NOTE—If necessary, dissolve USP Loperamide Hydrochloride RS in a minimal amount of methanol, and then dilute with *Medium* to final concentration.]

Sample solution: Filtered solution under test

Buffer: Transfer 3.0 g of triethylamine hydrochloride and 1.0 mL of phosphoric acid to a 1-L flask, and add 550 mL of water.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7 or 4.6-mm × 12.5-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 10 min

Standard stock solution: 0.44 mg/mL of USP Loperamide Hydrochloride RS in methanol. Use sonication as necessary to dissolve.

Standard solution: 0.0022 mg/mL of USP Loperamide Hydrochloride RS in *Medium*, from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size, discarding first few milliliters of the filtrate.

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Buffer: Transfer 3.0 g of triethylamine hydrochloride and 1.0 mL of phosphoric acid to a 1-L flask, and add 550 mL of water.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Solvent mixture, Ion pairing solution, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.002 mg/mL of USP Loperamide Related Compound F RS in *Mobile phase*
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between loperamide and loperamide related compound F
Tailing factor: NMT 2.0 for both peaks
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of loperamide *N*-oxide in the portion of Tablets taken:

$$\text{Result} = (r_T/r_S) \times (C_S/C_U) \times 100$$

r_T = sum of the peak responses of the *cis* and *trans* isomers of *N*-oxide from the *Sample solution*
r_S = peak response of loperamide related compound F from the *Standard solution*
C_S = concentration of USP Loperamide Related Compound F RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loperamide hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

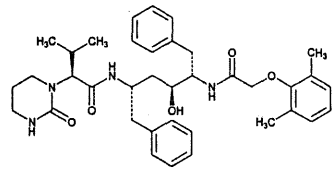
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Loperamide	1.0	—
Loperamide *trans*-*N*-oxide (loperamide related compound F)	1.5	2.0[a]
Loperamide *cis*-*N*-oxide[b]	1.7	

[a] For the sum of *trans* and *cis* isomers.
[b] (1*s*,4*r*)-4-(4-Chlorophenyl)-1-[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidine 1-oxide.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
• **Labeling:** Label chewable Tablets to indicate that they are to be chewed before swallowing. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP Reference Standards** ⟨11⟩
USP Loperamide Hydrochloride RS
USP Loperamide Related Compound F RS
Loperamide *trans*-*N*-oxide;
(1*r*,4*s*)-4-(4-Chlorophenyl)-1-[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidine 1-oxide.
$C_{29}H_{33}ClN_2O_3$ 493.04

Lopinavir



$C_{37}H_{48}N_4O_5$ 628.80
[1*S*-[1*R**(*R**),3*R**,4*R**]]-*N*-[4[[(2,6-Dimethylphenoxy) acetyl] amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]-tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetamide;
(α*S*)-Tetrahydro-*N*-[(α*S*)-α-[(2*S*,3*S*)-2-hydroxy-4-phenyl-3-[2-(2,6-xylyloxy)acetamido]butyl]phenethyl]-α-isopropyl-2-oxo-1(2*H*)-pyrimidineacetamide [192725-17-0].

DEFINITION

Lopinavir contains NLT 98.0% and NMT 102.0% of lopinavir ($C_{37}H_{48}N_4O_5$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)
• **B.** The retention time of the lopinavir peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Buffer: 2.7 g/L of monobasic potassium phosphate and 0.9 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0. Pass the solution through a suitable filter of 0.45-µm pore size.

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Diluent: Acetonitrile and water (1:1)
Solution A: Acetonitrile and *Buffer* (9:11)
Mobile phase: *Solution A*
Standard solution: 0.025 mg/mL of USP Lopinavir RS in *Diluent*
Sample solution: 0.025 mg/mL of Lopinavir in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 4-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 60 min
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 8000 theoretical plates
Capacity factor: NLT 15
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lopinavir ($C_{37}H_{48}N_4O_5$) in the portion of Lopinavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)
C_U = concentration of Lopinavir in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES: PROCEDURE 1**

[NOTE—For early-eluting impurities.]
Buffer, Diluent, and **Solution A:** Prepare as directed in the *Assay*.
Solution B: Acetonitrile and *Buffer* (3:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
60	100	0
61	0	100
81	0	100
82	100	0
100	100	0

System suitability solution: 0.5 mg/mL of USP Lopinavir System Suitability Mixture RS in *Diluent*
Standard solution: 0.005 mg/mL of USP Lopinavir RS in *Diluent*
Sample solution: 0.5 mg/mL of Lopinavir in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 4-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 100 min
[NOTE—Data collection is only for the first 60 min. The remaining gradient steps wash out the late-eluting impurities and re-equilibrate the column.]
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times are listed in *Table 2*.]
Suitability requirements
Resolution: NLT 1.2 between lopinavir *N*-formylphenoxyacetamide and lopinavir *N*-acetylphenoxyacetamide, *System suitability solution*
Capacity factor: NLT 15, *Standard solution*
Column efficiency: NLT 8000, *Standard solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Diluent, System suitability solution, Standard solution,* and *Sample solution*
Calculate the percentage of each lopinavir related impurity and unidentified impurity in the portion of Lopinavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of lopinavir from the *Standard solution*
C_S = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)
C_U = concentration of Lopinavir in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Table 2

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir free amine[a]	0.03	0.61	0.1
Lopinavir *N*-formylaminoalcohol[b]	0.07	0.80	0.2
Lopinavir divalinate[c]	0.10	0.65	0.1
Sulfolopinavir[d]	0.13	0.76	0.1
Lopinavir phenoxyacetamide[e]	0.25	0.96	0.1
Lopinavir *N*-formylphenoxyacetamide[f]	0.59	1.3	0.1
Lopinavir *N*-acetylphenoxyacetamide[g]	0.62	1.2	0.1
Lopinavir oxazine[h]	0.90	1.1	0.1
Lopinavir	1.00	—	—
Isolopinavir[i]	1.10	0.99	0.2
Lopinavir 2,4-phenoxy isomer[j]	1.13	0.97	0.1
Lopinavir D-valine diastereomer[k]	1.25	1.1	0.1
Z-Diacylethenediamine[l]	1.28	1.4	0.1

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Table 2 *(continued)*

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir (2*R*,4*R*) diastereomer[m]	1.32	1.0	0.1
Lopinavir (4*R*) epimer[n]	1.38	0.97	0.1
Any other individual impurity	—	1.0	0.1

[a] (*S*)-*N*-[(2*S*,4*S*,5*S*)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.
[b] (*S*)-*N*-[(2*S*,4*S*,5*S*)-5-Formamido-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.
[c] (2*S*,2′*S*)-*N*,*N*′-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis(3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide).
[d] (2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-{(*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido}-1,6-diphenylhexan-3-yl hydrogen sulfate.
[e] *N*-[(2*S*,3*S*,5*S*)-5-Amino-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.
[f] 2-(2,6-Dimethylphenoxy)-*N*-[(2*S*,3*S*,5*S*)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.
[g] *N*-[(2*S*,3*S*,5*S*)-5-Acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.
[h] *N*-{(*S*)-1-[(4*S*,6*S*)-4-Benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl}-2-(2,6-dimethylphenoxy)acetamide.
[i] (*S*)-*N*-{(2*S*,3*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-3-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[j] (*S*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,4-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[k] (*R*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[l] (*Z*)-*N*,*N*′-(Ethene-1,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].
[m] (*S*)-*N*-{(2*R*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[n] (*S*)-*N*-{(2*S*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.

• **ORGANIC IMPURITIES: PROCEDURE 2**

[NOTE—For late-eluting impurities.]

Buffer, Diluent, and **Solution A:** Prepare as directed in the *Assay*.

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: *Solution A* and *Solution B* (3:7)

System suitability solution: 0.5 mg/mL of USP Lopinavir System Suitability Mixture RS in *Diluent*

Standard solution: 0.005 mg/mL of USP Lopinavir RS in *Diluent*

Sample solution: 0.5 mg/mL in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 50 min

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times are listed in *Table 3*.]

Suitability requirements

Capacity factor: NLT 1.5

Column efficiency: NLT 3000

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Diluent, System suitability solution, Standard solution,* and *Sample solution*

Calculate the percentage of each lopinavir related impurity and unidentified impurity in the portion of Lopinavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of lopinavir from the *Standard solution*

C_S = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)

C_U = concentration of Lopinavir in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Table 3

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir	1.00	—	—
Lopinavir *O*-acyl[a]	1.49	0.77	0.1
Lopinavir (2*R*) epimer[b]	1.91	1.1	0.1
Lopinavir diamide[c]	4.39	1.4	0.1
Lopinavir *N*-acyl[d]	6.01	1.3	0.1
Lopinavir *O*-phenoxyactyl[e]	7.14	1.1	0.1
Lopinavir amino alcohol urea[f]	8.46	1.3	0.1
Any other individual impurity	—	1.0	0.1
Total impurities from Procedure 1 and Procedure 2	—	1.0	0.7[g]

[a] (*S*)-{(2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-[(*S*)-3-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)butanamido]-1,6-diphenylhexan-3-yl} 3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoate.
[b] (*S*)-*N*-{(2*R*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[c] *N*,*N*′-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].
[d] (*S*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-2-{3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2*H*)-yl}-3-methylbutanamide.
[e] (2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-{(*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido}-1,6-diphenylhexan-3-yl 2-(2,6-dimethylphenoxy)acetate.
[f] *N*,*N*′-(2*S*,2′*S*,3*S*,3′*S*,5*S*,5′*S*)-5,5′-Carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].
[g] Exclude from *Organic Impurities, Procedure 2*, lopinavir (4*R*) epimer and any other peak eluting prior to this peak because these are already monitored in *Procedure 1*.

Acceptance criteria: See *Table 2* and *Table 3*.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 4.4%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Lopinavir RS

USP Lopinavir System Suitability Mixture RS

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Lopinavir System Suitability Mixture contains lopinavir *N*-formylphenoxyacetamide, lopinavir *N*-acetylphenoxyacetamide, and several other minor components.

Lopinavir *N*-formylphenoxyacetamide is (2-(2,6-dimethylphenoxy)-*N*-[(2*S*,3*S*,5*S*)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.

$C_{29}H_{34}N_2O_4$ 474.59

Lopinavir *N*-acetylphenoxyacetamide is (*N*-[(2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

$C_{30}H_{36}N_2O_4$ 488.62

Lopinavir and Ritonavir Oral Solution

DEFINITION

Lopinavir and Ritonavir Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION

• **A.** The retention times of the lopinavir and ritonavir peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **LOPINAVIR AND RITONAVIR**

Buffer: 4.1 g/L of monobasic potassium phosphate in water

Solution A: Acetonitrile and *Buffer* (65:35)

Solution B: Acetonitrile and *Buffer* (50:50)

Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Buffer* (175:100:100:625). Separately filter the *Buffer* and the premixed solvents before combining them to make the *Mobile phase*.

Standard stock solution: 0.1 mg/mL each of USP Lopinavir RS and USP Ritonavir RS in *Solution B*

Standard solution: 0.025 mg/mL each of USP Lopinavir RS and USP Ritonavir RS in *Solution B* from the *Standard stock solution*

Sample stock solution: Nominally 4 mg/mL of lopinavir and 1 mg/mL of ritonavir in *Solution A* prepared as follows. Transfer a volume of Oral Solution equivalent to 400 mg of lopinavir and 100 mg of ritonavir to a 100-mL volumetric flask with the aid of several small portions of *Solution A*, and then dilute with *Solution A* to volume.

Sample solution: Nominally 0.05 mg/mL of lopinavir and 0.0125 mg/mL of ritonavir in *Solution B* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.2 for the ritonavir peak

Relative standard deviation: NMT 2.0% each for ritonavir and lopinavir

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

C_S = concentration of USP Lopinavir RS or USP Ritonavir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lopinavir or ritonavir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$)

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer A: 4.1 g/L of monobasic potassium phosphate in water

Buffer B: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate in water

Solution A: Acetonitrile and *Buffer A* (50:50)

Solution B: Acetonitrile, butyl alcohol, and *Buffer A* (15:5:80)

Solution C: Acetonitrile and *Buffer A* (65:35)

Mobile phase: Acetonitrile, tetrahydrofuran, butyl alcohol, and *Buffer B* (18:8:5:69). Adjust with 1 M phosphoric acid or 1 M potassium hydroxide, if necessary, to a pH of 6.3.

Standard stock solution: 0.1 mg/mL each of USP Lopinavir RS and USP Ritonavir RS in *Solution A*

Standard solution: 0.01 mg/mL each of USP Lopinavir RS and USP Ritonavir RS from *Standard stock solution* in *Solution B*

Peak identification solution: Transfer a weighed portion of Oral Solution to a crimp-top container. Add an amount of citric acid equivalent to 1% by weight of the Oral Solution taken and mix until dissolved. Seal the container, and heat at 50° for approximately 4 days. Use this degradation solution and follow the procedure described below in the *Sample stock solution* and *Sample solution* sections to prepare the *Peak identification solution*.

Sample stock solution: Transfer 5 mL of Oral Solution with the aid of several small portions of *Solution C* to a 100-mL volumetric flask, and dilute with *Solution C* to volume.

Sample solution: Dilute 25.0 mL of *Sample stock solution* with *Solution B* to 50.0 mL. Transfer 15.0 mL of this solution to a 50-mL centrifuge tube that has been previously rinsed with methanol and dried. Add 20.0 mL of *n*-heptane, and shake vigorously until a uniform emulsion is formed. Vent the tube periodically while shaking. Centrifuge the emulsion for about 5 min. Carefully remove the top heptane layer by aspiration, leaving the clear *Sample solution* layer. The middle viscous, cloudy layer should be considered part of the heptane layer for removal by aspiration. Precondition a strong anion-exchange cartridge (quaternary ammonium functionality on a styrene/divinylbenzene base) with a sorbent mass of 600 mg by rinsing the cartridge with 3 mL of methanol, then 3 mL of *Solution C*, and repeating these rinse steps. Dry the cartridge for about 10 min with the aid of a low vacuum. Transfer 5.0 mL of the clear *Sample solution* to the preconditioned cartridge. With the aid of a vacuum, slowly pass the *Sample solution* completely through the cartridge, collect the extract in a 5-mL volumetric flask, and then dilute with *Solution C* to volume.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm and 240 nm
Column: 4.6-mm × 15-cm; 3-µm packing L26
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: 2 times the retention time of lopinavir

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between the ritonavir and lopinavir peaks at 215 nm
Tailing factor: 0.8–1.2 for the ritonavir peak at 240 nm
Relative standard deviation: NMT 3.0% for the lopinavir peak at 215 nm; NMT 3.0% for the ritonavir peak at 240 nm

Analysis
Samples: *Standard solution*, *Peak identification solution*, and *Sample solution*
[NOTE—Determine the relative retention values (*r*) for the components listed in *Table 1* and *Table 2*, using the time measured at the first baseline deflection of the *Standard solution* chromatogram as the void volume (t_M).]
To identify the ritonavir impurities, determine the relative retention value from the 240-nm chromatogram relative to ritonavir (see *Table 1*). The *Peak identification solution* may also be used to identify ritonavir degradants. Unspecified ritonavir impurities are assigned according to the algorithm outlined in *Table 3*.
Calculate the percentage of each ritonavir impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of ritonavir from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1 (Lopinavir and Ritonavir Oral Solution)

Name	Relative Retention Value (*r*)	Relative Response Factor	Acceptance Criteria, NMT (%)
Ureidovaline[a]	0.03	—	—[b]
N-Deacylvaline ritonavir[c]	0.11	0.81	0.8
Glycerol carbamate analog[d]	0.14	0.62	0.2
Acetamidoalcohol[e]	0.15	—	—[b]
Hydroxypropyl carbamate analog[f]	0.24[g]	0.59	0.5
2,5-Thiazolylmethyldicarbamate[h]	0.24[i]	—	—[b]
Hydroxyritonavir[j]	0.36	0.86	0.3
Hydantoin aminoalcohol[k]	0.39	0.73	0.4
Ritonavir hydroperoxide[l]	0.44[i]	0.88	0.2
Ethyl carbamate analog[m]	0.45[i]	0.66	0.7
Hydantoin-oxazolidinone derivative[n]	0.50	—	—[b]
Ethyl analog[o]	0.64	—	—[b]
O-Acyl isomer[p]	0.74	1.1	0.2
BOC-aminoalcohol[q]	0.81	—	—[b]
Isobutoxycarbonyl aminoalcohol[r]	0.81	—	—[b]
Oxazolidinone derivative[s]	0.87	0.53	0.2
Ureidovaline isobutyl ester[t]	0.94	—	—[b]
Ritonavir	1.0	—	—
4-Hydroxy isomer[u]	1.05	—	—[b]
3*R*-Epimer[v]	1.11	—	—[b]
Aminoalcohol urea derivative[w]	1.14	—	—[b]
3*R*,5*R*-Epimer[x]	1.23	—	—[b]
5*R*-Epimer[y]	1.32	—	—[b]
Diacyl valine urea[z]	1.70	—	—[b]
Any unspecified ritonavir impurity	—	1.0	0.2

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Table 1 (Lopinavir and Ritonavir Oral Solution) *(continued)*

Name	Relative Retention Value (*r*)	Relative Response Factor	Acceptance Criteria, NMT (%)
Total ritonavir impurities, specified and unspecified	—	—	3.0[aa]

[a] [*N*-Methyl[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl-L-valine.
[b] These are process impurities which are included in this table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and are not included in the total impurities.
[c] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[d] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(2*S*)-2-(2,3-dihydroxypropoxycarbonylamino)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[e] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(2*S*)-2-(2-hydroxypropoxycarbonylamino)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[f] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[g] If two peaks appear with a relative retention value of 0.24, the second peak will be identified as 2,5-thiazolylmethyldicarbamate.
[h] 2,5-Thiazolylmethyldicarbamate.
[i] A single peak with a relative retention value of 0.44 should be reported as the ethyl carbamate analog due to possible coelution with ritonavir hydroperoxide impurity.
[j] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[2-(3-{[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)acetamido]-1,6-diphenylhexan-2-ylcarbamate.
[k] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.
[l] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-ethoxycarbonylamino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[m] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[n] (4*S*,5*S*)-Thiazol-5-ylmethyl 4-benzyl-5-{(*S*)-2-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl}-2-oxooxazolidine-3-carboxylate.
[o] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[p] (*S*)-{(2*S*,3*S*,5*S*)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl} 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[q] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[r] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[s] (*S*)-*N*-[(*S*)-1-[(4*S*,5*S*)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide.
[t] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[u] Thiazol-5-ylmethyl (2*S*,4*S*,5*S*)-4-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[v] Thiazol-5-ylmethyl (2*S*,3*R*,5*S*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[w] Bis(thiazol-5-ylmethyl) (2*S*,2′*S*,3*S*,3′*S*,5*S*,5′*S*)-5,5′-carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.
[x] Thiazol-5-ylmethyl (2*S*,3*R*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[y] Thiazol-5-ylmethyl (2*S*,3*S*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[z] (*S*)-*N*-[(2*S*,4*S*,5*S*)-5-(Thiazol-5-ylmethoxycarbonylamino)-4-hydroxy-1,6-diphenylhexan-2-yl]-2-{3-[(2*S*,4*S*,5*S*)-5-(thiazol-5-ylmethoxycarbonylamino)-4-hydroxy-1,6-diphenylhexan-2-yl]ureido}-3-methylbutanamide.
[aa] Disregard any peak less than 0.01% in the calculation of total impurities.

To identify the lopinavir impurities, determine the relative retention value from the 215-nm chromatogram relative to ritonavir (see *Table 2*). Compare the 215-nm chromatogram to the 240-nm chromatogram. Any impurity assigned as a ritonavir impurity at 240 nm that is also observed at 215 nm is discounted. Unspecified ritonavir impurities are assigned according to the algorithm outlined in *Table 3*.

Calculate the percentage of each unspecified lopinavir impurity at 215 nm in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of lopinavir from the *Standard solution*
C_S = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lopinavir in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Value (*r*)	Acceptance Criteria, NMT (%)
Lopinavir aminoalcohol[a]	0.06	—[b]
Lopinavir *N*-formylaminoalcohol[c]	0.12	—[b]
Lopinavir divalinate[d]	0.21	—[b]
Lopinavir phenoxyacetamide[e]	0.35	—[b]
Lopinavir *N*-formylphenoxyacetamide[f]	0.67	—[b]
Lopinavir *N*-acetylphenoxyacetamide[g]	0.69	—[b]
Lopinavir oxazine[h]	0.77	—[b]
Z-Diacylethenediamine[i]	0.92	—[b]
Ritonavir	1.0	—[b]
Isolopinavir[j]	1.18	—[b]
Lopinavir 2,4-dimethylphenoxy isomer[k]	1.21	—[b]
Lopinavir 4-epimer[l]	1.26	—[b]
Lopinavir D-valine diastereomer[m]	1.33	—[b]

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Table 2 *(continued)*

Name	Relative Retention Value (*r*)	Acceptance Criteria, NMT (%)
Lopinavir (2*R*,4*R*) diastereomer[n]	1.42	—[b]
Lopinavir 2-epimer[o]	1.79	—[b]
Any unspecified lopinavir impurity	—	0.2[p]
Total unspecified lopinavir impurities	—	0.5[p]

[a] (*S*)-*N*-[(2*S*,4*S*,5*S*)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.
[b] These are process impurities which are included in this table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and are not included in the total impurities.
[c] (*S*)-*N*-[(2*S*,4*S*,5*S*)-5-Formamido-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.
[d] (2*S*,2′*S*)-*N*,*N*′-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis{3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide}.
[e] *N*-[(2*S*,3*S*,5*S*)-5-Amino-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.
[f] 2-(2,6-Dimethylphenoxy)-*N*-[(2*S*,3*S*,5*S*)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.
[g] *N*-[(2*S*,3*S*,5*S*)-5-Acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.
[h] *N*-{(*S*)-1-[(4*S*,6*S*)-4-Benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl}-2-(2,6-dimethylphenoxy)acetamide.
[i] (*Z*)-*N*,*N*′-(Ethene-1,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].
[j] (*S*)-*N*-{(2*S*,3*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-3-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[k] (*S*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,4-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[l] (*S*)-*N*-{(2*S*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[m] (*R*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[n] (*S*)-*N*-{(2*R*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[o] (*S*)-*N*-{(2*R*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[p] Disregard any peak less than 0.01%.

For calculating and reporting impurities, follow the algorithm outlined in *Table 3*.

Table 3

Wavelength (nm)	Unspecified Impurity Observed	
240	Yes	No
215	Yes or No	Yes
Peak assigned to	Ritonavir	Lopinavir
Quantitation wavelength	240 nm	215 nm

SPECIFIC TESTS

- **ALCOHOL DETERMINATION** ⟨611⟩

Internal standard solution: Transfer 10.0 mL of butyl alcohol to a 200-mL volumetric flask and dilute with methanol to volume.
Internal standard identification solution: Dilute 5.0 mL of *Internal standard solution* with methanol to 100 mL.
Standard stock solution: 4.0% (v/v) of dehydrated alcohol in methanol
Standard solution: 0.4% (v/v) of dehydrated alcohol prepared as follows. Transfer 10.0 mL of *Standard stock solution* and 5.0 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with methanol to volume.
Sample stock solution: Transfer 5.0 mL of Oral Solution to a 50-mL volumetric flask with the aid of several portions of methanol, and dilute with methanol to volume.
Sample solution: Transfer 10.0 mL of *Sample stock solution* and 5.0 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused silica capillary; coated with a 1-µm film of liquid phase G16
Temperatures
Injection port: 185°
Detector: 220°
Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	10	145	6
145	20	200	9.75

Carrier gas: Helium
Flow rate: 4.5 mL/min
Makeup gas flow: 30 mL/min
Injection volume: 1 µL
Injection type: Split ratio 4:1
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.2 for the alcohol peak
Relative standard deviation: NMT 3.0% for the peak area ratio of alcohol to the internal standard
Analysis
Samples: *Internal standard identification solution*, *Standard solution*, and *Sample solution*
Calculate the percentage of the labeled amount of alcohol in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times D \times 100$$

R_U = peak response ratio of alcohol to butyl alcohol from the *Sample solution*
R_S = peak response ratio of alcohol to butyl alcohol from the *Standard solution*
C_S = concentration of dehydrated alcohol in the *Standard solution* (% v/v)
C_U = nominal concentration of alcohol in the Oral Solution (% v/v)
D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 85.0%–115.0% of the labeled amount of alcohol (C_2H_6O)

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL.

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at 2°–8°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lopinavir RS
  USP Ritonavir RS

Lopinavir and Ritonavir Tablets

DEFINITION
Lopinavir and Ritonavir Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION
- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **LOPINAVIR AND RITONAVIR**

Buffer 1: 4.1 g/L of monobasic potassium phosphate in water
Solution A: Acetonitrile and *Buffer 1* (50:50)
Buffer 2: 2.1 g/L of monobasic potassium phosphate in water
Solution B: Acetonitrile and 1-butanol (13:3)
Solution C: Acetonitrile, 1-butanol, *Buffer 1*, and water (65:15:10:10)
Standard solution: 6.25 µg/mL of USP Ritonavir RS and 25 µg/mL of USP Lopinavir RS in *Solution A*
Sample solution: Place a number of Tablets equivalent to 1000 mg of lopinavir and 250 mg of ritonavir in a 250-mL volumetric flask, add 25 mL of *Buffer 2*, and agitate to dissolve the Tablet coating, if necessary. Add 100 mL of *Solution B*, and shake mechanically until the Tablets are dissolved. Dilute with *Solution C* to volume. Centrifuge a portion of this solution, and then further dilute with *Solution A* to a nominal concentration of 6.25 µg/mL of ritonavir and 25 µg/mL of lopinavir.
Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Buffer 1* (175:100:100:625)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The elution order is ritonavir, then lopinavir.]
Suitability requirements
Capacity factor: 15–24 for the ritonavir peak
Tailing factor: 0.8–1.2 for the ritonavir peak
Theoretical plates: More than 5000 for the ritonavir peak
Relative standard deviation: NMT 2.0% for the ritonavir and lopinavir peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lopinavir or ritonavir from the *Sample solution*
r_S = peak response of lopinavir or ritonavir from the *Standard solution*
C_S = concentration of lopinavir or ritonavir in the *Standard solution* (µg/mL)
C_U = nominal concentration of lopinavir or ritonavir in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$)

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: 60 mM polyoxyethylene 10 lauryl ether (37.56 g/L) in water; 900 mL
Apparatus 2: 75 rpm
Time: 90 min
Mobile phase: Acetonitrile and 4.1 g/L potassium phosphate monobasic (55:45). Adjust with phosphoric acid to an apparent pH of 4.0 ± 0.05.
Standard solution: Dissolve USP Lopinavir RS in methanol to obtain a solution containing 2.6 mg/mL. Dissolve USP Ritonavir RS in methanol to obtain a solution containing 1.3 mg/mL. Combine portions of these solutions to make a solution containing approximately 0.104 mg/mL of lopinavir and 0.026 mg/mL of ritonavir in *Medium*.
Sample solutions: Pass a portion of the solution under test through a suitable filter. If necessary, dilute the solution with *Medium* to obtain a final sample solution containing approximately 0.104 mg/mL of lopinavir and 0.026 mg/mL of ritonavir.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between lopinavir and ritonavir
Tailing factor: 0.9–1.5 for the lopinavir and ritonavir peaks
Relative standard deviation: NMT 2.0% for the lopinavir and ritonavir peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response of lopinavir or ritonavir from the *Sample solution*
r_S = peak response of lopinavir or ritonavir from the *Standard solution*
C_S = concentration of USP Lopinavir RS or USP Ritonavir RS in the *Standard solution* (mg/mL)
L = Tablet label claim for lopinavir or ritonavir (mg)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) are dissolved.

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• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Buffer 1: 4.1 g/L of monobasic potassium phosphate in water

Solution A: *Buffer 1* and acetonitrile (50:50)

Buffer 2: 2.1 g/L of monobasic potassium phosphate in water

Solution B: Acetonitrile, 1-butanol, and *Buffer 1* (15:5:80)

Solution C: Acetonitrile, 1-butanol, *Buffer 1*, and water (65:15:10:10)

Solution D: Acetonitrile and 1-butanol (13:3)

Buffer solution: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate in water

Mobile phase: Acetonitrile, tetrahydrofuran,1-butanol, and *Buffer solution* (18:8:5:69). Adjust with 1 M phosphoric acid or 1 M potassium hydroxide, if necessary, to a pH of 6.3 ± 0.1.

Standard stock solution: 0.025 mg/mL of USP Ritonavir RS in *Solution A*

Standard solution: 2.5 µg/mL of USP Ritonavir RS in *Solution B* from *Standard stock solution*

Ritonavir degradant identification solution: Transfer two 5.0 mL portions of a 1 mg/mL solution of USP Ritonavir RS in *Solution A* to separate 50-mL volumetric flasks. Add 1 g of citric acid to one flask, and shake until dissolved. Heat both flasks at 80° for approximately 24 h. Cool the flasks, and add 13 mL of 1 N sodium hydroxide to the flask containing the citric acid. Dilute both flasks with *Solution B* to volume. Combine equal volumes of both solutions. This solution contains ritonavir and the ritonavir degradation products (*N*-deacylvaline ritonavir, hydantoin aminoalcohol, *O*-acyl isomer, and oxazolidinone derivative).

Ritonavir related compounds identification solution: 1 mg/mL of USP Ritonavir Related Compounds Mixture RS dissolved in *Solution C* and further diluted with *Solution B* to 0.5 mg/mL.

Sample solution: Place a number of Tablets equivalent to 1000 mg of lopinavir and 250 mg of ritonavir into a 250-mL volumetric flask. Add 25 mL of *Buffer 2*, and agitate to dissolve the Tablet coating, if necessary. Add 100 mL of *Solution D*, and shake mechanically until the Tablets are dissolved. Dilute with *Solution C* to volume. Centrifuge a portion of this solution, and further dilute with *Solution B* to a concentration of 2 mg/mL of lopinavir and 0.5 mg/mL of ritonavir.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Column: 4.6-mm × 15-cm; 3-µm packing L26

Column temperature: 60°

Detector: UV 240 nm

Injection volume: 50 µL

Flow rate: 1.0 mL/min

System suitability

Samples: *Ritonavir degradant identification solution*, *Ritonavir related compounds identification solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between the peaks for *O*-acyl isomer and oxazolidinone derivative, *Ritonavir degradant identification solution*. NLT 0.7 between the peaks for hydroxyritonavir and hydantoin aminoalcohol, *Ritonavir related compounds identification solution*

Capacity factor: NLT 10.8, *Standard solution*

Tailing factor: 0.8–1.2, *Standard solution*

Column efficiency: NLT 5000, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each ritonavir degradation product in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of individual degradation product from the *Sample solution*

r_S = peak response of ritonavir from the *Standard solution*

C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)

F = relative response factor

Acceptance criteria: See *Table 1*. [Note—Disregard all peaks eluting before the retention time of the *N*-deacylvaline ritonavir peak from the *Ritonavir degradant identification solution*.]

Table 1

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Deacylvaline ritonavir[a]	0.11	0.81	0.2
Acetamidoalcohol[b]	0.15	—	—*
2,5-Thiazolylmethyl-dicarbamate[c]	0.24	—	—*
Hydroxyritonavir[d]	0.36	0.86	0.3
Hydantoin aminoalcohol[e]	0.39	0.73	2.6
Ritonavir hydroperoxide[f]	0.44	0.88	0.2
Hydantoin-oxazolidinone derivative[g]	0.50	—	—*
Ethyl analog[h]	0.64	—	—*
O-Acyl isomer[i]	0.74	1.1	0.2
BOC-aminoalcohol[j]	0.81	—	—*
Isobutoxycarbonyl aminoalcohol[k]	0.81	—	—*
Oxazolidinone derivative[l]	0.87	0.53	0.3
Ureidovaline isobutyl ester[m]	0.94	—	—*
Ritonavir	1.0	—	—*
4-Hydroxy isomer[n]	1.05	—	—*
3*R*-Epimer[o]	1.11	—	—*
Aminoalcohol urea derivative[p]	1.14	—	—*
3*R*,5*R*-Epimer[q]	1.23	—	—*
5*R*-Epimer[r]	1.32	—	—*
Diacyl valine urea[s]	1.70	—	—*
Any unspecified impurity	—	1.0	0.2**

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Table 1 *(continued)*

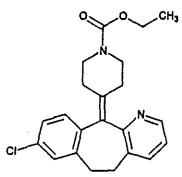
Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	3.5**

* Process impurities; for information only.
[a] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[b] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[c] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate.
[d] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-2-(3-{[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[e] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.
[f] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-(3-{[2-(2-hydroperoxypropan-2-yl) thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[g] (4*S*,5*S*)-Thiazol-5-ylmethyl 4-benzyl-5-{(*S*)-2-[(S)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl}-2-oxooxazolidine-3-carboxylate.
[h] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[i] (*S*)-{(2*S*,3*S*,5*S*)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy) carbonylamino]hexan-3-yl} 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[j] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[k] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[l] (*S*)-*N*-[(*S*)-1-[(4*S*,5*S*)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide.
[m] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[n] Thiazol-5-ylmethyl (2*S*,4*S*,5*S*)-4-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[o] Thiazol-5-ylmethyl (2*S*,3*R*,5*S*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[p] Bis(thiazol-5-ylmethyl) (2*S*,2′*S*,3*S*,3′*S*,5*S*,5′*S*)-5,5′-carbonylbis(azanediyl) bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.
[q] Thiazol-5-ylmethyl (2*S*,3*R*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[r] Thiazol-5-ylmethyl (2*S*,3*S*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl) methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[s] (3*S*,4*S*,6*S*,10*S*,13*S*,15*S*,16*S*)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.
** Disregard any peak less than 0.05%.

ADDITIONAL REQUIREMENTS

- **USP Reference Standards** ⟨11⟩
  USP Lopinavir RS
  USP Ritonavir RS
  USP Ritonavir Related Compounds Mixture RS

Loratadine



$C_{22}H_{23}ClN_2O_2$ 382.88

1-Piperidinecarboxylic acid, 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)-, ethyl ester;
Ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)-1-piperidinecarboxylate [79794-75-5].

DEFINITION

Loratadine contains NLT 98.5% and NMT 101.0% of loratadine ($C_{22}H_{23}ClN_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer A (0.01 M dibasic potassium phosphate): 1.74 g/L of anhydrous dibasic potassium phosphate in water
Buffer B (0.6 M dibasic potassium phosphate): 105 g/L of anhydrous dibasic potassium phosphate in water
0.05 N hydrochloric acid: Transfer 500 mL of water to a 1000-mL volumetric flask, add 83 mL of hydrochloric acid, and dilute with water to volume. Transfer 50 mL of this solution into a 1000-mL volumetric flask, and dilute with water to volume.
Mobile phase: Acetonitrile, methanol, and *Buffer A* (60:60:70). Adjust with 10% phosphoric acid to an apparent pH of 7.2.
Diluent: Transfer 400 mL of *0.05 N hydrochloric acid* and 80 mL of *Buffer B* to a 1-L volumetric flask. Dilute with a mixture of acetonitrile and methanol (1:1) to volume.
Standard solution: 0.4 mg/mL of USP Loratadine RS in *Diluent*
Sample solution: 0.4 mg/mL of Loratadine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 25°–35°
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of loratadine ($C_{22}H_{23}ClN_2O_2$) in the portion of Loratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%

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• **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended if 4,8-dichloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-one is a potential related compound.]

Mobile phase and **Diluent:** Proceed as directed in the *Assay*.

Standard solution: 0.8 µg/mL of USP Loratadine RS in *Diluent*

Sample solution: 0.4 mg/mL of Loratadine in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 25°–35°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 4.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Loratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of loratadine from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = concentration of Loratadine in the *Sample solution* (mg/mL)

F = relative response factor as listed in *Table 1*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluoroloratadine[a]	0.79	0.25	0.2
Loratadine	1.0	—	—
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.3

[a] Ethyl 4-(8-chloro-11-fluoro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl) piperidin-1-carboxylate.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: Dissolve 0.96 g of 1-pentanesulfonic acid sodium salt in 900 mL of water. Adjust with phosphoric acid solution (1 in 10) to a pH of 3.00 ± 0.05, and dilute with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
20	50	50
30	40	60
35	30	70
45	30	70
50	75	25

Standard stock solution: 0.1 mg/mL each of USP Loratadine RS, USP Loratadine Related Compound A RS, and USP Loratadine Related Compound B RS in methanol

Standard solution: 0.01 mg/mL each of USP Loratadine RS, USP Loratadine Related Compound A RS, and USP Loratadine Related Compound B RS prepared as follows. Transfer 1.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, add 2 mL of *Solution A*, and dilute with methanol to volume.

Sample solution: 10 mg/mL of Loratadine prepared as follows. Transfer 100 mg of Loratadine to a 10-mL volumetric flask, and dissolve in 2 mL of methanol. Add 2 mL of *Solution A*, and then dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between loratadine related compound A and loratadine related compound B

Relative standard deviation: NMT 10% for the loratadine peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Loratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*

r_S = peak area of loratadine from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = concentration of Loratadine in the *Sample solution* (mg/mL)

F = relative response factor as listed in *Table 3*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine related compound A	0.50	1.00	0.1
Loratadine related compound B	0.53	0.89	0.1
Loratadine related compound C[a]	0.70	0.60	0.1
Hydroxy deacyl analog[b]	0.75	0.46	0.1

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Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine	1.00	—	—
Dichlorobenzocycloheptapyridinone[c]	1.23	0.92	0.1
Hydroxyloratadine[d]	1.60	0.42	0.1
4-Chloroloratadine[e]	1.83	1.08	0.1
Any individual unknown impurity	—	1.0	0.10
Total impurities	—	—	0.3

[a] 8-Chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-one.
[b] 8-Chloro-5,6-dihydro-11-hydroxy-11-(1-methylpiperidin-4-yl)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
[c] 4,8-Dichloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-one.
[d] Ethyl 4-(8-chloro-11-hydroxy-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl) piperidin-1-carboxylate.
[e] Ethyl 4-(4,8-dichloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidin-1-carboxylate.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample at 100° to constant weight.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store between 2° and 30°.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Loratadine RS
  USP Loratadine Related Compound A RS
  8-Chloro-5,6-dihydro-11-(piperidin-4-ylidene)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
  $C_{19}H_{19}ClN_2$ 310.82
  USP Loratadine Related Compound B RS
  8-Chloro-5,6-dihydro-11-(*N*-methylpiperidin-4-ylidene)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
  $C_{20}H_{21}ClN_2$ 324.85

Loratadine Oral Solution

DEFINITION

Loratadine Oral Solution contains NLT 94.0% and NMT 105.0% of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
  Mobile phase: Acetonitrile and *Buffer* (3:7)
  Diluent: Acetonitrile and water (3:7)
  Internal standard solution: 0.3 mg/mL of USP Butylparaben RS in *Diluent*
  Standard stock solution: 1.0 mg/mL of USP Loratadine RS in acetonitrile
  Standard solution: Transfer 5.0 mL of *Internal standard solution*, 5.0 mL of *Standard stock solution*, and 12 mL of water into a 50-mL volumetric flask. Dilute with *Diluent* to volume.
  Sample solution: Transfer a portion of Oral Solution, nominally equivalent to 5 mg of loratadine, into a 50-mL volumetric flask. Pipet 5.0 mL of *Internal standard solution* into the flask, and dilute with *Diluent* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
  Column: 3.9-mm × 30-cm; 10-µm packing L11
  Column temperature: 20°–30°
  Flow rate: 2 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for butylparaben and loratadine are about 0.78 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 1.9 between loratadine and butylparaben
  Tailing factor: NMT 1.6 for the loratadine and butylparaben peaks
  Relative standard deviation: NMT 2%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of loratadine to the internal standard from the *Sample solution*
R_S = peak response ratio of loratadine to the internal standard from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 94.0%–105.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**
  Mobile phase: 4.3 g/L of sodium dodecyl sulfate in a mixture of acetonitrile and water (1:1). Adjust with phosphoric acid to a pH of 2.6 ± 0.1.
  Diluent: *Mobile phase* and water (2:1)
  System suitability solution 1: 2 µg/mL of USP Loratadine RS in *Diluent*
  System suitability solution 2: 0.2 µg/mL of USP Loratadine RS in *Diluent* from *System suitability solution 1*
  System suitability solution 3: Transfer an amount of Oral Solution, equivalent to 20 mg of loratadine, into a screw-cap glass container. Add 1 mL of 3% aqueous hydrogen peroxide and mix. Cap and heat at 65° for 18–24 h. Allow to cool to room temperature and then dilute 5 mL of the resulting solution with *Diluent* to 25 mL.
  Sample solution: Nominally 0.2 mg/mL of loratadine from a volume of Oral Solution in *Diluent*

USP Monographs

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°–40°
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution 1*, *System suitability solution 2*, and *System suitability solution 3*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between loratadine and 2-hydroxymethyl loratadine, *System suitability solution 3*
Tailing factor: 0.7–1.1, *System suitability solution 1*
Relative standard deviation: NMT 10%, *System suitability solution 2*
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity in the *Sample solution*
r_T = sum of all the peak responses in the *Sample solution*, excluding excipient peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Hydroxymethyl loratadine[a]	0.70	0.3
2-Hydroxymethyl loratadine[b]	0.84	0.3
Loratadine	1.0	—
Any other individual impurity	—	0.2
Total impurities	—	0.5

[a] Ethyl 4-[8-chloro-5,6-dihydro-4-(hydroxymethyl)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene]-1-piperidinecarboxylate.
[b] Ethyl 4-[8-chloro-5,6-dihydro-2-(hydroxymethyl)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene]-1-piperidinecarboxylate.

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^2 cfu/mL, and the total combined molds and yeasts count is NMT 5×10^1 cfu/mL. It meets the requirements for the absence of *Salmonella* species and *Escherichia coli*.
- **PH** ⟨791⟩: 2.2–3.1

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 2° and 25°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Butylparaben RS
USP Loratadine RS

Loratadine Tablets

DEFINITION
Loratadine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Buffer A: 0.01 M dibasic potassium phosphate (1.74 g/L of anhydrous dibasic potassium phosphate in water)
Buffer B: 0.6 M dibasic potassium phosphate (105 g/L of anhydrous dibasic potassium phosphate in water)
0.05 N hydrochloric acid: Transfer 500 mL of water into a 1000-mL volumetric flask, add 83 mL of hydrochloric acid, and dilute with water to volume. Transfer 50 mL of this solution into a 1000-mL volumetric flask and dilute with water to volume.
Mobile phase: Acetonitrile, methanol, and *Buffer A* (60:60:70). Adjust with 10% phosphoric acid to a pH of 7.2.
Diluent: Transfer 400 mL of *0.05 N hydrochloric acid* and 80 mL of *Buffer B* into a 1-L volumetric flask. Dilute with a mixture of acetonitrile and methanol (1:1) to volume.
Standard solution: 0.4 mg/mL of USP Loratadine RS in *Diluent*
Sample solution: Transfer 10 Tablets to a 250-mL volumetric flask, add 100 mL of *0.05 N hydrochloric acid*, and shake for 40 min or until the Tablets are completely disintegrated. Add 75 mL of a mixture of acetonitrile and methanol (1:1), and 20 mL of *Buffer B*, and mix for 5 min. Dilute with a mixture of acetonitrile and methanol (1:1) to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 25°–35°
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: NLT 3.5
Tailing factor: NMT 1.7
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

USP Monographs

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Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Standard solution: USP Loratadine RS at a known concentration in *Medium*
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*, if necessary
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: Maximum absorbance at about 280 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer A, Buffer B, 0.05 N hydrochloric acid, Mobile phase, Diluent, and **Sample solution:** Proceed as directed in the *Assay*.
Standard solution: 0.8 µg/mL of USP Loratadine RS in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 4.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of loratadine from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluoroloratadine[a, b]	0.79	—
Loratadine	1.0	—
Any other individual impurity	—	0.1
Total impurities	—	0.1

[a] This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.
[b] Ethyl 4-(8-chloro-11-fluoro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl) piperidin-1-carboxylate.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 2° and 30°. Protect from excessive moisture if packaged in blisters.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Loratadine RS

Loratadine Chewable Tablets

DEFINITION

Loratadine Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Diluent: Chloroform and methanol (1:1)
Standard solution: 5 mg/mL of USP Loratadine RS in *Diluent*
Sample solution: Transfer a quantity of ground Chewable Tablets, equivalent to 25 mg of loratadine, to a centrifuge tube. Add 5 mL of *Diluent*, mix for 30 min, then centrifuge for 5 min. Use the clear supernatant.
Application volume: 5 µL
Developing solvent system: Ethyl ether and diethylamine (40:1)
Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Protect solutions containing loratadine from light.
Buffer A (phosphate buffer pH 7.2)**:** Dissolve 4.35 g of dibasic potassium phosphate in 950 mL of water. Add 1 mL of triethylamine, adjust with 10% phosphoric acid or 10% potassium hydroxide to a pH of 7.2, and dilute with water to 1 L.
Buffer B (0.6 M dibasic potassium phosphate)**:** 105 g/L of dibasic potassium phosphate in water
Diluent: Transfer 400 mL of 0.05 N hydrochloric acid and 80 mL of *Buffer B* to a 1-L volumetric flask. Dilute with a mixture of acetonitrile and methanol (1:1) to volume.
Mobile phase: Acetonitrile, methanol and *Buffer A* (40:10:50)
Standard solution: 0.4 mg/mL of USP Loratadine RS in *Diluent*
Sample solution: Nominally 0.4 mg/mL of loratadine in *Diluent*. Prepare by transferring 20 Chewable Tablets to a suitable volumetric flask. Add *Diluent* to 40% of the volume

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of the flask, and shake for 40 min. Dilute with *Diluent* to volume, and filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 15 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.7
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 10% phosphoric acid to a pH of 2.80 ± 0.05.
Mobile phase: Acetonitrile, methanol, and *Buffer* (40:30:35)
Standard solution: 10 µg/mL of USP Loratadine RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.7
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Protect solutions containing loratadine from light.
Mobile phase, Diluent, and **Sample solution:** Prepare as directed in the *Assay*.
System suitability solution: 0.8 µg/mL each of USP Loratadine Related Compound A RS, USP Loratadine Related Compound B RS, and USP Loratadine Related Compound C RS in *Diluent*
Standard solution: 0.8 µg/mL of USP Loratadine RS in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10%, *Standard solution*
Resolution: NLT 5.5 between loratadine related compound A and loratadine related compound C; NLT 2.0 between loratadine related compound C and loratadine related compound B, *System suitability solution*
Tailing factor: NMT 2.0 for loratadine related compound A, *System suitability solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of loratadine from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.
Reporting level for impurities: 0.05%

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine related compound A	0.20	1.0	0.05
Loratadine related compound C	0.32	0.63	0.10
Loratadine related compound B[a]	0.39	1.0	—

USP Monographs

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 20° and 25°.
- **LABELING:** Label it to indicate that the Chewable Tablets are to be chewed before swallowing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Loratadine RS
  USP Loratadine Related Compound A RS
  8-Chloro-5,6-dihydro-11-(piperidin-4-ylidene)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
  $C_{19}H_{19}ClN_2$ 310.82
  USP Loratadine Related Compound B RS
  8-Chloro-5,6-dihydro-11-(*N*-methylpiperidin-4-ylidene)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
  $C_{20}H_{21}ClN_2$ 324.85
  USP Loratadine Related Compound C RS
  8-Chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-one.
  $C_{14}H_{10}ClNO$ 243.69

Loratadine Orally Disintegrating Tablets

DEFINITION

Loratadine Orally Disintegrating Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Assay, Procedure 1* or *Assay, Procedure 2*.▲ 2S (USP41)

ASSAY

[NOTE—Matrix effects have been observed that affect the extraction of loratadine. Depending on the composition of the Tablet, use *Assay, Procedure 1* or *Assay, Procedure 2*.]

Change to read:

- **PROCEDURE 1**

Buffer: 2.72 g/L of monobasic potassium phosphate in water. Adjust with 5 N sodium hydroxide solution to a pH of 6.50 ± 0.05, and filter.
Mobile phase: Acetonitrile and *Buffer* (70:30)
Diluent: Acetonitrile and *Buffer* (40:60)
Standard solution: 0.1 mg/mL of USP Loratadine RS in *Mobile phase*
Sample solution: Transfer 10 Tablets into a 500-mL volumetric flask, add 400 mL of acetonitrile, and stir for 10 min. Sonicate the solution for 10 min, and stir for another 10 min. Dilute with acetonitrile to volume, and mix. Dilute an aliquot of the resulting solution with *Diluent* to obtain a solution that has a concentration of about 0.1 mg/mL, based on the label claim. Pass a portion of this solution through a polyvinylidene fluoride (PVDF) filter of 0.45-µm pore size, and discard the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ 2S (USP41)
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loratadine ▲($C_{22}H_{23}ClN_2O_2$)▲ 2S (USP41) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of loratadine from the *Sample solution*
r_S = peak response of loratadine from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

Change to read:

- **PROCEDURE 2**

Buffer: 2.28 g/L of dibasic potassium phosphate trihydrate
Mobile phase: Methanol, *acetonitrile*, and *Buffer* (6:6:7), adjusted with 10% phosphoric acid to an apparent pH of 7.2
Diluent: Methanol and water (1:1)
System suitability solution: 0.8 µg/mL each of USP Loratadine Related Compound A RS, USP Loratadine Related Compound B RS, and USP Loratadine Related Compound C RS in *Diluent*
Standard solution: 0.4 mg/mL of USP Loratadine RS in *Diluent*. [NOTE—The solution may be sonicated for 5 min to aid in dissolution.]
Sample solution: Transfer a quantity of Tablets into a 250-mL volumetric flask so that the final concentration is 0.4 mg/mL, based on the label claim. Add 50 mL of water, and sonicate, if necessary, to disperse the Tablets. Add 50 mL of methanol, and shake to dissolve. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ 2S (USP41)
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 15 µL for the *Standard solution* and *Sample solution*; 50 µL for the *System suitability solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for loratadine related compound A, loratadine related compound C, loratadine related compound B, and loratadine are about 0.26, 0.31, 0.42, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.2 between loratadine related compound A and loratadine related compound C; NLT 1.2 between loratadine related compound C and loratadine related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loratadine ▲($C_{22}H_{23}ClN_2O_2$)▲ 2S (USP41) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of loratadine from the *Sample solution*
r_S = peak response of loratadine from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩
Test 1
Stage 1: All 6 Tablets completely disintegrate in 1 min.
Stage 2: NLT 16 of 18 Tablets completely disintegrate in 1 min.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Disintegration Test 2*.
Analysis: Place a stainless steel wire clip on each Tablet to prevent the Tablet from floating.
Acceptance criteria: NMT 30 s

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: Simulated gastric fluid without enzymes; 900 mL, deaerated
Apparatus 1: 50 rpm
Time: 6 min
Standard solution: Prepare a solution of USP Loratadine RS in *Medium* at a concentration similar to that expected in the *Sample solution*.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Analytical wavelength: 278 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of USP Loratadine RS from the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of loratadine (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of loratadine ▲($C_{22}H_{23}ClN_2O_2$)▲ 2S (USP41) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1**
Use *Organic Impurities, Procedure 1* if *Assay, Procedure 1* is used.
Buffer, Mobile phase, and **Diluent:** Prepare as directed in *Assay, Procedure 1*.
Sensitivity solution: 0.05 µg/mL of USP Loratadine RS in *Mobile phase*
Standard solution: 0.5 µg/mL of USP Loratadine RS in *Mobile phase*
Sample solution: Transfer 10 Tablets to a 500-mL volumetric flask, add 400 mL of acetonitrile, and stir for about 10 min. Sonicate the solution for 10 min, and stir for another 10 min. Dilute with acetonitrile to volume, and mix. Dilute an aliquot of the resulting solution with *Diluent* to obtain a solution that has a concentration of about 0.1 mg/mL, based on the label claim. Centrifuge the solution for about 10 min, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of loratadine from the *Standard solution*
C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

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Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine related compound C	0.5	0.64	0.2
Loratadine	1.0	—	—
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.3

• **ORGANIC IMPURITIES, PROCEDURE 2**

Use *Organic Impurities, Procedure 2* if *Assay, Procedure 2* is used.

Buffer, Mobile phase, Diluent, System suitability solution, and Sample solution: Prepare as directed in *Assay, Procedure 2*.

Sensitivity solution: 0.04 µg/mL of USP Loratadine RS in *Diluent*

Standard solution: 0.8 µg/mL of USP Loratadine RS in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

[NOTE—The relative retention times for loratadine related compound A, loratadine related compound C, loratadine related compound B, and loratadine are about 0.26, 0.31, 0.42, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.2 between loratadine related compound A and loratadine related compound C; NLT 1.2 between loratadine related compound C and loratadine related compound B, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 4.0%, *Standard solution*

Signal-to-noise ratio: NLT 3.0, *Sensitivity solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of loratadine from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine related compound A	0.26	0.9	0.1
Loratadine related compound B[a]	0.42	—	—
Unspecified impurity[a]	0.76	—	—
Loratadine	1.0	—	—
Unspecified impurity[a]	1.5	—	—
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.1

[a] These impurities are controlled in the drug substance and are listed here for information only. These impurities are not included when determining total impurities.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 20° and 25°.

• **LABELING:** The labeling states with which *Organic Impurities* and *Assay* procedure the article complies, if other than *Procedure 1*. When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Loratadine RS

USP Loratadine Related Compound A RS

8-Chloro-5,6-dihydro-11-(piperidin-4-ylidene)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

$C_{19}H_{19}ClN_2$ 310.82

USP Loratadine Related Compound B RS

8-Chloro-5,6-dihydro-11-(*N*-methylpiperidin-4-ylidene)-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

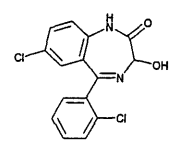
$C_{20}H_{21}ClN_2$ 324.85

USP Loratadine Related Compound C RS

8-Chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-one.

$C_{14}H_{10}ClNO$ 243.69

Lorazepam



$C_{15}H_{10}Cl_2N_2O_2$ 321.16

2*H*-1,4-Benzodiazepin-2-one, 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-, (±)-;

(±)-7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one [846-49-1].

DEFINITION

Lorazepam contains NLT 98.0% and NMT 102.0% of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$), calculated on the dried basis.

USP Monographs

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, glacial acetic acid, and water (50: 1.2: 50)
Diluent: Methanol and water (75:25)
Standard solution: 0.1 mg/mL of USP Lorazepam RS in *Diluent*
Sample solution: 0.1 mg/mL of Lorazepam in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 5°
Sample chamber: 4°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Collect data for at least 50 min.
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) in the portion of Lorazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lorazepam from the *Sample solution*
r_S = peak response of lorazepam from the *Standard solution*
C_S = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)
C_U = concentration of Lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **ORGANIC IMPURITIES**

Mobile phase and **Diluent:** Proceed as directed in the *Assay*.
System suitability solution: 3.2 mg/mL of USP Lorazepam RS and 32 µg/mL each of USP Lorazepam Related Compound A RS, USP Lorazepam Related Compound B RS, USP Lorazepam Related Compound C RS, USP Lorazepam Related Compound D RS, and USP Lorazepam Related Compound E RS in *Diluent*
Standard solution: 32 µg/mL of USP Lorazepam RS in *Diluent*
Sample solution: 3.2 mg/mL of Lorazepam in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 5°
Sample chamber: 4°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Collect data for at least 50 min.
Suitability requirements
Resolution: NLT 1.2 between lorazepam related compound A and lorazepam related compound E, *System suitability solution*
Tailing factor: NMT 2.0 for lorazepam, *Standard solution*
Relative standard deviation: NMT 5% for lorazepam, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Lorazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of lorazepam from the *Standard solution*
C_S = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)
C_U = concentration of Lorazepam in the *Sample solution* (mg/mL)
F = relative response factor for any given impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lorazepam	1.0	1.0	—
Lorazepam related compound D	1.4	1.0	0.15
Lorazepam related compound A	1.7	1.0	0.10
Lorazepam related compound E	1.9	1.3	0.15
Lorazepam related compound C	2.1	1.0	0.30
Lorazepam related compound B	5.5	1.0	0.01
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.75

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

USP Monographs

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lorazepam RS
USP Lorazepam Related Compound A RS
7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-acetoxy-2*H*-1,4-benzodiazepin-2-one.
$C_{17}H_{12}Cl_2N_2O_3$ 363.20
USP Lorazepam Related Compound B RS
2-Amino-2′,5-dichlorobenzophenone.
$C_{13}H_9Cl_2NO$ 266.12
USP Lorazepam Related Compound C RS
6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxaldehyde.
$C_{15}H_8Cl_2N_2O$ 303.14
USP Lorazepam Related Compound D RS
6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxylic acid.
$C_{15}H_8Cl_2N_2O_2$ 319.14
USP Lorazepam Related Compound E RS
6-Chloro-4-(*o*-chlorophenyl)-2-quinazoline methanol.
$C_{15}H_{10}Cl_2N_2O$ 305.16

Lorazepam Injection

DEFINITION
Lorazepam Injection is a sterile solution of Lorazepam in a suitable medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 0.05 M monobasic ammonium phosphate
Mobile phase: Methanol and *Buffer* (50:50). Adjust with ammonium hydroxide to a pH of 6.5.
System suitability solution: 0.04 mg/mL of lorazepam and 32 µg/mL each of USP Lorazepam Related Compound C RS and USP Lorazepam Related Compound D RS in *Mobile phase*
Standard stock solution: 1 mg/mL of USP Lorazepam RS in methanol
Standard solution: 0.16 mg/mL of USP Lorazepam RS in *Mobile phase* from the *Standard stock solution*
Sample solution: Nominally 0.16 mg/mL of lorazepam from Injection, diluted with *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 10 to 15-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of lorazepam
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for lorazepam related compound D, lorazepam, and lorazepam related compound C are 0.7, 1.0, and 2.7, respectively.]
Suitability requirements
Resolution: NLT 1.2 between lorazepam related compound D and lorazepam; NLT 1.2 between lorazepam and lorazepam related compound C, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lorazepam from the *Sample solution*
r_S = peak response of lorazepam from the *Standard solution*
C_S = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution 1: Prepare as directed for the *Standard solution* in the *Assay*.
Standard solution 2: 3.2 µg/mL each of USP Lorazepam Related Compound C RS and USP Lorazepam Related Compound D RS in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution 1*
[NOTE—The relative retention times for lorazepam related compound D, lorazepam, and lorazepam related compound C are 0.7, 1.0, and 2.7, respectively.]
Suitability requirements
Resolution: NLT 1.2 between lorazepam related compound D and lorazepam; NLT 1.2 between lorazepam and lorazepam related compound C, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution 1*
Analysis
Samples: *Sample solution* and *Standard solution 2*. Do not include as an impurity any peak from the *Sample solution* that has a retention time shorter than that of the lorazepam related compound D peak from *Standard solution 2*.
Calculate the percentage of lorazepam related compound C and lorazepam related compound D in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lorazepam related compound C or lorazepam related compound D from the *Sample solution*
r_S = peak response of the corresponding related compound from *Standard solution 2*
C_S = concentration of the corresponding related compound in *Standard solution 2* (µg/mL)
C_U = nominal concentration of lorazepam in the *Sample solution* (µg/mL)

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Calculate the percentage of any other impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*
r_T = peak response of lorazepam from the *Sample solution*

Acceptance criteria: NMT 4.0% of all impurities

• **LIMIT OF LORAZEPAM RELATED COMPOUND B**

Buffer: 0.05 M monobasic ammonium phosphate. Adjust with ammonium hydroxide to a pH of 6.5.
Mobile phase: Methanol and *Buffer* (55:45)
Diluent: Methanol and *Buffer* (50:50)
Standard solution: 0.16 µg/mL of USP Lorazepam Related Compound B RS in *Diluent*. Sonicate to dissolve if necessary.
Sample solution: Nominally 0.16 mg/mL of lorazepam from Injection, diluted with *Diluent*. Sonicate to dissolve if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
Run time: 1.5 times the retention time of lorazepam related compound B
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Signal-to-noise ratio: NLT 10
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lorazepam related compound B in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lorazepam related compound B from the *Sample solution*
r_S = peak response of lorazepam related compound B from the *Standard solution*
C_S = concentration of USP Lorazepam Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Contains NMT 10^2 USP Endotoxin Units/mg of lorazepam
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store in a refrigerator.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Lorazepam RS
USP Lorazepam Related Compound B RS
2-Amino-2′,5-dichlorobenzophenone.
$C_{13}H_9Cl_2NO$ 266.12
USP Lorazepam Related Compound C RS
6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxaldehyde.
$C_{15}H_8Cl_2N_2O$ 303.14
USP Lorazepam Related Compound D RS
6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxylic acid.
$C_{15}H_8Cl_2N_2O_2$ 319.14

Lorazepam Oral Concentrate

DEFINITION

Lorazepam Oral Concentrate contains NLT 90.0% and NMT 110.0% of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, glacial acetic acid, and water (45: 0.2: 55)
System suitability solution: 0.1 mg/mL each of USP Lorazepam RS and USP Lorazepam Related Compound E RS in methanol
Standard solution: 0.05 mg/mL of USP Lorazepam RS in methanol
Sample solution: Nominally 0.05 mg/mL of lorazepam prepared as follows. Transfer a suitable volume of Oral Concentrate to a volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode-array detector in the range of 220–400 nm.
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for lorazepam and lorazepam related compound E are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between lorazepam and lorazepam related compound E, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) in the portion of Oral Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase: Methanol and 0.05 M monobasic ammonium phosphate (64:36)

Diluent: Methanol and 0.05 M monobasic ammonium phosphate (50:50). Adjust with ammonium hydroxide to a pH of 6.5.

System suitability solution: 0.04 mg/mL of USP Lorazepam RS, and 0.032 mg/mL each of USP Lorazepam Related Compound C RS and USP Lorazepam Related Compound D RS in *Diluent*

Standard stock solution: 1.0 mg/mL of USP Lorazepam RS in methanol

Standard solution 1: 0.16 µg/mL of lorazepam from the *Standard stock solution* in *Diluent*

Standard solution 2: 0.16 µg/mL of USP Lorazepam Related Compound B RS, and 3.2 µg/mL each of USP Lorazepam Related Compound C RS and USP Lorazepam Related Compound D RS in *Mobile phase*

Sample solution: Nominally 0.16 mg/mL of lorazepam prepared as follows. Transfer a suitable volume of Oral Concentrate to a volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 10- to 15-cm; packing L1

Flow rate: 0.7 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution 1*

Suitability requirements

Resolution: NLT 1.2 between lorazepam related compound D and lorazepam; NLT 1.2 between lorazepam and lorazepam related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0% for lorazepam, *Standard solution 1*

Analysis

Samples: *Standard solution 2* and *Sample solution*

[NOTE—Disregard peaks eluting prior to lorazepam related compound D.]

Calculate the percentage of lorazepam related compound B, lorazepam related compound C, and lorazepam related compound D in the portion of Oral Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lorazepam related compound B, lorazepam related compound C, or lorazepam related compound D from the *Sample solution*

r_S = peak response of the corresponding related compound from *Standard solution 2*

C_S = concentration of the corresponding related compound in *Standard solution 2* (mg/mL)

C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lorazepam related compound D	0.8	4.0[a]
Lorazepam	1.0	—
Lorazepam related compound C	2.3	4.0[a]
Lorazepam related compound B	2.9	0.1

[a] Includes the sum of lorazepam related compound C and lorazepam related compound D.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store in a refrigerator.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Lorazepam RS

USP Lorazepam Related Compound B RS

2-Amino-2′,5-dichlorobenzophenone.

$C_{13}H_9Cl_2NO$ 266.12

USP Lorazepam Related Compound C RS

6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxaldehyde.

$C_{15}H_8Cl_2N_2O$ 303.14

USP Lorazepam Related Compound D RS

6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxylic acid.

$C_{15}H_8Cl_2N_2O_2$ 319.14

USP Lorazepam Related Compound E RS

6-Chloro-4-(*o*-chlorophenyl)-2-quinazoline methanol.

$C_{15}H_{10}Cl_2N_2O$ 305.16

Lorazepam Tablets

DEFINITION

Lorazepam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Sample: Stir a portion of finely powdered Tablets, equivalent to 15 mg of lorazepam, with 40 mL of acetone for 5 min. Pass through very retentive filter paper pre-washed with acetone. Evaporate the filtrate to dryness on a steam bath with the aid of a current of air. Dissolve the residue in 1 mL of acetone, and add 20 mL of 2,2,4-trimethylpentane. Heat the solution on a hot plate to a gentle boil, and evaporate to a volume of about 10 mL. Remove the solution from the hot plate, and evaporate to dryness with the aid of a current of air. Dry the residue under vacuum at 60° for 1 h.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Methanol and water (85:15)

USP Monographs

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Mobile phase: Acetonitrile, glacial acetic acid, and water (40: 0.4: 60)
Standard solution: 0.1 mg/mL of USP Lorazepam RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of lorazepam prepared as follows. Transfer 20 Tablets to a 100-mL volumetric flask, add 50 mL of *Diluent*, sonicate for 10 min, and shake by mechanical means for 20 min. Dilute with *Diluent* to volume, mix, and centrifuge a portion of the solution at 2000 rpm for 10 min. Dilute a portion of the clear supernatant with *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Times: 30 and 60 min
Mobile phase and **Chromatographic system:** Prepare as directed in the *Assay*, except use an *Injection volume* of 50 µL.
Standard solution: USP Lorazepam RS at a known concentration in *Medium*. Initially, use a volume of alcohol not exceeding 10% of the final volume of the *Standard solution* to dissolve the Reference Standard.
Sample solution: Sample per *Dissolution* ⟨711⟩.
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 60% (*Q*) of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) is dissolved in 30 min. NLT 80% (*Q*) of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) is dissolved in 60 min.

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Diluent, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sample solution: Nominally, 0.1 mg/mL of lorazepam prepared as follows. Place 1 Tablet in a volumetric flask of appropriate size, based on the labeled quantity, in mg, of lorazepam in the Tablet. Add a volume of *Diluent* equal to about 50% of the volume of the flask, sonicate for 10 min, and shake by mechanical means for 20 min. Dilute with *Diluent* to volume, mix, and centrifuge a portion of the solution for 10 min at 2000 rpm.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) in the portion of the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer: 67.7 g/L of sodium acetate trihydrate in water. Adjust with glacial acetic acid to a pH of 5.0 ± 0.05.
Mobile phase: Acetonitrile, glacial acetic acid, and water (50: 1.2: 50)
Diluent: Methanol and *Buffer* (75:25)
Standard solution: 1.6 µg/mL of USP Lorazepam RS in *Diluent*
Peak identification solution: 0.16 mg/mL of USP Lorazepam RS, 1.6 µg/mL each of USP Lorazepam Related Compound A RS, USP Lorazepam Related Compound B RS, USP Lorazepam Related Compound C RS, USP Lorazepam Related Compound D RS, and USP Lorazepam Related Compound E RS in *Diluent*
Sample solution: Nominally 0.16 mg/mL of lorazepam prepared as follows. Transfer a weighed amount of lorazepam, equivalent to 21.3 mg from powdered Tablets, to a 25-mL volumetric flask. Add 20 mL of *Diluent*, and stir for 15 min. Do not dilute to volume. Centrifuge at 2000 rpm for 15 min. Pass the supernatant through a polyethersulfone membrane of 0.45-µm pore size. Dilute a portion of the filtrate with *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC. Use an instrument equipped with a sample compartment chiller maintained at 4°.
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 5°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: At least 50 min
System suitability
Samples: *Standard solution* and *Peak identification solution*
[Note—See *Table 1* for the approximate relative retention times.]
Suitability requirements
Resolution: NLT 1.2 between lorazepam related compound A and lorazepam related compound E, *Peak identification solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for lorazepam from the *Standard solution*
C_S = concentration of lorazepam in the *Standard solution* (mg/mL)
C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)
F = relative response factor for any given impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lorazepam	1.0	1.0	—
Lorazepam related compound D[a]	1.4	1.0	0.5
Lorazepam related compound A[b,c]	1.7	—	—
Lorazepam related compound E[d]	1.9	1.3	0.5
Lorazepam related compound C[e]	2.1	1.0	3.0
Lorazepam related compound B[f]	5.5	1.0	0.1
Any individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	4.0

[a] 6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxylic acid.
[b] 7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-acetoxy-2*H*-1,4-benzodiazepin-2-one.
[c] Lorazepam related compound A is included only for peak identification purposes. It is not quantified and should not be included in the total impurities calculation.
[d] 6-Chloro-4-(*o*-chlorophenyl)-2-quinazoline methanol.
[e] 6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxaldehyde.
[f] 2-Amino-2′,5-dichlorobenzophenone.

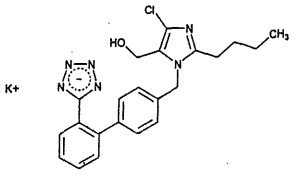
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lorazepam RS
  USP Lorazepam Related Compound A RS
  7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-acetoxy-2*H*-1,4-benzodiazepin-2-one.
  $C_{17}H_{12}Cl_2N_2O_3$ 363.20
  USP Lorazepam Related Compound B RS
  2-Amino-2′,5-dichlorobenzophenone.
  $C_{13}H_9Cl_2NO$ ▲266.12▲ (ERR 1-Oct-2018)
  USP Lorazepam Related Compound C RS
  6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxaldehyde.
  $C_{15}H_8Cl_2N_2O$ ▲303.14▲ (ERR 1-Oct-2018)
  USP Lorazepam Related Compound D RS
  6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxylic acid.
  $C_{15}H_8Cl_2N_2O_2$ ▲319.14▲ (ERR 1-Oct-2018)
  USP Lorazepam Related Compound E RS
  6-Chloro-4-(*o*-chlorophenyl)-2-quinazoline methanol.
  $C_{15}H_{10}Cl_2N_2O$ 305.16

Losartan Potassium



$C_{22}H_{22}ClKN_6O$ 461.00
1*H*-Imidazole-5-methanol, 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-, monopotassium salt;
2-Butyl-4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl] imidazole-5-methanol, monopotassium salt [124750-99-8].

DEFINITION

Losartan Potassium contains NLT 98.5% and NMT 101.0% of losartan potassium ($C_{22}H_{22}ClKN_6O$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020) or ⟨197K⟩: Meets the requirements

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
  Sample solution: 10 µg/mL in methanol
  Acceptance criteria: Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**
  Solution A: 0.1% solution of phosphoric acid in water
  Solution B: Acetonitrile
  Mobile phase: *Solution B* and *Solution A* (2:3)
  Standard solution: 0.25 mg/mL of USP Losartan Potassium RS in methanol
  Sample solution: 0.25 mg/mL of Losartan Potassium in methanol
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.0-mm × 25-cm; packing L1
  Column temperature: 35°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 5600 theoretical plates
  Tailing factor: NMT 1.4
  Relative standard deviation: NMT 0.5%

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of losartan potassium ($C_{22}H_{22}ClKN_6O$) in the portion of Losartan Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the anhydrous, solvent-free basis

IMPURITIES
• **ORGANIC IMPURITIES**
Solution A: 0.1% solution of phosphoric acid in water
Solution B: Acetonitrile
System suitability solution: 0.3 mg/mL of USP Losartan Potassium RS and 2 µg/mL of triphenylmethanol in methanol
Sample solution: 0.3 mg/mL of Losartan Potassium in methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
25	10	90
35	10	90
45	75	25
50	75	25

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for losartan and triphenylmethanol are 1.0 and 1.9, respectively. The typical retention time for triphenylmethanol is 20 min.]
Suitability requirements
Tailing factor: NMT 1.6
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of losartan potassium ($C_{22}H_{22}ClKN_6O$) taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses for all peaks

Acceptance criteria
Individual impurities: NMT 0.2%
Total impurities: NMT 0.5%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%. If labeled as an amorphous form, NMT 5.0%.

ADDITIONAL REQUIREMENTS
• **LABELING:** Where it is an amorphous form, the label so indicates.
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Losartan Potassium RS

Losartan Potassium Tablets

DEFINITION
Losartan Potassium Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 1.25 mg/mL of monobasic potassium phosphate and 1.5 mg/mL of dibasic sodium phosphate in water. The resulting pH is approximately 7.0. Pass the solution through a PTFE or equivalent filter of 0.45-µm pore size, and degas before use.
Solution A: Acetonitrile and *Buffer* (15:85)
Solution B: Use acetonitrile.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	40	60
11	80	20
15	80	20

System suitability stock solution: Dissolve 12 mg of USP Losartan Potassium RS in a 50-mL volumetric flask, first using 5 mL of water, followed by 5 mL of 0.1 N hydrochloric acid. Place the flask in a 105° oven for 1–2 h, and allow to cool to room temperature. Pipet 5 mL of 0.1 N sodium hydroxide into the flask, and dilute with water to volume. Adjust with either 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 6.0. [NOTE—The resulting solution contains the 1*H*-dimer and 2*H*-dimer, and the resulting solution may be cloudy.]
System suitability solution: Add 3 mL of acetonitrile to 7 mL of *System suitability stock solution* to clear the cloudy solution, and mix well.
Standard solution: 0.25 mg/mL of USP Losartan Potassium RS in *Solution A*. Pass through a PTFE or equivalent filter of 0.45-µm pore size.
Sample stock solution: Transfer 10 Tablets to a 500-mL volumetric flask, add *Solution A* to fill the flask to about 50% of the final volume, and sonicate with intermittent shaking for 15 min. Sonicate for an additional 10 min. Dilute with *Solution A* to volume, and mix well.

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Sample solution: 0.25 mg/mL of losartan potassium in *Solution A* from the *Sample stock solution*. Mix well. Pass an aliquot of the solution through a PTFE filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 3.9-mm × 15-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the losartan, 1*H*-dimer, and 2*H*-dimer peaks; *System suitability solution*
Resolution: NLT 2.0 between the 1*H*-dimer and 2*H*-dimer, *System suitability solution*
Column efficiency: NLT 3000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan from the *Sample solution*
r_S = peak response of losartan from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1
Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: (*L*/1000) mg/mL of USP Losartan Potassium RS in *Medium*, where *L* is the Tablet label claim, in mg
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Analysis: Determine the amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved by using one of the following procedures:
Instrumental conditions
Analytical wavelength: Maximum absorbance at about 256 nm
Path length: See *Table 2* or make the appropriate dilution of the solutions with *Medium* to be within the linearity range of the spectrophotometer.

Table 2

Tablet Strength (mg/Tablet)	Cell Size (cm)
25	1.0
50	0.5
100	0.2

Blank: *Medium*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Chromatographic procedure
Solution A: 0.1% v/v phosphoric acid in water
Mobile phase: Acetonitrile and *Solution A* (40:60)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.0-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of losartan

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: 1.4 g/L of anhydrous monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.3 ± 0.1.
Mobile phase: Methanol, acetonitrile, and *Buffer* (20:20:60)
Standard solution: 0.028 mg/mL of USP Losartan Potassium RS in *Medium*
Sample solution
For Tablets labeled to contain 25 mg: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
For Tablets labeled to contain 50 and 100 mg: Pass a portion of the solution under test through a suitable filter

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of 0.45-µm pore size. Further dilute the filtrate with *Medium* to prepare a 0.028-mg/mL solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (*Q*) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 30 min for 25-mg and 50-mg Tablet strengths, and 45 min for 100-mg Tablet strength
Buffer: 0.025 M phosphoric acid. Adjust with 1 N sodium hydroxide to a pH of 2.15.
Mobile phase: Acetonitrile and *Buffer* (400:600)
Standard stock solution: 0.27 mg/mL of USP Losartan Potassium RS prepared as follows. Add methanol to USP Losartan Potassium RS to fill about 10% of the volume of the flask, and add *Medium* to fill about 50% of the volume of the flask. Sonicate for NLT 15 min. Cool to room temperature, and dilute with *Medium* to volume.
Standard solution: Prepare as directed in *Table 3* from the *Standard stock solution*.

Table 3

Tablet Strength (mg/Tablet)	Concentration (mg/mL)
25	0.027
50	0.054
100	0.108

Sample solution: Pass a portion of the solution under test through a suitable polyethylene filter of 10-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of losartan from the *Sample solution*
r_S = peak response of losartan from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved for 25-mg and 50-mg Tablet strengths. NLT 80% (*Q*) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved for 100-mg Tablet strength.

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Buffer: Dissolve 1.36 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
Diluent: Dissolve 17.42 g of dibasic potassium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 8.0. Dilute with water to a volume of 1000 mL, and mix well. Further dilute with water (1 in 10), and mix well.
Mobile phase: Acetonitrile and *Buffer* (60:40)
Standard solution: 0.05 mg/mL of USP Losartan Potassium RS in *Diluent*
Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask, add about 65 mL of *Diluent*, and shake mechanically for 30 min. Dilute with *Diluent* to volume, and mix well.
Sample solution: 0.05 mg/mL of losartan potassium in *Diluent* from the *Sample stock solution*. Filter an aliquot of the solution, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 10-µm packing L7
Flow rate: 1.4 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) in the portion of the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan from the *Sample solution*
r_S = peak response of losartan from the *Standard solution*

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C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Prepare as directed in the *Assay*.

Standard stock solution: Use the *Standard solution*, prepared as directed in the *Assay*.

Standard solution: 2.5 µg/mL of USP Losartan Potassium RS in *Solution A* from the *Standard stock solution*

Sensitivity solution: Dilute 1 mL of the *Standard solution* to 10 mL in *Solution A*.

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Tailing factor: NMT 2.0 for the losartan, 1*H*-dimer, and 2*H*-dimer peaks; *System suitability solution*

Resolution: NLT 2.0 between the 1*H*-dimer and 2*H*-dimer, *System suitability solution*

Column efficiency: NLT 3000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10 for the losartan peak from the first injection. If this is not met, then the *Signal-to-noise ratio* must be greater than 3 with a relative standard deviation of area counts less than 25% for three replicate injections, *Sensitivity solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Identify the peaks using the relative retention times provided in *Table 4*.]

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of losartan from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Losartan	1.0	—
1*H*-Dimer[a]	2.4	0.5
2*H*-Dimer[b]	2.9	0.5

Table 4 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities[c]	—	1.0

[a] 5-[4′-({2-Butyl-5-[(5-{4′-[(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl}-1*H*-tetrazol-1-yl)methyl]-4-chloro-1*H*-imidazol-1-yl}methyl)biphenyl-2-yl]tetrazol, potassium salt.

[b] 5-[4′-({2-Butyl-5-[(5-{4′-[(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl}-2*H*-tetrazol-2-yl)methyl]-4-chloro-1*H*-imidazol-1-yl}methyl)biphenyl-2-yl]tetrazol, potassium salt.

[c] The total impurities include the sum of all the specified impurities and the sum of all the unspecified impurities. Disregard peaks less than 0.1%.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in tightly closed containers, protected from light, at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Losartan Potassium RS

Losartan Potassium and Hydrochlorothiazide Tablets

DEFINITION

Losartan Potassium and Hydrochlorothiazide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amounts of losartan potassium ($C_{22}H_{22}ClKN_6O$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer A: 2.76 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Buffer B: 1.25 g/L of monobasic potassium phosphate and 1.5 g/L of dibasic sodium phosphate in water. The pH of the resulting solution is about 7.0–7.5.

Diluent: Acetonitrile and *Buffer A* (3:2)

Solution A: Acetonitrile and *Buffer B* (7:93)

Solution B: Use acetonitrile.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	92	8
28	38	62
30	100	0
35	100	0

Standard solution: Transfer USP Losartan Potassium RS and USP Hydrochlorothiazide RS into a suitable volumetric flask, and dissolve in *Diluent* (50% of the volume of the flask). Dilute with *Buffer A* to volume to obtain a solution having concentrations as directed in *Table 2*. Pass a portion of the

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solution through a PTFE or equivalent filter of 0.45-µm pore size.

Table 2

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Concentration of USP Losartan Potassium RS (mg/mL)	Concentration of USP Hydrochlorothiazide RS (mg/mL)
50/12.5	0.4	0.1
100/12.5	0.4	0.05
100/25	0.4	0.1

Sample stock solution: Transfer 10 Tablets into a suitable volumetric flask and add *Diluent* as directed in *Table 3*. Mix well and mechanically shake or stir until the solid is dispersed. Dilute with *Buffer A* to volume, and sonicate.

Table 3

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Flask Size (mL)	Volume of Diluent (mL)
50/12.5	250	210
100/12.5	500	420
100/25	500	420

Sample solution: Dilute a portion of the *Sample stock solution* first with acetonitrile (20% of the volume of the flask) and then with *Buffer A*, to obtain a solution having nominal concentrations as directed in *Table 4*. Pass a portion of this solution through a PTFE or equivalent filter of 0.45-µm pore size, and use the filtrate.

Table 4

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Concentration of USP Losartan Potassium RS (mg/mL)	Concentration of USP Hydrochlorothiazide RS (mg/mL)
50/12.5	0.4	0.1
100/12.5	0.4	0.05
100/25	0.4	0.1

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 15-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: Less than 2.5 for the losartan peak
Relative standard deviation: Less than 2.0% for both hydrochlorothiazide and losartan peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response of losartan or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of losartan potassium or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1
Medium: Water; 900 mL, deaerated
Apparatus 1: 100 rpm
Time: 30 min for both losartan and hydrochlorothiazide
Buffer: Dissolve 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Buffer* (2:3)
Losartan potassium stock solution: 0.44 mg/mL of USP Losartan Potassium RS in *Medium*
Hydrochlorothiazide stock solution: 0.14 mg/mL of USP Hydrochlorothiazide RS prepared by dissolving in methanol (10% of the volume of the flask). Dilute with *Medium* to volume.
Standard solution: Transfer the appropriate volumes of *Losartan potassium stock solution* and *Hydrochlorothiazide stock solution* to a 100-mL volumetric flask according to the dilution schemes in *Table 5*. Dilute with *Medium* to volume.

Table 5

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Aliquot of Losartan Potassium Stock Solution (mL)	Aliquot of Hydrochlorothiazide Stock Solution (mL)
50/12.5	12.5	10.0
100/12.5	25.0	10.0
100/25	25.0	20.0

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 10-µm packing L7
Column temperature: 35°
Flow rate: 2.3 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2 between the hydrochlorothiazide and losartan peaks
Relative standard deviation: NMT 2.0% for both the hydrochlorothiazide and losartan peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

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$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of losartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response of losartan or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (*Q*) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) and NLT 75% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium, Apparatus 1, and **Time:** Proceed as directed in *Test 1*.

Buffer: Dissolve 1.78 g of dibasic sodium phosphate dihydrate in 1 L of water. Adjust with phosphoric acid to a pH of 6.5.

Mobile phase: Acetonitrile and *Buffer* (32:68)

Diluent: Acetonitrile and water (40:60)

Standard stock solution 1: 1.1 mg/mL of USP Losartan Potassium RS in *Diluent*. Sonication may be necessary for complete dissolution.

Standard stock solution 2: 0.28 mg/mL of USP Hydrochlorothiazide RS in *Diluent*. Sonication may be necessary for complete dissolution.

Standard solution: Transfer appropriate volumes of *Standard stock solution 1* and *Standard stock solution 2* to a 100-mL volumetric flask according to the dilution schemes in *Table 6*. Dilute with *Medium* to volume.

Table 6

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Aliquot of Standard Stock Solution 1 (mL)	Aliquot of Standard Stock Solution 2 (mL)
50/12.5	5	5
100/12.5	10	5
100/25	10	10

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Autosampler temperature: 8°
Flow rate: 1.2 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for both the hydrochlorothiazide and losartan peaks

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (1/L) \times V \times 100$$

r_U = peak response of losartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response of losartan or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (*Q*) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) and NLT 80% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Buffer A: Prepare as directed in the *Assay*.

Buffer B: Dissolve 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Diluent: Prepare a mixture of acetonitrile and *Buffer A* (3:2).

Mobile phase: Acetonitrile and *Buffer B* (2:3)

Standard stock solution 1: 0.46 mg/mL of USP Losartan Potassium RS prepared by dissolving in *Diluent* (50% of the total volume of the flask). Mechanically shake for 15 min or until dissolved. Dilute with *Buffer A* to volume.

Standard stock solution 2: 0.35 mg/mL of USP Hydrochlorothiazide RS prepared by dissolving in *Diluent* (50% of the total volume of the flask). Mechanically shake for 15 min or until dissolved. Dilute with *Buffer A* to volume.

Standard solution: Transfer aliquots of *Standard stock solution 1* and *Standard stock solution 2* into a suitable volumetric flask, and add *Diluent*, up to 42% of the total volume of the flask. Dilute with *Buffer A* to volume, mix well, and sonicate for 2 min to obtain a solution having concentrations based on Tablet strength as listed in *Table 7*. Pass a portion of the solution through a PTFE or equivalent filter of 0.45-µm pore size, and use the filtrate.

Table 7

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Concentration of USP Losartan Potassium RS (mg/mL)	Concentration of USP Hydrochlorothiazide RS (mg/mL)
50/12.5	0.06	0.014
100/12.5	0.06	0.007
100/25	0.06	0.014

Sample stock solution: Transfer 1 Tablet into a suitable volumetric flask, and add *Diluent* as directed in *Table 8*. Mix well, and mechanically shake for 30 min or until the solid is finely dispersed. Dilute with *Buffer A* to volume, and mix well.

Table 8

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Flask Size (mL)	Volume of Diluent (mL)
50/12.5	100	50
100/12.5	200	100
100/25	200	100

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Sample solution: Dilute 10 mL of the *Sample stock solution* in a 100-mL volumetric flask, with 45 mL of *Diluent,* and then dilute with *Buffer A* to volume. Pass an aliquot of the solution through a PTFE or equivalent filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 10-µm packing L7
Column temperature: 35°
Flow rate: 2.3 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2 between the hydrochlorothiazide and losartan peaks
Relative standard deviation: NMT 2.0% for both the hydrochlorothiazide and losartan peaks

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response of losartan or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Losartan Potassium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of losartan potassium or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• Organic Impurities

Buffer A, Buffer B, Diluent, Solution A, Solution B, Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*

Chlorothiazide standard solution: 0.1 mg/mL of USP Chlorothiazide RS prepared by dissolving in *Diluent* (50% of the volume of the flask). Dilute with *Buffer A* to volume, and sonicate.

Benzothiadiazine related compound A standard solution: 0.1 mg/mL of USP Benzothiadiazine Related Compound A RS prepared by dissolving in *Diluent* (50% of the volume of the flask). Dilute with *Buffer A* to volume, and sonicate.

Stressed losartan solution: [Note—This solution contains the degradates 1-*H*-dimer and 2-*H*-dimer and losartan potassium.] Weigh 12 mg of the USP Losartan Potassium RS in a 50-mL flask. Dissolve in 5 mL of water. Pipet 5.0 mL of 0.1 N hydrochloric acid into this solution, and place it in an oven at 105° for 1–2 h. Remove from the oven and allow to cool to room temperature. Pipet 5.0 mL of 0.1 N sodium hydroxide into the flask, and dilute with water to volume.

Diluted standard solution: Dilute portions of the *Standard solution* and *Benzothiadiazine related compound A standard solution* first with acetonitrile (30% of the volume of the flask), then with *Buffer A* to obtain a solution having nominal concentrations based on Tablet strength as listed in *Table 9.*

Table 9

Tablet Strength Losartan Potassium/ Hydrochloro-thiazide (mg)	Concentration of USP Losartan Potassium RS (µg/mL)	Concentration of USP Hydrochloro-thiazide RS (µg/mL)	Concentration of USP Benzothia-diazine Related Compound A RS (µg/mL)
50/12.5	4	1	1
100/12.5	4	0.5	1
100/25	4	1	1

System suitability solution: Dissolve weighed quantities of USP Losartan Potassium RS and USP Hydrochlorothiazide RS in a suitable volumetric flask in *Diluent* (50% of the volume of the flask). Add the *Stressed losartan solution,* about 25% of the volume of the flask, into the same flask. Transfer appropriate amounts of *Chlorothiazide standard solution* and *Benzothiadiazine related compound A standard solution* into the same flask, and dilute with *Buffer A* to volume to obtain a solution having a known concentration of about 0.4 mg/mL of losartan, 0.1 mg/mL of hydrochlorothiazide, and 0.001 mg/mL each of benzothiadiazine related compound A and chlorothiazide. Adjust with phosphoric acid to a pH of 2.5, and mix well. Pass an aliquot of the solution through a PTFE or equivalent filter of 0.45-µm pore size, and use the filtrate.

Limit of quantitation solution: Pipet 5.0 mL of the *Diluted standard solution* into a 50-mL volumetric flask. Add 15 mL of acetonitrile, dilute with *Buffer A* to volume, and mix well.

System suitability
Samples: *Standard solution, Diluted standard solution, System suitability solution,* and *Limit of quantitation solution*
Suitability requirements
Resolution: Greater than 1.5 between chlorothiazide and benzothiadiazine related compound A; greater than 1.5 between the benzothiadiazine related compound A and hydrochlorothiazide peak, *System suitability solution*
Tailing factor: Less than 2.5 for the losartan peak, *Standard solution*
Relative standard deviation: Less than 2.0% for both the hydrochlorothiazide and losartan peaks, *Standard solution;* less than 10.0% for both the hydrochlorothiazide and losartan peaks, *Diluted standard solution*
Signal-to-noise ratio: NLT 10 for each component from the first injection. If this is not met, then the signal-to-noise ratio must be greater than 3 with a relative standard deviation of area counts less than 25% for three replicate injections, *Limit of quantitation solution*

Analysis
Samples: *Sample solution* and *Diluted standard solution*
[Note—The run time is about 1.6 times the retention time of the losartan peak. Identify the peaks using the relative retention times provided in *Table 10.*]
Calculate the percentage of benzothiadiazine related compound A (expressed as hydrochlorothiazide equivalent) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A from the *Diluted standard solution*

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C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Diluted standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of hydrochlorothiazide, 298
M_{r2} = molecular weight of benzothiadiazine related compound A, 286

Calculate the percentage of each specified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of losartan from the *Diluted standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Diluted standard solution* (mg/mL)
C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

For Tablet strengths of 50/12.5 and 100/25 for losartan potassium/hydrochlorothiazide, respectively, calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of losartan from the *Diluted standard solution*
C_S = concentration of USP Losartan Potassium RS in the *Diluted standard solution* (mg/mL)
C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

For a Tablet strength of 100/12.5 for losartan potassium/hydrochlorothiazide, calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Diluted standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Diluted standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria See *Table 10*.

Table 10

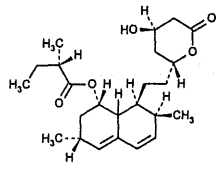
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorothiazide[a]	0.57	—
Benzothiadiazine related compound A	0.69	1.0
Hydrochlorothiazide	1.0	—
Losartan	2.7	—
1-*H*-Dimer[b]	3.3	0.5
2-*H*-Dimer[c]	3.5	0.5
Total impurities[d]	—	2.0

[a] This process impurity (not a degradation product) is related to hydrochlorothiazide and is controlled in the drug substance.
[b] Related to losartan potassium: 5-[4′-([2-butyl-5-[(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl]-1*H*-tetrazol-1-yl]methyl]-4-chloro-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl]tetrazol, potassium salt.
[c] Related to losartan potassium: 5-[4′-([2-butyl-5-[(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl]-2*H*-tetrazol-2-yl]methyl]-4-chloro-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl]tetrazol, potassium salt.
[d] Total impurities include the sum of all the specified impurities and the unspecified impurities that are equal to or greater than 0.1%.

ADDITIONAL REQUIREMENTS

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **PACKAGING AND STORAGE:** Preserve in tightly closed containers protected from light, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzothiadiazine Related Compound A RS
  4-Amino-6-chloro-1,3-benzenedisulfonamide.
  $C_6H_8ClN_3O_4S_2$ 285.73
  USP Chlorothiazide RS
  USP Hydrochlorothiazide RS
  USP Losartan Potassium RS

Lovastatin



$C_{24}H_{36}O_5$ 404.54
Butanoic acid, 2-methyl-, 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)-ethyl]-1-naphthalenyl ester, [1*S*-[1α(*R**),3α,7β,8β(2*S**,4*S**),8aβ]]-.
(*S*)-2-Methylbutyric acid, 8-ester with (4*R*,6*R*)-6-[2-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one [75330-75-5].

» Lovastatin contains not less than 98.5 percent and not more than 101.0 percent of $C_{24}H_{36}O_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers under nitrogen in a cold place.
USP Reference standards ⟨11⟩—
USP Lovastatin RS
USP Lovastatin Related Compound A RS
(1*S*,3*S*,4a*R*,7*S*,8*S*,8a*S*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl (*S*)-2-methylbutanoate.
$C_{24}H_{38}O_5$ 406.56

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests ⟨197⟩, Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: acetonitrile.
Specific rotation ⟨781S⟩: between +324° and +338°.
Test solution: 5 mg per mL, in acetonitrile.
Loss on drying ⟨731⟩—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 0.3% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

Limit of lovastatin related compound A—
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and 0.01 M phosphoric acid (13:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve accurately weighed quantities of USP Lovastatin RS and USP Lovastatin Related Compound A RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution containing 2.0 µg of each per mL.
Standard solution—Dissolve an accurately weighed quantity of USP Lovastatin RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 2.0 µg per mL.
Test solution—Transfer about 25 mg of Lovastatin, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The column temperature is maintained at 40°. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for lovastatin and 1.3 for lovastatin related compound A; and the resolution, *R,* between lovastatin and lovastatin related compound A is not less than 6.0. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of lovastatin related compound A in the portion of Lovastatin taken by the formula:

$$2.5F(C/W)(r_U/r_S)$$

in which F is the response factor for lovastatin related compound A and is equal to 1.6; C is the concentration, in µg per mL, of USP Lovastatin RS in the *Standard solution;* W is the weight, in mg, of Lovastatin in the *Test solution;* r_U is the peak response for lovastatin related compound A obtained from the *Test solution;* and r_S is the peak response for lovastatin obtained from the *Standard solution:* not more than 0.5% of lovastatin related compound A is found.

Chromatographic purity—
Solution A—Prepare a 0.001 M phosphoric acid solution, adjusted with 1 M sodium hydroxide to a pH of 4.0.
Solution B—Use acetonitrile.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve accurately weighed quantities of USP Lovastatin RS and compactin in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution containing 2.0 µg of each per mL.
Standard solution—Dissolve an accurately weighed quantity of USP Lovastatin RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 2.0 µg per mL.
Test solution—Transfer about 25 mg of Lovastatin, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 4.0-mm × 12.5-cm column that contains 4-µm packing L1. The column temperature is maintained at 40°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–2	60	40	isocratic
2–5	60→45	40→55	linear gradient
5–8	45	55	isocratic
8–16	45→10	55→90	linear gradient
16–25	10	90	isocratic
25–27	10→60	90→40	linear gradient
27–35	60	40	isocratic

Chromatograph the *System suitability solution* and the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.00 for lovastatin and 0.85 for compactin; the resolution, *R,* between lovastatin and compactin is not less than 3.5; and the relative standard deviation for replicate injections is not more than 5%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of each impurity in the portion of Lovastatin taken by the formula:

$$2.5(C/W)(r_i/r_S)F$$

in which C is the concentration, in µg per mL, of USP Lovastatin RS in the *Standard solution;* W is the weight, in mg, of Lovastatin in the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* r_S is the peak response for lovastatin obtained from the *Standard solution;* and F is the response factor for each impurity and is equal to 1.4 for the impurity with a relative retention time of about 0.73 and 1.0 for all other impurities. Disregard any peak with less than 0.04%: not more than 0.2% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—
Dilute phosphoric acid—Transfer 1 mL of phosphoric acid to a 1-L volumetric flask, and dilute with water to volume.

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Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Dilute phosphoric acid* (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Lovastatin RS in acetonitrile to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Transfer about 30 mg of Lovastatin, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

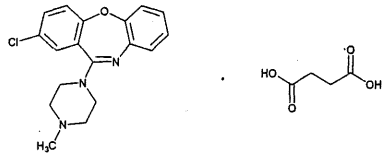
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.4, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{36}O_5$ in the portion of Lovastatin taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Lovastatin RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Loxapine Succinate



$C_{18}H_{18}ClN_3O \cdot C_4H_6O_4$ 445.90

Butanedioic acid, compd. with 2-chloro-11-(4-methyl-1-piperazinyl)dibenz[*b,f*][1,4]oxazepine (1:1);
2-Chloro-11-(4-methyl-1-piperazinyl)dibenz[*b,f*][1,4] oxazepine succinate (1:1) [27833-64-3].

DEFINITION

Loxapine Succinate contains NLT 98.5% and NMT 101.0% of $C_{18}H_{18}ClN_3O \cdot C_4H_6O_4$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 20 µg/mL in 0.01 N hydrochloric acid

ASSAY

• **PROCEDURE**

Sample: 400 mg of Loxapine Succinate

Analysis: Dissolve in 80 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Calculate the percentage of $C_{18}H_{18}ClN_3O \cdot C_4H_6O_4$ in the portion of Loxapine Succinate taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

V	= sample titrant volume (mL)
B	= blank titrant volume (mL)
N	= normality of titrant (mEq/mL)
F	= equivalence factor, 22.29 mg/mEq
W	= sample weight (mg)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

• **Procedure**

Buffer: 3.9 g/L of ammonium acetate in water. Adjust with 20% acetic acid in water or 6 N ammonium hydroxide to a pH of 7.3.

Mobile phase: Acetonitrile and *Buffer* (3:7)

Diluent: Acetonitrile and *Buffer* (7:3)

Standard solution: 10 µg/mL of USP Loxapine Succinate RS and 1.5 µg/mL each of USP Amoxapine RS and USP Loxapine Related Compound A RS in *Diluent.* [NOTE—Amoxapine has been included in the *Standard solution* for peak identification purposes only.]

Sample solution: 1 mg/mL of Loxapine Succinate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 5-cm; 2.7-µm packing L1

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection size: 10 µL

Run time: 2 times the retention time of loxapine succinate

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between loxapine succinate and loxapine related compound A

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Loxapine Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U	= peak response of each impurity from the *Sample solution*
r_S	= peak response of loxapine succinate from the *Standard solution*
C_S	= concentration of USP Loxapine Succinate RS in the *Standard solution* (mg/mL)
C_U	= concentration of Loxapine Succinate in the *Sample solution* (mg/mL)
F	= relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1.*

USP Monographs

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Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxapine[a]	0.19	1.3	0.15
Chlorodibenzoxazepinone[b]	0.45	0.70	0.15
Loxapine succinate	1.0	—	—
Loxapine related compound A[c]	1.2	1.2	0.15
Any unspecified individual impurity	—	1.0	0.10

[a] 2-Chloro-11-(piperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine.
[b] 2-Chlorodibenzo[*b,f*]-1,4-oxazepin-11-one.
[c] 3-Chloro-11-(4-methylpiperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxapine RS
  USP Loxapine Related Compound A RS
  3-Chloro-11-(4-methylpiperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine.
  $C_{18}H_{18}ClN_3O$ 327.81
  USP Loxapine Succinate RS

Loxapine Capsules

DEFINITION
Loxapine Capsules contain an amount of loxapine succinate ($C_{18}H_{18}ClN_3O \cdot C_4H_6O_4$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of loxapine ($C_{18}H_{18}ClN_3O$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Dissolve 4 g of tetramethylammonium chloride in 800 mL of water, and add 200 mL of acetonitrile and 1 mL of phosphoric acid.
Standard solution: 0.136 mg/mL of USP Loxapine Succinate RS prepared as follows. Dissolve a suitable quantity of USP Loxapine Succinate RS in 0.01 N hydrochloric acid in a suitable volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Nominally 0.1 mg/mL of loxapine prepared as follows. Weigh the contents of NLT 20 Capsules. Transfer a portion of the contents, nominally equivalent to NLT 50 mg of loxapine, to a suitable volumetric flask. Add 10% of the flask volume of 0.1 N hydrochloric acid. Shake, and sonicate for 5 min. Dilute with *Mobile phase* to volume, and filter. Discard the first 8 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Flow rate: 1.6 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loxapine ($C_{18}H_{18}ClN_3O$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Loxapine Succinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of loxapine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of loxapine, 327.81
M_{r2} = molecular weight of loxapine succinate, 445.90

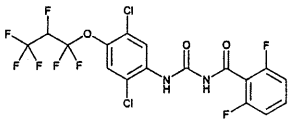
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Loxapine Succinate RS at a known concentration in *Medium*
Sample solution: Dilute with water as needed.
Instrumental conditions
Mode: UV
Analytical wavelength: 254 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 75% (*Q*) of the labeled amount of loxapine ($C_{18}H_{18}ClN_3O$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Loxapine Succinate RS

Lufenuron



$C_{17}H_8Cl_2F_8N_2O_3$ 511.15
Benzamide, *N*-[[[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]amino]carbonyl]-2,6-difluoro-; 1-[2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea [103055-07-8].

DEFINITION
Lufenuron contains NLT 98.0% and NMT 102.0% of lufenuron ($C_{17}H_8Cl_2F_8N_2O_3$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1 mL of phosphoric acid diluted with water to 1 L

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions, and equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	30	70
5	30	70
15	10	90
17	10	90

Diluent: Acetonitrile and water (70:30)

System suitability solution: 0.4 mg/mL of USP Lufenuron RS and 0.14 mg/mL of USP Lufenuron Related Compound G RS in *Diluent*. Sonicate if necessary to facilitate dissolution.

Standard stock solution: 0.4 mg/mL of USP Lufenuron RS in *Diluent*. Sonicate if necessary to facilitate dissolution.

Standard solution: 0.04 mg/mL of USP Lufenuron RS in *Diluent* from *Standard stock solution*

Sample stock solution: 0.4 mg/mL of Lufenuron in *Diluent*. Sonicate if necessary to facilitate dissolution.

Sample solution: 0.04 mg/mL of Lufenuron in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 255 nm

Column: 4-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lufenuron related compound G and lufenuron are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between lufenuron related compound G and lufenuron, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lufenuron ($C_{17}H_8Cl_2F_8N_2O_3$) in the portion of Lufenuron taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lufenuron RS in the *Standard solution* (mg/mL)

C_U = concentration of Lufenuron in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, Diluent, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Identification solution: 1.2 µg/mL of USP Lufenuron Related Compound B RS and 1.6 µg/mL of USP Lufenuron Related Compound C RS in *Diluent*

Diluted standard solution: 0.4 µg/mL of USP Lufenuron RS in *Diluent* from *Standard solution*

Sample solution: 0.4 mg/mL of Lufenuron in *Diluent*. Sonicate if necessary to facilitate dissolution.

Analysis

Samples: *Identification solution*, *Diluted standard solution*, and *Sample solution*

Chromatograph the *Identification solution*, and identify the components on the basis of their relative retention times, given in *Table 2*.

Calculate the percentage of each impurity in the portion of Lufenuron taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of lufenuron from the *Diluted standard solution*

C_S = concentration of USP Lufenuron RS in the *Diluted standard solution* (µg/mL)

C_U = concentration of Lufenuron in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lufenuron related compound B	0.3	0.77	0.3
Lufenuron related compound C	0.7	0.77	0.4
Lufenuron	1.0	—	—
Any other individual impurity	—	1.0	0.20
Total impurities	—	—	1.0

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 0.5%

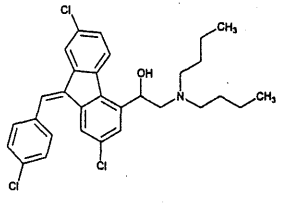
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

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- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Lufenuron RS
  USP Lufenuron Related Compound B RS
  N-[(2,5-Dichloro-4-hydroxyphenyl)carbamoyl]-2,6-difluorobenzamide.
  $C_{14}H_8Cl_2F_2N_2O_3$ 361.13
  USP Lufenuron Related Compound C RS
  N-[3-Chloro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenylcarbamoyl]-2,6-difluorobenzamide.
  $C_{17}H_9ClF_8N_2O_3$ 476.71
  USP Lufenuron Related Compound G RS
  2,5-Dichloro-4-[3-(2,6-difluorobenzoyl)ureido]phenyl phenyl carbonate.
  $C_{21}H_{12}Cl_2F_2N_2O_5$ 481.23

Lumefantrine



$C_{30}H_{32}Cl_3NO$ 528.94
(±)-2,7-Dichloro-9-[(*Z*)-*p*-chlorobenzylidine]-α[(dibutylamino)methyl]-fluorene-4-methanol [82186-77-4].

DEFINITION
Lumefantrine contains NLT 98.0% and NMT 102.0% of lumefantrine ($C_{30}H_{32}Cl_3NO$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the lumefantrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: Dissolve 5.65 g of sodium 1-hexanesulfonate and 2.75 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 2.3 before dilution with water to a final volume of 1000 mL.
  Solution A: Acetonitrile and *Buffer* (300:700)
  Solution B: Acetonitrile and 2-propanol (540:460)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
1.2	65	35
6.0	50	50
6.4	30	70
10.0	25	75
15.0	10	90
15.1	65	35
20.0	65	35

System suitability stock solution: 10 µg/mL of USP Lumefantrine Related Compound A RS prepared as follows. Transfer a suitable quantity of USP Lumefantrine Related Compound A RS to a volumetric flask, dissolve in 10% volume dichloromethane, and dilute with acetonitrile to volume.
System suitability solution: 1 mg/mL of USP Lumefantrine RS and 1 µg/mL of USP Lumefantrine Related Compound A RS prepared as follows. Transfer 10 mg of USP Lumefantrine RS to a 10-mL volumetric flask, and dissolve in 1 mL of dichloromethane. Add 1.0 mL of the *System suitability stock solution*, and dilute with acetonitrile to volume.
Standard solution: 1 mg/mL of USP Lumefantrine RS prepared as follows. Transfer a suitable quantity of USP Lumefantrine RS to a volumetric flask, dissolve in 10% volume of dichloromethane, and dilute with acetonitrile to volume.
Sample solution: 1 mg/mL of Lumefantrine prepared as follows. Transfer a suitable quantity of Lumefantrine to a volumetric flask, dissolve in 10% volume of dichloromethane, and dilute with acetonitrile to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 50-mm; 1.8-µm packing L1
Column temperature: Beginning of column, 50°; end of column, 35°
Flow rate: 2.5 mL/min
Injection volume: 2.5 µL
Run time: 20 min
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for lumefantrine related compound A and lumefantrine are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.3 between lumefantrine and lumefantrine related compound A, *System suitability solution*
Tailing factor: NMT 2.1, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lumefantrine ($C_{30}H_{32}Cl_3NO$) in the portion of Lumefantrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

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• **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, System suitability stock solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard stock solution: 50 µg/mL of USP Lumefantrine RS prepared as follows. Transfer a suitable quantity of USP Lumefantrine RS into a volumetric flask, dissolve in 10% volume of dichloromethane, and dilute with acetonitrile to volume.

Standard solution: 1 µg/mL of USP Lumefantrine RS in acetonitrile from the *Standard stock solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of desbutyl lumefantrine or any other impurity in the portion of Lumefantrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of desbutyl lumefantrine or any other impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

F = relative response factor

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbutyl lumefantrine[a]	0.68	1.1	0.05
Lumefantrine	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.3

[a] (*Z*)-2-(Butylamino)-1-(2,7-dichloro-9-(4-chlorobenzylidene)-9*H*-fluoren-4-yl) ethanol.

SPECIFIC TESTS

• **CLARITY OF SOLUTION**

Hydrazine sulfate solution: Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, and dissolve in and dilute with water to volume. Allow to stand for 4–6 h before use.

Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25 mL of water, insert the glass stopper, and mix to dissolve.

Primary opalescent suspension: Transfer 25.0 mL of *Hydrazine sulfate solution* to the *Methanamine solution* in the 100-mL glass-stoppered flask. Allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Stock opalescence suspension: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. This suspension should not be used beyond 24 h after preparation.

Sample solution: Dissolve 1.0 g of Lumefantrine in dichloromethane, and dilute with dichloromethane to 10.0 mL.

Standard suspension: Transfer 5.0 mL of *Stock opalescence suspension* to a 100-mL volumetric flask, and dilute with water to volume. Prepare only if the *Sample solution* is not as clear as water or dichloromethane.

Analysis

Samples: *Sample solution*, *Standard suspension*, water, and dichloromethane

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension* and dichloromethane to separate matching test tubes. Compare the *Sample solution*, *Standard suspension*, water, and dichloromethane in diffused daylight, viewing vertically against a black background. The diffusion of light must be such that the *Standard suspension* can readily be distinguished from dichloromethane. If the *Sample solution* is as clear as water or dichloromethane, it is not necessary to prepare the *Standard suspension*.

Acceptance criteria: The *Sample solution* shows the same or more clarity than water, dichloromethane, or the *Standard suspension*.

ADDITIONAL REQUIREMENTS

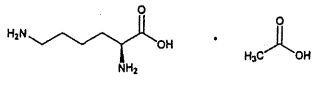
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Lumefantrine RS

USP Lumefantrine Related Compound A RS
(*RS,Z*)-2-(Dibutylamino)-2-(2,7-dichloro-9-(4-chlorobenzylidene)-9*H*-fluoren-4-yl)ethanol.
$C_{30}H_{32}Cl_3NO$ 528.94

Lysine Acetate



$C_6H_{14}N_2O_2 \cdot C_2H_4O_2$ 206.24

L-Lysine monoacetate [57282-49-2].

DEFINITION

Lysine Acetate contains NLT 98.0% and NMT 102.0% of L-lysine acetate ($C_6H_{14}N_2O_2 \cdot C_2H_4O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample: 100 mg of Lysine Acetate

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the *Blank* determination.

Calculate the percentage of lysine acetate ($C_6H_{14}N_2O_2 \cdot C_2H_4O_2$) in the *Sample* taken:

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$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 103.1 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.4%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Standard solution: 0.50 mL of 0.020 N hydrochloric acid
  Sample: 0.73 g of Lysine Acetate
  Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Standard solution: 0.10 mL of 0.020 N sulfuric acid
  Sample: 0.33 g of Lysine Acetate
  Acceptance criteria: NMT 0.03%
- **IRON** ⟨241⟩: NMT 30 ppm
- **RELATED COMPOUNDS**
  Standard solution: 0.05 mg/mL of USP L-Lysine Acetate RS in water
  [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]
  Sample solution: 10 mg/mL of Lysine Acetate in water
  System suitability solution: 0.4 mg/mL each of USP L-Lysine Acetate RS and USP Arginine Hydrochloride RS
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Isopropyl alcohol and ammonium hydroxide (7:3)
  Spray reagent: 0.2 g of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
  System suitability
  Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.
  Analysis
  Samples: *Standard solution*, *System suitability solution*, and *Sample solution*
  Dry the plate between 100° and 105° until the ammonia completely disappears. Spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
  Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
  Individual impurities: NMT 0.5%
  Total impurities: NMT 2.0%

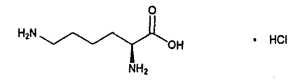
SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 100 mg/mL in water
  Acceptance criteria: +8.4° to +9.9°
- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 80° for 3 h: it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Arginine Hydrochloride RS
  USP L-Lysine Acetate RS

Lysine Hydrochloride



$C_6H_{14}N_2O_2 \cdot HCl$ 182.65
L-Lysine hydrochloride [657-27-2].

DEFINITION

Lysine Hydrochloride contains NLT 98.5% and NMT 101.5% of L-lysine hydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**
  Sample: 90 mg of Lysine Hydrochloride
  Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Endpoint detection: Potentiometric
  Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with the *Titrant*. Perform the *Blank* determination.
  Calculate the percentage of lysine hydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 91.33 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

OTHER COMPONENTS

- **CONTENT OF CHLORIDE**
  Sample: 350 mg of Lysine Hydrochloride
  Blank: 140 mL of water
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N silver nitrate VS
  Endpoint detection: Visual
  Analysis: Transfer the *Sample* to a porcelain casserole, and add 140 mL of water and 1 mL of dichlorofluorescein TS. Titrate with the *Titrant* until the silver chloride flocculates and the mixture acquires a faint pink color. Perform the *Blank* determination.
  Calculate the percentage of chloride (Cl) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)

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F = equivalency factor, 35.45 mg/mEq
W = *Sample* weight (mg)

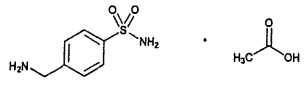
Acceptance criteria: 19.0%–19.6%

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Standard solution: 0.10 mL of 0.020 N sulfuric acid
  Sample: 0.33 g of Lysine Hydrochloride
  Acceptance criteria: NMT 0.03%
- **IRON** ⟨241⟩: NMT 30 ppm
- **RELATED COMPOUNDS**
  Standard solution: 0.05 mg/mL of USP L-Lysine Hydrochloride RS in water. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]
  Sample solution: 10 mg/mL of Lysine Hydrochloride in water
  System suitability solution: 0.4 mg/mL each of USP L-Lysine Hydrochloride RS and USP Arginine Hydrochloride RS
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Isopropyl alcohol and ammonium hydroxide (7:3)
  Spray reagent: 0.2 g of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
  System suitability
  Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.
  Analysis
  Samples: *Standard solution*, *System suitability solution*, and *Sample solution*
  Dry the plate between 100° and 105° until the ammonia completely disappears. Spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
  Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
  Individual impurities: NMT 0.5%
  Total impurities: NMT 2.0%

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 80 mg/mL in 6 N hydrochloric acid
  Acceptance criteria: +20.4° to +21.4°
- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 3 h: it loses NMT 0.4% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Arginine Hydrochloride RS
  USP L-Lysine Hydrochloride RS

Mafenide Acetate



$C_7H_{10}N_2O_2S \cdot C_2H_4O_2$ 246.28
Benzenesulfonamide, 4-(aminomethyl)-, monoacetate; α-Amino-*p*-toluenesulfonamide monoacetate [13009-99-9].

DEFINITION
Mafenide Acetate contains NLT 98.0% and NMT 102.0% of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The R_F value of the principal spot of the *Identification solution* corresponds to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**
  Standard solution: 200 µg/mL of USP Mafenide Acetate RS in 0.01 N hydrochloric acid
  Sample stock solution: 2 mg/mL of Mafenide Acetate
  Sample solution: 200 µg/mL of Mafenide Acetate prepared as follows. Pipet 10 mL of the *Sample stock solution* into a 100-mL volumetric flask containing 1 mL of 1 N hydrochloric acid, and dilute with water to volume.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: 267 nm
  Cell: 1 cm
  Blank: 0.01 N hydrochloric acid
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*
  Calculate the percentage of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$) in the portion of Mafenide Acetate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Mafenide Acetate RS in the *Standard solution* (µg/mL)
C_U = concentration of Mafenide Acetate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **SELENIUM** ⟨291⟩
  Sample: 200 mg
  Acceptance criteria: NMT 30 ppm
- **ORGANIC IMPURITIES**
  Standard solution A: 500 µg/mL of USP Mafenide Acetate RS in methanol
  Standard solution D: 500 µg/mL of USP Mafenide Related Compound A RS in methanol
  [NOTE—USP Mafenide Related Compound A RS is 4-formylbenzenesulfonamide.]
  Standard solutions: Quantitatively dilute portions of *Standard solution A* with methanol to obtain *Standard*

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solution B and *Standard solution C*. Similarly, quantitatively dilute portions of *Standard solution D* with methanol to obtain *Standard solution E* and *Standard solution F*. The compositions are shown in *Table 1*.

Table 1

Standard Solution	Dilution	Concentration (µg/mL)	Percentage (%, for comparison with test specimen)
A	(undiluted)	500	1.0
B	5 in 10	250	0.5
C	1 in 5	100	0.2
D	(undiluted)	500	1.0
E	5 in 10	250	0.5
F	1 in 5	100	0.2

Sample solution: 50 mg/mL of Mafenide Acetate in methanol
Identification solution: 500 µg/mL from the *Sample solution* in methanol
Ninhydrin solution: 300 mg of ninhydrin in 100 mL of butyl alcohol. Add 3 mL of glacial acetic acid.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Ethyl acetate, methanol, and isopropylamine (77:20:3)
Spray reagent: *Ninhydrin solution*
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Identification solution*
Apply the *Samples* separately to the chromatographic plate. Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm, circulating air. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* at the R_f value corresponding to those of the principal spots in the chromatograms of *Standard solutions D*, *E*, and *F*. Spray the plate with *Spray reagent*, heat the plate at 105° for 5 min, and examine the plate. Compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* to those of the principal spots in the chromatograms of *Standard solutions A*, *B*, and *C*.
Acceptance criteria: No secondary spot, observed by both visualizations, from the chromatogram of the *Sample solution* is larger or more intense than the principal spots obtained from *Standard solution B* (0.5%) and *Standard solution E* (0.5%). The sum of the intensities of all secondary spots obtained from the *Sample solution* corresponds to NMT 1.0%.

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: Between 162° and 171°, but the range between the beginning and end of melting does not exceed 4°.
• **PH** ⟨791⟩
Sample solution: 100 mg/mL
Acceptance criteria: 6.4–6.8
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mafenide Acetate RS
USP Mafenide Related Compound A RS
4-Formylbenzenesulfonamide.
$C_7H_7NO_3S$ 185.20

Mafenide Acetate Cream

DEFINITION
Mafenide Acetate Cream is Mafenide Acetate in a water-miscible, oil-in-water cream base, containing suitable preservatives. It contains NLT 90.0% and NMT 110.0% of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$), in terms of the labeled amount of mafenide ($C_7H_{10}N_2O_2S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Sample solution: Proceed as directed in the *Assay*.
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL,** *Acetate* ⟨191⟩
Sample solution: Place about 1 g in a 60-mL separatory funnel, and add 20 mL of chloroform to dissolve it. Add 20 mL of water, shake for 2 min, allow the layers to separate completely, and discard the lower chloroform layer. Repeat this washing with another 20-mL portion of chloroform, and discard the chloroform washing. Centrifuge the aqueous layer. Use a 1-mL aliquot of the supernatant with 2 mL of water for test *A* and a 3-mL aliquot of the supernatant test *B*.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Standard solution: 200 µg/mL of USP Mafenide Acetate RS in 0.01 N hydrochloric acid
Sample solution: Nominally 200 µg/mL of mafenide acetate prepared as follows. Transfer a portion of Cream containing 100 mg of mafenide acetate to a 60-mL separator, and add 20 mL of chloroform to dissolve it. Add 20 mL of water, shake for 2 min, allow the layers to separate completely, and discard the lower chloroform layer. Repeat this washing with two separate 20-mL portions of chloroform, and discard the chloroform washings. Pass the aqueous phase through a dry filter into a 100-mL volumetric flask. Rinse the separator and the filter with water, passing all rinses through the filter, and add water to volume. Centrifuge 30 mL, then pipet 20 mL of the clear supernatant into a 100-mL volumetric flask. Add 1 mL of 1 N hydrochloric acid, and add water to volume.
Instrumental conditions
Mode: UV
Analytical wavelength: 267 nm
Cell: 1 cm
Blank: 0.01 N hydrochloric acid
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*

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Calculate the percentage of the labeled amount of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$) in the portion of Cream taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Mafenide Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of mafenide acetate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mafenide Acetate RS

Mafenide Acetate for Topical Solution

DEFINITION

Mafenide Acetate for Topical Solution contains NLT 98.0% and NMT 102.0% of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 6.8 g of monobasic potassium phosphate and 1.0 g of sodium 1-hexanesulfonate in 800 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute to 1000 mL.
Mobile phase: Acetonitrile and *Solution A* (1:9)
Standard solution: 1 mg/mL of USP Mafenide Acetate RS in *Mobile phase*. Sonicate to dissolve if necessary.
Sample solution: Nominally 1 mg/mL of mafenide acetate prepared as follows. Constitute the Topical Solution as directed in the labeling. Transfer a volume of the constituted Topical Solution, equivalent to 25 mg of mafenide acetate, to a 25-mL volumetric flask. Using sonication, dissolve in 12 mL of *Mobile phase*. Dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 267 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$) in the portion of the constituted Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mafenide Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mafenide acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

OTHER COMPONENTS

- **CONTENT OF ACETIC ACID**

Internal standard solution: 0.5% (v/v) of propionic acid in water
Standard stock solution: Transfer 50 mL of water to a 100-mL volumetric flask, insert a stopper, and weigh. Add 0.5 mL of glacial acetic acid to the flask, insert the stopper, weigh, and calculate, by difference, the amount of acetic acid added. Dilute with water to volume.
Standard solution: 530 µg/mL of acetic acid prepared as follows. Add 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution* to a 100-mL volumetric flask containing 200 mg of oxalic acid. Dilute with water to volume.
Sample solution: Nominally 2 mg/mL of mafenide acetate prepared as follows. Constitute the Topical Solution as directed in the labeling. Transfer a volume of the constituted Topical Solution, equivalent to 200 mg of mafenide acetate, to a 100-mL volumetric flask containing 200 mg of oxalic acid. Pipet 10.0 mL of *Internal standard solution* into the flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 60-m fused-silica capillary column coated with a 0.5-µm layer of acid-deactivated phase G35
Temperatures
Injection: 250°
Detector: 250°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	0	150	11
150	25	240	10
240	25	150	1

Carrier gas: Helium
Flow rate: 40 cm/s
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between acetic acid and propionic acid
Relative standard deviation: NMT 6.0% for peak response ratios

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetic acid in the portion of the constituted Topical Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetic acid to propionic acid from the *Sample solution*
R_S = peak response ratio of acetic acid to propionic acid from the *Standard solution*
C_S = concentration of acetic acid in the *Standard solution* (mg/mL)
C_U = nominal concentration of mafenide acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 22.0%–26.8%

IMPURITIES
• **ORGANIC IMPURITIES**
Solution A: Dissolve 6.8 g of monobasic potassium phosphate and 1.0 g of sodium 1-hexanesulfonate in 800 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute to 1000 mL.
Mobile phase: Acetonitrile and *Solution A* (1:9)
Standard stock solution: 25 µg/mL of USP Mafenide Related Compound A RS in *Mobile phase*
[NOTE—USP Mafenide Related Compound A RS is 4-formylbenzenesulfonamide.]
System suitability solution: 1.0 mg/mL of USP Mafenide Acetate RS and 10 µg/mL of USP Mafenide Related Compound A RS from *Standard stock solution* in *Mobile phase*. Initially dissolve the USP Mafenide Acetate RS using 20% of the final volume by sonication in 2 mL of *Mobile phase*, add the appropriate volume of USP Mafenide Related Compound A RS, and dilute to final volume.
Standard solution A: 5 µg/mL of USP Mafenide Related Compound A RS from *Standard stock solution* in *Mobile phase*
Standard solution B: 1 µg/mL of USP Mafenide Related Compound A RS from *Standard solution A* in *Mobile phase*
Sample solution: Proceed as directed in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 267 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 3 times the retention time of mafenide
System suitability
Samples: *System suitability solution*, *Standard solution A*, and *Standard solution B*
Suitability requirements
Resolution: NLT 3.0 between mafenide acetate and mafenide related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution A*
Analysis
Samples: *Mobile phase*, *Standard solution A*, and *Sample solution*
Chromatograph the *Standard solution* and adjust the integration parameters so that the response is 5%–15% of full-scale deflection. Disregard the peaks corresponding to those obtained from the *Mobile phase*.
Calculate the percentage of each impurity in the portion of the constituted Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of mafenide related compound A from *Standard solution A*
C_S = concentration of USP Mafenide Related Compound A RS in *Standard solution A* (µg/mL)
C_U = nominal concentration of mafenide acetate in the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurity: NMT 0.5%
Total impurities: NMT 1.0%

SPECIFIC TESTS
• **PH** ⟨791⟩
Sample solution: Nominally 100 mg/mL
Acceptance criteria: 6.4–6.8
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, at controlled room temperature. For prepared solutions, use within 48 h of preparation.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mafenide Acetate RS
USP Mafenide Related Compound A RS
4-Formylbenzenesulfonamide.
$C_7H_7NO_3S$ 185.20

Magaldrate

Aluminum magnesium hydroxide sulfate $(Al_5Mg_{10}(OH)_{31}(SO_4)_2 \cdot xH_2O)$.
Aluminum magnesium hydroxide sulfate, hydrate [74978-16-8].
Anhydrous 1097.38

» Magaldrate is a chemical combination of aluminum and magnesium hydroxides and sulfate, corresponding approximately to the formula:

$$Al_5Mg_{10}(OH)_{31}(SO_4)_2 \cdot xH_2O.$$

It contains the equivalent of not less than 90.0 percent and not more than 105.0 percent of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Magaldrate RS

Identification—
A: Dissolve about 600 mg in 20 mL of 3 N hydrochloric acid, add 3 drops of methyl red TS and about 30 mL of water, and heat to boiling. Add 6 N ammonium hydroxide until the color just changes to yellow, continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* ⟨191⟩.
B: Wash the precipitate obtained in *Identification* test *A* with 50 mL of hot ammonium chloride solution (1 in 50), then dissolve the precipitate in 15 mL of 3 N hydrochloric acid: the solution responds to the tests for *Aluminum* ⟨191⟩.

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C: Its X-ray diffraction pattern (see *X-ray Diffraction* ⟨941⟩) in the d-spacings region below 0.257 nm (2.57 angstrom units) conforms to that of USP Magaldrate RS.
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the test for absence of *Escherichia coli.*
Loss on drying ⟨731⟩—Dry it at 200° for 4 hours: it loses between 10.0% and 20.0% of its weight.
Soluble chloride—Boil 1 g of it, accurately weighed, with 50.0 mL of water for 5 minutes, cool, add water to restore the original volume, mix, and filter. To 25.0 mL of the filtrate add 0.1 mL of potassium chromate TS, and titrate with 0.10 N silver nitrate until a persistent pink color is obtained: not more than 5.0 mL of 0.10 N silver nitrate is required (3.5%).
Soluble sulfate ⟨221⟩—A 2.5-mL portion of the filtrate obtained in the test for *Soluble chloride* shows no more sulfate than corresponds to 1.0 mL of 0.020 N sulfuric acid (1.9%).
Sodium—Transfer 2 g of it, accurately weighed, to a 100-mL volumetric flask, place in an ice bath, add 5 mL of nitric acid, and swirl to dissolve. Allow to warm to room temperature, dilute with water to volume, and mix. Filter, if necessary, to obtain a clear solution. Dilute 10.0 mL of the filtrate with water to 100.0 mL: the emission intensity of this solution, determined with a suitable flame photometer at 589 nm and corrected for background transmission at 580 nm, is not greater than that produced by a standard containing 2.2 µg of Na per mL, similarly measured (0.11%).
Arsenic, *Method I* ⟨211⟩: 8 ppm.
Magnesium hydroxide content—Dissolve about 100 mg, accurately weighed, in 3 mL of dilute hydrochloric acid (1 in 10), and dilute with water to about 200 mL. Add, with stirring, 1 g of ammonium chloride, 20 mL of triethanolamine, 10 mL of ammonia–ammonium chloride buffer TS, and 0.1 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $Mg(OH)_2$: between 49.2% and 66.6% of $Mg(OH)_2$ is found, calculated on the dried basis.

Aluminum hydroxide content—
Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum.*
Procedure—Dissolve about 100 mg of Magaldrate, accurately weighed, in 3 mL of dilute hydrochloric acid (1 in 10), and dilute with water to about 30 mL. Add, with stirring, 25.0 mL of *Edetate disodium titrant,* mix, and allow to stand for 5 minutes. Then add 20 mL of acetic acid-ammonium acetate buffer TS, 60 mL of alcohol, and 2 mL of dithizone TS, and titrate with 0.05 M zinc sulfate to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of $Al(OH)_3$: between 32.1% and 45.9% of $Al(OH)_3$ is found, calculated on the dried basis.

Sulfate content—
Chromatographic column—Transfer 15 mL of strongly acidic 50- to 100-mesh styrene-divinylbenzene cation-exchange resin to a 1-cm inside diameter glass column. Wash the resin with 30 mL of water.
Indicator solution—Prepare a solution in water containing 2 mg of sodium alizarinsulfonate per mL.
Magnesium acetate solution—Dissolve 26.8 g of magnesium acetate in 500 mL of water.
0.05 M Barium chloride—Dissolve 12.2 g of barium chloride in about 900 mL of water, adjust with 1 N hydrochloric acid to a pH of 3.0, dilute with water to 1000 mL, and mix. Standardize this solution as follows: Transfer 10.0 mL of 0.1 N sulfuric acid VS to a 125-mL conical flask. Adjust by adding *Magnesium acetate solution* to a pH of 3.0. Add 25 mL of methanol and 3 or 4 drops of *Indicator solution.* Add from a buret an accurately measured volume of 8 to 9 mL of 0.05 M barium chloride. Add an additional 4 drops of *Indicator solution,* and titrate slowly until the yellow color disappears and a pink tinge is visible. Calculate the molarity of the barium chloride titrant taken by the formula:

$$5(N/V)$$

in which N is the normality of the sulfuric acid; and V is the volume, in mL, of titrant consumed.
Test preparation—Transfer about 875 mg of Magaldrate, accurately weighed, to a 25-mL volumetric flask. Dissolve in 10 mL of water and 5 mL of glacial acetic acid, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to the chromatographic column and wash the column with 15 mL of water, collecting the eluate in a 125-mL conical flask (*Test preparation*).
Procedure—Add to the *Test preparation* 5 mL of *Magnesium acetate solution,* 32 mL of methanol, and 3 or 4 drops of *Indicator solution.* Add from a buret an accurately measured volume of 5.0 to 5.5 mL of 0.05 M barium chloride. Add an additional 3 drops of *Indicator solution,* and titrate slowly until the yellow color disappears and a pink tinge is visible. Each mL of 0.05 M barium chloride is equivalent to 4.803 mg of sulfate (SO_4): between 16.0% and 21.0% of SO_4 is found, calculated on the dried basis.
Assay—Transfer about 3 g of Magaldrate, accurately weighed, to a 250-mL beaker, add 100.0 mL of 1 N hydrochloric acid VS, and stir until the solution becomes clear. Titrate the excess acid with 1 N sodium hydroxide VS to a pH of 3.0, determined potentiometrically. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 1 N hydrochloric acid is equivalent to 35.40 mg of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Magaldrate and Simethicone Oral Suspension

» Magaldrate and Simethicone Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magaldrate [$Al_5Mg_{10}(OH)_{31}(SO_4)_2$], and an amount of polydimethylsiloxane [$-(CH_3)_2SiO-]_n$ that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

Packaging and storage—Preserve in tight containers, and keep from freezing.
USP Reference standards ⟨11⟩—
USP Magaldrate RS
USP Polydimethylsiloxane RS

Identification—
A: Dissolve an amount of Oral Suspension, equivalent to about 800 mg of magaldrate, in 20 mL of 3 N hydrochloric acid, dilute with water to about 50 mL, add 3 drops of methyl red TS, and proceed as directed in *Identification* test *A* under *Magaldrate,* beginning with "and heat to boiling."
B: Wash the precipitate obtained in *Identification* test *A* with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid. Divide this solution into two portions: the dropwise addition of 6 N ammonium hydroxide to one portion yields a gelatinous white precipitate, which does not dissolve in an excess of 6 N ammonium

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hydroxide. The dropwise addition of 1 N sodium hydroxide to the other portion yields a gelatinous white precipitate, which dissolves in an excess of 1 N sodium hydroxide, leaving some turbidity.

C: Transfer an amount of Oral Suspension, equivalent to about 1 g of magaldrate, to a 100-mL centrifuge tube. Add about 60 mL of water, insert the cap, and shake for 3 minutes. Centrifuge the suspension, and discard the supernatant. Repeat the washing of the residue with three 60-mL portions of water. Transfer the residue to a 250-mL beaker, and heat on a steam bath to dryness: the X-ray diffraction pattern (see *X-ray Diffraction* ⟨941⟩), in the d-spacings region below 2.57 angstrom units, of the residue so obtained conforms to that of USP Magaldrate RS.

D: The IR absorption spectrum, in the 7- to 15-µm region, determined in a 0.1-mm cell, of the *Assay preparation* prepared as directed in the *Assay for polydimethylsiloxane* exhibits maxima only at the same wavelengths as that of the *Standard preparation* prepared as directed in the *Assay for polydimethylsiloxane.*

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli.*

Acid-neutralizing capacity ⟨301⟩—The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.8(0.0282M)$$

in which 0.0282 is the theoretical acid-neutralizing capacity, in mEq per mg, of magaldrate; and M is the quantity, in mg, of the labeled amount of magaldrate.

Magnesium hydroxide content—

Test preparation—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 1 g of magaldrate, to a 100-mL volumetric flask, add 30 mL of dilute hydrochloric acid (1 in 10), shake to dissolve, dilute with water to volume, and mix.

Procedure—Transfer 10.0 mL of *Test preparation* to a 400-mL beaker, and proceed as directed in the test for *Magnesium hydroxide content* under *Magaldrate,* beginning with "and dilute with water to about 200 mL." Not less than 492 mg and not more than 666 mg of magnesium hydroxide [$Mg(OH)_2$] per g of the labeled amount of magaldrate is found.

Aluminum hydroxide content—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum.*

Test preparation—Prepare as directed in the test for *Magnesium hydroxide content.*

Procedure—Transfer 10.0 mL of *Test preparation* and 20 mL of water to a 250-mL beaker, and proceed as directed for *Procedure* in the test for *Aluminum hydroxide content* under *Magaldrate,* beginning with "Add, with stirring, 25.0 mL of *Edetate disodium titrant.*" Not less than 321 mg and not more than 459 mg of aluminum hydroxide [$Al(OH)_3$] per g of the labeled amount of magaldrate is found.

Other requirements—Evaporate a volume of Oral Suspension, equivalent to about 5 g of magaldrate, on a steam bath to dryness: the residue so obtained meets the requirements of the tests for *Arsenic* under *Magaldrate.*

Assay for magaldrate—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 3 g of magaldrate, to a beaker. Add 100.0 mL of 1 N hydrochloric acid VS, and mix, using a magnetic stirrer to achieve dissolution. Titrate the excess acid with 1 N sodium hydroxide VS to a pH of 3.0, determined potentiometrically. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 1 N hydrochloric acid is equivalent to 35.40 mg of magaldrate [$Al_5Mg_{10}(OH)_{31}(SO_4)_2$].

Assay for polydimethylsiloxane—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 250 mg of simethicone, to a 200-mL centrifuge bottle. Add an equal volume of hydrochloric acid, swirl to dissolve the Oral Suspension, add 25.0 mL of hexanes, and immediately close the bottle securely with a cap having an inert liner. Shake the bottle for 30 minutes, and centrifuge the mixture until a clear supernatant layer is obtained (*Assay preparation*). Prepare a *Standard preparation* of USP Polydimethylsiloxane RS in hexanes having a known concentration of about 10 mg per mL. Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 0.1-mm cells at the wavelength of maximum absorbance at about 7.9 µm and at the wavelengths of minimum absorbance at about 7.5 µm and 8.3 µm, with a suitable IR spectrophotometer, using hexanes as the blank. Draw a linear baseline between the two minima, and determine the absorbances for the *Standard preparation* and the *Assay preparation* with respect to the baseline, making any necessary correction for the blank. Calculate the quantity, in mg, of $[-(CH_3)_2SiO-]_n$ in the portion of Oral Suspension taken by the formula:

$$25C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Polydimethylsiloxane RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation,* respectively.

Magaldrate and Simethicone Chewable Tablets

Former title: Magaldrate and Simethicone Tablets

» Magaldrate and Simethicone Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magaldrate [$Al_5Mg_{10}(OH)_{31}(SO_4)_2$], and an amount of polydimethylsiloxane [$-(CH_3)_2SiO-]_n$ that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label the Chewable Tablets to indicate that they are to be chewed before being swallowed.

USP Reference standards ⟨11⟩—
USP Magaldrate RS
USP Polydimethylsiloxane RS

Identification—

A: Transfer a quantity of powdered Chewable Tablets, equivalent to about 2 g of magaldrate, to a 100-mL centrifuge tube. Add about 60 mL of water, cap, and shake for 3 minutes. Centrifuge the suspension, and discard the supernatant. Repeat the washing with three more 60-mL portions of water. Transfer the residue to a 250-mL beaker, and heat on a steam bath to dryness: the residue so obtained meets the requirements of the *Identification* tests under *Magaldrate.*

B: The IR absorption spectrum, in the 7- to 11-µm region, determined in a 0.5-mm cell, of the *Assay preparation*

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prepared as directed in the *Assay for polydimethylsiloxane,* exhibits maxima only at the same wavelengths as that of the *Standard preparation* containing about 2 mg of USP Polydimethylsiloxane RS per mL prepared as directed in the *Assay for polydimethylsiloxane.*

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Chewable Tablets meet the requirements of the test for absence of *Escherichia coli.*

Uniformity of dosage units ⟨905⟩**:** meet the requirements for *Weight Variation* with respect to magaldrate.

Acid-neutralizing capacity—Proceed as directed under *Acid-neutralizing Capacity* ⟨301⟩. The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.8(0.0282M)$$

in which 0.0282 is the theoretical acid-neutralizing capacity, in mEq per mg, of magaldrate, and M is the quantity, in mg, of the labeled amount of magaldrate.

Magnesium hydroxide content—

Test preparation—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 g of magaldrate, to a 100-mL volumetric flask, add 30 mL of dilute hydrochloric acid (1 in 10), shake for 15 minutes, dilute with water to volume, and mix.

Procedure—Transfer 10.0 mL of the *Test preparation* to a 400-mL beaker, and proceed as directed in the test for *Magnesium hydroxide content* under *Magaldrate,* beginning with "and dilute with water to about 200 mL." Not less than 492 mg and not more than 666 mg of magnesium hydroxide [$Mg(OH)_2$] per g of the labeled amount of magaldrate is found.

Aluminum hydroxide content—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum.*

Test preparation—Prepare as directed in the test for *Magnesium hydroxide content.*

Procedure—Transfer 10.0 mL of *Test preparation* and 20 mL of water to a 250-mL beaker, and proceed as directed for *Procedure* in the test for *Aluminum hydroxide content* under *Magaldrate,* beginning with "Add, with stirring, 25.0 mL of *Edetate disodium.*" Not less than 321 mg and not more than 459 mg of aluminum hydroxide [$Al(OH)_3$] per g of the labeled amount of magaldrate is found.

Assay for magaldrate—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6 g of magaldrate, to a 200-mL volumetric flask. Add 100.0 mL of 2 N hydrochloric acid VS, and swirl by mechanical means for 30 minutes. Dilute with water to volume, mix, and filter. Transfer 100.0 mL of the filtrate to a beaker. Titrate the excess acid with 1 N sodium hydroxide VS to a pH of 3.0, determined potentiometrically. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 2 N hydrochloric acid is equivalent to 70.80 mg of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Assay for polydimethylsiloxane—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of simethicone, to a 60-mL separator. Add 10.0 mL of hexanes and 25 mL of 6 N hydrochloric acid, cap the separator, and shake by mechanical means for not less than 2 hours. Allow to stand for about 10 minutes, and drain off as much of the lower, aqueous layer as possible without removing any of the unseparated interphase. Add 25 mL of 4 N sodium hydroxide to the separator, cap it, and shake by mechanical means for 1 hour. Transfer the mixture from the separator to a 50-mL centrifuge tube, cap, and centrifuge to obtain clear layers. Transfer not less than 5 mL of the clear upper hexanes layer to a test tube containing about 0.5 g of anhydrous sodium sulfate. Cap the tube, shake vigorously, and allow to stand to obtain a clear supernatant (*Assay preparation*). Prepare three *Standard preparations* in hexanes having known concentrations of about 1.6, 2.0, and 2.4 mg of USP Polydimethylsiloxane RS per mL, respectively. Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparations* in a 0.5-mm cell at the wavelength of maximum absorbance at about 1260 cm^{-1} with an IR spectrophotometer, using hexanes as the blank. [NOTE—Between each measurement, rinse the cell with heptane, empty, and dry it.] Plot the absorbances for the *Standard preparations* versus concentration, in mg per mL, of USP Polydimethylsiloxane RS, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C, in mg per mL, of polydimethylsiloxane in the *Assay preparation.* Calculate the quantity, in mg, of $[-(CH_3)_2SiO-]_n$ in the portion of Chewable Tablets taken by multiplying C by 10.

Milk of Magnesia

$Mg(OH)_2$ 58.32

Magnesium hydroxide.

Magnesium hydroxide [1309-42-8].

» Milk of Magnesia is a suspension of Magnesium Hydroxide. Milk of Magnesia, Double-Strength Milk of Magnesia, and Triple-Strength Milk of Magnesia contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $Mg(OH)_2$, the labeled amount being 80, 160, and 240 mg of $Mg(OH)_2$ per mL, respectively. It may contain not more than 0.05 percent of a volatile oil or a blend of volatile oils, suitable for flavoring purposes.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 35°. Avoid freezing.

Labeling—Double- or Triple-Strength Milk of Magnesia is so labeled, or may be labeled as 2× or 3× Concentrated Milk of Magnesia, respectively.

Identification—A solution of the equivalent of 1 g of regular-strength Milk of Magnesia in 2 mL of 3 N hydrochloric acid meets the requirements of the tests for *Magnesium* ⟨191⟩.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli.*

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.8(0.0343M)$$

in which 0.0343 is the theoretical acid-neutralizing capacity, in mEq, of $Mg(OH)_2$; and M is the quantity, in mg, of $Mg(OH)_2$ in the specimen tested, based on the labeled quantity.

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Soluble alkalies—Centrifuge about 50 mL of Milk of Magnesia. Dilute 5.0 mL of the clear supernatant with 40 mL of water. Add 1 drop of methyl red TS, and titrate the solution with 0.10 N sulfuric acid to the production of a persistent pink color: not more than 1.0 mL of the acid is required. Where the specimen is Double- or Triple-Strength Milk of Magnesia, not more than 2.0 or 3.0 mL of the acid is required, respectively.
Carbonate and acid-insoluble matter—To the equivalent of 1 g of regular-strength Milk of Magnesia add 2 mL of 3 N hydrochloric acid: not more than a slight effervescence occurs, and the solution is not more than slightly turbid.
Assay—Transfer an accurately measured quantity of Milk of Magnesia, previously shaken in its original container, equivalent to about 800 mg of magnesium hydroxide, to a 250-mL volumetric flask. Dissolve in 30 mL of 3 N hydrochloric acid, dilute with water to volume, and mix. Filter, if necessary, and transfer 25.0 mL of the filtrate to a beaker containing 75 mL of water, and mix. Adjust the reaction of the solution with 1 N sodium hydroxide to a pH of 7 (using pH indicator paper; see *Indicator and Test Papers* under *Reagents*, in the section *Reagents, Indicators, and Solutions*), add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $Mg(OH)_2$.

Magnesia Tablets

DEFINITION
Magnesia Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of magnesium hydroxide [$Mg(OH)_2$].

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: Crush several Tablets, and dissolve 1 g of the powder in 20 mL of 3 N hydrochloric acid.
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 250 mg of magnesium hydroxide, to a 100-mL volumetric flask. Dissolve in 10 mL of 3 N hydrochloric acid, and dilute with water to volume. Filter, if necessary, and transfer 25.0 mL of the filtrate to a beaker containing 75 mL of water.
Analysis: Adjust the reaction of the solution with 1 N sodium hydroxide to a pH of 7 (using pH indicator paper; see *Reagents, Indicators, and Solutions—Indicator and Test Papers*), and add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $Mg(OH)_2$.
Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩
Time: NMT 10 min, simulated gastric fluid TS being substituted for water in the test
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS
• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩
Analysis: NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated by the formula:

$$\text{Result} = (F_M \times M) \times 0.8$$

F_M = theoretical acid-neutralizing capacity of $Mg(OH)_2$, 0.0343 mEq
M = quantity of $Mg(OH)_2$ in the sample tested, based on the labeled quantity (mg)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Magnesium Carbonate

Carbonic acid, magnesium salt, basic; or, Carbonic acid, magnesium salt (1:1), hydrate;
Magnesium carbonate, basic; or, Magnesium carbonate (1:1) hydrate [23389-33-5].
Anhydrous 84.31
[546-93-0].

DEFINITION
Magnesium Carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate. It contains the equivalent of NLT 40.0% and NMT 43.5% of magnesium oxide (MgO).

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩**:** When treated with 3 N hydrochloric acid, it dissolves with effervescence, and the resulting solution meets the requirements.

ASSAY
• **PROCEDURE**
Sample: 1 g
Analysis: Dissolve the *Sample* in 30.0 mL of 1 N sulfuric acid VS, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide VS. Perform the blank determination. Calculate the volume, V_S, of 1 N sulfuric acid, in mL, consumed by the *Sample*:

$$\text{Result} = (V_B - V_A) \times N_{NaOH}$$

V_B = volume of 1 N sodium hydroxide consumed by the blank determination (mL)
V_A = volume of 1 N sodium hydroxide consumed by the *Sample* (mL)
N_{NaOH} = exact normality of the sodium hydroxide solution

Calculate the volume of 1 N sulfuric acid, V_{Ca}, in mL, consumed by calcium, which is present in the portion of Magnesium Carbonate taken for the *Assay*:

$$\text{Result} = (W \times L_{Ca})/(F_{Ca} \times 100)$$

W = weight of Magnesium Carbonate taken (mg)
L_{Ca} = content of calcium as determined in the test for *Limit of Calcium* (%)
F_{Ca} = weight of Ca that is equivalent to each mL of 1 N sulfuric acid, 20.04 mg

Calculate the percentage of magnesium oxide (MgO) in the portion of Magnesium Carbonate taken:

$$\text{Result} = (V_S - V_{Ca}) \times F_{MgO}/W \times 100$$

V_S = volume of 1 N sulfuric acid consumed by the *Sample*, as calculated above (mL)

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V_{Ca} = volume of 1 N sulfuric acid consumed by calcium, as calculated above (mL)
F_{MgO} = weight of MgO that is equivalent to each mL of 1 N sulfuric acid, 20.15 mg
W = weight of Magnesium Carbonate taken (mg)

Acceptance criteria: 40.0%–43.5% of MgO

IMPURITIES

• **SOLUBLE SALTS**
Sample: 2.0 g
Analysis: Mix the *Sample* with 100 mL of a mixture of equal volumes of *n*-propyl alcohol and water. Heat the mixture to the boiling point with constant stirring, cool to room temperature, dilute with water to 100 mL, and filter. Evaporate 50 mL of the filtrate on a steam bath to dryness, and dry at 105° for 1 h.
Acceptance criteria: The weight of the residue does not exceed 10 mg (NMT 1.0%).

• **ACID-INSOLUBLE SUBSTANCES**
Sample: 5.0 g
Analysis: Mix the *Sample* with 75 mL of water, add hydrochloric acid in small portions, with agitation, until no more of the magnesium carbonate dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, and ignite.
Acceptance criteria: The weight of the ignited residue does not exceed 2.5 mg (NMT 0.05%).

• **ARSENIC,** *Method I* ⟨211⟩
Test preparation: 750 mg in 25 mL of 3 N hydrochloric acid
Acceptance criteria: NMT 4 ppm

• **LIMIT OF CALCIUM**
[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a calcium standard stock solution is described below. Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument.]
Dilute hydrochloric acid: Dilute 100 mL of hydrochloric acid with water to 1000 mL.
Lanthanum solution: To 58.65 g of lanthanum oxide add 400 mL of water, and add, gradually with stirring, 250 mL of hydrochloric acid. Stir until dissolved, and dilute with water to 1000 mL.
Standard solutions: Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in a minimum amount of hydrochloric acid, and dilute with water to volume. Transfer 1.0, 5.0, 10.0, and 15.0 mL of this stock solution to separate 1000-mL volumetric flasks, each containing 20 mL of *Lanthanum solution* and 40 mL of *Dilute hydrochloric acid*. Dilute with water to volume. These *Standard solutions* contain 1.0, 5.0, 10.0, and 15.0 µg/mL of calcium, respectively.
Blank solution: Transfer 4 mL of *Lanthanum solution* and 10 mL of *Dilute hydrochloric acid* to a 200-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 250 mg of Magnesium Carbonate to a beaker, add 30 mL of *Dilute hydrochloric acid*, and stir until dissolved, heating if necessary. Transfer the solution so obtained to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution*, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Lamp: Calcium hollow-cathode
Flame: Nitrous oxide–acetylene
Analytical wavelength: Calcium emission line at 422.7 nm
Analysis
Samples: *Standard solutions*, *Blank solution*, and *Sample solution*
Using the *Blank solution* as blank, determine the concentration, C, in µg/mL, of calcium in the *Sample solution* using the calibration graph.
Calculate the percentage of calcium in the portion of Magnesium Carbonate taken:

$$\text{Result} = (V/W \times C \times F) \times 100$$

V = volume of the *Sample solution* (mL)
W = weight of Magnesium Carbonate taken (mg)
C = as defined above
F = conversion factor from µg/mL to mg/mL, 0.001

Acceptance criteria: NMT 0.45%

• **IRON** ⟨241⟩
Test preparation: Boil 50 mg with 5 mL of 2 N nitric acid for 1 min. Cool, dilute with water to 45 mL, add 2 mL of hydrochloric acid, and mix.
Acceptance criteria: NMT 200 ppm

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Magnesium Carbonate and Citric Acid for Oral Solution

DEFINITION
Magnesium Carbonate and Citric Acid for Oral Solution contains a dry mixture of Magnesium Carbonate and Citric Acid that when constituted as directed in the labeling yields a solution that contains NLT 90.0% and NMT 110.0% of the labeled amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: Meets the requirements

• **B.** The retention time of the citrate peak of the *Sample solution* corresponds to that of *Standard solution 1*, as obtained in the test for *Content of Anhydrous Citric Acid*.

ASSAY

• **PROCEDURE**
Sample solution: Transfer a volume of constituted oral solution, equivalent to 18.7 g of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$), to a 1000-mL volumetric flask. Add 200 mL of 1 N hydrochloric acid, swirl, and allow to stand for 10 min. Dilute with water to volume. Stir by mechanical means for 30 min.
Analysis: Transfer 10.0 mL of the *Sample solution* to a 250-mL beaker. Add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS until the last hint of violet disappears (blue endpoint). Each mL of 0.05 M edetate disodium is equivalent to 7.520 mg of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).
Acceptance criteria: 90.0%–110.0%

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OTHER COMPONENTS

• **CONTENT OF ANHYDROUS CITRIC ACID**

Mobile phase, Standard solution 1, Chromatographic system, and System suitability: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution: Transfer an appropriate volume of the constituted oral solution into a suitable volumetric flask, and proceed as directed for the *Sample solution* in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Assay Preparation for Citric Acid/Citrate Assay.*

Analysis

Samples: *Standard solution 1* and *Sample solution*

Proceed as directed for *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure.*

Calculate the percentage of anhydrous citric acid ($C_6H_8O_7$) in relation to the labeled amount of magnesium citrate in the volume of constituted oral solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of citrate from the *Sample solution*
r_S = peak area of citrate from *Standard solution 1*
C_S = concentration of citrate in *Standard solution 1* (mg/mL)
C_U = nominal concentration of magnesium citrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of anhydrous citric acid, 192.12
M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10

Acceptance criteria: 76.6%–107.8% of the labeled amount of magnesium citrate

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (NMT 0.01%).

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (NMT 0.015%).

• **TARTARIC ACID**

Sample solution: Constitute as directed in the labeling.

Analysis: To 10 mL of *Sample solution* in a test tube, add 1 mL of glacial acetic acid and 3 mL of a solution of potassium acetate (1 in 2). Shake the mixture vigorously, then gently rub the inner wall of the test tube with a glass rod for a few min, and allow to stand for 1 h.

Acceptance criteria: No white, crystalline precipitate soluble in 6 N ammonium hydroxide is formed.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the test for absence of *Escherichia coli* and *Salmonella* species.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** The label contains directions for constitution of the powder and states the equivalent amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$) in a given volume of the oral solution obtained after constitution.

Magnesium Carbonate, Citric Acid, and Potassium Citrate for Oral Solution

DEFINITION

Magnesium Carbonate, Citric Acid, and Potassium Citrate for Oral Solution contains a dry mixture of Magnesium Carbonate, Citric Acid, and Potassium Citrate that when constituted as directed in the labeling yields a solution that contains NLT 90.0% and NMT 110.0% of the labeled amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the citrate peak of the *Sample solution* corresponds to that of *Standard solution 1*, as obtained in the test for *Content of Anhydrous Citric Acid.*

ASSAY

• **PROCEDURE**

Sample solution: Transfer a volume of constituted oral solution, equivalent to 1.9 g of magnesium citrate to a 100-mL volumetric flask and dilute with water to volume.

Analysis: Transfer 10.0 mL of the *Sample solution* to a beaker. While stirring, add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS until the last hint of violet disappears (blue endpoint). Each mL of 0.05 M edetate disodium is equivalent to 7.520 mg of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF ANHYDROUS CITRIC ACID**

Mobile phase, Chromatographic system, and System suitability: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Standard solution: 0.02 mg/mL of anhydrous citric acid, from USP Citric Acid RS in a freshly prepared 1 mM sodium hydroxide

Sample solution: Transfer a volume of constituted oral solution equivalent to 500 mg of magnesium citrate, to a suitable volumetric flask, and dilute quantitatively, and stepwise if necessary, with a freshly prepared 1 mM sodium hydroxide to obtain a solution having a concentration of 0.02 mg/mL of magnesium citrate, based on the label claim. Pass through a filter of 0.5-µm or finer pore size, and use the filtrate.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of anhydrous citric acid ($C_6H_8O_7$) in relation to the labeled amount of magnesium citrate in the volume of constituted oral solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of citrate from the *Sample solution*
r_S = peak area of citrate from the *Standard solution*
C_S = concentration of citrate in the *Standard solution* (mg/mL)
C_U = nominal concentration of magnesium citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 126.1%–154.4% of the labeled amount of magnesium citrate

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

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IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Sample solution: Constitute as directed in the labeling.
  Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (NMT 0.01%).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Sample solution: Constitute as directed in the labeling.
  Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (NMT 0.015%).
- **TARTARIC ACID**
  Sample solution: Constitute as directed in the labeling.
  Analysis: To 10 mL of *Sample solution* in a test tube, add 1 mL of glacial acetic acid and 3 mL of a solution of potassium acetate (1 in 2). Shake the mixture vigorously, then gently rub the inner wall of the test tube with a glass rod for a few min, and allow to stand for 1 h.
  Acceptance criteria: No white, crystalline precipitate soluble in 6 N ammonium hydroxide is formed.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g. It meets the requirements of the test for absence of *Escherichia coli.*
- **pH** ⟨791⟩
  Sample solution: Constitute as directed in the labeling.
  Acceptance criteria: 3.3–4.3

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers. Store at controlled room temperature.
- **LABELING:** The label specifies the directions for the constitution of the powder and states the equivalent amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

Magnesium Carbonate and Sodium Bicarbonate for Oral Suspension

» Magnesium Carbonate and Sodium Bicarbonate for Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of $MgCO_3$ and $NaHCO_3$. It may contain suitable flavors.

Packaging and storage—Preserve in tight containers.

Identification—

A: Place about 1 g in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

B: The solution remaining in the flask responds to the tests for *Magnesium* ⟨191⟩ and for *Sodium* ⟨191⟩.

Minimum fill ⟨755⟩: meets the requirements.

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.8(0.024M) + 0.8(0.0119S)$$

in which 0.024 and 0.0119 are the theoretical acid-neutralizing capacities, in mEq, of $MgCO_3$ and $NaHCO_3$, respectively; and M and S are the quantities, in mg, of $MgCO_3$ and $NaHCO_3$ in the specimen tested, based on the labeled quantities.

Assay for magnesium carbonate—Transfer an accurately weighed portion of Magnesium Carbonate and Sodium Bicarbonate for Oral Suspension, equivalent to about 4.2 g of $MgCO_3$, to a 500-mL volumetric flask. Add 200 mL of 1 N hydrochloric acid, and mix. When dissolved, dilute with water to volume, and mix. Transfer 10.0 mL of this stock solution to a suitable container, dilute with water to 100 mL, add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium consumed is equivalent to 4.216 mg of $MgCO_3$.

Assay for sodium bicarbonate—

Standard preparations—Dissolve a suitable quantity of sodium chloride, previously dried at 125° for 30 minutes and accurately weighed, in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 600 µg per mL. On the day of use, further dilute this solution quantitatively with water to obtain three solutions containing 6.0, 12.0, and 18.0 µg of sodium chloride per mL, respectively.

Assay preparation—Transfer an accurately measured volume of the stock solution remaining from the *Assay for magnesium carbonate,* equivalent to about 180 mg of $NaHCO_3$, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of the resulting solution to a 1000-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the sodium emission line at about 589.0 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a sodium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg of sodium chloride per mL, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in µg per mL, of sodium chloride equivalent in the *Assay preparation.* Calculate the quantity, in g, of $NaHCO_3$ in the portion of Magnesium Carbonate and Sodium Bicarbonate for Oral Suspension taken by the formula:

$$(84.01/58.44)(5C/V)$$

in which 84.01 and 58.44 are the molecular weights of sodium bicarbonate and sodium chloride, respectively; C is the concentration, in µg per mL, of sodium chloride equivalent in the *Assay preparation;* and V is the volume, in mL, of the stock solution remaining from the *Assay for magnesium carbonate* taken.

Magnesium Chloride

$MgCl_2 \cdot 6H_2O$	203.30
Magnesium chloride, hexahydrate [7791-18-6].	
Anhydrous	95.21
[7786-30-3].	

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DEFINITION
Magnesium Chloride contains NLT 98.0% and NMT 101.0% of $MgCl_2 \cdot 6H_2O$.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: 50 mg/mL
• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: 50 mg/mL
[NOTE—Acidify the *Sample solution* with diluted nitric acid before adding 6 N ammonium hydroxide.]

ASSAY
• **PROCEDURE**
Sample: 450 mg
Analysis: Dissolve the *Sample* in 25 mL of water, add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M disodium edetate is equivalent to 10.17 mg of $MgCl_2 \cdot 6H_2O$.
Acceptance criteria: 98.0%–101.0%

IMPURITIES
• **INSOLUBLE MATTER**
Sample: 20 g
Analysis: Dissolve the *Sample* in 200 mL of water, heat to boiling, and digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, dry at 115°, and determine the weight of the residue.
Acceptance criteria: NMT 0.005%
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample: 10 g
Acceptance criteria: It shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid (0.005%).
• **BARIUM**
Sample: 1 g
Analysis: Dissolve the *Sample* in 10 mL of water, and add 1 mL of 2 N sulfuric acid.
Acceptance criteria: No turbidity is produced within 2 h.
• **LIMIT OF CALCIUM**
[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a calcium standard stock solution is described below. Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument.]
Dilute hydrochloric acid: Dilute 100 mL of hydrochloric acid with water to 1000 mL.
Lanthanum solution: To 58.65 g of lanthanum oxide add 400 mL of water, and add, gradually with stirring, 250 mL of hydrochloric acid. Stir until dissolved, and dilute with water to 1000 mL.
Standard solutions: Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in a minimum amount of hydrochloric acid, and dilute with water to volume. Transfer 1.0, 5.0, 10.0, and 15.0 mL of this stock solution to separate 1000-mL volumetric flasks, each containing 20 mL of *Lanthanum solution* and 40 mL of *Dilute hydrochloric acid*. Dilute with water to volume. These *Standard solutions* contain 1.0, 5.0, 10.0, and 15.0 µg/mL of calcium, respectively.
Blank solution: Transfer 4 mL of *Lanthanum solution* and 10 mL of *Dilute hydrochloric acid* to a 200-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 10.0 g of Magnesium Chloride to a 200-mL volumetric flask, and add water to dissolve. Add 4 mL of *Lanthanum solution*, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Lamp: Calcium hollow-cathode
Flame: Nitrous oxide–acetylene
Analytical wavelength: Calcium emission line at 422.7 nm
Analysis
Samples: *Standard solutions*, *Blank solution*, and *Sample solution*.
Determine the concentration, C, in µg/mL, of calcium in the *Sample solution* using the calibration graph.
Calculate the percentage of calcium in the portion of Magnesium Chloride taken:

$$\text{Result} = (V/W \times C \times F) \times 100$$

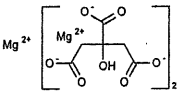
V = volume of the *Sample solution* (mL)
W = weight of Magnesium Chloride taken (mg)
C = as defined above
F = conversion factor from µg/mL to mg/mL, 0.001

Acceptance criteria: NMT 0.01%
• **POTASSIUM**
Sample solution: 5 g
Analysis: Dissolve the *Sample* in 5 mL of water, and add 0.2 mL of sodium bitartrate TS.
Acceptance criteria: No turbidity is produced within 5 min.
• **ALUMINUM** ⟨206⟩ (where it is labeled as intended for use in hemodialysis)
Test preparation: Prepare as directed in the chapter, using 2.0 g.
Acceptance criteria: NMT 1 ppm

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 50 mg/mL in carbon dioxide-free water
Acceptance criteria: 4.5–7.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Where Magnesium Chloride is intended for use in hemodialysis, it is so labeled.

Magnesium Citrate



$C_{12}H_{10}Mg_3O_{14}$ 451.11
1,2,3-Propanetricarboxylic acid, hydroxy-, magnesium salt (2:3);
Magnesium citrate (3:2) [3344-18-1].

DEFINITION
Magnesium Citrate contains NLT 14.5% and NMT 16.4% of magnesium (Mg), calculated on the dried basis.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample: 10 mg/mL
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩
Sample: 80 mg/mL

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Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Sample: 400 mg

Analysis: Dissolve the *Sample* in 50 mL of water. Add 20 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary correction. From the volume of 0.05 M edetate disodium consumed, deduct the volume of 0.05 M edetate disodium corresponding to the amount of calcium in the portion of Magnesium Citrate taken, based on the amount of calcium found in the test for *Limit of Calcium*. Each mg of Ca is equivalent to 0.25 mL of 0.05 M edetate disodium. The difference is the volume of 0.05 M edetate disodium consumed by the magnesium. Each mL of 0.05 M edetate disodium is equivalent to 1.215 mg of Mg.

Acceptance criteria: 14.5%–16.4% on the dried basis

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample: 300 mg

Acceptance criteria: It shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.05%).

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample: 100 mg

Acceptance criteria: It shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.2%).

- **ARSENIC,** *Method I* ⟨211⟩**:** NMT 3 ppm
- **IRON** ⟨241⟩

Test preparation: Boil 50 mg with 5 mL of 2 N nitric acid for 1 min. Cool, dilute with water to 45 mL, and add 2 mL of hydrochloric acid.

Acceptance criteria: NMT 200 ppm

- **LIMIT OF CALCIUM**

[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a calcium standard stock solution is described below. Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument.]

Dilute hydrochloric acid: Dilute 100 mL of hydrochloric acid with water to 1000 mL.

Lanthanum solution: To 58.65 g of lanthanum oxide add 400 mL of water, and add, gradually with stirring, 250 mL of hydrochloric acid. Stir until dissolved, and dilute with water to 1000 mL.

Standard solutions: Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in a minimum amount of hydrochloric acid, and dilute with water to volume. Transfer 1.0, 5.0, 10.0, and 15.0 mL of this stock solution to separate 1000-mL volumetric flasks, each containing 20 mL of *Lanthanum solution* and 40 mL of *Dilute hydrochloric acid*. Dilute with water to volume. These *Standard solutions* contain 1.0, 5.0, 10.0, and 15.0 µg/mL of calcium, respectively.

Sample solution: Transfer 250 mg of Magnesium Citrate to a beaker, add 30 mL of *Dilute hydrochloric acid*, and stir until dissolved. Transfer the solution to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution*, and dilute with water to volume.

Blank solution: Transfer 4 mL *of Lanthanum solution* and 10 mL of *Dilute hydrochloric acid* to a 200-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow–cathode

Flame: Nitrous oxide–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank solution*

Determine the concentration, C, in µg/mL, of calcium in the *Sample solution* using the calibration graph.

Calculate the percentage of calcium in the portion of Magnesium Citrate taken:

$$\text{Result} = (V/W \times C \times F) \times 100$$

V = volume of the *Sample solution* (mL)
W = weight of Magnesium Citrate taken (mg)
C = concentration of calcium in the *Sample solution* (µg/mL)
F = conversion from µg/mL to mg/mL, 0.001

Acceptance criteria: NMT 1.0% on the dried basis

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 5.0–9.0, in a suspension (50 mg/mL)
- **LOSS ON DRYING** ⟨731⟩ Dry 1 g in a mechanical convection oven at 135° for 16 h, then to constant weight: it loses NMT 29% of its weight, except that where it is labeled as anhydrous, it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Magnesium Citrate that loses NMT 2.0% of its weight in the test for *Loss on Drying* may be labeled as Anhydrous Magnesium Citrate.

Magnesium Citrate Oral Solution

DEFINITION

Magnesium Citrate Oral Solution is a sterilized or pasteurized solution containing NLT 7.59 g of anhydrous citric acid ($C_6H_8O_7$) and an amount of magnesium citrate equivalent to NLT 1.55 g and NMT 1.9 g of magnesium oxide (MgO) in each 100 mL of Oral Solution.

Prepare Magnesium Citrate Oral Solution as follows.

Magnesium Carbonate	15 g
Anhydrous Citric Acid	27.4 g
Syrup	60 mL
Talc	5 g
Lemon Oil	0.1 mL
Potassium Bicarbonate	2.5 g
Purified Water, a sufficient quantity to make	350 mL

Dissolve the *Anhydrous Citric Acid* in 150 mL of hot *Purified Water* in a suitable dish, slowly add the *Magnesium Carbonate* previously mixed with 100 mL of *Purified Water*, and stir until it is dissolved. Add the *Syrup*, heat the mixed liquids to the boiling point, and immediately add the *Lemon Oil* previously triturated with *Talc*. Filter the mixture, while hot, into a strong bottle of suitable capacity previously rinsed with boiling *Purified Water*. Add boiled *Purified Water* to bring the preparation to final volume. Use Purified Cotton as a stopper for the bottle, and allow to cool. Add the

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Potassium Bicarbonate, and immediately insert the stopper in the bottle securely. Shake the solution occasionally until the *Potassium Bicarbonate* is dissolved, cap the bottle, and sterilize or pasteurize the solution.

Alternatively, 30 g of citric acid containing 1 molecule of water of hydration, equivalent to 27.4 g of *Anhydrous Citric Acid*, may be used in the foregoing formula. In this process, replace the 2.5 g of *Potassium Bicarbonate* with 2.1 g of sodium bicarbonate, preferably in tablet form. The Oral Solution may be further carbonated by the use of carbon dioxide under pressure.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩**:** Meets the requirements
- **B.**

Sample: 5 mL

Analysis: To the *Sample* add 1 mL of potassium permanganate TS and 5 mL of mercuric sulfate TS, and heat the solution.

Acceptance criteria: A white precipitate is formed.

ASSAY

- **CITRIC ACID**

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Assay preparation: Transfer 10 mL of Oral Solution that has been previously freed from excessive carbon dioxide by repeated pouring to a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Assay Preparation for Citric Acid/Citrate Assay.*

Analysis: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩,*Procedure.*

Calculate the quantity, g, of anhydrous citric acid ($C_6H_8O_7$) in 100 mL of Oral Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times F$$

r_U	= peak area of citrate from the *Assay preparation*
r_S	= peak area of citrate from *Standard preparation 1*
C_S	= concentration of citrate in *Standard preparation 1* (µg/mL)
V	= final volume of Oral Solution, 100 mL
D	= dilution factor
M_{r1}	= molecular weight of citric acid ($C_6H_8O_7$), 192.12
M_{r2}	= molecular weight of citrate ($C_6H_5O_7$), 189.10
F	= conversion factor, 10^{-6} g/µg

Acceptance criteria: NLT 7.59 g in 100 mL of Oral Solution

- **MAGNESIUM OXIDE**

Sample: 50.0 mL of Oral Solution that has been previously freed from excessive carbon dioxide by repeated pouring

Analysis: Transfer the *Sample* to a 100-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of the resulting solution to a beaker containing 150 mL of water heated to 70°–80°, and add 1 mL of ammonium chloride TS and 3 mL of ammonium hydroxide. Mix, and slowly add 8 mL of 8-hydroxyquinoline TS with stirring. After standing for 30 min, filter through a sintered-glass crucible, previously dried and weighed, and wash the precipitate with ten 10-mL portions of water. Dry the crucible and contents at 105° for 3 h, cool, and weigh. Determine the equivalent of magnesium oxide (MgO) in 100 mL of Oral Solution by multiplying the weight of $C_{18}H_{12}MgN_2O_2 \cdot 2H_2O$ so obtained by 4.624.

Acceptance criteria: 1.55–1.9 g in 100 mL of Oral Solution

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample: 2.0 mL

Acceptance criteria: 0.01%; it shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid.

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample: 2.0 mL

Acceptance criteria: 0.015%; it shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid.

- **TARTARIC ACID**

Sample: 10 mL

Analysis: Place the *Sample* in a test tube, add 1 mL of glacial acetic acid and 3 mL of a solution of potassium acetate (1 in 2), shake the mixture vigorously, then gently rub the inner wall of the test tube with a glass rod for a few min, and allow to stand for 1 h.

Acceptance criteria: No white, crystalline precipitate soluble in 6 N ammonium hydroxide is formed.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in bottles NLT 200 mL in capacity. Store at controlled room temperature or in a cool place.

Magnesium Citrate for Oral Solution

DEFINITION

Magnesium Citrate for Oral Solution, when constituted as directed in the labeling, yields a solution that contains NLT 90.0% and NMT 110.0% of the labeled amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the citrate peak of the *Sample solution* corresponds to that of *Standard solution 1*, as obtained in the test for *Content of Anhydrous Citric Acid.*

ASSAY

- **PROCEDURE**

Sample solution: Transfer a volume of constituted oral solution, equivalent to 18.7 g of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$), to a 1000-mL volumetric flask. Add 200 mL of 1 N hydrochloric acid, swirl, and allow to stand for 10 min. Dilute with water to volume. Stir by mechanical means for about 30 min.

Analysis: Transfer 10.0 mL of the *Sample solution* to a 250-mL beaker. Add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS until the last hint of violet disappears (blue endpoint). Each mL of 0.05 M edetate disodium is equivalent to 7.520 mg of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **CONTENT OF ANHYDROUS CITRIC ACID**

Mobile phase, Standard solution 1, Chromatographic system, and System suitability: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution: Transfer an appropriate volume of the constituted oral solution into a suitable volumetric flask, and proceed as directed for the *Sample solution* in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate).*

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Analysis
Samples: *Standard solution 1* and *Sample solution*
Proceed as directed for *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure.*
Calculate the percentage of anhydrous citric acid ($C_6H_8O_7$) in relation to the labeled amount of magnesium citrate in the volume of constituted oral solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of citrate from the *Sample solution*
r_S = peak area of citrate from *Standard solution 1*
C_S = concentration of citrate in *Standard solution 1* (mg/mL)
C_U = nominal concentration of magnesium citrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of anhydrous citric acid, 192.12
M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10

Acceptance criteria: 76.6%–93.7% of the labeled amount of magnesium citrate

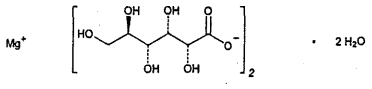
PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (NMT 0.01%).
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (NMT 0.015%).
• **TARTARIC ACID**
Sample solution: Constitute as directed in the labeling.
Analysis: To 10 mL of *Sample solution* in a test tube, add 1 mL of glacial acetic acid and 3 mL of a solution of potassium acetate (1 in 2). Shake the mixture vigorously, then gently rub the inner wall of the test tube with a glass rod for a few min, and allow to stand for 1 h.
Acceptance criteria: No white, crystalline precipitate soluble in 6 N ammonium hydroxide is formed.

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the test for absence of *Escherichia coli* and *Salmonella* species.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** The label contains directions for constitution of the powder and states the equivalent amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$) in a given volume of the oral solution obtained after constitution.

Magnesium Gluconate



$C_{12}H_{22}MgO_{14}$ 414.60
$C_{12}H_{22}MgO_{14} \cdot 2H_2O$ 450.64
D-Gluconic acid, magnesium salt (2:1), hydrate;
Magnesium D-gluconate (1:2) hydrate;
Magnesium D-gluconate (1:2) dihydrate [59625-89-7].
Anhydrous [3632-91-5].

DEFINITION
Magnesium Gluconate contains NLT 98.0% and NMT 102.0% of anhydrous magnesium gluconate ($C_{12}H_{22}MgO_{14}$), calculated on the anhydrous basis.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩**:** A 100-mg/mL solution meets the requirements.
• **B. THIN-LAYER CHROMATOGRAPHY**
Standard solution: 10 mg/mL of USP Potassium Gluconate RS
Sample solution: 10 mg/mL of Magnesium Gluconate, heating in a water bath at 60°, if necessary, to dissolve
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent.* Heat the plate at 110° for about 10 min.
Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution.*

ASSAY
• **PROCEDURE**
Sample: 800 mg of Magnesium Gluconate
Blank: 20 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 20 mL of water. Add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with *Titrant* to a blue endpoint. Perform the *Blank* determination.
Calculate the percentage of magnesium gluconate ($C_{12}H_{22}MgO_{14}$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100]$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = *Titrant* molarity (mM/mL)
F = equivalency factor, 414.6 mg/mM
W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

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IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard solution: 0.7 mL of 0.020 N hydrochloric acid
Sample: 1.0 g
Acceptance criteria: NMT 0.05%

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard solution: 1.0 mL of 0.020 N sulfuric acid
Sample: 2.0 g
Acceptance criteria: NMT 0.05%

• **REDUCING SUBSTANCES**
Sample: 1.0 g of Magnesium Gluconate
Blank: 10 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.1 N iodine VS
Back titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.
Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = *Back titrant* volume consumed by the *Blank* (mL)
V_S = *Back titrant* volume consumed by the *Sample* (mL)
N = *Back titrant* normality (mEq/mL)
F = equivalency factor, 27 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

• **PH** ⟨791⟩
Sample solution: 50 mg/mL
Acceptance criteria: 6.0–7.8

• **WATER DETERMINATION,** *Method Ib* ⟨921⟩
Test preparation: Proceed as directed in the chapter. Allow 30 min for solubilization of Magnesium Gluconate and for the reaction to reach completion.
Blank: Use the same volume of *Reagent* as that of the *Test preparation* but without the specimen.
Analysis
Samples: *Test preparation* and *Blank*
Calculate the water content, in mg, of the Magnesium Gluconate taken:

$$\text{Result} = F \times (X_B - X) \times R$$

X_B = volume of standardized *Water–Methanol Solution* required to neutralize the unconsumed *Reagent* in the *Blank* determination (mL)

The other terms are as defined in *Water Determination, Method Ib* ⟨921⟩.
Acceptance criteria: 3.0%–12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Potassium Gluconate RS

Magnesium Gluconate Tablets

DEFINITION

Magnesium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of magnesium gluconate ($C_{12}H_{22}MgO_{14}$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: A filtered solution in water from powdered Tablets, equivalent to 100 mg/mL of magnesium gluconate
Acceptance criteria: Meet the requirements

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**
Standard solution: 10 mg/mL of USP Potassium Gluconate RS
Sample solution: Equivalent to 10 mg/mL of magnesium gluconate from a dilution of the *Sample solution* obtained for the *Identification* test *A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.
Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

ASSAY

• **PROCEDURE**
Sample: A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 800 mg of magnesium gluconate
Blank: Proceed as directed in the *Analysis* without the *Sample*.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a crucible, and ignite, gently at first, until free from carbon. Cool the crucible, add 25 mL of water and 5 mL of hydrochloric acid, and stir. Heat on a steam bath for 5 min. Filter, rinsing the filter with several portions of water. Dilute the combined filtrate and washings with water to 150 mL. Add ammonia–ammonium chloride buffer TS until the solution is neutral to litmus. Add an excess of 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with *Titrant* to a blue endpoint. Perform a *Blank* determination.
Calculate the percentage of the labeled amount of magnesium gluconate ($C_{12}H_{22}MgO_{14}$) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

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V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 414.6 mg/mM
W = nominal amount of magnesium gluconate in the *Sample* taken (mg)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: Solution having a known concentration of magnesium in the *Medium*
Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 285.2 nm
Lamp: Magnesium hollow-cathode
Flame: Air–acetylene
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the concentration of magnesium (Mg) in the *Sample solution* in comparison with a *Standard solution*.
Calculate the percentage of the labeled amount of magnesium gluconate ($C_{12}H_{22}MgO_{14}$) dissolved:

$$\text{Result} = (C \times D \times V/L) \times (M_r/A_r) \times 100$$

C = determined concentration of magnesium in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label amount of magnesium gluconate (mg/Tablet)
M_r = molecular weight of magnesium gluconate, 414.60
A_r = atomic weight of magnesium, 24.31

Tolerances: NLT 80.0% (Q) of the labeled amount of magnesium gluconate ($C_{12}H_{22}MgO_{14}$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Potassium Gluconate RS

Magnesium Hydroxide

$Mg(OH)_2$ 58.32
Magnesium hydroxide.
Magnesium hydroxide [1309-42-8].

» Magnesium Hydroxide, dried at 105° for 2 hours, contains not less than 95.0 percent and not more than 100.5 percent of $Mg(OH)_2$.

Packaging and storage—Preserve in tight containers.

Identification—A 1 in 20 solution in 3 N hydrochloric acid responds to the tests for *Magnesium* ⟨191⟩.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the test for absence of *Escherichia coli*.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 2.0% of its weight.
Loss on ignition ⟨733⟩—Ignite it at 800°, increasing the heat gradually, to constant weight: it loses between 30.0% and 33.0% of its weight.
Soluble salts—Boil 2.0 g with 100 mL of water for 5 minutes in a covered beaker, filter while hot, cool, and dilute the filtrate with water to 100 mL. Titrate 50 mL of the diluted filtrate with 0.10 N sulfuric acid, using methyl red TS as the indicator: not more than 2.0 mL of the acid is consumed. Evaporate 25 mL of the diluted filtrate to dryness, and dry at 105° for 3 hours: not more than 10 mg of residue remains.
Carbonate—Boil a mixture of 0.10 g with 5 mL of water, cool, and add 5 mL of 6 N acetic acid: not more than a slight effervescence is observed.

Limit of calcium—
Dilute hydrochloric acid , *Lanthanum solution*, *Standard preparations*, and *Blank solution*—Prepare as directed in the test for *Limit of calcium* under *Magnesium Carbonate*.
Test preparation—Transfer 250 mg of Magnesium Hydroxide, previously dried, to a beaker, add 30 mL of *Dilute hydrochloric acid*, and stir until dissolved, heating if necessary. Transfer the solution so obtained to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution*, dilute with water to volume, and mix.
Procedure—Proceed as directed in the test for *Limit of calcium* under *Magnesium Carbonate:* the limit is 1.5%.
Limit of lead— [NOTE—When water is specified as a diluent, use deionized ultra-filtered water.]
Blank solution—Transfer 3.0 mL of nitric acid to a 50-mL volumetric flask, and dilute with water to volume.
Thallium internal standard 20 ppb— [NOTE—Use this solution only if an ICP–MS instrument is used. This internal standard is added in-line via a mixing block between the sample probe and the spray chamber.]Dilute 20.0 mL of a commercially prepared thallium ICP standard solution (1000 ppb) with water to 1 L.
Dilute nitric acid—Dilute 2.0 mL of nitric acid with water to 100 mL.
Standard stock solution 100 ppb—Prepare this solution fresh every two months. Quantitatively dilute an accurately measured volume of a commercially prepared lead ICP standard (1000 ppm) with *Dilute nitric acid* to obtain a solution containing 10 ppm of lead. Further dilute this solution with *Dilute nitric acid* to obtain a solution containing 1000 ppb of lead. Transfer 10.0 mL of this solution to a separate 100-mL volumetric flask, add 2.0 mL of nitric acid, and dilute with water to volume.
Standard solutions—Prepare these solutions fresh weekly. [NOTE—The concentrations specified below are recommended if an ICP–MS instrument is used. If an ICP–AES instrument is used, the concentrations of the *Standard solutions* may be modified to adapt to the working range of the instrument.] Transfer 5.0 mL of the *Standard stock solution 100 ppb* to a 50-mL volumetric flask, add 3.0 mL of nitric acid, and dilute with water to volume *(Standard lead solution 10 ppb)*. Transfer 5.0 mL of *Standard lead solution 10 ppb* to a 50-mL volumetric flask, add 3.0 mL of nitric acid, and dilute with water to volume *(Standard lead solution 1 ppb)*.
Test solution— [NOTE—The concentration specified below is recommended if an ICP–MS instrument is used. If an ICP–AES instrument is used, the concentration of the *Test solution* may be modified to adapt to the working range of the instrument.] Accurately weigh about 0.25 g of Magnesium

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Hydroxide. Cautiously add 3.0 mL of nitric acid, and mix until the sample is dissolved. Accurately transfer this solution to a 50-mL volumetric flask, and dilute with water to volume.

Procedure (see *Plasma Spectrochemistry* ⟨730⟩)—The inductively coupled plasma–mass spectrometer (ICP–MS) is equipped with a quadrupole mass spectrometer and an ion detector maintained under vacuum. The instrument should read all isotopes for lead (206, 207, and 208 amu) and the thallium internal standard (205 amu), and should report the total lead content using the most naturally abundant isotope at 208 amu. Alternatively, lead could be determined using an inductively coupled plasma–atomic emission spectrometer (ICP–AES) by measuring the emission at 220.353 nm, with the settings optimized as directed by the manufacturer. [NOTE—To minimize matrix interference when using an ICP–AES instrument, it is recommended that the method of standard additions be used.]

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Generate the calibration curve using the *Blank solution, Standard lead solution 1 ppb,* and *Standard lead solution 10 ppb:* a linear regression coefficient is not less than 0.999.

Aspirate the *Test solution,* at least in duplicate, and calculate the amount of lead using the calibration curve. Report the average reading as the lead content of the sample. Calculate the content of lead in the portion of Magnesium Hydroxide taken: not more than 0.00015% (1.5 ppm) is found.

Assay—Transfer about 75 mg of Magnesium Hydroxide, previously dried and accurately weighed, to a conical flask. Add 2 mL of 3 N hydrochloric acid, and swirl to dissolve. Add 100 mL of water, adjust the reaction of the solution to a pH of 7 (using pH indicator paper; see *Indicator and Test Papers* under *Reagents* in the section *Reagents, Indicators, and Solutions*) with 1 N sodium hydroxide, add 5 mL of ammonia-ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $Mg(OH)_2$.

Magnesium Hydroxide Paste

» Magnesium Hydroxide Paste is an aqueous paste of Magnesium Hydroxide. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of magnesium hydroxide [$Mg(OH)_2$], the labeled amount being not less than 28.0 percent and not more than 70.0 percent of magnesium hydroxide.

Packaging and storage—Preserve in tight containers.

Identification—One g of Paste dissolved in 10 mL of 3 N hydrochloric acid responds to the tests for *Magnesium* ⟨191⟩.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Its total aerobic microbial count does not exceed 400 cfu per g, and it meets the requirements of the test for absence of *Escherichia coli.*

Soluble alkalies—Accurately weigh a portion of Paste, equivalent to about 7.75 g of magnesium hydroxide, and mix with 75.0 mL of water. Transfer about 25 mL of this diluted Paste to a filter, and reject the first 5 mL of the filtrate. [NOTE—Retain the remaining diluted Paste for the test for *Carbonate and acid-insoluble matter.*] Dilute 5 mL of the clear filtrate with 40 mL of water. Add 1 drop of methyl red TS, and titrate the solution with 0.10 N sulfuric acid to the production of a persistent pink color: not more than 1.0 mL of the acid is required.

Soluble salts—To 5.0 mL of the clear filtrate obtained in the test for *Soluble alkalies* add 3 drops of sulfuric acid, evaporate on a steam bath to dryness, and ignite gently to constant weight: the residue weighs not more than 12 mg.

Carbonate and acid-insoluble matter—To 1 mL of the diluted Paste obtained in the test for *Soluble alkalies* add 2 mL of 3 N hydrochloric acid: not more than a slight effervescence occurs, and the solution is not more than slightly turbid.

Limit of calcium—

Dilute hydrochloric acid, Lanthanum solution, Standard preparations, and *Blank solution*—Prepare as directed in the test for *Limit of calcium* under *Magnesium Carbonate.*

Test preparation—Transfer a portion of the Paste, equivalent to 250 mg of $Mg(OH)_2$, to a beaker, add 30 mL of *Dilute hydrochloric acid,* and stir until dissolved, heating if necessary. Transfer the solution so obtained to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution,* dilute with water to volume, and mix.

Procedure—Proceed as directed in the test for *Limit of calcium* under *Magnesium Carbonate:* the limit is 1.5%.

Limit of lead—

Blank solution, Thallium internal standard 20 ppb, Dilute nitric acid, Standard stock solution 100 ppb, and *Standard solutions*—Proceed as directed in the test for *Limit of lead* under *Magnesium Hydroxide.*

Test solution—Accurately weigh an amount of Paste equivalent to 0.25 g of magnesium hydroxide. Cautiously add 3.0 mL of nitric acid, and mix until the sample is dissolved. Accurately transfer this solution to a 50-mL volumetric flask, and dilute with water to volume. [NOTE—This concentration is recommended if an ICP–MS instrument is used. If an ICP–AES instrument is used, the concentration of the *Test solution* may be modified to adapt to the working range of the instrument.]

Procedure—Proceed as directed in the test for *Limit of lead* under *Magnesium Hydroxide.* Calculate the content of lead in the portion of Paste taken based on the content of magnesium hydroxide in the Paste, as determined in the *Assay:* not more than 0.00015% (1.5 ppm) is found.

Assay—Transfer an accurately weighed portion of Paste, equivalent to about 250 mg of magnesium hydroxide, to a 100-mL volumetric flask. Dissolve in 10 mL of 3 N hydrochloric acid, dilute with water to volume, and mix. Filter, if necessary, and transfer 25.0 mL of the filtrate to a beaker containing 75 mL of water, and mix. Adjust the reaction of the solution to a pH of 7 (using pH indicator paper; see *Indicator and Test Papers* under *Reagents* in the section *Reagents, Indicators, and Solutions*) with 1 N sodium hydroxide, add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].

Magnesium Oxide

MgO 40.30

Magnesium oxide [1309-48-4].

DEFINITION

Magnesium Oxide, after ignition, contains NLT 96.0% and NMT 100.5% of magnesium oxide (MgO).

USP Monographs

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IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩**:** A solution in diluted hydrochloric acid meets the requirements.

ASSAY

• **PROCEDURE**

Diluted ammonium hydroxide: Dilute 67 g of ammonium hydroxide (about 75 mL) with water to 100 mL.

Buffer: Prepare ammonium chloride buffer pH 10 as follows. In a 100-mL volumetric flask, dissolve 5.4 g of ammonium chloride in 20 mL of water, add 35 mL of *Diluted ammonium hydroxide,* and dilute with water to volume.

Sample stock solution: Ignite a sample of Magnesium Oxide to a constant weight in the temperature range of (800°–900°) ± 25°. Weigh 320 mg of the ignited sample into a 100-mL volumetric flask, dissolve in 20 mL of 2 N hydrochloric acid, and dilute with water to volume.

Sample solution: Transfer 20.0 mL of the *Sample stock solution* to a 500-mL flask, and dilute with water to 300 mL. The *Sample solution* is equivalent to 64 mg of ignited Magnesium Oxide.

Analysis: To the *Sample solution,* add 10 mL of *Buffer* and 50 mg of eriochrome black T-sodium chloride indicator. Heat the sample to 40°, and titrate to a blue endpoint with 0.1 M edetate disodium VS. Perform a blank determination, and make any necessary correction to determine the volume of 0.1 M edetate disodium consumed (V_S).

Calculate the volume of 0.1 M edetate disodium, V_{Ca}, in mL, consumed by calcium, which is present in the portion of Magnesium Oxide taken:

$$V_{Ca} = (W \times L_{Ca})/(F_{Ca} \times 100)$$

W = amount of ignited Magnesium Oxide in the *Sample solution* (mg)
L_{Ca} = content of calcium as determined in the test for *Limit of Calcium* (%)
F_{Ca} = weight of calcium equivalent to each mL of 0.1 M edetate disodium, 4.008 mg

Calculate the percentage of magnesium oxide (MgO) in the portion of Magnesium Oxide taken:

$$\text{Result} = (V_S - V_{Ca}) \times (F_{MgO}/W) \times 100$$

V_S = volume of 0.1 M edetate disodium consumed by the *Sample solution* (mL)
V_{Ca} = volume of 0.1 M edetate disodium consumed by calcium (mL)
F_{MgO} = weight of Magnesium Oxide equivalent to each mL of 0.1 M edetate disodium, 4.030 mg
W = amount of the ignited Magnesium Oxide in the *Sample solution* (mg)

Acceptance criteria: 96.0%–100.5% after ignition

IMPURITIES

• **FREE ALKALI AND SOLUBLE SALTS**

Sample solution: Boil 2.0 g with 100 mL of water for 5 min in a covered beaker, and filter while hot. Allow to cool, and dilute with water to 100 mL.

Analysis 1: To 50 mL of the *Sample solution* add methyl red TS, and titrate with 0.10 N sulfuric acid.

Acceptance criteria 1: NMT 2.0 mL of the acid is consumed.

Analysis 2: Evaporate 25 mL of the remaining *Sample solution* to dryness, and dry at 105° for 1 h.

Acceptance criteria 2: NMT 10 mg of residue remains (NMT 2.0%).

• **ACID-INSOLUBLE SUBSTANCES**

Sample: 2 g

Analysis: Mix the *Sample* with 75 mL of water, add hydrochloric acid in small portions with agitation until no more dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, and ignite.

Acceptance criteria: The weight of the ignited residue is NMT 2 mg (NMT 0.1%).

• **LIMIT OF CALCIUM**

[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a calcium standard stock solution is described below. Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument.]

Dilute hydrochloric acid: Dilute 100 mL of hydrochloric acid with water to 1000 mL.

Lanthanum solution: To 58.65 g of lanthanum oxide, add 400 mL of water, and add, gradually with stirring, 250 mL of hydrochloric acid. Stir until dissolved, and dilute with water to 1000 mL.

Standard solutions: Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in a minimum amount of hydrochloric acid, and dilute with water to volume. Transfer 1.0, 5.0, 10.0, and 15.0 mL of this stock solution to separate 1000-mL volumetric flasks, each containing 20 mL of the *Lanthanum solution* and 40 mL of *Dilute hydrochloric acid,* and dilute with water to volume. These *Standard solutions* contain 1.0, 5.0, 10.0, and 15.0 µg/mL of calcium, respectively.

Sample solution: Transfer 250 mg of Magnesium Oxide, freshly ignited for 1 h in the temperature range of (800°–900°) ± 25°, to a beaker. Add 30 mL of *Dilute hydrochloric acid,* and stir until dissolved, heating if necessary. Transfer the solution so obtained to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution,* and dilute with water to volume.

Blank solution: Transfer 4 mL of the *Lanthanum solution* and 10 mL of *Dilute hydrochloric acid* to a 200-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Nitrous oxide–acetylene

Analysis

Samples: *Standard solutions, Sample solution,* and *Blank solution*

Using the calibration graph, determine the concentration, C, in µg/mL, of calcium in the *Sample solution.*

Calculate the percentage of calcium in the portion of Magnesium Oxide taken:

$$\text{Result} = [(V/W) \times C \times F] \times 100$$

V = volume of the *Sample solution* (mL)
W = weight of Magnesium Oxide (mg)
C = concentration of calcium in the *Sample solution* (µg/mL)
F = conversion from µg/mL to mg/mL, 0.001

Acceptance criteria: NMT 1.1%

• **IRON** ⟨241⟩

Test preparation: Boil 40 mg with 5 mL of 2 N nitric acid for 1 min. Cool, dilute with water to 50 mL, and mix. Dilute

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25 mL of this solution with water to 45 mL, and add 2 mL of hydrochloric acid.
Acceptance criteria: NMT 0.05%

SPECIFIC TESTS
- **Loss on Ignition** ⟨733⟩
Sample: 500–1000 mg
Analysis: Transfer the *Sample* to a tared platinum crucible, and ignite in the temperature range of (800°–900°) ± 25° to constant weight.
Acceptance criteria: NMT 10.0%
- **Bulk Density and Tapped Density of Powders,** *Bulk Density, Method I* ⟨616⟩**:** Using the procedure specified in the chapter, determine the bulk density of Magnesium Oxide.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate its bulk density. The indicated density may be in the form of a range.

Magnesium Oxide Capsules

DEFINITION
Magnesium Oxide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of magnesium oxide (MgO).

IDENTIFICATION
- **A. Identification Tests—General,** *Magnesium* ⟨191⟩
Sample solution: Transfer the contents of 1 Capsule to a beaker. Add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 min, and filter. Use the filtrate.
Acceptance criteria: Meet the requirements

ASSAY
- **Procedure**
Eriochrome black indicator solution: Dissolve 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol.
Sample solution: Mix the contents of NLT 20 Capsules. Transfer a portion of the powder, equivalent to 500 mg of magnesium oxide, to a beaker. Add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume.
Analysis: Transfer 20.0 mL of the *Sample solution* to a 400-mL beaker. Add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of *Eriochrome black indicator solution*. Cool the solution to 3°–4° by immersion of the beaker in an ice bath. Remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the *Sample solution*, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.05 M edetate disodium consumed is equivalent to 2.015 mg of MgO.
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Analysis: Using atomic absorption spectrophotometry at a wavelength of 285.2 nm, determine the amount of MgO dissolved, using filtered portions of the solution under test, suitably diluted with *Medium* if necessary, in comparison with a standard solution having a known concentration of magnesium in the same *Medium*.
Tolerances: NLT 75% (*Q*) of the labeled amount of MgO is dissolved.
- **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS
- **Acid-Neutralizing Capacity** ⟨301⟩**:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT 85.0% of the expected mEq value calculated from the results of the *Assay* is obtained. Each mg of MgO has an expected acid-neutralizing capacity value of 0.0492 mEq.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.

Magnesium Oxide Tablets

DEFINITION
Magnesium Oxide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of magnesium oxide (MgO).

IDENTIFICATION
- **A. Identification Tests—General,** *Magnesium* ⟨191⟩
Sample solution: Finely powder 1 Tablet. Transfer the powder to a beaker, add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 min, and filter. Use the filtrate.
Acceptance criteria: Meet the requirements

ASSAY
- **Procedure**
Eriochrome black indicator solution: Dissolve 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol.
Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 500 mg of magnesium oxide, to a beaker. Add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume.
Analysis: Transfer 20.0 mL of the *Sample solution* to a 400-mL beaker. Add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of *Eriochrome black indicator solution*. Cool the solution to 3°–4° by immersion of the beaker in an ice bath. Remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the *Sample solution*, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.05 M edetate disodium consumed is equivalent to 2.015 mg of MgO.
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Analysis: Using atomic absorption spectrophotometry at a wavelength of 285.2 nm, determine the amount of MgO

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dissolved, using filtered portions of the solution under test, suitably diluted with *Medium* if necessary, in comparison with a standard solution having a known concentration of magnesium in the same *Medium*.

Tolerances: NLT 75% (*Q*) of the labeled amount of MgO is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

• **Acid-Neutralizing Capacity** ⟨301⟩ (where Tablets are labeled as intended for antacid use)**:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT 85.0% of the expected mEq value calculated from the results of the *Assay* is obtained. Each mg of MgO has an expected acid-neutralizing capacity value of 0.0492 mEq.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

Magnesium Phosphate

$Mg_3(PO_4)_2 \cdot 5H_2O$ 352.93
Phosphoric acid, magnesium salt (2:3), pentahydrate; Magnesium phosphate (3:2) pentahydrate [10233-87-1].
$Mg_3(PO_4)_2$ 262.86
[7757-87-1].

DEFINITION

Magnesium Phosphate, ignited at 425° to constant weight, contains NLT 98.0% and NMT 101.5% of $Mg_3(PO_4)_2$.

IDENTIFICATION

• **A.**

Sample: 200 mg

Analysis: Dissolve the *Sample* in 10 mL of 2 N nitric acid, and add, dropwise, ammonium molybdate TS.

Acceptance criteria: A greenish-yellow precipitate of ammonium phosphomolybdate is formed, and it is soluble in 6 N ammonium hydroxide.

• **B. Identification Tests—General,** *Magnesium* ⟨191⟩

Sample solution: Dissolve 0.1 g in 0.7 mL of 1 N acetic acid and 20 mL of water. Add 1 mL of ferric chloride TS, allow to stand for 5 min, and filter. Use 5 mL of the filtrate.

Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**

Sample: 200 mg, previously ignited at 425° to constant weight

Analysis: Dissolve the *Sample* in a mixture of 25 mL of water and 10 mL of 2 N nitric acid. Filter, if necessary. Wash any precipitate, add sufficient 6 N ammonium hydroxide to the filtrate to produce a slight precipitate, and then dissolve the precipitate by the addition of 1 mL of 2 N nitric acid. Adjust the temperature to 50°, add 75 mL of ammonium molybdate TS, and maintain the temperature at 50° for 30 min, stirring occasionally. Wash the precipitate once or twice with water by decantation, using 30–40 mL each time and passing the washings through a filter. Transfer the precipitate to the filter, and wash with potassium nitrate solution (1 in 100) until the last washing is not acid to litmus. Transfer the precipitate and filter to the precipitation vessel. Add 50 mL of water and 40.0 mL of 1 N sodium hydroxide VS, and agitate until the precipitate is dissolved. Add phenolphthalein TS, and then titrate the excess alkali with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide is equivalent to 5.716 mg of $Mg_3(PO_4)_2$.

Acceptance criteria: 98.0%–101.5% on the previously ignited basis

IMPURITIES

• **Acid-Insoluble Substances**

[Note—Perform if an insoluble residue remains in the test for *Carbonate*.]

Analysis: Filter the solution, wash well with hot water until the last washing is free from chloride, and ignite the residue.

Acceptance criteria: The weight of the residue does not exceed 4 mg (NMT 0.2%).

• **Soluble Substances**

Sample: 2.0 g

Analysis: Digest the *Sample* with 100 mL of water on a steam bath for 30 min. Cool, add sufficient water to restore the original volume, mix, and filter. Evaporate 50 mL of the filtrate to dryness, and ignite gently to constant weight.

Acceptance criteria: The weight of the residue does not exceed 15 mg (NMT 1.5%).

• **Carbonate**

Sample: 2.0 g

Analysis: Mix the *Sample* with 20 mL of water, and add hydrochloric acid, dropwise, to dissolve.

Acceptance criteria: No effervescence occurs when the acid is added.

• **Chloride and Sulfate,** *Chloride* ⟨221⟩

Sample: 0.50 g

Analysis: Dissolve the *Sample* in 50 mL of 2 N nitric acid, and add 1 mL of silver nitrate TS.

Acceptance criteria: The turbidity does not exceed that produced by 1.0 mL of 0.020 N hydrochloric acid (NMT 0.14%).

• **Chloride and Sulfate,** *Sulfate* ⟨221⟩

Sample: 0.50 g

Analysis: Dissolve the *Sample* in the smallest possible amount of 3 N hydrochloric acid, dilute with water to 48 mL, and add 2 mL of barium chloride TS.

Acceptance criteria: The turbidity does not exceed that produced by 3.0 mL of 0.020 N sulfuric acid (NMT 0.6%).

• **Limit of Nitrate**

Sample: 0.20 g

Analysis: Mix the *Sample* with 5 mL of water, and add just sufficient hydrochloric acid to dissolve. Dilute with water to 10 mL, add 0.1 mL of indigo carmine TS, then add, with stirring, 10 mL of sulfuric acid.

Acceptance criteria: The blue color persists for NLT 5 min.

• **Arsenic,** *Method I* ⟨211⟩

Test preparation: Prepare as directed in the chapter, using 1.0 g and dissolving it first in just a sufficient amount of 3 N hydrochloric acid (about 9 mL).

Acceptance criteria: NMT 3 ppm

• **Barium**

Sample: 2.0 g

Analysis: Mix the *Sample* with 40 mL of water. Heat, add hydrochloric acid, dropwise, to dissolve, and then add 1 mL of acid in excess. Cool, dilute with water to 50 mL, and filter. To 5 mL of the filtrate add 1 mL of potassium sulfate TS.

Acceptance criteria: No turbidity is produced within 15 min.

• **Calcium**

Sample: 0.50 g

Analysis: Mix the *Sample* with 15 mL of water. Heat, and add sufficient hydrochloric acid, in small portions, to dissolve. Cool, add 6 N ammonium hydroxide, in small portions, to produce a slight permanent precipitate, then add 2 mL of 6 N acetic acid. Dilute with water to 25 mL, and filter. To 10 mL of the filtrate add 2 mL of ammonium oxalate TS.

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Acceptance criteria: NMT a slight turbidity is produced within 5 min.

• **DIBASIC SALT AND MAGNESIUM OXIDE**
Sample: Ignite about 2.5 g to constant weight and use 2 g of the ignited salt.
Analysis: Dissolve the *Sample* by warming with 50.0 mL of 1 N hydrochloric acid VS. Cool, add 1 or 2 drops of methyl orange TS, and slowly titrate the excess 1 N hydrochloric acid VS with 1 N sodium hydroxide VS to a yellow color, vigorously shaking the mixture during the titration.
Acceptance criteria: 14.8–15.4 mL of 1 N hydrochloric acid is consumed for each g of the ignited salt.

• **LEAD** ⟨251⟩
Test preparation: Dissolve 1.0 g in 20 mL of 3 N hydrochloric acid, evaporate on a steam bath to 10 mL, dilute with water to 20 mL, and cool.
Analysis: Proceed as directed in the chapter, using 5 mL of *Diluted Standard Lead Solution* (5 µg of Pb).
Acceptance criteria: NMT 5 ppm

SPECIFIC TESTS

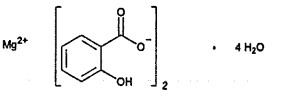
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the test for the absence of *Escherichia coli.*

• **LOSS ON IGNITION** ⟨733⟩**:** Ignite a sample at 425° to constant weight; it loses 20.0%–27.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Magnesium Salicylate



$C_{14}H_{10}MgO_6 \cdot 4H_2O$ 370.59

$C_{14}H_{10}MgO_6$ 298.54

Magnesium, bis(2-hydroxybenzoato-O^1,O^2)-, tetrahydrate; Magnesium salicylate (1:2), tetrahydrate [18917-95-8]. Anhydrous [18917-89-0].

DEFINITION

Magnesium Salicylate contains NLT 98.0% and NMT 103.0% of magnesium salicylate ($C_{14}H_{10}MgO_6 \cdot 4H_2O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

• **C. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩**:** Meets the requirements

ASSAY

• **PROCEDURE**
Mobile phase: Methanol, phosphoric acid, and water (40:0.1:60), prepared by adding 1 mL of phosphoric acid to a solution containing 400 mL of methanol and 600 mL of water
Standard solution: 0.5 mg/mL of USP Magnesium Salicylate RS, in *Mobile phase*
Sample solution: 0.5 mg/mL of Magnesium Salicylate, in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 236 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.10%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of magnesium salicylate ($C_{14}H_{10}MgO_6 \cdot 4H_2O$) in the portion of Magnesium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Magnesium Salicylate RS in the *Standard solution* (mg/mL)
C_U = concentration of Magnesium Salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–103.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase: Methanol, phosphoric acid, and water (40:0.1:60), prepared by adding 1 mL of phosphoric acid to a solution containing 400 mL of methanol and 600 mL of water
Standard stock solution: 0.25 mg/mL of USP Magnesium Salicylate RS, 0.25 mg/mL of USP Salicylic Acid Related Compound A RS, 0.125 mg/mL of USP Salicylic Acid Related Compound B RS, and 0.05 mg/mL of USP Phenol RS, in *Mobile phase*
Standard solution: 0.005 mg/mL of USP Magnesium Salicylate RS, 0.005 mg/mL of USP Salicylic Acid Related Compound A RS, 0.0025 mg/mL of USP Salicylic Acid Related Compound B RS, and 0.001 mg/mL of USP Phenol RS, in *Mobile phase* prepared from *Standard stock solution*
Sample solution: 5 mg/mL of Magnesium Salicylate, in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 212 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between any two peaks
Relative standard deviation: NMT 3% for each peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid related compound A, salicylic acid related compound B, and phenol in the portion of Magnesium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of salicylic acid related compound A, salicylic acid related compound B, or phenol from the *Sample solution*
r_S = peak response of salicylic acid related compound A, salicylic acid related compound B, or phenol from the *Standard solution*
C_S = concentration of USP Salicylic Acid Related Compound A RS, USP Salicylic Acid Related Compound B RS, or USP Phenol RS in the *Standard solution* (mg/mL)
C_U = concentration of Magnesium Salicylate in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Magnesium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of salicylic acid related compound B from the *Standard solution*
C_S = concentration of USP Salicylic Acid Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Magnesium Salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A	0.3	0.1
Phenol	0.4	0.02
Salicylic acid related compound B	0.6	0.05
Salicylic acid	1.0	—
Any other individual impurity	—	0.05
Total impurities	—	0.2

SPECIFIC TESTS

• **MAGNESIUM CONTENT**
Sample solution: Transfer 800 mg of Magnesium Salicylate to a 200-mL volumetric flask. Dissolve in and dilute with water to volume. Stir the solution continuously for 15 min, and filter, discarding the first 10 mL of the filtrate, into a flask.
Titrimetric system
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Transfer 50.0 mL of the *Sample solution* to a 250-mL conical flask. Add 50 mL of water, 5 mL of ammonia–ammonium chloride buffer TS, and 0.15 mL of eriochrome black TS. Titrate with *Titrant* to a blue endpoint. Each mL of *Titrant* is equivalent to 1.215 mg of magnesium.
Acceptance criteria: 6.3%–6.7% of magnesium
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 17.5%–21.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in tight containers at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Magnesium Salicylate RS
USP Phenol RS
USP Salicylic Acid Related Compound A RS
4-Hydroxybenzoic acid.
$C_7H_6O_3$ 138.12
USP Salicylic Acid Related Compound B RS
4-Hydroxyisophthalic acid.
$C_8H_6O_5$ 182.13

Magnesium Salicylate Tablets

DEFINITION

Magnesium Salicylate Tablets contain an amount of magnesium salicylate ($C_{14}H_{10}MgO_6 \cdot 4H_2O$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of anhydrous magnesium salicylate ($C_{14}H_{10}MgO_6$).

IDENTIFICATION

• **A.** The IR absorption spectrum of a potassium bromide dispersion of a quantity of finely powdered Tablets exhibits maxima at the same wavelengths as those of a similar preparation of USP Magnesium Salicylate RS.
• **B. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: Prepare a 50-mg/mL magnesium salicylate solution from Tablets, and filter.
Acceptance criteria: Meet the requirements
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Methanol, trifluoroacetic acid, and water (40: 0.1: 60), prepared by adding 1 mL of trifluoroacetic acid to a solution containing 400 mL of methanol and 600 mL of water
Standard solution: 0.05 mg/mL of anhydrous USP Magnesium Salicylate RS in *Mobile phase*
Sample stock solution: Nominally 0.5 mg/mL of anhydrous magnesium salicylate from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 75% of the flask volume of *Mobile phase*, and sonicate for 10 min. Allow the solution to cool to room temperature and then dilute with *Mobile phase* to volume.
Sample solution: Nominally 0.05 mg/mL of anhydrous magnesium salicylate in *Mobile phase*, from the *Sample stock solution*. Pass through a suitable filter of 0.20-µm pore size, discarding the first 2–3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Column temperature: 30°
Flow rate: 0.2 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.8
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous magnesium salicylate ($C_{14}H_{10}MgO_6$) in the portion of Tablets taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of anhydrous USP Magnesium Salicylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anhydrous magnesium salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 120 min
Standard solution: USP Salicylic Acid RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium* if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum wavelength at about 296 nm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous magnesium salicylate ($C_{14}H_{10}MgO_6$) dissolved:

$$\text{Result} = (A_U/A_S) \times (1/L) \times (M_{r1}/M_{r2}) \times C_S \times V \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
L = nominal concentration of anhydrous magnesium salicylate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of anhydrous magnesium salicylate, 298.54
M_{r2} = twice the molecular weight of salicylic acid, 276.24
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of anhydrous magnesium salicylate ($C_{14}H_{10}MgO_6$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase: Methanol, trifluoroacetic acid, and water (40: 0.1: 60), prepared by adding 1 mL of trifluoroacetic acid to a solution containing 400 mL of methanol and 600 mL of water
System suitability solution: 0.5 mg/mL of USP Magnesium Salicylate RS, 0.5 µg/mL of USP Salicylic Acid Related Compound A RS, 0.5 µg/mL of USP Salicylic Acid Related Compound B RS, and 0.5 µg/mL of USP Phenol RS, in *Mobile phase*
Standard solution: 2.5 µg/mL of anhydrous USP Magnesium Salicylate RS in *Mobile phase*
Sample solution: Nominally 2.5 mg/mL of anhydrous magnesium salicylate from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 80% of the flask volume of *Mobile phase*, and sonicate for 15 min. Allow the solution to cool to room temperature and then dilute with *Mobile phase* to volume. Centrifuge the solution, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Column temperature: 30°
Flow rate: 0.2 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between any two peaks, *System suitability solution*
Relative standard deviation: NMT 3%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of magnesium salicylate from the *Standard solution*
C_S = concentration of anhydrous USP Magnesium Salicylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anhydrous magnesium salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A	0.3	—[a]
Phenol	0.4	—[a]
Salicylic acid related compound B	0.6	—[a]
Salicylic acid	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] These are process impurities, which are included in the table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Magnesium Salicylate RS
USP Phenol RS
USP Salicylic Acid RS
USP Salicylic Acid Related Compound A RS
4-Hydroxybenzoic acid.
$C_7H_6O_3$ 138.12
USP Salicylic Acid Related Compound B RS
4-Hydroxyisophthalic acid.
$C_8H_6O_5$ 182.13

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Magnesium Sulfate

$MgSO_4 \cdot xH_2O$
Sulfuric acid magnesium salt (1:1), hydrate;
Magnesium sulfate (1:1) monohydrate 138.36
[14168-73-1].
Magnesium sulfate (1:1) heptahydrate 246.47
[10034-99-8].
Anhydrous 120.37
[7487-88-9].

DEFINITION

Magnesium Sulfate, rendered anhydrous by ignition, contains NLT 99.0% and NMT 100.5% of $MgSO_4$.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩ and *Sulfate* ⟨191⟩
Sample solution: 50 mg/mL
Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**
Sample: 250 mg of the ignited Magnesium Sulfate obtained in the test for *Loss on Ignition*
Titrimetric system
Mode: Direct titration
Titrant: 0.05M edetate sodium VS
Endpoint detection: Colorimetric
Analysis: Dissolve the *Sample* in 100 mL of water and the minimum amount of 3 N hydrochloric acid required for a clear solution. Adjust the reaction of the solution (using pH indicator paper; see *Reagents, Indicators, and Solutions—Reagents—Indicator and Test Papers*) with 1 N sodium hydroxide to a pH of 7, add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and titrate with the *Titrant* to a blue endpoint. Caclulate the perecentage of $MgSO_4$ in the portion of the ignited Magnesium Sulfate taken:

$$\text{Result} = [V \times N \times F \times 100]/W$$

V = *Sample* titrant volume (mL)
N = titrant molarity (mmol/mL)
F = equivalency factor, 120.36 mg/mmol
W = weight of *Sample* (mg)

Acceptance criteria: 99.0%–100.5% on the anhydrous by ignition basis

IMPURITIES

• **LIMIT OF CHLORIDE** ⟨221⟩
Sample: 1.0 g
Acceptance criteria: The *Sample* shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.014%).
• **LIMIT OF IRON** ⟨241⟩
Magnesium Sulfate intended for use in preparing nonparenteral dosage forms
Sample solution: Dissolve 0.50 g in 40 mL of water.
Analysis: Proceed as directed in the test for *Iron* ⟨241⟩.
Acceptance criteria: NMT 20 µg/g
Magnesium Sulfate intended for use in preparing parenteral dosage forms
[NOTE—Rinse all glassware used in this test with *Dilute hydrochloric acid.*]
Dilute hydrochloric acid: 1 mL of hydrochloric acid diluted with water to 1000 mL
Solution A: 500 mg/mL of ammonium acetate in water
Solution B: 13.4 mg/mL of ascorbic acid in water. [NOTE—Use this solution on the day prepared.]
Color reagent: 3.8 mg/mL of 3-(2-pyridyl)-5,6-di-(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid, disodium salt in *Solution A*. Shake by mechanical means if necessary. Use this solution on the day prepared.
Standard stock solution: 1.0 µg/mL of iron, from *Standard Iron Solution* in *Dilute hydrochloric acid*
Standard solutions: To three separate 50-mL volumetric flasks transfer 2.0, 5.0, and 10.0 mL of *Standard stock solution*, and dilute each with *Dilute hydrochloric acid* to 35 mL. These solutions contain 2.0, 5.0, and 10.0 µg of iron, respectively.
Sample solution: Transfer 10.0 g of Magnesium Sulfate to a 50-mL volumetric flask, add *Dilute hydrochloric acid* to 35 mL, and sonicate, if necessary, to dissolve.
Blank: Transfer 35 mL of *Dilute hydrochloric acid* to a 50-mL volumetric flask.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 594 nm
Analysis
Samples: *Standard solutions, Blank,* and *Sample solution*
To each of the flasks containing the *Standard solutions,* the *Sample solution,* and the *Blank* add 5 mL of *Solution B* and 5 mL of *Color reagent*. Dilute each solution with *Dilute hydrochloric acid* to volume, mix, and allow to stand for 10 min.
Plot the absorbance values of the *Standard solutions* versus their iron contents in µg and draw the straight line best fitting the three plotted points. From the graph, determine the iron content, C, in µg, of the *Sample solution.*
Calculate the content, in µg/g, of iron in the portion of Magnesium Sulfate taken:

$$\text{Result} = C/W$$

C = content of iron in the *Sample solution* in µg, determined from the graph
W = weight of Magnesium Sulfate in the *Sample solution* (g)

Acceptance criteria: NMT 0.5 µg/g
• **SELENIUM** ⟨291⟩
Test solution: 200 mg in 50 mL of 0.25 N nitric acid
Acceptance criteria: NMT 30 µg/g

SPECIFIC TESTS

• **PH** ⟨791⟩
Sample solution: 50 mg/mL
Acceptance criteria: 5.0–9.2
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 2 h: the anhydrous form loses NMT 2% of its weight.
• **LOSS ON IGNITION** ⟨733⟩
Sample: 1 g
Analysis: Weigh the *Sample* in a crucible, heat at 105° for 2 h, then ignite in a muffle furnace at 450 ± 25° to constant weight.
Acceptance criteria
Monohydrate: Loses 13.0%–16.0% of its weight
Dried form: Loses 22.0%–28.0% of its weight
Heptahydrate: Loses 40.0%–52.0% of its weight

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** The label states whether it is the monohydrate, the dried form, or the heptahydrate. Magnesium Sulfate

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intended for use in preparing parenteral dosage forms is so labeled. Magnesium Sulfate not intended for use in preparing parenteral dosage forms is so labeled. In addition, it may be labeled also as intended for use in preparing nonparenteral dosage forms.

Magnesium Sulfate Injection

DEFINITION

Magnesium Sulfate Injection is a sterile solution of Magnesium Sulfate in Water for Injection. It contains magnesium sulfate ($MgSO_4$) equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩ and *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Sample: A known volume of Injection equivalent to 250 mg of anhydrous magnesium sulfate

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis

Sample: *Sample*

Transfer the *Sample* to a beaker, and dilute with water to 100 mL. Adjust with 1 N sodium hydroxide to a pH of 7 (using pH indicator paper; see *Reagents, Indicators, and Solutions—Indicators and Indicator Test Papers*). Add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS. Titrate with *Titrant* to a blue endpoint. Each mL of *Titrant* consumed is equivalent to 12.32 mg of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$).

Acceptance criteria: 93.0%–107.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.09 USP Endotoxin Unit/mg of magnesium sulfate.
- **pH** ⟨791⟩

Sample solution: 50 mg/mL

Acceptance criteria: 5.5–7.0

- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Magnesium Sulfate in Dextrose Injection

DEFINITION

Magnesium Sulfate in Dextrose Injection is a sterile solution of Magnesium Sulfate and Dextrose in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) and NLT 90.0% and NMT 110.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$).

IDENTIFICATION

- **A.**

Sample solution 1: A suitable volume of Injection equivalent to 50 mg/mL of dextrose

Analysis 1: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria 1: A copious red precipitate of cuprous oxide is formed.

Analysis 2: Proceed as directed in *Identification Tests—General* ⟨191⟩, *Magnesium.*

Acceptance criteria 2: Meets the requirements

ASSAY

- **MAGNESIUM SULFATE**

Sample: A known volume of Injection equivalent to 250 mg of anhydrous magnesium sulfate

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a beaker, and dilute with water to 100 mL. Adjust with 1 N sodium hydroxide to a pH of 7 using pH indicator paper (see *Reagents, Indicators, and Solutions—Indicator and Test Papers*), and add 5 mL of ammonia-ammonium chloride buffer TS and 0.15 mL of eriochrome black TS. Titrate with *Titrant* to a blue endpoint. Each mL of *Titrant* is equivalent to 12.32 mg of magnesium sulfate ($MgSO_4 \cdot 7H_2O$).

Acceptance criteria: 93.0%–107.0% of the labeled amount of magnesium sulfate ($MgSO_4 \cdot 7H_2O$)

- **DEXTROSE**

Sample solution: Nominally 2 g/100 mL of dextrose prepared as follows. Transfer a suitable volume of Injection to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩). Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times \alpha)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

α = observed angular rotation of the *Sample solution* (°)

l = length of the polarimeter tube, decimeter

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution*, g/100 mL

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–110.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$)

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IMPURITIES

- **Limit of 5-Hydroxymethylfurfural and Related Substances**

Sample solution: Nominally 2 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in water from a suitable volume of Injection containing 1.0 g of dextrose in water

Instrumental conditions

Mode: UV

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Acceptance criteria: Absorbance of the *Sample solution* is NMT 0.25.

SPECIFIC TESTS

- **Bacterial Endotoxins Test** ⟨85⟩: NMT 0.039 USP Endotoxin Unit/mg of magnesium sulfate
- **pH** ⟨791⟩: 3.5–6.5
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type 1 or Type II glass.

Magnesium Trisilicate

$2MgO \cdot 3SiO_2 \cdot xH_2O$(anhydrous) 260.86

Silicic acid ($H_4Si_3O_8$), magnesium salt (1:2), hydrate.

Magnesium silicate hydrate ($Mg_2Si_3O_8 \cdot xH_2O$) [39365-87-2].

Anhydrous [14987-04-3].

» Magnesium Trisilicate is a compound of Magnesium Oxide and silicon dioxide with varying proportions of water. It contains not less than 20.0 percent of magnesium oxide (MgO) and not less than 45.0 percent of silicon dioxide (SiO_2).

Packaging and storage—Preserve in well-closed containers.

Identification—

A: Mix about 500 mg with 10 mL of 3 N hydrochloric acid, filter, and neutralize the filtrate to litmus paper with 6 N ammonium hydroxide: the neutralized filtrate responds to the tests for *Magnesium* ⟨191⟩.

B: Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with Magnesium Trisilicate, and again fuse: silica floats about in the bead, producing, upon cooling, an opaque bead with a web-like structure.

Water Determination, *Method III* ⟨921⟩ —Weigh accurately about 1 g in a tared platinum crucible provided with a cover. Gradually apply heat to the crucible at first, then strongly ignite to constant weight: it loses between 17.0% and 34.0% of its weight.

Soluble salts—Boil 10.0 g with 150 mL of water for 15 minutes. Cool to room temperature, allow the mixture to stand for 15 minutes, filter with the aid of suction, transfer the filtrate to a 200-mL volumetric flask, dilute with water to volume, and mix. Evaporate 50.0 mL of this solution, representing 2.5 g of the Trisilicate, in a tared platinum dish to dryness, and ignite gently to constant weight: the weight of the residue does not exceed 38.0 mg (1.5%).

Chloride ⟨221⟩—A 20-mL portion of the diluted filtrate prepared in the test for *Soluble salts,* representing 1 g of Magnesium Trisilicate, shows no more chloride than corresponds to 0.75 mL of 0.020 N hydrochloric acid (0.055%).

Sulfate—Treat the residue obtained in the test for *Soluble salts* with 2 mL of hydrofluoric acid, and evaporate on a steam bath to dryness. Mix the residue with water, transfer to a filter, and wash, using approximately 50 mL of water for the complete procedure. Heat the filtrate to boiling, and add 0.1 mL of hydrochloric acid and 5 mL of barium chloride TS. Maintain the mixture near its boiling point for 1 hour, filter, wash the precipitate thoroughly with water, dry, and ignite to constant weight: the weight of the residue does not exceed 30 mg (0.5%).

Free alkali—Add 2 drops of phenolphthalein TS to 20 mL of the diluted filtrate prepared in the test for *Soluble salts,* representing 1 g of the Trisilicate: if a pink color is produced, not more than 1.0 mL of 0.10 N hydrochloric acid is required to discharge it.

Arsenic, *Method I* ⟨211⟩: 8 ppm.

Acid-consuming capacity—Weigh accurately about 200 mg into a glass-stoppered, 125-mL conical flask. Add 30.0 mL of 0.1 N hydrochloric acid VS and 20.0 mL of water. Place the flask in a bath maintained at 37°, and shake the mixture occasionally during a period of 4 hours but leave the mixture undisturbed during the last 15 minutes of the heating period. Cool to room temperature. To 25.0 mL of the supernatant add methyl red TS, and titrate the excess acid with 0.1 N sodium hydroxide VS. One g of Magnesium Trisilicate, calculated on the anhydrous basis, consumes not less than 140 mL and not more than 160 mL of 0.10 N hydrochloric acid.

Assay for magnesium oxide—Weigh accurately about 1.5 g, and transfer to a 250-mL conical flask. Add 50.0 mL of 1 N sulfuric acid VS, and digest on a steam bath for 1 hour. Cool to room temperature, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide VS. Each mL of 1 N sulfuric acid is equivalent to 20.15 mg of MgO.

Assay for silicon dioxide—Transfer about 700 mg of Magnesium Trisilicate, accurately weighed, to a small platinum dish. Add 10 mL of 1 N sulfuric acid, and heat on a steam bath to dryness, leaving the dish uncovered. Treat the residue with 25 mL of water, and digest on a steam bath for 15 minutes. Decant the supernatant through an ashless filter paper, with the aid of suction, and wash the residue, by decantation, three times with hot water, passing the washings through the filter paper. Finally transfer the residue to the filter, and wash thoroughly with hot water. Transfer the filter paper and its contents to the platinum dish previously used. Heat to dryness, incinerate, ignite strongly for 30 minutes, cool, and weigh. Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh: the loss in weight represents the weight of SiO_2.

Ratio of SiO_2 to MgO—Divide the percentage of SiO_2 obtained in the *Assay for silicon dioxide* by the percentage of MgO obtained in the *Assay for magnesium oxide:* the quotient obtained is between 2.10 and 2.37.

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Magnesium Trisilicate Tablets

» Magnesium Trisilicate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $Mg_2Si_3O_8$.

Packaging and storage—Preserve in well-closed containers.

Identification—

A: Powder 1 Tablet, add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate so obtained responds to the tests for *Magnesium* ⟨191⟩.

B: Wash the solids on the filter obtained in *Identification* test *A* with hot ammonium chloride solution (1 in 50), add 10 mL of 3 N hydrochloric acid, and filter. Transfer the filter paper and contents to a small platinum dish, ignite, cool in a desiccator, and weigh. Moisten the residue with water, and add 6 mL of hydrofluoric acid. Evaporate to dryness, ignite for 5 minutes, cool in a desiccator, and weigh: a loss of more than 10% in relation to the weight of the residue from the initial ignition indicates SiO_2.

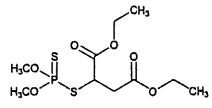
Disintegration ⟨701⟩: 10 minutes, simulated gastric fluid TS being substituted for water in the test.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 g of magnesium trisilicate, to a beaker, add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume, and mix. Transfer 20.0 mL of this solution to a 400-mL beaker, add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the assay solution, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 6.521 mg of $Mg_2Si_3O_8$.

Malathion



$C_{10}H_{19}O_6PS_2$ 330.36

Butanedioic acid, [(dimethoxyphosphinothioyl)-thio]-, diethyl ester, (±)-;

Diethyl (±)-mercaptosuccinate, *S*-ester with *O,O*-dimethyl phosphorodithioate [121-75-5].

DEFINITION

Malathion contains NLT 98.0% and NMT 102.0% of malathion ($C_{10}H_{19}O_6PS_2$).

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

ASSAY

• **Procedure**

Mobile phase: Methanol and water (50:30)

Internal standard solution: 0.6 mg/mL of propylparaben in *Mobile phase*

Standard solution: 10 mg/mL of USP Malathion RS and 0.06 mg/mL of propylparaben from *Internal standard solution* in methanol

Sample solution: 10 mg/mL of Malathion and 0.06 mg/mL of propylparaben from *Internal standard solution* in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for propylparaben and malathion are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 for propylparaben and malathion

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of malathion ($C_{10}H_{19}O_6PS_2$) in the portion of Malathion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of malathion to the internal standard from the *Sample solution*

R_S = peak response ratio of malathion to the internal standard from the *Standard solution*

C_S = concentration of USP Malathion RS in the *Standard solution* (mg/mL)

C_U = concentration of Malathion in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **Limit of Isomalathion**

Mobile phase: Methanol and water (50:30)

Standard solution: 0.1 mg/mL of USP Isomalathion RS in methanol

Sample solution: 20 mg/mL of Malathion in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 30-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

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[NOTE—The relative retention times for isomalathion and malathion are about 0.5 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isomalathion in the portion of Malathion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isomalathion from the *Sample solution*

r_S = peak response of isomalathion from the *Standard solution*

C_S = concentration of USP Isomalathion RS in the *Standard solution* (mg/mL)

C_U = concentration of Malathion in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: Between 1.220 and 1.240
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Isomalathion RS

Butanedioic acid, [[methoxy(methylthio)phosphinyl]thio]-, diethyl ester.

$C_{10}H_{19}O_6PS_2$ 330.36

USP Malathion RS

Malathion Lotion

DEFINITION

Malathion Lotion is Malathion in a suitable isopropyl alcohol vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of malathion ($C_{10}H_{19}O_6PS_2$).

IDENTIFICATION

- **A.** The retention time of the major peak for malathion of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Methyl ethyl ketone and *n*-hexane (4:1)

Internal standard solution: 2 mg/mL of parathion in *Solution A*

Standard stock solution: 2 mg/mL of USP Malathion RS in *Solution A*

Standard solution: 0.4 mg/mL of USP Malathion RS from the *Standard stock solution* and 0.4 mg/mL of parathion from the *Internal standard solution* in *Solution A*

Sample solution: Nominally 0.4 mg/mL of malathion and 0.4 mg/mL of parathion in *Solution A*, prepared as follows. Transfer a volume of Lotion, equivalent to 10 mg of malathion, to a 25-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; packed with 5% G6 liquid phase on 110- to 120-mesh support S1A

Temperatures

Injector: 230°

Detector: 250°

Column: 190°

Carrier gas: Dry nitrogen

Flow rate: 15 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for malathion and parathion are 1.0 and about 1.3, respectively.]

Suitability requirements

Resolution: NLT 3.0 between malathion and parathion

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of malathion ($C_{10}H_{19}O_6PS_2$) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of malathion to the internal standard from the *Sample solution*

R_S = peak response ratio of malathion to the internal standard from the *Standard solution*

C_S = concentration of USP Malathion RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of malathion in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **CONTENT OF ISOPROPYL ALCOHOL**

Internal standard solution: Ethyl acetate and dehydrated alcohol (4:1)

Standard solution: Transfer 2.0 mL of isopropyl alcohol and 5.0 mL of *Internal standard solution* to a 200-mL volumetric flask, and dilute with ethyl acetate to volume.

Sample solution: Transfer a volume of Lotion, equivalent to 2.0 mL of isopropyl alcohol, to a 200-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with ethyl acetate to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; packed with 110- to 120-mesh support S2

Temperatures

Injector: 200°

Detector: 220°

Column: 130°

Carrier gas: Dry nitrogen

Flow rate: 7 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0% for the peak response ratio of isopropyl alcohol to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isopropyl alcohol (C_3H_8O) in the portion of Lotion taken:

USP Monographs

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$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of isopropyl alcohol to the internal standard from the *Sample solution*
R_S = peak response ratio of isopropyl alcohol to the internal standard from the *Standard solution*
C_S = concentration of isopropyl alcohol in the *Standard solution* (mg/mL)
C_U = nominal concentration of isopropyl alcohol in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–110%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight glass containers.
- **LABELING:** The labeling states the percentage (v/v) of isopropyl alcohol in the Lotion.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Malathion RS

Manganese Chloride

$MnCl_2 \cdot 4H_2O$ 197.91
Manganese chloride ($MnCl_2$) tetrahydrate;
Manganese (2+) chloride tetrahydrate [13446-34-9].
Anhydrous 125.84
[7773-01-5].

DEFINITION
Manganese Chloride contains NLT 98.0% and NMT 101.0% of $MnCl_2$, calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Yields white, curdy precipitate of silver chloride with silver nitrate TS, which is insoluble in nitric acid. After being washed with water, this precipitate is soluble in a slight excess of 6 N ammonium hydroxide.
- **B. IDENTIFICATION TESTS—GENERAL,** *Manganese* ⟨191⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**
  Sample: 425 mg
  Analysis: Transfer the *Sample* to a 400-mL beaker, dissolve in 25 mL of water, add 300 mg of ammonium chloride and 0.5 g of hydroxylamine hydrochloride, and swirl to dissolve. Warm slightly on a hot plate, and dilute with water to 100 mL. Add 3 mL of triethanolamine and stir the solution, preferably using a magnetic stirrer. Begin the titration by adding 25 mL of 0.05 M edetate disodium VS, then add 10 mL of ammonia–ammonium chloride buffer TS, and 1 mL of eriochrome black TS. Continue to titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 6.292 mg of $MnCl_2$.
  Acceptance criteria: 98.0%–101.0% of $MnCl_2$ on the dried basis

IMPURITIES
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Sample: 2.0 g
  Acceptance criteria: Shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.005%).
- **IRON** ⟨241⟩
  Sample solution: 2.0 g in 40 mL of water
  Acceptance criteria: NMT 5 ppm
- **ZINC**
  Sample solution: Dissolve 1 g in a mixture of 48 mL of water and 2 mL of sulfuric acid.
  Analysis: To the *Sample solution*, add, slowly and with constant agitation, 1 mL of potassium ferrocyanide solution (1 in 100).
  Acceptance criteria: No turbidity is produced within 5 min.

SPECIFIC TESTS
- **INSOLUBLE MATTER**
  Sample solution: Transfer 10 g to a 250-mL beaker, add 150 mL of water, cover the beaker, and heat to boiling.
  Analysis: Digest the hot *Sample solution* on a steam bath for 1 h, and pass through a tared filtering crucible of fine pore size. Rinse the beaker with hot water, passing the rinsings through the filter, and finally wash the filter with additional hot water. Dry the filter at 105°.
  Acceptance criteria: The residue weighs NMT 0.5 mg (0.005%).
- **SUBSTANCES NOT PRECIPITATED BY AMMONIUM SULFIDE**
  Sample solution: Dissolve 2.0 g in 90 mL of water, add 5 mL of ammonium hydroxide, and warm the solution to 80°. Pass a stream of hydrogen sulfide through the solution for 30 min. Dilute with water to 100 mL, mix, and allow the precipitate to settle. Decant the supernatant through a filter of fine pore size, and transfer 50.0 mL to an evaporating dish that previously has been ignited and tared.
  Analysis: Evaporate the filtrate to dryness, cool, add 0.5 mL of sulfuric acid, heat gently to remove the excess acid, and ignite at 800 ± 25° for 15 min.
  Acceptance criteria: The weight of the residue is NMT 2.0 mg (NMT 0.2% as sulfate).
- **pH** ⟨791⟩
  Sample solution: 10 g in 200 mL of carbon dioxide- and ammonia-free water
  Acceptance criteria: 3.5–6.0
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 50° for 2 h, then raise the temperature to 150° for 24 h: it loses 36.0%–38.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

Manganese Chloride Injection

» Manganese Chloride Injection is a sterile solution of Manganese Chloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of manganese (Mn).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.
Labeling—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.
Identification—The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 279 nm when tested as directed for *Procedure* in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.45 USP Endotoxin Unit per µg of manganese.
pH ⟨791⟩: between 1.5 and 2.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

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Assay—

Sodium chloride solution—Dissolve 1.8 g of sodium chloride in water, dilute with water to 2000 mL, and mix.

Manganese stock solution—Transfer 1.000 g of manganese to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, dilute with 0.1 N hydrochloric acid to volume, and mix. This solution contains 1000 µg of manganese per mL. Store in a polyethylene bottle.

Standard preparations—Pipet 10 mL of the *Manganese stock solution* into a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 4.0, 5.0, and 6.0 mL, respectively, of this solution to separate 50-mL volumetric flasks, containing 10 mL of *Sodium chloride solution*, dilute the contents of each flask with water to volume, and mix. These *Standard preparations* contain, respectively, 1.6, 2.0, and 2.4 µg of manganese per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 1 mg of manganese, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the initial dilution, and add sufficient *Sodium chloride solution* to bring the total sodium chloride content in this flask to 9 mg. Dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the manganese emission line of 279 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a manganese hollow-cathode lamp and an air–acetylene flame, using a dilution of *Sodium chloride solution* (1:5) as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of manganese, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in µg per mL, of manganese in the *Assay preparation*. Calculate the quantity, in mg, of manganese in each mL of the Injection taken by the formula:

$$0.5C/V$$

in which C is the concentration, in µg per mL, of manganese in the *Assay preparation*; and V is the volume, in mL, of Injection taken.

Delete the following:

▲Manganese Chloride for Oral Solution

» Manganese Chloride for Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of manganese (Mn). It may contain one or more suitable flavors, sweetening agents, thickening agents, and stabilizers.

Packaging and storage—Preserve in tight, light-resistant, single-dose containers.

Labeling—The label contains directions for constitution of the powder and states the amount of manganese in a given volume of the Oral Solution obtained after constitution.

Identification—It meets the requirements of the tests for *Chloride* ⟨191⟩ and for *Manganese* ⟨191⟩.

Uniformity of dosage units ⟨905⟩—

FOR POWDER PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—

FOR POWDER PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH ⟨ 791 ⟩ : between 6.0 and 8.0, when constituted to 300 mL with water.

Osmolality and Osmolarity ⟨ 785 ⟩ : 230 mOsmol pH 6.0 to 8.0.

Assay—

Sodium chloride solution, Manganese stock solution, and *Standard preparations*—Prepare as directed in the *Assay* under *Manganese Chloride Injection.*

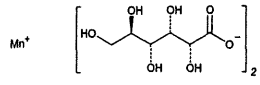
Assay preparation—Constitute the Manganese Chloride for Oral Solution as directed in the labeling. Transfer about 25 mL, accurately measured, of the constituted Manganese Chloride for Oral Solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Proceed as directed for *Assay preparation* in the *Assay* under *Manganese Chloride Injection,* beginning with "Pipet 10 mL of this solution."

Procedure—Proceed as directed in the *Assay* under *Manganese Chloride Injection.* Calculate the quantity, in µg, of manganese in each mL of the constituted for Oral Solution taken by the formula:

$$500C/V$$

in which C is the concentration, in µg per mL, of the manganese in the *Assay preparation;* and V is the volume, in mL, of the constituted Manganese Chloride for Oral Solution taken. ▲ (USP 1-May-2020)

Manganese Gluconate



$C_{12}H_{22}MnO_{14}$ 445.23
$C_{12}H_{22}MnO_{14} \cdot 2H_2O$ 481.26

Bis(D-gluconato-O^1,O^2) manganese;
Manganese D-gluconate (1:2).
Anhydrous [6485-39-8].

DEFINITION

Manganese Gluconate is dried or contains two molecules of water of hydration. It contains NLT 98.0% and NMT 102.0% of manganese gluconate ($C_{12}H_{22}MnO_{14}$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Manganese* ⟨191⟩**:** A 50-mg/mL solution meets the requirements.
- **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Manganese Gluconate, heating in a water bath at 60°, if necessary, to dissolve

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

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Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Sample: 700 mg of Manganese Gluconate

Blank: 50 mL of water

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 50 mL of water. Add 1 g of ascorbic acid and 10 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with the *Titrant* until the solution is deep blue in color. Perform the blank determination.

Calculate the percentage of manganese gluconate ($C_{12}H_{22}MnO_{14}$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

M = actual molarity of the *Titrant* (mmol/mL)

F = equivalency factor, 445.2 mg/mmol

W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Standard: 0.70 mL of 0.020 N hydrochloric acid

Sample: 1.0 g of Manganese Gluconate

Acceptance criteria: NMT 0.05%

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Standard: 4.0 mL of 0.020 N sulfuric acid

Sample: 2.0 g of Manganese Gluconate

Acceptance criteria: NMT 0.2%

• **LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Ascorbic acid–sodium iodide solution: 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

Trioctylphosphine oxide solution: 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone. [**CAUTION**—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

Standard solution: Transfer 5.0 mL of lead nitrate stock solution TS, to a 100-mL volumetric flask. Dilute with water to volume. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask. Add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution*, and it contains 2.0 µg/mL of lead.

Sample solution: To a 50-mL volumetric flask add 1.0 g of Manganese Gluconate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

Blank: To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 283.3 nm

Lamp: Lead hollow-cathode

Flame: Air–acetylene

System suitability

Samples: *Standard solution* and *Blank*

Suitability requirements: The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and the *Sample solution*. Use the *Blank* to set the instrument to zero.

Acceptance criteria: The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 10 ppm).

• **REDUCING SUBSTANCES**

Sample: 1.0 g of Manganese Gluconate

Blank: Proceed as directed in the *Analysis*, omitting the *Sample*.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Residual titration

Titrant: 0.1 N iodine VS

Back-titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the blank determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)

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V_S = *Back-titrant* volume consumed by the *Sample* (mL)
N = *Back-titrant* normality (mEq/mL)
F = equivalency factor, 27 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩
Analysis: Proceed as directed in the chapter. Maintain the mixture containing the *Test preparation* at 50°, and stir for 30 min before titrating with the *Reagent*.
Acceptance criteria
Where labeled as the dried form: 3.0%–9.0%
Where labeled as the dihydrate: 6.0%–9.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The label indicates whether it is the dried or the dihydrate form.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Potassium Gluconate RS

Manganese Sulfate

$MnSO_4 \cdot H_2O$ 169.02
Sulfuric acid, manganese(2+) salt (1:1) monohydrate; Manganese(2+) sulfate (1:1) monohydrate [10034-96-5].
Anhydrous 151.00
[7785-87-7].

DEFINITION
Manganese Sulfate contains NLT 98.0% and NMT 102.0% of $MnSO_4 \cdot H_2O$.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Manganese* ⟨191⟩
Sample solution: 100 mg/mL
Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩
Sample solution: 100 mg/mL
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
Sample: 350 mg
Analysis: Dissolve the *Sample* in 200 mL of water. Add 10 mg of ascorbic acid. Begin the titration by adding 25 mL of 0.05 M edetate disodium VS using a suitable buret, then add 10 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS. Complete the titration with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 8.451 mg of $MnSO_4 \cdot H_2O$.
Acceptance criteria: 98.0%–102.0%

IMPURITIES
- **SUBSTANCES NOT PRECIPITATED BY AMMONIUM SULFIDE**
Sample: 2.0 g
Analysis: Dissolve the *Sample* in 90 mL of water, add 5 mL of ammonium hydroxide, warm the solution, and pass hydrogen sulfide through the solution for about 30 min. Dilute with water to 100 mL, mix, and allow the precipitate to settle. Decant the supernatant through a filter, transfer 50 mL of the clear filtrate to a tared dish, evaporate to dryness, and ignite, gently at first and finally at 800 ± 25°.
Acceptance criteria: The weight of the residue does not exceed 5 mg (NMT 0.5%).

SPECIFIC TESTS
- **LOSS ON IGNITION** ⟨733⟩
Analysis: Ignite a sample at 450° to constant weight: it loses 10.0%–13.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

Change to read:

Manganese Sulfate Injection

▲The omission of this monograph has been postponed. As of August 1, 2019, this monograph remains official USP text.▲ (RB 1-Aug-2019)

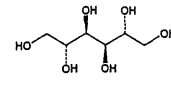
» Manganese Sulfate Injection is a sterile solution of Manganese Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of manganese (Mn).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.
Labeling—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.
Identification—The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 279 nm when tested as directed for *Procedure* in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.45 USP Endotoxin Unit per µg of manganese.
pH ⟨791⟩**:** between 2.0 and 3.5.
Particulate Matter in Injections ⟨788⟩**:** meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Sodium chloride solution, Manganese stock solution, and *Standard preparations*—Prepare as directed in the *Assay* under *Manganese Chloride Injection*.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 1 mg of manganese, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Manganese Chloride Injection*.

Mannitol

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (‣ ◂) to specify this fact.



$C_6H_{14}O_6$ 182.17

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D-Mannitol [69-65-8].

DEFINITION

Mannitol contains NLT 97.0% and NMT 102.0% of mannitol ($C_6H_{14}O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

If the spectra shows differences, proceed as directed.

Standard solution: Dissolve 25 mg of USP Mannitol RS in a glass vial with 0.25 mL of distilled water without heating. The solution is clear. Evaporate to dryness by one of the following methods. Heat in a microwave oven with a power range of 600–700 W for 20 min, or heat in an oven at 100° for 1 h, then gradually apply vacuum until a dry residue is obtained. Non-sticky, white, or slightly yellowish powders are obtained.

Sample solution: Dissolve 25 mg of Mannitol in a glass vial with 0.25 mL of distilled water without heating. The solution is clear. Evaporate to dryness by one of the following methods. Heat in a microwave oven with a power range of 600–700 W for 20 min, or heat in an oven at 100° for 1 h, then gradually apply vacuum until a dry residue is obtained. Non-sticky, white, or slightly yellowish powders are obtained.

Analysis: Record new spectra using the residues from the *Standard solution* and the *Sample solution*.

ASSAY

- **PROCEDURE**

Mobile phase: Degassed water

System suitability solution A: 25.0 mg/mL each of sorbitol and USP Mannitol RS

System suitability solution B: 1.0 mg/mL each of maltitol and isomalt

Standard solution A: 50.0 mg/mL of USP Mannitol RS

Standard solution B: Dilute 2.0 mL of the *Sample solution* with water to 100.0 mL.

Standard solution C: Dilute 0.5 mL of *Standard solution B* with water to 20.0 mL.

Sample solution: 50.0 mg/mL of Mannitol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L19

Temperatures

Detector: 40° (maintain at a constant temperature)

Column: 85 ± 2°

Flow rate: 0.5 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of the mannitol peak. [NOTE—The retention time for mannitol is about 20 min.]

System suitability

Samples: *System suitability solution A*, *System suitability solution B*, *Standard solution B*, and *Standard solution C*

Suitability requirements

Resolution: NLT 2.0 between sorbitol and mannitol, *System suitability solution A*

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of mannitol ($C_6H_{14}O_6$) in the portion of Mannitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from *Standard solution A*

C_S = concentration of USP Mannitol RS in *Standard solution A* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **RELATED SUBSTANCES**

Mobile phase, System suitability solution A, System suitability solution B, Standard solution B, Standard solution C, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution B*, *Standard solution C*, and *Sample solution*

Acceptance criteria: See *Table 1* for the relative retention times.

Table 1

Name	Relative Retention Time
Isomalt (1st peak)	0.60
Maltitol	0.69
Isomalt (2nd peak)	0.73
Mannitol	1.0
Sorbitol	1.2

[NOTE—Impurity A—Sorbitol; Impurity B—Maltitol; Impurity C—Isomalt.]

[NOTE—Isomalt elutes in two peaks.]

[NOTE—Coelution of impurity B and the second peak of impurity C may be observed.]

Disregard limit: NMT 0.05%; any peak NMT the area of the principal peak obtained with *Standard solution C*

Sorbitol: NMT 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

Sum of isomalt and maltitol: NMT 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

Unspecified impurities: NMT 0.10% for each impurity; NMT twice the area of the principal peak obtained with *Standard solution C*

Total impurities: NMT 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

- **REDUCING SUGARS**

Ferric sulfate solution: Dissolve 50 g of ferric sulfate in an excess of water, add 200 mL of sulfuric acid, and dilute with water to 1000 mL.

Copper sulfate solution: 69.2 mg/mL of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water

Sodium potassium tartrate solution: Dissolve 173 g of sodium potassium tartrate ($C_4H_4KNaO_6 \cdot 4H_2O$) and 50 g of sodium hydroxide in 400 mL of water. Heat to boiling, allow to cool, and dilute with carbon dioxide-free water to 500 mL.

Cupri-tartaric solution: Mix equal volumes of *Copper sulfate solution* and *Sodium potassium tartrate solution* immediately before use.

Sample: 7.0 g

Analysis: To the *Sample* add 13 mL of water. Boil gently with 40 mL of *Cupri-tartaric solution* for 3 min, and allow to stand for about 2 min. A precipitate is formed. Pass through a sintered-glass filter (10–16 µm) coated with diatomaceous earth or a sintered-glass filter (5–10 µm). Wash the precipitate with hot water (at about 50°–60°) until the

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washing is no longer alkaline, and pass the washings through the filter described above. Discard all the filtrate at this step. Immediately dissolve the precipitate in 20 mL of *Ferric sulfate solution*, pass through the filter described above in a clean flask, and wash the filter with 15–20 mL of water. Combine the washings and the filtrate, heat to 80°, and titrate with 0.02 M potassium permanganate VS.

Acceptance criteria: NMT 0.1%, expressed as glucose; NMT 3.2 mL of 0.02 M potassium permanganate VS is required to change the color of the solution. The green color turns to pink, and the color persists at least 10 s.

• **NICKEL**

Sample solution: Suspend 10.0 g of Mannitol in 30 mL of dilute acetic acid [115–125 g/L of acetic acid ($C_2H_4O_2$)], add water, and shake to dissolve. Dilute with water to 100.0 mL. Add 2.0 mL of a saturated solution of ammonium pyrrolidinedithiocarbamate ($C_5H_{12}N_2S_2$) (about 10 g/L) and 10.0 mL of water-saturated methyl isobutyl ketone ($C_6H_{12}O$, 4-methyl-2-pentanone), and then shake for 30 s protected from bright light. Allow the layers to separate, and use the methyl isobutyl ketone layer.

Blank solution: Treat water-saturated methyl isobutyl ketone as described for preparation of the *Sample solution*, omitting the mannitol.

Standard solutions: Prepare three reference solutions in the same manner as the *Sample solution* but adding 0.5, 1.0, and 1.5 mL, respectively, of nickel standard solution TS [10 ppm nickel (Ni)] in addition to the 10.0 g of the substance to be examined.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 232.0 nm

Lamp: Nickel hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Sample solution* and *Standard solutions*

Set the zero of the instrument using the blank. Record the average of the steady readings for each of the *Standard solutions* and the *Sample solution*. Between each measurement rinse with water, and ascertain that the reading returns to zero with the blank. Plot the absorbances of the *Standard solutions* and the *Sample solution* versus the added quantity of nickel. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of nickel in the *Sample solution*.

Acceptance criteria: NMT 1 µg/g

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩, *Class I*

Melting point: 165°–170°

• **APPEARANCE OF SOLUTION**

Hydrazine sulfate solution: 10.0 mg/mL of hydrazine sulfate. Allow to stand for 4–6 h.

Methenamine solution: 2.5 g of methenamine in 25 mL of water, in a ground-glass-stoppered flask

Primary opalescent suspension: To the *Methenamine solution*, add 25.0 mL of the *Hydrazine sulfate solution*. Mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opalescence standard: Dilute 15.0 mL of the *Primary opalescent suspension* with water to 1000.0 mL. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspension: To 5.0 mL of *Opalescence standard* add 95.0 mL of water. Mix, and shake before use.

Standard solution: Pipet 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, and 2.4 mL of cupric sulfate CS into a 1-L volumetric flask. Dilute with 1% (w/v) hydrochloric acid to volume.

Sample solution: 100.0 mg/mL of Mannitol

Analysis: Compare the color, clarity, and opalescence of equal volumes of the *Reference suspension*, *Standard solution*, and *Sample solution*.

Acceptance criteria: The *Sample solution* is clear and colorless; its clarity is the same as that of water, or its opalescence is not more pronounced than that of the *Reference suspension*, and it is not more intensely colored than the *Standard solution*.

• **LOSS ON DRYING** ⟨731⟩

Sample: 1.000 g

Analysis: Dry the *Sample* at 105° for 4 h.

Acceptance criteria: NMT 0.5%

• **CONDUCTIVITY**

Sample: 20.0 g

Analysis: Dissolve the *Sample* in carbon dioxide-free water prepared from distilled water by heating to 40°–50°, and dilute with the same solvent to 100 mL. After cooling, measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Acceptance criteria: NMT 20 µS/cm at 25°

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count (TAMC) is NMT 10^3 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g. It meets the requirements of the test for absence of *Escherichia coli*.

If intended for use in the manufacture of parenteral dosage forms, the TAMC is NMT 10^2 cfu/g.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins, less than 4 IU/g for parenteral dosage forms with a concentration of 100 g/L or less of mannitol, and less than 2.5 IU/g for parenteral dosage forms with a concentration of more than 100 g/L of mannitol

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING**

The label states, where applicable, the maximum concentration of bacterial endotoxins.

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Mannitol RS

Mannitol Injection

» Mannitol Injection is a sterile solution, which may be supersaturated, of Mannitol in Water for Injection. It may require warming or autoclaving before use if crystallization has occurred. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of mannitol ($C_6H_{14}O_6$). It contains no antimicrobial agents.

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Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL. The label also states that it should be warmed before use to dissolve any crystals that may have formed.
USP Reference standards ⟨11⟩—
USP Mannitol RS

Identification—
A: Evaporate a portion of Injection on a steam bath to dryness, and dry the residue at 105° for 4 hours. To 3 mL of freshly prepared solution of catechol in water (1 in 10) add 6 mL of sulfuric acid with cooling. Place 3 mL of this solution in each of two separate test tubes. To one tube add 0.3 mL of water (reagent blank) and to the other add 0.3 mL of a solution of it in water (1 in 10). Heat the tubes over an open flame for about 30 seconds: the solution in the tube containing mannitol is dark pink or wine red, and the solution in the tube containing the reagent blank is light pink.
B: The retention time for the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Specific rotation ⟨781⟩—+137° to +145°. Transfer an accurately measured volume of Injection, equivalent to about 1 g of mannitol as determined by the *Assay,* to a 100-mL volumetric flask. Add 40 mL of a 1-in-10 ammonium molybdate solution, previously filtered if necessary. Add 20 mL of 1 N sulfuric acid, and dilute with water to volume.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.04 USP Endotoxin Unit per mg of mannitol where the labeled amount of mannitol in the Injection is 10% or less, and not more than 2.5 USP Endotoxin Units per g of mannitol where the labeled amount of mannitol in the Injection is greater than 10%.
pH ⟨791⟩: between 4.5 and 7.0, determined potentiometrically, on a portion to which 0.30 mL of saturated potassium chloride solution has been added for each 100 mL, and which previously has been diluted with water, if necessary, to a concentration of not more than 5% of mannitol.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Use degassed water.
Resolution solution—Dissolve sorbitol and USP Mannitol RS in water to obtain a solution having concentrations of about 4.8 mg per mL of each.
Chromatographic system (see Chromatography ⟨621⟩*)*—The liquid chromatograph is equipped with a refractive index detector that is maintained at a constant temperature and a 4-mm × 25-cm column that contains packing L19. The column temperature is maintained at a temperature between 30° and 85° controlled within ±2° of the selected temperature, and the flow rate is about 0.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%. In a similar manner, chromatograph the *Resolution solution*: the resolution, *R,* between the sorbitol and mannitol peaks is not less than 2.0.
Standard preparation—Dissolve an accurately weighed quantity of USP Mannitol RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 5 mg per mL.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of mannitol, to a 100-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Separately inject equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of mannitol ($C_6H_{14}O_6$) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken; and the other terms are as defined therein.

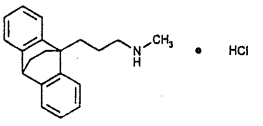
Mannitol in Sodium Chloride Injection

» Mannitol in Sodium Chloride Injection is a sterile solution of Mannitol and Sodium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of $C_6H_{14}O_6$ and NaCl. It contains no antimicrobial agents.

Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
USP Reference standards ⟨11⟩—
USP Mannitol RS

Identification—
A: Evaporate a portion of Injection on a steam bath to dryness, and dry the residue at 105° for 4 hours: the residue responds to the *Identification* test *A* under *Mannitol Injection.*
B: It responds to the tests for *Sodium* ⟨191⟩ and for *Chloride* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.04 USP Endotoxin Unit per mg of mannitol.
pH ⟨791⟩: between 4.5 and 7.0.
Other requirements—It meets the requirements for *Packaging and storage* under *Mannitol Injection.* It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay for mannitol—Proceed with Injection as directed in the *Assay* under *Mannitol Injection.*
Assay for sodium chloride—Proceed with Injection as directed in the *Assay* under *Sodium Chloride Injection.*

Maprotiline Hydrochloride



$C_{20}H_{23}N \cdot HCl$ 313.86

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9,10-Ethanoanthracene-9(10*H*)-propanamine, *N*-methyl-, hydrochloride;
N-Methyl-9,10-ethanoanthracene-9(10*H*)-propylamine hydrochloride [10347-81-6].

DEFINITION

Maprotiline Hydrochloride contains NLT 98.0% and NMT 102.0% of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*

Sample solution: 5 mg/mL
Acceptance criteria: Responds to test *A* and test *B*

ASSAY

- **PROCEDURE**

Ammonia solution: 70-g/L ammonium hydroxide solution
Mobile phase: Dissolve 0.58 g of ammonium acetate in 200 mL of water, and add 2 mL of *Ammonia solution*, 150 mL of 2-propanol, and 650 mL of methanol. Adjust with drops of glacial acetic acid to a pH of 8.1.
System suitability solution: 1 mg/mL of USP Maprotiline Hydrochloride RS and 0.1 mg/mL of USP Maprotiline Related Compound D RS in *Mobile phase*
Standard solution: 1.0 mg/mL of USP Maprotiline Hydrochloride RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Maprotiline Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 272 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Autosampler temperature: 4°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of maprotiline
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for maprotiline related compound D and maprotiline are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: 1.8–3.2 between the maprotiline related compound D and maprotiline peaks, *System suitability solution*. [NOTE—If necessary, adjust the pH of *Mobile phase*, in increments of 0.1 pH unit, by adding a 50% (v/v) solution of acetic acid if the *Resolution* is less than 1.8, or by adding the *Ammonia solution* if the *Resolution* is greater than 3.2.]
Tailing factor: 0.9–2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$) in the portion of Maprotiline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Maprotiline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Maprotiline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES, PROCEDURE 1**

Mobile phase: Dissolve 0.6 g of ammonium acetate in 200 mL of water, and add 2 mL of a 70-g/L ammonia solution, 150 mL of 2-propanol, and 650 mL of methanol. Adjust with diluted glacial acetic acid to a pH of 8.3.
System suitability solution: 1 mg/mL of USP Maprotiline Hydrochloride RS and 0.1 mg/mL of USP Maprotiline Related Compound D RS in *Mobile phase*
Standard stock solution: 0.2 mg/mL of USP Maprotiline Hydrochloride RS in methanol
Standard solution: 0.002 mg/mL of USP Maprotiline Hydrochloride RS in *Mobile phase*, from *Standard stock solution*
Sample solution: 1 mg/mL of Maprotiline Hydrochloride in *Mobile phase*. [NOTE—A small quantity of methanol, NMT 10% of the final volume, can be used to aid the dissolution.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 272 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Autosampler temperature: 4°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of maprotiline
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: 1.8–3.2 between the maprotiline related compound D and maprotiline peaks, *System suitability solution*. [NOTE—If necessary, adjust the pH of the *Mobile phase*, in increments of 0.1 pH unit, by adding a 50% (v/v) solution of acetic acid if the *Resolution* is less than 1.8, or by adding a 70-g/L ammonia solution if the *Resolution* is greater than 3.2.]
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Maprotiline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of any impurity from the *Sample solution*
r_S = peak area of maprotiline from the *Standard solution*
C_S = concentration of USP Maprotiline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Maprotiline Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 1*)

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Acceptance criteria: See *Table 1*. Disregard any peak representing less than 0.05% of the area of the main peak.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maprotiline acrylaldehyde analog[a]	0.3	—	0.2[b]
Maprotiline dimer[c]	0.5	1.16	0.2
Desmethylmaprotiline[d]	0.7	1.15	0.2
Maprotiline related compound D	0.9	1.0	0.2
Maprotiline	1.0	—	—
N-Methylmaprotiline[e]	1.2	1.0	0.2
Any individual unknown impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)acrylaldehyde.
[b] Determined in the test for *Organic Impurities, Procedure 2*.
[c] *N*-Methyl-*N*,*N*-bis[3-(9,10-dihydro-9,10-ethanoanthracen-9-yl)propyl]amine.
[d] 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)propan-1-amine.
[e] 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-*N*,*N*-dimethylpropan-1-amine.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Ammonium bicarbonate solution: 3.95 g/L of ammonium bicarbonate solution. Adjust with 2 M ammonium hydroxide to a pH of 8.1.

Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Ammonium bicarbonate solution* (80: 32: 4.5: 20)

Standard stock solution: 0.2 mg/mL of USP Maprotiline Hydrochloride RS in methanol

Standard solution: 0.002 mg/mL of USP Maprotiline Hydrochloride RS in *Mobile phase* from *Standard stock solution*

Sample solution: 1.0 mg/mL of Maprotiline Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Temperatures

Autosampler: 4°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of maprotiline

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for maprotiline acrylaldehyde analog and maprotiline are 0.27 and 1.0, respectively.]

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of maprotiline acrylaldehyde analog in the portion of Maprotiline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of maprotiline acrylaldehyde analog from the *Sample solution*

r_S = peak response of maprotiline from the *Standard solution*

C_S = concentration of USP Maprotiline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Maprotiline Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor of maprotiline acrylaldehyde analog, 2.2

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Sample: Dry under vacuum at 80° to constant weight.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Maprotiline Hydrochloride RS

USP Maprotiline Related Compound D RS

3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-*N*-methylprop-2-en-1-amine hydrochloride.

$C_{20}H_{21}N \cdot HCl$ 311.85

Maprotiline Hydrochloride Tablets

DEFINITION

Maprotiline Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$).

IDENTIFICATION

Delete the following:

▲• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 20 mg/mL of USP Maprotiline Hydrochloride RS in methanol

Sample solution: Transfer a portion of powdered Tablets, equivalent to 100 mg of maprotiline hydrochloride, to a glass-stoppered centrifuge tube. Add 5.0 mL of methanol to the tube, sonicate for 10 min, shake by mechanical means for 10 min, and centrifuge.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel that has been prewashed with chloroform by allowing chloroform to travel the full length of the plate, and dried at 100° for 30 min

Application volume: 5 µL

Developing solvent system: Secondary butyl alcohol, ethyl acetate, and 2 N ammonium hydroxide (6:3:1)

Analysis

Samples: *Standard solution* and *Sample solution*

In a suitable chromatographic chamber, place a volume of the *Developing solvent system* sufficient to develop a chromatogram. Place a beaker containing 25 mL of

USP Monographs

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ammonium hydroxide in the bottom of the chamber, and allow it to equilibrate for 1 h. Apply *Samples* and allow the spots to dry. Develop the chromatograms until the solvent front has moved three-fourths of the length of the plate, remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to hydrogen chloride vapor for 30 min, and expose it to a high-intensity UV light irradiator (1000 to 1600 watts) for 5 min. [**CAUTION**—UV irradiators emit UV radiation that is harmful to eyes and skin.] Compare the chromatograms under long-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ▲2S (USP41)

Add the following:

▲• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲2S (USP41)

• **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

▲Ammonia solution: 70-g/L ammonium hydroxide solution

Mobile phase: Dissolve 0.6 g of ammonium acetate in 200 mL of water, and then add 2 mL of *Ammonia solution*, 150 mL of 2-propanol, and 650 mL of methanol.

System suitability solution: 1.0 mg/mL of USP Maprotiline Hydrochloride RS and 0.1 mg/mL of USP Maprotiline Related Compound D RS in *Mobile phase*

Standard solution: 1.0 mg/mL of USP Maprotiline Hydrochloride RS in *Mobile phase*

Sample stock solution: Nominally 2.0 mg/mL of maprotiline hydrochloride in *Mobile phase* prepared as follows. Transfer NLT 4 Tablets into a suitable volumetric flask and add 80% of the flask volume of *Mobile phase*. Shake for 10 min and then sonicate with occasional shaking until the Tablets disintegrate. Cool and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size. Discard the first 2 mL of the filtrate.

Sample solution: Nominally 1.0 mg/mL of maprotiline hydrochloride in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 272 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L3

Autosampler temperature: 4°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2.5 times the retention time of maprotiline

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for maprotiline related compound D and maprotiline are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.8 between the maprotiline related compound D and maprotiline peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Maprotiline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of maprotiline hydrochloride in the *Sample solution* (mg/mL) ▲2S (USP41)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Medium: Dilute hydrochloric acid (7 in 1000); 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: USP Maprotiline Hydrochloride RS in *Medium*

Sample solution: ▲Pass portions of the solution under test through a suitable filter and dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*. ▲2S (USP41)

Instrumental conditions

Mode: UV

Analytical wavelengths: Minimum absorbance at about 268 nm; maximum absorbance at about 272 nm

Analysis

Samples: *Standard solution* and *Sample solution*

▲Calculate the percentage of the labeled amount of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Maprotiline Hydrochloride RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*, if needed

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet) ▲2S (USP41)

Tolerances: NLT 75% (Q) of the labeled amount of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

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IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Ammonia solution, Mobile phase, System suitability solution, Sample stock solution, and **Sample solution:** Prepare as directed in the *Assay*.

Standard solution: 0.002 mg/mL of USP Maprotiline Hydrochloride RS in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.

Detector: UV 272 nm

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for maprotiline related compound D and maprotiline are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.8 between the maprotiline related compound D and maprotiline peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*

r_S = peak response of maprotiline hydrochloride from the *Standard solution*

C_S = concentration of USP Maprotiline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of maprotiline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria, NMT (%)
Any individual degradation product	0.2
Total degradation products	3.0▲ 2S (USP41)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. ▲Protect from light. Store at controlled room temperature.▲ 2S (USP41)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Maprotiline Hydrochloride RS

▲USP Maprotiline Related Compound D RS

3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-*N*-methylprop-2-en-1-amine hydrochloride.

$C_{20}H_{21}N \cdot HCl$ 311.85▲ 2S (USP41)

Marbofloxacin Compounded Oral Suspension, Veterinary

DEFINITION

Marbofloxacin Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of marbofloxacin ($C_{17}H_{19}FN_4O_4$).

Prepare Marbofloxacin Compounded Oral Suspension, Veterinary 25 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Marbofloxacin powder	2.5 g
Purified Water	A small amount
Vehicle: 1:1 mixture of Ora-Plus[a] and Ora-Sweet[a]; or Ora-Blend[a], a sufficient quantity to make	100 mL

[a] Perrigo Pharmaceuticals, Allegan, MI.

Wet the *Marbofloxacin powder* with a small amount of *Purified Water* and triturate to make a smooth paste. Add the *Vehicle* to make the mortar contents pourable. Transfer the contents of the mortar stepwise and quantitatively to a calibrated container using the *Vehicle*. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Solution A: 10 mM ammonium formate containing 0.1% (v/v) formic acid

Solution B: Methanol containing 0.1% formic acid

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
0.5	90	10
23.0	70	30
23.5	70	30
23.6	0	100
26	0	100
26.1	90	10
30	90	10

Standard solution: 0.4 mg/mL of marbofloxacin in *Solution A*

Sample solution: Transfer 1.6 mL of Oral Suspension, Veterinary to a 100-mL volumetric flask, dilute with *Solution A* to volume, and mix well. Pass through a PVDF filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV-Vis 327 nm

Columns

Guard: Packing L1

Analytical: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

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[NOTE—The retention time for marbofloxacin is about 6.2 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of marbofloxacin ($C_{17}H_{19}FN_4O_4$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of marbofloxacin from the *Sample solution*

r_S = peak response of marbofloxacin from the *Standard solution*

C_S = concentration of marbofloxacin in the *Standard solution* (mg/mL)

C_U = nominal concentration of marbofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

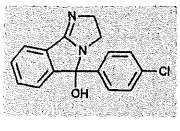
- **pH** ⟨791⟩: 6.2–7.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant, plastic containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator (2°–8°) or at controlled room temperature. [NOTE—A slight darkening in yellow color may occur in the suspension with 1:1 Ora-Plus and Ora-Sweet that does not affect the strength of the preparation.]
- **LABELING:** Label it to indicate that it is for veterinary use only. Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

Delete the following:

▲Mazindol



$C_{16}H_{13}ClN_2O$ 284.74

3*H*-Imidazo[2,1-a]isoindol-5-ol, 5-(4-chlorophenyl)-2,5-dihydro-, (±)-.

(±)-5-(*p*-Chlorophenyl)-2,5-dihydro-3*H*-imidazo[2,1-a]isoindol-5-ol [22232-71-9].

» Mazindol contains not less than 98.0 percent and not more than 102.0 percent of $C_{16}H_{13}ClN_2O$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨ 11 ⟩ —

USP Mazindol RS

Clarity and color of solution—A 1 in 100 solution of Mazindol in a mixture of chloroform and methanol (9:1) is clear and not darker in color than a solution prepared by mixing equal volumes of *Matching Fluid C* (see *Color and Achromicity* ⟨631⟩) and water.

Identification—

A: Infrared Absorption ⟨197K⟩.

B: Ultraviolet Absorption ⟨197U⟩—

Solution: 10 µg per mL.

Medium: 0.6 N hydrochloric acid.

Absorptivities at 272 nm, calculated on the dried basis, do not differ by more than 3.0%.

Loss on drying ⟨ 731 ⟩ —Dry it in vacuum at 60° for 2 hours: it loses not more than 0.5% of its weight.

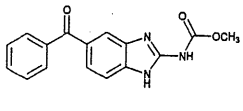
Residue on ignition ⟨ 281 ⟩ : not more than 0.1%.

Sulfate ⟨ 221 ⟩ —Triturate a 500-mg portion with 10 mL of water in a mortar. Filter the suspension through a water-washed filter, and rinse the mortar and filter with 30 mL of water, collecting the combined filtrate and washings in a 50-mL color-comparison tube. The filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).

Chromatographic purity—Dissolve 10 mg in 2.0 mL of a mixture of chloroform and methanol (9:1) to obtain the test solution. Dissolve a suitable quantity of USP Mazindol RS in a mixture of chloroform and methanol (9:1) to obtain a Standard solution having a concentration of 5.0 mg per mL. Dilute portions of this solution quantitatively and stepwise with the mixture of chloroform and methanol (9:1) to obtain a series of diluted standard solutions having concentrations of 0.100, 0.050, 0.025, and 0.0125 mg per mL, respectively. Separately apply a 20-µL portion of the test solution and 20-µL portions of the Standard solution and each diluted standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, alcohol, and ammonium hydroxide (80:20:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the chromatograms show principal spots at about the same R_F value. Estimate the concentration of any secondary spots present in the chromatogram from the test solution by comparison with the diluted standard solutions: the principal spots from the 0.100, 0.050, 0.025, and 0.0125 mg per mL dilutions are equivalent to 2.0%, 1.0%, 0.50%, and 0.25% of impurities, respectively. No individual impurity is greater than 1.0%, and the sum of the impurities is not greater than 2.0%.

Assay—Transfer about 230 mg of Mazindol, accurately weighed, to a suitable flask, dissolve in 40 mL of glacial acetic acid, add 3 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to an emerald-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 28.47 mg of $C_{16}H_{13}ClN_2O$. ▲ (USP 1-May-2020)

Mebendazole



$C_{16}H_{13}N_3O_3$ 295.29

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Carbamic acid, (5-benzoyl-1*H*-benzimidazol-2-yl), methyl ester;
Methyl 5-benzoyl-2-benzimidazolecarbamate [31431-39-7].

DEFINITION

Mebendazole contains NLT 98.0% and NMT 102.0% of mebendazole ($C_{16}H_{13}N_3O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Solution A: 7.5 g/L of ammonium acetate
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
15	70	30
20	10	90
25	10	90
26	80	20
30	80	20

Diluent: Acetonitrile and water (50:50)
System suitability solution: 50 µg/mL of USP Mebendazole RS and 2.5 µg/mL of USP Mebendazole Related Compound D RS in *Diluent*. Sonicate in *Diluent* using 80% of the final volume at 45°–50° for 10 min. Dilute with *Diluent* to final volume.
Standard solution: 0.05 mg/mL of USP Mebendazole RS in *Diluent*. Sonicate in *Diluent* using 80% of the final volume at 45°–50° for 10 min. Dilute with *Diluent* to final volume.
Sample solution: 0.05 mg/mL of Mebendazole in *Diluent*. Sonicate in *Diluent* using 80% of the final volume at 45°–50° for 10 min. Dilute with *Diluent* to final volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times of mebendazole and mebendazole related compound D are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 5.0 between mebendazole and mebendazole related compound D, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mebendazole ($C_{16}H_{13}N_3O_3$) in the portion of Mebendazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mebendazole from the *Sample solution*
r_S = peak response of mebendazole from the *Standard solution*
C_S = concentration of USP Mebendazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Mebendazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay*.
Standard solution: 2.5 µg/mL of USP Mebendazole RS in *Diluent*
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 5.0 between the mebendazole and mebendazole related compound D peaks
Relative standard deviation: NMT 1.0% for the mebendazole peak and NMT 5.0% for the mebendazole related compound D peak
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Mebendazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of mebendazole from the *Standard solution*
C_S = concentration of USP Mebendazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Mebendazole in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Amino mebendazole[a]	0.46	1.0	0.25
2-Hydroxy mebendazole[b]	0.53	1.0	0.25
2-Amino-1-methyl mebendazole[c]	0.67	1.0	0.25
Mebendazole	1.0	—	—
Mebendazole related compound D	1.1	1.0	0.25
Ethyl mebendazole[d]	1.3	1.0	0.25

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Toluoyl mebendazole[e]	1.4	1.0	0.25
Mebendazole dimer[f]	1.6	0.71	0.5
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 2-Amino-5-benzoylbenzimidazole.
[b] 5-Benzoyl-2-hydroxybenzimidazole.
[c] 2-Amino-5-benzoyl-1-methylbenzimidazole.
[d] Ethyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate.
[e] Methyl 5-(4-toluoyl)-1*H*-benzimidazol-2-ylcarbamate.
[f] 1,3-Bis(5-benzoylbenzimidazol-2-yl)urea.

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Mebendazole RS
USP Mebendazole Related Compound D RS
Methyl 5-benzoyl-1-methylbenzimidazol-2-ylcarbamate.
$C_{17}H_{15}N_3O_3$ 309.32

Mebendazole Oral Suspension

» Mebendazole Oral Suspension is Mebendazole in an aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mebendazole ($C_{16}H_{13}N_3O_3$).

Packaging and storage—Preserve in tight containers at controlled room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Mebendazole RS
Identification—Mix a quantity of Oral Suspension, equivalent to about 200 mg of mebendazole, with 20 mL of a mixture of chloroform and 96 percent formic acid (19:1). Proceed as directed for *Identification* under *Mebendazole Tablets*, beginning with "Warm the suspension on a water bath for a few minutes." The specified result is obtained.
pH ⟨791⟩: between 6.0 and 7.0.

Assay—

Standard preparation—Transfer about 10 mg of USP Mebendazole RS, accurately weighed, to a 100-mL volumetric flask, and add 90 mL of chloroform, 7 mL of isopropyl alcohol, and 2 mL of 96 percent formic acid. Agitate until the solid has dissolved, add isopropyl alcohol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix to obtain a solution having a known concentration of about 5 µg per mL.

Assay preparation 1—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 1000 mg of mebendazole, to a 100-mL volumetric flask, dilute with 96 percent formic acid to volume, and mix. Transfer 10.0 mL of this mixture to a second 100-mL volumetric flask, add 40 mL of 96 percent formic acid, and heat in a water bath at a temperature of 50° for 15 minutes. Cool, add water to volume, mix, and pass through a medium-porosity, sintered-glass filter. Transfer 10.0 mL of the filtrate to a 250-mL separator, and add 50 mL of water and 50 mL of chloroform. Shake for about 2 minutes, allow the phases to separate, and transfer the chloroform layer to a second 250-mL separator. Wash the aqueous layer with two 10-mL portions of chloroform, add the chloroform washings to the second separator, and discard the aqueous layer. Wash the combined chloroform solutions with a mixture of 4 mL of 1 N hydrochloric acid and 50 mL of a 1 in 10 solution of 96 percent formic acid in water, and transfer the chloroform layer to a 100-mL volumetric flask. Extract the aqueous washing with two 10-mL portions of chloroform, add these chloroform extracts to the chloroform solution in the volumetric flask, add 2 mL of 96 percent formic acid and 7 mL of isopropyl alcohol, dilute with chloroform to volume, and mix. Transfer 5.0 mL of this solution to another 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix.

Assay preparation 2 (where the Oral Suspension is packaged in syringes calibrated to deliver stated increments of mebendazole)—Express an increment of Oral Suspension to a volumetric flask of an appropriate nominal volume so that when diluted with 96 percent formic acid to volume a mixture containing about 10 mg of mebendazole per mL is obtained. Transfer 10.0 mL of this mixture to a 100-mL volumetric flask, add 40 mL of 96 percent formic acid, and heat in a water bath at a temperature of 50° for 15 minutes. Proceed as directed for *Assay preparation 1* beginning with "Cool, add water to volume."

Procedure—Mix 90 mL of chloroform with 2 mL of 96 percent formic acid in a 100-mL volumetric flask, add isopropyl alcohol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix to obtain a reagent blank. Concomitantly determine the absorbances of the relevant *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 247 nm with a spectrophotometer, using the reagent blank to set the instrument. Calculate the quantity, in mg, of mebendazole ($C_{16}H_{13}N_3O_3$) in the portion of Oral Suspension taken to prepare *Assay preparation 1* by the formula:

$$200C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Mebendazole RS in the *Standard preparation*; and A_U and A_S are the absorbances of *Assay preparation 1* and the *Standard preparation*, respectively. Where appropriate, calculate the quantity, in mg, of mebendazole ($C_{16}H_{13}N_3O_3$) in the increment of Oral Suspension taken to prepare *Assay preparation 2* by the formula:

$$20{,}000(C/V)(A_U/A_S)$$

in which V is the volume, in mL, of the volumetric flask into which the increment of Oral Suspension was expressed; A_U is the absorbance of *Assay preparation 2;* and the other terms are as defined above.

Mebendazole Tablets

DEFINITION

Mebendazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mebendazole ($C_{16}H_{13}N_3O_3$).

USP Monographs

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IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 7.5 g/L of ammonium acetate in *water*
Solution B: *Acetonitrile*
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
15	70	30
20	10	90
25	10	90
26	80	20
30	80	20

Diluent: *N,N*-Dimethyl formamide
Standard solution: 0.05 mg/mL of USP Mebendazole RS in *Diluent*. Sonicate, if necessary, to dissolve.
Sample solution: Nominally 0.05 mg/mL of mebendazole in *Diluent*, prepared as follows. Transfer a suitable quantity of mebendazole from NLT 20 finely powdered Tablets into a suitable volumetric flask. Add *Diluent* to about 80% of the flask volume and sonicate for 30 min. Dilute with *Diluent* to volume and pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mebendazole ($C_{16}H_{13}N_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mebendazole from the *Sample solution*
r_S = peak response of mebendazole from the *Standard solution*
C_S = concentration of USP Mebendazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mebendazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid containing 1.0% sodium lauryl sulfate; 900 mL
Apparatus 2: 75 rpm
Time: 120 min
Solution A: Dissolve 8.0 g of sodium hydroxide in 2 L of *water*. Add 3.0 g of sodium lauryl sulfate, and mix. Add 20 mL of *phosphoric acid*, and adjust with *phosphoric acid* to a pH of 2.5.
Mobile phase: *Acetonitrile* and *Solution A* (3:7)
Standard solution: 0.5 mg/mL of USP Mebendazole RS prepared as follows. Transfer the appropriate amount of USP Mebendazole RS to a volumetric flask. Add 20% of the final volume of formic acid, and dissolve. Dilute with *methanol* to volume. Dilute a portion of this solution with *Medium* to obtain a solution having a known concentration similar to the expected concentration in the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 3-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis: Determine the percentage of mebendazole ($C_{16}H_{13}N_3O_3$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of mebendazole ($C_{16}H_{13}N_3O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.1 mg/mL of USP Mebendazole RS and 0.002 mg/mL of USP Mebendazole Related Compound D RS in *Diluent*. Sonicate, if necessary, to dissolve.
Standard solution: 0.002 mg/mL of USP Mebendazole RS in *Diluent*. Sonicate, if necessary, to dissolve.
Sample solution: Nominally 1 mg/mL of mebendazole in *Diluent*, prepared as follows. Transfer a suitable quantity of mebendazole from NLT 20 powdered Tablets into a suitable volumetric flask. Add *Diluent* to about 40% of the flask volume and sonicate for about 30 min. Dilute with *Diluent* to volume and pass through a suitable filter of 0.45-µm pore size.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between mebendazole and mebendazole related compound D, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

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r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of mebendazole from the *Standard solution*
C_S = concentration of USP Mebendazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mebendazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

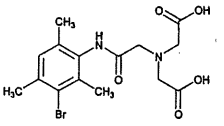
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mebendazole	1.0	—
Mebendazole related compound D[a]	1.1	—
Any individual unspecified impurity	—	0.2
Total impurities	—	1.0

[a] This is a process-related impurity and not included in the total impurities.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mebendazole RS
  USP Mebendazole Related Compound D RS
  Methyl 5-benzoyl-1-methylbenzimidazol-2-yl carbamate.
  $C_{17}H_{15}N_3O_3$ 309.32

Mebrofenin



$C_{15}H_{19}BrN_2O_5$ 387.23
Glycine, *N*-[2-[(3-bromo-2,4,6,-trimethylphenyl)amino]-2-oxoethyl]-*N*-(carboxymethyl)-;
[[[(3-Bromomesityl)carbamoyl]methyl]imino]diacetic acid [78266-06-5].

DEFINITION
Mebrofenin contains NLT 97.0% and NMT 101.0% of mebrofenin ($C_{15}H_{19}BrN_2O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**

Sample solution: Dissolve 100 mg of Mebrofenin in 40 mL of dimethylformamide in a conical flask with the aid of sonication if necessary. Add 3 drops of thymol blue TS.
Analysis: Titrate the *Sample solution* with 0.1 N sodium methoxide (in toluene) VS to a blue endpoint while flushing the flask with a gentle stream of nitrogen. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 19.36 mg of mebrofenin ($C_{15}H_{19}BrN_2O_5$).
Acceptance criteria: 97.0%–101.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF NITRILOTRIACETIC ACID**

Mobile phase: Add 10 mL of a solution (1 in 4) of tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 M phosphoric acid to a pH of 7.5 ± 0.1. Transfer this solution to a 1000-mL volumetric flask, add 90 mL of methanol, and dilute with water to volume.
Diluent: 10 mg/mL of cupric nitrate solution
Standard stock solution: 0.5 mg/mL of nitrilotriacetic acid in dilute ammonium hydroxide (1 in 20)
Standard solution: 0.05 mg/mL of nitrilotriacetic acid from a suitable volume of *Standard stock solution* in *Diluent*. [NOTE—Prepare fresh on the day of use.]
Sample solution: 10 mg/mL of Mebrofenin in *Diluent*. Sonicate, if necessary, to dissolve. [NOTE—Prepare fresh on the day of use.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—Peaks containing copper may be present.]
Suitability requirements
Resolution: NLT 1.7 between the major peak and any other peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nitrilotriacetic acid in the portion of Mebrofenin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of copper nitrilotriacetic acid from the *Sample solution*
r_S = peak response of copper nitrilotriacetic acid from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer: Prepare a mixture of equal volumes of 0.025 M monobasic potassium phosphate and 0.025 M dibasic sodium phosphate.
Mobile phase: Mix 400 mL of *Buffer* with 600 mL of methanol in a 1-L volumetric flask. Dilute with water to volume. Adjust with 1 N hydrochloric acid to a pH of 5.0 ± 0.1.
Sample solution: 0.5 mg/mL of Mebrofenin in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: Twice the retention time of mebrofenin
System suitability
Sample: *Sample solution*

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Suitability requirements
Capacity factor, *k'*: NLT 1.2
Column efficiency: NLT 200 plates
Tailing factor: NMT 4.0
Relative standard deviation: NMT 2.0% for the mebrofenin peak

Analysis
Sample: *Sample solution*
Calculate the percentage of impurities in the portion of Mebrofenin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

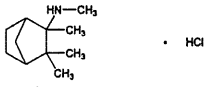
r_U = sum of the peak responses of the individual impurities
r_T = total of all of the peak responses in the chromatogram

Acceptance criteria: NMT 3%

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩**:** 185°–200°, but the range between the beginning and end of melting does not exceed 4°.
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at 100° for 3 h.
  Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mebrofenin RS

Mecamylamine Hydrochloride



$C_{11}H_{21}N \cdot HCl$ 203.75
Bicyclo[2.2.1]heptan-2-amine, *N*,2,3,3-tetramethyl-, hydrochloride.
N,2,3,3-Tetramethyl-2-norbornanamine hydrochloride [826-39-1].

» Mecamylamine Hydrochloride contains not less than 98.0 percent and not more than 100.5 percent of $C_{11}H_{21}N \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Mecamylamine Hydrochloride RS
USP Mecamylamine Related Compound A RS
N,1,7,7-Tetramethyl bicyclo [2.2.1]heptan-2-amine.
$C_{11}H_{21}N$ 167.29

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: It responds to the tests for *Chloride* ⟨191⟩.

Acidity—Dissolve 5.0 g in 100 mL of methanol, and titrate potentiometrically with 0.10 N alcoholic potassium hydroxide to an apparent pH of 5.5, using a calomel-glass electrode system and a potentiometer previously standardized with pH 5.0 neutralized phthalate buffer (see *Solutions* in the section *Reagents, Indicators, and Solutions*): after correction for the volume of alkali consumed by 100 mL of methanol, not more than 0.55 mL of 0.10 N alcoholic potassium hydroxide is required.
Loss on drying ⟨731⟩—Dry it at a pressure not exceeding 5 mm of mercury at 105° for 1 hour: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.5%.

Limit of residual solvents—
Diluent—Prepare a mixture of dimethyl sulfoxide and water (2:1).
Internal standard solution—Prepare a solution of absolute alcohol in *Diluent* having a known concentration of about 15 µL per mL.
Standard stock solution—Transfer 50 mL of the *Diluent* to a 100-mL volumetric flask, add 0.64 mL of isopropyl alcohol, dilute with *Diluent* to volume, and mix.
Standard solution—Pipet 1.0 mL of the *Standard stock solution* into a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix (*Solution 1*). Transfer about 500 mg of sodium chloride, accurately weighed, to a headspace vial, add 1.5 mL of *Solution 1* and 1.5 mL of the *Internal standard solution*, and mix.
Test solution—Transfer about 150 mg of Mecamylamine Hydrochloride, accurately weighed, to a headspace vial, add about 500 mg of sodium chloride, 1.5 mL of *Diluent*, and 1.5 mL of the *Internal standard solution*, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m capillary column whose internal wall is coated with a 1.0-µm film of liquid phase G16. This column is joined with a 0.53-mm × 25-m capillary column whose internal wall is coated with a 5.0-µm film of liquid phase G1. The G16 column is connected to the detector, and the G1 column is connected to the injector. The injection port temperature is maintained at about 100°; the detector temperature is maintained at about 210°; and the column temperature is maintained at 50° for 10 minutes, then increased at a rate of 5° per minute to 110°, then increased at a rate of 30° per minute to 210°, and maintained for 5 minutes at 210°. Nitrogen is used as the carrier gas, flowing at a rate of about 6.5 mL per minute. The split flow is 15 mL per minute.
Procedure—Allow the *Standard solution*, the *Internal standard solution*, and the *Test solution* to stand for 20 minutes at 90°. Separately inject equal volumes (about 1 mL) of the headspace of the *Standard solution*, the *Internal standard solution*, and the *Test solution* into the gas chromatograph, record the chromatograms, and measure the peak responses of the internal standard and isopropyl alcohol. Calculate the quantity, in ppm, of isopropyl alcohol in the portion of Mecamylamine Hydrochloride taken by the formula:

$$150(R_U)(W_S)/(R_S)(W_U)$$

in which W_S is the amount, in ppm, of isopropyl alcohol in the *Standard solution*; W_U is the weight, in mg, of Mecamylamine Hydrochloride taken to prepare the *Test solution*; and R_U and R_S are the peak response ratios of isopropyl alcohol to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively: not more than 2000 ppm of isopropyl alcohol is found.

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Related compounds—

Internal standard solution—Proceed as directed in the *Assay*.

Solution 1—Prepare a solution of *dl*-camphene and USP Mecamylamine Related Compound A RS in the *Internal standard solution* containing 625 µg of each per mL.

System suitability solution—Transfer about 125 mg of USP Mecamylamine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 1 mL of *Solution 1*, dilute with *Internal standard solution* to volume, and mix.

Test solution—Use the *Assay preparation*.

Chromatographic system—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record peak responses as directed for *Procedure:* the resolution, *R*, between the mecamylamine and mecamylamine related compound A is not less than 5; the column efficiency is not less than 4000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 1 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of each impurity in the portion of Mecamylamine Hydrochloride taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses for all the peaks: not more than 0.5% of mecamylamine related compound A is found; not more than 0.5% of *dl*-camphene is found; and not more than 1.0% of total impurities is found.

Chloride content—Dissolve about 500 mg, accurately weighed, in 5 mL of water. Add 5 mL of glacial acetic acid, 50 mL of methanol, and 1 drop of eosin Y TS, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl: the content is between 17.0% and 17.8%.

Assay—

Internal standard solution—Transfer about 600 mg of sodium hydroxide pellets to a 1 L volumetric flask, dissolve in about 800 mL of methanol. Add an accurately weighed quantity of about 1.7 g of biphenyl to the flask, and dilute with methanol to volume.

Standard preparation—Dissolve an accurately weighed quantity of USP Mecamylamine Hydrochloride RS in *Internal standard solution*, and dilute with *Internal standard solution*, quantitatively and stepwise if necessary, to obtain a solution having a known concentration of about 2.5 mg per mL.

Assay preparation—Transfer about 125 mg of Mecamylamine Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector connected to a 0.53-mm × 30-m capillary column, coated with a 1.5-µm film of liquid phase G27. The injection port temperature is maintained at about 200°, the detector temperature is maintained at about 280°, and the column temperature is at 120° for 15 minutes then increased at 25° per minute to 250° and maintained for 7 minutes at 250°. Nitrogen is used as the carrier gas at 7.4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 4000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject equal volumes (about 1 µL) of the *Assay preparation* and the *Standard preparation* into the gas chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{11}H_{21}N \cdot HCl$ in the portion of Mecamylamine Hydrochloride taken by the formula:

$$50C(R_U/R_S)$$

in which *C* is the concentration of USP Mecamylamine Hydrochloride RS, in mg per mL, in the *Standard preparation;* and R_U and R_S are the peak response ratios of mecamylamine hydrochloride to the internal standard biphenyl obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mecamylamine Hydrochloride Tablets

» Mecamylamine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mecamylamine hydrochloride ($C_{11}H_{21}N \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Mecamylamine Hydrochloride RS

Identification—

A: To a quantity of powdered Tablets, equivalent to about 75 mg of mecamylamine hydrochloride, add 50 mL of chloroform, and triturate the mixture for 5 minutes. Filter, and evaporate the filtrate on a steam bath with the aid of a current of air to dryness: the IR absorption spectrum of a potassium bromide dispersion of a portion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mecamylamine Hydrochloride RS.

B: A portion of the residue obtained in *Identification* test *A* responds to the tests for *Chloride* ⟨191⟩.

Dissolution ⟨711⟩—

Medium: water; 750 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{11}H_{21}N \cdot HCl$ dissolved using the following procedure.

Diluent—Prepare a solution of triethylamine in alcohol (1:100).

Internal standard solution—Prepare a solution of biphenyl in *Diluent* having a concentration of 82.5 µg per mL.

Standard solution—Prepare a solution of USP Mecamylamine Hydrochloride RS and biphenyl in *Diluent* having concentrations of 8.25 µg per mL of each.

Test solution—[NOTE—Condition the solid-phase extraction column specified in this procedure in the following manner. Wash the column with 5 mL of water, then with 5 mL of *Diluent*, and finally with two 5-mL portions of water.] Transfer by pipetting 25.0 mL of the solution under test through a freshly conditioned solid-phase extraction column containing L1 packing with a sorbent-mass to column volume ratio of 360 mg per 5 mL, or equivalent. Wash the pipet and the solid-phase extraction column with two 5-mL portions of water. Discard the filtrate. Elute the solid-phase extraction column with two 4-mL portions of *Diluent*, and collect the eluate in a 10-mL volumetric flask containing 1.0 mL of *Internal standard solution*. Dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a splitless injection system, and a 0.53-mm × 30-m

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analytical column coated with a 1-5-µm layer of phase G27. The carrier gas is helium at a flow rate of 5.2 mL per minute. The detector and column temperatures are maintained at 250° and 150°, respectively. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2, and the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount in mg, of $C_{11}H_{21}N \cdot HCl$ dissolved by the formula:

$$0.3C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Mecamylamine Hydrochloride RS in the *Standard solution*; and R_U and R_S are the peak response ratios of the mecamylamine hydrochloride peak to the internal standard peak obtained from the *Test solution* and *Standard solution*, respectively.

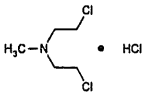
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{11}H_{21}N \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Place 1 Tablet in the digestion flask, and proceed as directed under *Nitrogen Determination, Method II* ⟨461⟩. Each mL of 0.01 N sulfuric acid is equivalent to 2.038 mg of mecamylamine hydrochloride.

Assay—Weigh and finely powder not fewer than 30 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of mecamylamine hydrochloride, to a glass-stoppered, 125-mL conical flask. Add about 25 mL of water, insert the stopper in the flask, and shake by mechanical means for 20 minutes. Transfer the contents of the flask to a 250-mL separator with the aid of small portions of water. Add 1 mL of 1 N sodium hydroxide and 5 g of sodium chloride, and extract the mixture successively with two 50-mL and three 25-mL portions of ether. Wash the combined ether extracts with three 10-mL portions of water, and wash, in turn, the combined water washes with a 10-mL portion of ether, adding it to the washed combined ether extracts. Transfer the ether phase to a 250-mL conical flask containing 25.0 mL of 0.02 N sulfuric acid VS, and evaporate the ether on a steam bath. Cool the solution, add methyl red TS, and titrate the excess acid with 0.02 N sodium hydroxide VS. Each mL of 0.02 N sulfuric acid is equivalent to 4.075 mg of mecamylamine hydrochloride ($C_{11}H_{21}N \cdot HCl$).

Mechlorethamine Hydrochloride



$C_5H_{11}Cl_2N \cdot HCl$ 192.51

Ethanamine, 2-chloro-*N*-(2-chloroethyl)-*N*-methyl-, hydrochloride.

2,2′-Dichloro-*N*-methyldiethylamine hydrochloride [55-86-7].

» Mechlorethamine Hydrochloride contains not less than 97.5 percent and not more than 100.5 percent of $C_5H_{11}Cl_2N \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—The label bears a warning that great care should be taken to prevent inhaling particles of Mechlorethamine Hydrochloride and exposing the skin to it.

USP Reference standards ⟨11⟩—
USP Mechlorethamine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Transfer 100 mg to a test tube containing 1 mL of sodium thiosulfate solution (prepared by dissolving 1 g of sodium thiosulfate and 100 mg of sodium carbonate in 40 mL of water), shake, allow to stand for 2 hours, then add 1 drop of iodine TS: the color of free iodine remains.

Melting range ⟨741⟩**:** between 108° and 111°.

pH ⟨791⟩**:** between 3.0 and 5.0, in a solution (1 in 500).

Water Determination, *Method I* ⟨921⟩**:** not more than 0.4%.

Ionic chloride content—Dissolve about 30 mg, accurately weighed, in 30 mL of water contained in a beaker. Add 5 mL of nitric acid and stir. Titrate with 0.02 N silver nitrate VS to a potentiometric endpoint, using a silver combination electrode. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary correction. Each mL of 0.02 N silver nitrate is equivalent to 0.709 mg of ionic chloride: not less than 18.0% and not more than 19.3% of ionic chloride is found.

Assay—Transfer about 100 mg of Mechlorethamine Hydrochloride, accurately weighed, to a 125-mL conical flask. Add 100 mg of sodium bicarbonate and 20.0 mL of 0.1 N sodium thiosulfate VS. Allow to stand for 2½ hours, add 3 mL of starch TS, and titrate the excess sodium thiosulfate with 0.1 N iodine VS. Each mL of 0.1 N sodium thiosulfate is equivalent to 9.626 mg of $C_5H_{11}Cl_2N \cdot HCl$.

Mechlorethamine Hydrochloride for Injection

» Mechlorethamine Hydrochloride for Injection is a sterile mixture of Mechlorethamine Hydrochloride with Sodium Chloride or other suitable diluent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mechlorethamine hydrochloride ($C_5H_{11}Cl_2N \cdot HCl$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.* The label bears a warning that great care should be taken to prevent inhaling particles of Mechlorethamine Hydrochloride for Injection and exposing the skin to it.

USP Reference standards ⟨11⟩—
USP Mechlorethamine Hydrochloride RS

Completeness of solution ⟨641⟩—A 0.10-g portion dissolves in 10 mL of carbon dioxide-free water to yield a clear solution.

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

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Identification—It meets the requirements of the *Identification* tests under *Mechlorethamine Hydrochloride.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 12.5 USP Endotoxin Units per mg of mechlorethamine hydrochloride.
pH ⟨791⟩: between 3.0 and 5.0, in a solution (1 in 50).
Water Determination, *Method I* ⟨921⟩: not more than 1.0%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements for *Sterility Tests* ⟨71⟩ and *Uniformity of Dosage Units* ⟨905⟩.

Assay—

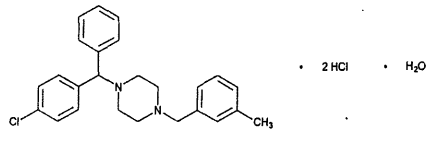
Assay preparation—Select a counted number of not fewer than 10 containers of Mechlorethamine Hydrochloride for Injection, equivalent to about 100 mg of mechlorethamine hydrochloride. Dissolve the contents of each container in water, and transfer the resulting solutions to a 250-mL conical flask.

Procedure—Immediately proceed as directed in the *Assay* under *Mechlorethamine Hydrochloride,* beginning with "Add 100 mg of sodium bicarbonate." Calculate the average content, in mg, of mechlorethamine hydrochloride ($C_5H_{11}Cl_2N \cdot HCl$) per container of Mechlorethamine Hydrochloride for Injection taken by the formula:

$$9.626(V/N)$$

in which V is the volume, in mL, of 0.1 N sodium thiosulfate consumed; and N is the number of containers selected to prepare the *Assay preparation.*

Meclizine Hydrochloride



$C_{25}H_{27}ClN_2 \cdot 2HCl \cdot H_2O$ 481.89
$C_{25}H_{27}ClN_2 \cdot 2HCl$ 463.88
Piperazine, 1-[(4-chlorophenyl)phenylmethyl]-4-[(3-methylphenyl)methyl]-, dihydrochloride, monohydrate; 1-(*p*-Chloro-α-phenylbenzyl)-4-(*m*-methylbenzyl)piperazine dihydrochloride monohydrate [31884-77-2].
Anhydrous [1104-22-9].

DEFINITION

Meclizine Hydrochloride contains NLT 97.0% and NMT 102.0% of $C_{25}H_{27}ClN_2 \cdot 2HCl$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Choride* ⟨191⟩

Sample solution: Dissolve 25 mg in a mixture of 3 mL of 2 N nitric acid and 5 mL of alcohol.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 1.5 g of sodium 1-heptanesulfonate in 300 mL of water, and mix this solution with 700 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 4.
Standard solution: 0.1 mg/mL of USP Meclizine Hydrochloride RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Meclizine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.3 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of meclizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$) in the portion of Meclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of meclizine from the *Sample solution*
r_S = peak response of meclizine from the *Standard solution*
C_S = concentration of USP Meclizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Meclizine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2. Procedure 2* is recommended when the isomeclizine impurity may be present.]
Mobile phase: Dissolve 1.5 g of sodium 1-heptanesulfonate in 300 mL of water, and mix this solution with 700 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 4.
System suitability solution: 0.01 mg/mL each of USP Meclizine Hydrochloride RS and 4-chlorobenzophenone in *Mobile phase*
Standard solution: 2.5 µg/mL of USP Meclizine Hydrochloride RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Meclizine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.3 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The elution order is meclizine, followed by 4-chlorobenzophenone.]

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Suitability requirements
Resolution: NLT 2.0 between meclizine hydrochloride and 4-chlorobenzophenone, *System suitability solution*
Column efficiency: NLT 1800 theoretical plates, determined from the analyte peak, *Standard solution*
Tailing factor: NMT 1.5 for the analyte peak, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Allow the *Sample solution* to elute for NLT three times the retention time of meclizine hydrochloride.
Calculate the percentage of each impurity in the portion of Meclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of meclizine from the *Standard solution*
C_S = concentration of USP Meclizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Meclizine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor, 0.72 for the 4-chlorobenzophenone peak and 1.0 for all other peaks

Acceptance criteria
Any individual impurity: NMT 0.5%
Total impurities: NMT 1.0%

• **ORGANIC IMPURITIES, PROCEDURE 2**
Mobile phase: Dissolve 5 g of sodium 1-heptanesulfonate in 1000 mL of water, and mix 600 mL of this solution with 400 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 4.0 ± 0.1.
System suitability solution: 2.5 µg/mL each of USP Meclizine Hydrochloride RS, USP Meclizine Related Compound A RS, and USP Meclizine Related Compound B RS in *Mobile phase*
Standard solution: 2.5 µg/mL of USP Meclizine Hydrochloride RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Meclizine Hydrochloride in *Mobile phase*. [NOTE—Store this solution no longer than 24 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 2.0 mL/min
Injection size: 30 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between meclizine related compound B and meclizine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 6.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Meclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of meclizine from the *Standard solution*
C_S = concentration of USP Meclizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Meclizine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak eluting before 1.75 min.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
3-Methylbenzyl alcohol	0.11	1.0	0.10
1,4-Bis(3-methylbenzyl) piperazine	0.22	0.73	0.10
4-Chlorobenzhydrol[a]	0.53	1.3	0.15
Meclizine *o*-chloro isomer[b]	0.81	1.0	0.10
Isomeclizine (meclizine *o*-methyl isomer)[c]	0.90	1.1	0.15
Meclizine	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] USP Meclizine Related Compound A.
[b] 1-[2-Chlorophenyl)(phenyl)methyl]-4-(3-methylbenzyl) piperazine.
[c] USP Meclizine Related Compound B.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Meclizine Hydrochloride RS
USP Meclizine Related Compound A RS
4-Chlorobenzhydrol.
$C_{13}H_{11}ClO$ 218.68
USP Meclizine Related Compound B RS
Isomeclizine
1-[(4-Chlorophenyl)(phenyl)methyl]-4-(2-methylbenzyl) piperazine dihydrochloride monohydrate.
$C_{25}H_{27}ClN_2 \cdot 2HCl \cdot H_2O$ 481.88

Meclizine Hydrochloride Tablets

» Meclizine Hydrochloride Tablets contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of meclizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$).

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Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Meclizine Hydrochloride RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

B: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Adsorbent: 0.5-mm layer of chromatographic silica gel mixture.
Test solution—Extract a quantity of finely powdered Tablets, equivalent to about 125 mg of meclizine hydrochloride, by shaking for 15 minutes with 50 mL of methanol.
Standard solution—Prepare a solution of USP Meclizine Hydrochloride RS in methanol, containing 2.5 mg per mL.
Application volume: 50 µL.
Developing solvent system: a mixture of cyclohexane, toluene, and diethylamine (15:3:2).
Procedure—Proceed as directed in the chapter, except to place the plate in a developing chamber that contains and has been equilibrated with *Developing solvent system.*

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.

Determine the amount of $C_{25}H_{27}ClN_2 \cdot 2HCl$ dissolved by employing the following method.

Mobile phase—Prepare a suitable degassed and filtered mixture of water and methanol (55:45) that contains 0.69 g of monobasic sodium phosphate in each 100 mL and is adjusted with phosphoric acid, if necessary, to a pH of 4.0.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm analytical column that contains packing L9. The flow rate is about 2 mL per minute. Chromatograph replicate injections of the Standard solution, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%.

Procedure—Inject about 100 µL of a filtered portion of the solution under test, suitably diluted with *Mobile phase,* if necessary, into the chromatograph, record the chromatogram, and measure the response for the major peak. Determine the amount of $C_{25}H_{27}ClN_2 \cdot 2HCl$ dissolved from the peak response obtained in comparison with the peak response obtained from a Standard solution having a known concentration of USP Meclizine Hydrochloride RS in a mixture of *Medium* and *Mobile phase* (1:1), similarly chromatographed. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to dissolve USP Meclizine Hydrochloride RS prior to dilution.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{25}H_{27}ClN_2 \cdot 2HCl$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Procedure for content uniformity—Place 1 Tablet in a 100-mL volumetric flask, add 50 mL of dilute hydrochloric acid (1 in 100), shake by mechanical means for 30 minutes, add the dilute acid to volume, and filter, discarding the first 20 mL of the filtrate. Dilute quantitatively and stepwise with the same acid to obtain a solution having a concentration of about 15 µg of meclizine hydrochloride per mL. Similarly, prepare a Standard solution of USP Meclizine Hydrochloride RS in dilute hydrochloric acid (1 in 100) having a known concentration of about 15 µg per mL. Concomitantly determine the absorbances of the solution from the Tablet and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 232 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 100) as the blank. Calculate the quantity, in mg, of meclizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$) in the Tablet taken by the formula:

$$(T/D)C(A_U/A_S)$$

in which T is the quantity, in mg, of meclizine hydrochloride in the Tablet; D is the concentration, in µg per mL, of meclizine hydrochloride in the solution from the Tablet, on the basis of the labeled quantity per Tablet and the extent of dilution; C is the concentration, in µg per mL, of USP Meclizine Hydrochloride RS in the Standard solution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Related compounds—

Mobile phase and *Buffer pH 7.5*—Prepare as directed in the *Assay.*

Standard solution—Dissolve an accurately weighed quantity of USP Meclizine Hydrochloride RS in *Mobile phase,* sonicating for about 5 minutes or until the material is dissolved, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.025 mg per mL. [NOTE—This solution is stable for 72 hours when stored at controlled room temperature protected from light.]

Sensitivity solution—Dilute an aliquot of the *Standard solution* with *Diluent* to obtain a solution containing about 1.25 µg per mL. [NOTE—Prepare this solution fresh daily.]

Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of meclizine hydrochloride based on the label claim, to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase* and shake by mechanical means for not less than 30 minutes. Dilute with *Mobile phase* to volume, mix, allow to settle for about 15 minutes, and pass through a 0.45-µm nylon filter, discarding the first 5 mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay.* Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency, N, is not less than 1200 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Sensitivity solution,* and record the peak responses as directed for *Procedure:* the signal-to-noise ratio, S/N, is not less than 10.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph. Allow the *Test solution* to elute for not less than two times the retention time of meclizine hydrochloride. Record the chromatograms and measure all of the peak areas. Calculate the percentage of each impurity relative to the labeled content of meclizine hydrochloride in the portion of the Tablets taken by the formula:

$$100(1/F)(C_S/C_T)(r_i/r_S)$$

in which F is the relative response factor, which is equal to 0.72 for the 4-chlorobenzophenone peak eluting at a relative retention time of about 0.23 and equal to 1.0 for all other peaks; C_T is the concentration, in mg per mL, of meclizine hydrochloride in the *Test solution,* based on the label claim; C_S is the concentration, in mg per mL, of meclizine hydrochloride in the *Standard solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the response of the meclizine peak obtained from the *Standard solution:* not more than 0.5% of any individual impurity is found; and not

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more than 1.0% of total impurities is found. Reporting level for impurities is 0.1%.

Assay—

Buffer pH 7.5—Dissolve 1.32 g of dibasic ammonium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 7.5 ± 0.05.

Mobile phase—Prepare a mixture of *Buffer pH 7.5*, methanol, and acetonitrile (350:325:325). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Meclizine Hydrochloride RS in *Mobile phase*, sonicating for about 5 minutes or until the material is dissolved, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.125 mg per mL. [NOTE—This solution is stable for 72 hours when stored at controlled room temperature protected from light.]

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of meclizine hydrochloride based on the label claim, to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and shake by mechanical means for not less than 30 minutes. Dilute with *Mobile phase* to volume, mix, and filter through a 0.45-µm nylon filter, discarding the first 5 mL of the filtrate.

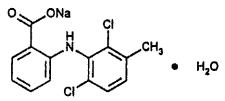
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 232-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L11. The column temperature is maintained at 30°, and the flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of the labeled amount of meclizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$) in each Tablet taken by the formula:

$$100(CV/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of meclizine hydrochloride in the *Standard preparation*; V is the volume, in mL, of the *Assay preparation*; W is the quantity, in mg, of meclizine hydrochloride based on the label claim, taken to prepare the *Assay preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Meclofenamate Sodium



$C_{14}H_{10}Cl_2NNaO_2 \cdot H_2O$ 336.15

Benzoic acid, 2-[(2,6-dichloro-3-methylphenyl)amino]-, monosodium salt, monohydrate.

Monosodium *N*-(2,6-dichloro-*m*-tolyl)anthranilate monohydrate [6385-02-0].

Anhydrous 318.13

» Meclofenamate Sodium contains not less than 97.0 percent and not more than 103.0 percent of $C_{14}H_{10}Cl_2NNaO_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Meclofenamate Sodium RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 25 µg per mL.
Medium: 0.01 N hydrochloric acid in methanol.
Absorptivities at 242 nm, 279 nm, and 336 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

Change to read:

C: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 1 in 40,000.
Medium: 0.1 N sodium hydroxide.
Absorptivities at 279 nm and 317 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

Water Determination, *Method I* ⟨921⟩: between 4.8% and 5.8%.

Copper—

Standard copper solution—Dissolve 1000 mg of copper wire in 6 mL of nitric acid in a 1 L volumetric flask. Add 8 mL of hydrochloric acid, dilute with water to volume, and mix. Dilute this solution quantitatively and stepwise with water to obtain a *Standard copper solution* having a known concentration of 0.6 µg per mL.

Test solution—Transfer 2 g of Meclofenamate Sodium, accurately weighed, to a 100-mL volumetric flask, and add 1 drop of ammonium hydroxide. Dissolve in water, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard copper solution* and the *Test solution* at the copper emission line at about 325 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a copper hollow-cathode lamp, using water as the blank. Adjust the operating conditions to obtain about 70% full-scale detector response with the *Standard copper solution*. The detector response obtained with the *Test solution* is not greater than that obtained with the *Standard copper solution* (0.003%).

Chromatographic purity—

Standard solutions—Dissolve an accurately weighed quantity of USP Meclofenamate Sodium RS in methanol to obtain a solution containing 20 mg per mL (*Standard solution A*). Dilute 1.0 mL of *Standard solution A* with sufficient methanol to obtain 200 mL of solution (*Standard solution B*).

Test solution—Dissolve 200 mg of Meclofenamate Sodium in 10.0 mL of methanol.

Procedure—Apply 10-µL portions of *Standard solution A*, *Standard solution B*, and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩)

USP Monographs

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coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methylene chloride, methyl ethyl ketone, and glacial acetic acid (50:48:2) until the solvent front has moved about eight-tenths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: the chromatograms show a principal spot at about the same R_f value, and any secondary spot, if present in the chromatogram from the *Test solution* is not more intense than the principal spot obtained from *Standard solution B* (0.5%).

Assay—Transfer about 350 mg of Meclofenamate Sodium, accurately weighed, to a 125-mL separator, add 10 mL of water, and mix to dissolve. To this solution add 3 mL of 3 N hydrochloric acid, shake, and extract with three 30-mL portions of chloroform, collecting the chloroform extracts in an evaporating flask. Evaporate the chloroform extracts to dryness. Dissolve the residue in 5 mL of dimethyl sulfoxide and 25 mL of methanol. Mix, add 5 drops of phenolphthalein TS, and titrate the mixture with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 31.81 mg of $C_{14}H_{10}Cl_2NNaO_2$.

Meclofenamate Sodium Capsules

» Meclofenamate Sodium Capsules contain an amount of $C_{14}H_{10}Cl_2NNaO_2$ equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of meclofenamic acid ($C_{14}H_{11}Cl_2NO_2$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Meclofenamate Sodium RS

Identification—Prepare a solution of Capsule contents in methanol containing 20 mg per mL, and filter. The clear filtrate so obtained meets the requirements of the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, the solvent mixture consisting of methylene chloride, methyl ethyl ketone, and glacial acetic acid (50:48:2).

Dissolution ⟨711⟩—
Medium: 0.05 M pH 7.5 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of meclofenamic acid ($C_{14}H_{11}Cl_2NO_2$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 279 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Meclofenamate Sodium RS in the same *Medium*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{14}H_{11}Cl_2NO_2$ is dissolved in 45 minutes.

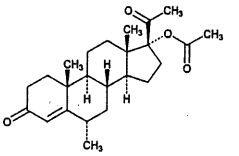
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of meclofenamic acid, to a 200-mL volumetric flask. Add 0.01 N hydrochloric acid in methanol to volume, and mix. Filter, discarding the first 20 mL of the filtrate. Transfer 10.0 mL of the filtrate to a 100-mL volumetric flask, add 0.01 N hydrochloric acid in methanol to volume, and mix. Dissolve an accurately weighed quantity of USP Meclofenamate Sodium RS in 0.01 N hydrochloric acid in methanol to obtain a solution having a known concentration of about 27 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 336 nm, with a suitable spectrophotometer, using 0.01 N hydrochloric acid in methanol as the blank. Calculate the quantity, in mg, of meclfenamate acid ($C_{14}H_{11}Cl_2NO_2$) in the portion of Capsule contents taken by the formula:

$$2C(296.15/318.13)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Meclofenamate Sodium RS in the Standard solution; 296.15 and 318.13 are the molecular weights of meclofenamic acid and meclofenamate sodium, respectively; and A_U and A_S are the absorbances of the solution from the Capsule contents and the Standard solution, respectively.

Medroxyprogesterone Acetate



$C_{24}H_{34}O_4$ 386.52
Pregn-4-ene-3,20-dione, 17-(acetyloxy)-6-methyl-, (6α)-;
17-Hydroxy-6α-methylpregn-4-ene-3,20-dione acetate [71-58-9].

DEFINITION
Medroxyprogesterone Acetate contains NLT 97.0% and NMT 103.0% of medroxyprogesterone acetate ($C_{24}H_{34}O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Analytical wavelength: 241 nm
Sample solution: 10 µg/mL in alcohol
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 2.0%.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (40:60)
Standard solution: 1 mg/mL of USP Medroxyprogesterone Acetate RS in acetonitrile
Sample solution: 1 mg/mL of Medroxyprogesterone Acetate in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of Medroxyprogesterone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Medroxyprogesterone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase: Acetonitrile and water (60:40)
System suitability solution: 40 µg/mL each of megestrol acetate and USP Medroxyprogesterone Acetate RS in *Mobile phase*
Standard solution: 50 µg/mL of USP Medroxyprogesterone Acetate RS in *Mobile phase*
Sample solution: 2.5 mg/mL of Medroxyprogesterone Acetate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between megestrol acetate and medroxyprogesterone acetate, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution and Sample solution*
Calculate the percentage of each impurity in the portion of Medroxyprogesterone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of medroxyprogesterone acetate from the *Standard solution*
C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Medroxyprogesterone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurity: NMT 1.0%
Total impurities: NMT 1.5%

• **LIMIT OF MEDROXYPROGESTERONE ACETATE RELATED COMPOUND A**
Standard solution: 20 mg/mL of USP Medroxyprogesterone Acetate RS and 0.1 mg/mL of USP Medroxyprogesterone Acetate Related Compound A RS in methylene chloride
Sample solution: 20 mg/mL of Medroxyprogesterone Acetate in methylene chloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Hexanes, *tert*-butyl methyl ether, and tetrahydrofuran (45:45:10)
Spray reagent: 200 mg/mL of *p*-toluenesulfonic acid in alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about 10 cm. Allow the plate to air-dry, and develop the chromatogram again until the solvent front has moved about 10 cm. Allow the plate to dry at 120° for 10 min. Spray the plate with *Spray reagent*. Heat the plate for 10 min at 120°, and examine the plate under UV light at 365 nm.
Acceptance criteria: NMT 0.5%; any blue fluorescent spot with an R_F value higher than that of the principal spot due to medroxyprogesterone acetate of the *Sample solution* is not more intense than the corresponding blue fluorescent spot of the *Standard solution*.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in dioxane
Acceptance criteria: +45° to +51°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Medroxyprogesterone Acetate RS
USP Medroxyprogesterone Acetate Related Compound A RS
4,5β-Dihydromedroxyprogesterone acetate.
$C_{24}H_{36}O_4$ 388.54

Medroxyprogesterone Acetate Injectable Suspension

DEFINITION

Medroxyprogesterone Acetate Injectable Suspension is a sterile suspension of Medroxyprogesterone Acetate in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$).

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Transfer a volume of Injectable Suspension, equivalent to 50 mg of medroxyprogesterone acetate, to a centrifuge tube, centrifuge, decant the supernatant, and wash the solids with two 15-mL portions of water, discarding the water washings. Dissolve the solids in 10 mL of chloroform, transfer to a small beaker, evaporate the chloroform on a steam bath, and dry the residue at 105° for 3 h.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: 700 mL of butyl chloride, 300 mL of hexane, both previously saturated with water, and 80 mL of acetonitrile. The acetonitrile concentration may be varied to meet *System suitability* requirements and to provide elution times of about 12 and 15 min for progesterone and medroxyprogesterone acetate, respectively. Pass the solution through a membrane filter of 1 µm or less pore size.

Internal standard solution: 0.25 mg/mL of progesterone in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Medroxyprogesterone Acetate RS in *Internal standard solution*

Sample solution: Nominally 0.4 mg/mL of medroxyprogesterone acetate in *Internal standard solution*, prepared as follows. Transfer a volume of Injectable Suspension, equivalent to 50 mg of medroxyprogesterone acetate, to a suitable container. Transfer 25 mL of chloroform into the container, shake for 20 min, and centrifuge. Transfer 4 mL of the chloroform layer into a suitable container, and evaporate to dryness. Dissolve the residue in 20 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 2-mm × 25-cm; 5-µm packing L3

Flow rate: The *Mobile phase* is maintained at a flow rate capable of giving the required resolution and suitable elution times.

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between progesterone and medroxyprogesterone acetate

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of Injectable Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of medroxyprogesterone acetate to the internal standard from the *Sample solution*

R_S = peak area ratio of medroxyprogesterone acetate to the internal standard from the *Standard solution*

C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.0–7.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Medroxyprogesterone Acetate RS

Medroxyprogesterone Acetate Tablets

DEFINITION

Medroxyprogesterone Acetate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Triturate a number of Tablets, equivalent to about 25 mg of medroxyprogesterone acetate, with 15 mL of chloroform. Filter, evaporate the chloroform on a steam bath, and dry the residue at 105° for 3 h.

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (40:60)

Standard solution: 1 mg/mL of USP Medroxyprogesterone Acetate RS in acetonitrile

Sample solution: Finely powder NLT 20 Tablets. Weigh a portion of the powder, equivalent to 25 mg of medroxyprogesterone acetate, into a 50-mL glass centrifuge tube. Transfer 25 mL of acetonitrile into the tube, shake to wet the powder thoroughly, sonicate for NLT 10 min, and centrifuge. Use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.5% sodium lauryl sulfate; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Acetonitrile and water (60:40)
Sodium lauryl sulfate stock solution: Transfer 180.0 g of sodium lauryl sulfate to a 2000-mL volumetric flask. Add 1500 mL of water, and stir until dissolved. [NOTE—Several hours of stirring are required.] Dilute with water to volume.
Standard stock solution: 70 mg of USP Medroxyprogesterone Acetate RS in 140 mL of *Sodium lauryl sulfate stock solution*. Dilute with water to 250 mL. [NOTE—It may be necessary to sonicate the solution to bring the Reference Standard into solution before dilution with water.] Prepare the *Standard stock solution* fresh daily.
Standard solution: Transfer a 20-mL aliquot of *Standard stock solution* into a 1-L volumetric flask. Add 40 mL of *Sodium lauryl sulfate stock solution*, and dilute with water to volume. This solution is stable for up to 7 days.
Sample solution: Withdraw 15 mL of the solution under test and filter, discarding the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 8-cm; packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) dissolved using the responses from the *Sample solution* and *Standard solution*.
Tolerances: NLT 50% (*Q*) of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Diluent: Alcohol and water (3:1)
Standard solution: 15 µg/mL of USP Medroxyprogesterone Acetate RS in *Diluent*
Sample solution: Nominally 15 µg/mL of medroxyprogesterone acetate in *Diluent* prepared as follows. Transfer 1 Tablet to a volumetric flask, dilute with *Diluent* to volume, and shake for 15 min. Filter, and quantitatively dilute a portion of the filtrate as needed.
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: Maximum at about 242 nm
Cell: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the Tablet taken:

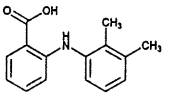
$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of medroxyprogesterone acetate in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Medroxyprogesterone Acetate RS

Mefenamic Acid



$C_{15}H_{15}NO_2$ 241.29
Benzoic acid, 2-(2,3-dimethylphenyl)amino-;
N-2,3-Xylylanthranilic acid [61-68-7].

DEFINITION
Mefenamic Acid contains NLT 98.0% and NMT 102.0% of mefenamic acid ($C_{15}H_{15}NO_2$), calculated on the dried basis.

IDENTIFICATION
• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K or 197A▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 50 mM monobasic ammonium phosphate. Adjust with 3 M ammonium hydroxide to a pH of 5.0.
Mobile phase: Acetonitrile, tetrahydrofuran, and *Buffer* (23:7:20)
Standard solution: 0.2 mg/mL of USP Mefenamic Acid RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Mefenamic Acid in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 8200 theoretical plates
Tailing factor: NMT 1.6
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mefenamic acid ($C_{15}H_{15}NO_2$) in the portion of Mefenamic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of mefenamic acid from the *Sample solution*
r_S = peak response of mefenamic acid from the *Standard solution*
C_S = concentration of USP Mefenamic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Mefenamic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.01 mg/mL of USP Mefenamic Acid RS in *Mobile phase*
Sensitivity solution: 0.3 µg/mL of USP Mefenamic Acid RS in *Mobile phase* from the *Standard solution*
Sample solution: 1 mg/mL of Mefenamic Acid in *Mobile phase*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 1.6, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Mefenamic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of mefenamic acid from the *Standard solution*
C_S = concentration of USP Mefenamic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Mefenamic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: The reporting threshold is 0.03%.
Any impurity: NMT 0.07%
Total impurities: NMT 0.5%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mefenamic Acid RS

Mefenamic Acid Capsules

DEFINITION

Mefenamic Acid Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of mefenamic acid ($C_{15}H_{15}NO_2$).

IDENTIFICATION

Delete the following:

▲• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Sample solution: Nominally 1 mg/mL of mefenamic acid from Capsule contents, in a mixture of chloroform and methanol (3:1). Shake vigorously and filter.
Developing solvent system: Chloroform, ethyl acetate, and glacial acetic acid (75:25:1). *Ordinary Impurities* ⟨466⟩ visualization technique 17 is used.
Acceptance criteria: Meet the requirements ▲(USP 1-May-2019)

Add the following:

▲• **A.** The UV spectrum of the mefenamic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲(USP 1-May-2019)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Solution A: 50 mM solution of monobasic ammonium phosphate in water. Adjust with 3 M ammonium hydroxide to a pH of 5.0.
Mobile phase: Acetonitrile, tetrahydrofuran, and *Solution A* (23:7:20)
Standard solution: 0.2 mg/mL of USP Mefenamic Acid RS in *Mobile phase*
Sample solution: Nominally 0.2 mg/mL of mefenamic acid in *Mobile phase* prepared as follows. Transfer a suitable amount of mefenamic acid from the contents of NLT 20 Capsules to an adequate volumetric flask. Add 2% of final volume of tetrahydrofuran, and sonicate for about 5 min with occasional mixing. Dilute with *Mobile phase* to the final volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm. ▲(USP 1-May-2019)
Column: 4.6-mm × 25-cm; ▲5-µm ▲(USP 1-May-2019) packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 8200 theoretical plates
Tailing factor: NMT 1.6
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mefenamic acid ($C_{15}H_{15}NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mefenamic acid from the *Sample solution*
r_S = peak response of mefenamic acid from the *Standard solution*

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C_S = concentration of USP Mefenamic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mefenamic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
0.05 M tris buffer: Dissolve 60.5 g of tris(hydroxymethyl) aminomethane in 6 L of water, and dilute with water to 10 L. Adjust with phosphoric acid to a pH of 9.0 ± 0.05. To a second container, transfer about 6 L of this solution, add 100 g of sodium lauryl sulfate, and mix to dissolve the solid material. Transfer this solution back into the first container, and mix.
Medium: *0.05 M tris buffer*; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
▲**Solution A, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*, making any necessary volumetric adjustments.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mefenamic acid ($C_{15}H_{15}NO$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

r_U = peak response of mefenamic acid from the *Sample solution*
r_S = peak response of mefenamic acid from the *Standard solution*
C_S = concentration of USP Mefenamic Acid RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*
L = label claim (mg/Capsule) ▲(USP 1-May-2019)

Tolerances: NLT 75% (Q) of the labeled amount of mefenamic acid ($C_{15}H_{15}NO$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Add the following:

▲• **Organic Impurities**
Solution A, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 0.01 mg/mL of USP 2,3-Dimethylaniline RS and 1 mg/mL of USP Mefenamic Acid RS in *Mobile phase*
Sensitivity solution: 0.5 µg/mL of USP Mefenamic Acid RS in *Mobile phase*
Standard solution: 0.01 mg/mL of USP Mefenamic Acid RS in *Mobile phase*
Sample solution: Nominally 1 mg/mL of mefenamic acid in *Mobile phase* from the contents of NLT 20 Capsules. Sonicate to dissolve if necessary.
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 2 between 2,3-dimethylaniline and mefenamic acid, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*
r_S = peak response of mefenamic acid from the *Standard solution*
C_S = concentration of USP Mefenamic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mefenamic acid in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,3-Dimethylaniline[a]	0.6	—
Mefenamic acid	1.0	—
Any individual degradation product	—	0.16
Total degradation products	—	1.0

[a] This is a process impurity and is not included in the total degradation products. ▲(USP 1-May-2019)

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in tight containers.
▲Store at 20°–25°, excursions permitted between 15° and 30°.▲(USP 1-May-2019)

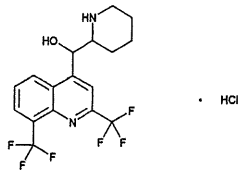
Change to read:

• **USP Reference Standards** ⟨11⟩
▲USP 2,3-Dimethylaniline RS
2,3-Dimethylaniline.
$C_8H_{11}N$ 121.18▲(USP 1-May-2019)
USP Mefenamic Acid RS

USP Monographs

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Mefloquine Hydrochloride



$C_{17}H_{16}F_6N_2O \cdot HCl$ 414.77

4-Quinolinemethanol, α-2-piperidinyl-2,8-bis(trifluoro methyl)-, monohydrochloride, (*R*,S**)- (±)-;
DL-*erythro*-α-2-Piperidyl-2,8-bis(trifluoromethyl)-4-quin olinemethanol monohydrochloride [51773-92-3].

DEFINITION

Mefloquine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{17}H_{16}F_6N_2O \cdot HCl$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY

- **PROCEDURE**

Solution A: 1.5 g/L of sodium hydrogen sulfate in water
Mobile phase: Dissolve 1 g of tetraheptylammonium bromide in a 1000-mL mixture of acetonitrile, methanol, and *Solution A* (2:1:2).
System suitability solution: 4 µg/mL each of USP Mefloquine Hydrochloride RS and USP Mefloquine Related Compound A RS in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Mefloquine Hydrochloride RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Mefloquine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Guard column: 4-mm × 3-cm; C18 (recommended)
Column: 4.0-mm × 25-cm; 5-µm packing L1
Column temperature: 25°
Flow rate: 0.8 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for mefloquine related compound A and mefloquine are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between mefloquine related compound A and mefloquine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) in the portion of Mefloquine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mefloquine from the *Sample solution*
r_S = peak response of mefloquine from the *Standard solution*
C_S = concentration of USP Mefloquine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Mefloquine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase: Dissolve 1 g of tetraheptylammonium bromide in a 1-L mixture of a 1.5-g/L solution of sodium hydrogen sulfate, acetonitrile, and methanol (2:2:1).
System suitability solution: 4 µg/mL each of USP Mefloquine Hydrochloride RS and USP Mefloquine Related Compound A RS in *Mobile phase*. [NOTE—Mefloquine related compound A is *threo*-mefloquine.]
Sample stock solution: 4 mg/mL of Mefloquine Hydrochloride in *Mobile phase*
Sample solution: 4 µg/mL from the *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Guard column: 4-mm × 2.5-cm; 5-µm packing L1
Column: 4.0-mm × 25-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection size: 20 µL. [NOTE—Equilibrate the column with *Mobile phase* at a flow rate of 0.8 mL/min for 30 min.]
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for mefloquine related compound A and mefloquine are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between mefloquine related compound A and mefloquine
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample stock solution* and *Sample solution*
Record the chromatogram for a time that is 10 times the retention time of the main peak.
Acceptance criteria: The response of the mefloquine related compound A peak in the *Sample stock solution* is NMT twice the area of the main peak of the *Sample solution* (0.2%). The response of any other individual peak, other than the main peak of the *Sample stock solution*, is NMT that of the main peak of the *Sample solution* (0.1%); and the sum of the responses of any such peaks of the *Sample stock solution* is NMT five times the response of the main peak of the *Sample solution* (0.5%). [NOTE—Exclude the main peak and any other peak producing a response of less than 0.2 times (0.02%) the main peak of the *Sample solution*.]

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 50 mg/mL in methanol
Acceptance criteria: –0.2° to +0.2°

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 15° and 30°.

USP Monographs

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mefloquine Hydrochloride RS
USP Mefloquine Related Compound A RS
threo-Mefloquine.
$C_{17}H_{16}F_6N_2O \cdot HCl$ 414.78

Mefloquine Hydrochloride Tablets

DEFINITION
Mefloquine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Diluent, Standard solution, and **Sample solution:** Proceed as directed in the *Assay*.
Blank: *Diluent*

ASSAY
• **PROCEDURE**
Buffer: 2.7 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Diluent: Methanol and water (23:27)
Mobile phase: Methanol, acetonitrile, and *Buffer* (13:10:27)
Standard solution: 0.05 mg/mL of USP Mefloquine Hydrochloride RS in *Diluent*
Sensitivity solution: 0.025 µg/mL of USP Mefloquine Hydrochloride RS in *Diluent*
Sample stock solution: Transfer a suitable number of Tablets to a volumetric flask, dilute with methanol (approximately 80% of the total volume), shake for 30 min, allow to sit for 1 h, and dilute with methanol to volume to obtain a solution having a concentration of 2.5 mg/mL of mefloquine hydrochloride.
Sample solution: Nominally 0.05 mg/mL of mefloquine hydrochloride in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm
Column: 4.6-mm × 15-cm; 5-µm packing L68
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Column efficiency: NLT 4000 theoretical plates, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Signal-to-noise ratio: NLT 5, *Sensitivity solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mefloquine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mefloquine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard stock solution: 0.2 mg/mL of USP Mefloquine Hydrochloride RS in *Medium*. A small amount of methanol, not exceeding 5% of the final volume, may be used to help solubilize mefloquine.
Standard solution: 0.04 mg/mL of USP Mefloquine Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: Dilute a portion of the solution under test with *Medium* (1:5), and pass a portion through a suitable filter of 0.8-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 285 nm
Cell length: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of USP Mefloquine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: 0.278 mg/mL of USP Mefloquine Hydrochloride RS in *Medium*. A small amount of methanol, not exceeding 2.5% of the final volume, may be used to help solubilize mefloquine.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 284 nm
Cell length: 0.2 cm
Blank: *Medium*

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Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of USP Mefloquine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Diluent, Mobile phase, Standard solution, Sensitivity solution, Sample stock solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of mefloquine hydrochloride from the *Standard solution*
C_S = concentration of USP Mefloquine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mefloquine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

[NOTE—Do not include the threo isomer, a process impurity monitored in the drug substance, in the calculation of total impurities. Disregard any peak less than 0.05%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Specified (unidentified)	0.67	0.15
Specified (unidentified)	0.70	0.15
threo-Mefloquine (DL-*threo*-α-2-piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol)	0.75	—
Specified (unidentified)	0.84	0.25
Mefloquine hydrochloride	1.0	—
Any other unknown individual impurity	—	0.15
Total impurities	—	0.50

ADDITIONAL REQUIREMENTS

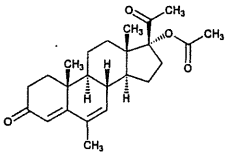
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Mefloquine Hydrochloride RS

Megestrol Acetate



$C_{24}H_{32}O_4$ 384.51

Pregna-4,6-diene-3,20-dione, 17-(acetyloxy)-6-methyl-; 17-Hydroxy-6-methylpregna-4,6-diene-3,20-dione acetate [595-33-5].

DEFINITION

Megestrol Acetate contains NLT 97.0% and NMT 103.0% of megestrol acetate ($C_{24}H_{32}O_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (55:45)

Diluent: Acetonitrile and water (40:60)

Internal standard solution: 0.8 mg/mL of propylparaben in acetonitrile

Standard stock solution: 1 mg/mL of USP Megestrol Acetate RS in acetonitrile

Standard solution: 80 µg/mL each of USP Megestrol Acetate RS and propylparaben in *Diluent* from the *Standard stock solution* and *Internal standard solution*, respectively

Sample stock solution: 1 mg/mL of Megestrol Acetate in acetonitrile

Sample solution: 80 µg/mL each of Megestrol Acetate and propylparaben in *Diluent* from the *Sample stock solution* and *Internal standard solution*, respectively

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for propylparaben and megestrol acetate are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8.0 between propylparaben and megestrol acetate

Relative standard deviation: NMT 2.0% for the peak response ratio of megestrol acetate to propylparaben

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of megestrol acetate ($C_{24}H_{32}O_4$) in the portion of Megestrol Acetate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of megestrol acetate to propylparaben from the *Sample solution*
R_S = peak response ratio of megestrol acetate to propylparaben from the *Standard solution*
C_S = concentration of USP Megestrol Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Megestrol Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%, with a platinum dish being used and ignition at 600 ± 25°

SPECIFIC TESTS
• **COMPLETENESS OF SOLUTION** ⟨641⟩
Sample: 500 mg in 10 mL of acetone
Acceptance criteria: Meets the requirements
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 213°–220°, but the range between the beginning and the end of melting does not exceed 3°.
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL in chloroform
Acceptance criteria: +8.8° to +12.0° (t = 20°)
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Megestrol Acetate RS

Megestrol Acetate Oral Suspension

DEFINITION
Megestrol Acetate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$).

IDENTIFICATION
• **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 4.0 mg/mL of USP Megestrol Acetate RS in chloroform
Sample solution: Transfer Oral Suspension, equivalent to 160 mg of megestrol acetate, to a separatory funnel, add 50 mL of water and 40 mL of chloroform, and shake. Allow the phases to separate, and discard the aqueous layer.
Developing solvent system: Chloroform and ethyl acetate (4:1)

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (11:9)
Standard solution: 80 µg/mL of USP Megestrol Acetate RS in *Mobile phase*
Sample solution: A volume of Oral Suspension diluted with *Mobile phase* to obtain nominally 80 µg/mL of megestrol acetate
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2500 theoretical plates
Tailing factor: NMT 1.4
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{32}O_4$ in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Megestrol Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.5% sodium lauryl sulfate in water; 900 mL
Apparatus 2: 25 rpm
Time: 30 min
Detector: UV 292 nm
Standard solution: 45 mg of USP Megestrol Acetate RS in a 250-mL volumetric flask. Add 12 mL of methanol, and place the flask in a warm water bath until the solid is dissolved. Dilute with *Medium* to volume. The final concentration is 180 µg/mL of megestrol acetate. Dilute with *Medium*, if necessary.
Sample solution: Transfer to the surface of the *Medium* in the dissolution vessel an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to 160 mg of megestrol acetate. At the sampling time, withdraw a volume of the solution under test and pass through a suitable filter with 0.45-µm pore size. Dilute with *Medium*, if necessary.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{32}O_4$ released:

$$\text{Result} = (A_U/A_S) \times (C_S/V) \times V_D \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of Oral Suspension taken
V_D = volume of *Medium*, 900 mL
L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{32}O_4$ is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.5% sodium lauryl sulfate in water; 900 mL
Apparatus 2: 25 rpm
Time: 30 min
Detector: UV 292 nm, using 0.5-cm pathlength cuvettes

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Standard solution: 45 mg of USP Megestrol Acetate RS in a 250-mL volumetric flask. Add 5 mL of methanol. Dilute with *Medium* to volume. Transfer 10 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume. The final concentration is 18 µg/mL.
Sample solution: [NOTE—Use a separate syringe for each vessel.] Withdraw more than 10 mL of the Oral Suspension, using a 10-mL syringe with a long cannula. Remove air bubbles from the syringe. Adjust the volume to the 10-mL mark on the syringe, and remove the needle. Wipe the tip of the syringe, and weigh (gross weight). Operate the apparatus, and rapidly dispense the Oral Suspension to the side of the vessel at about halfway from the bottom. Similarly dispense the Oral Suspension into other vessels. Weigh each syringe after dispensing the sample (tare weight). Record sample weights. After completion of the dissolution, pass an aliquot through a suitable nylon filter with 0.45-µm pore size, and dilute 2.0 mL of the filtrate with *Medium* to 50.0 mL to obtain a solution having a theoretical concentration of about 18 µg/mL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{32}O_4$ released:

$$\text{Result} = (A_U/A_S) \times (C_S/W) \times V_D \times d \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
W = weight of the Oral Suspension taken (mg)
V_D = volume of the *Medium* in the dissolution vessel, 900 mL
d = density of the Oral Suspension (mg/mL), obtained by dividing the weight of Oral Suspension taken by 10 mL
L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{32}O_4$ is dissolved.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.5% sodium lauryl sulfate in degassed water; 900 mL. [NOTE—Use ultrapure sodium lauryl sulfate with an *Assay* content of NLT 99.0%.]
Apparatus 2: 50 rpm
Time: 30 min
Determine the amount of $C_{24}H_{32}O_4$ dissolved by using the following method.
Mobile phase: Proceed as directed in the *Assay*.
Standard solution: 0.46 mg/mL of USP Megestrol Acetate RS in *Mobile phase*
Sample solution: Proceed as directed for *Test 2*, introducing the sample into the vessel over a 10- to 15-s period (about 1 mL/s).
Chromatographic system: Proceed as directed in the *Assay*.
Injection size: 10 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{32}O_4$ released:

$$\text{Result} = (r_U/r_S) \times (C_S/W) \times V_D \times d \times (100/L)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
W = weight of the Oral Suspension taken (mg)
V_D = volume of the *Medium* in the dissolution vessel, 900 mL
d = density of the Oral Suspension (mg/mL), obtained by dividing the weight of Oral Suspension taken by 10 mL
L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{32}O_4$ is dissolved.
• **DELIVERABLE VOLUME** ⟨698⟩: Meets the requirements

SPECIFIC TESTS
• **PH** ⟨791⟩: 3.0–4.7

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **LABELING:** When more than one test for *Dissolution* is given, the labeling states the test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Megestrol Acetate RS

Megestrol Acetate Tablets

DEFINITION
Megestrol Acetate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$).
[NOTE—Megestrol Acetate Tablets labeled solely for veterinary use are exempt from the requirements of the *Dissolution* test.]

IDENTIFICATION
• **A.**
Sample solution: Grind a suitable number of Tablets in a known volume of chloroform, NLT 10 mL, to obtain a solution containing 4 mg/mL of megestrol acetate.
Analysis: Filter the *Sample solution* into a beaker. Pipet 0.6 mL of the filtrate into a stainless steel grinding vial containing 500 mg of potassium bromide, dry with a current of air, grind, pellet, and record the IR spectrum.
Acceptance criteria: The IR absorption spectrum of the potassium bromide dispersion so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Megestrol Acetate RS.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (55:45)
Diluent: Acetonitrile and water (40:60)
Internal standard solution: 0.8 mg/mL of propylparaben in acetonitrile
Standard stock solution: 1 mg/mL of USP Megestrol Acetate RS in acetonitrile
Standard solution: 80 µg/mL each of USP Megestrol Acetate RS and propylparaben in *Diluent* from the *Standard stock solution* and *Internal standard solution*, respectively
Sample solution: Nominally 80 µg/mL of megestrol acetate prepared as follows. Transfer the equivalent of 80 mg of megestrol acetate from powdered Tablets (NLT 20 Tablets) to a 100-mL volumetric flask. Add 10 mL of water, and shake for 10 min. Add 75 mL of acetonitrile, shake for 30 min, then dilute with acetonitrile to volume. Place a 25-mL aliquot in a glass-stoppered 35-mL centrifuge tube, insert the stopper, and centrifuge for 10 min. Transfer 5.0 mL of the supernatant and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for propylparaben and megestrol acetate are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 8.0 between propylparaben and megestrol acetate
Relative standard deviation: NMT 2.0% for the peak response ratio of megestrol acetate to propylparaben
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of megestrol acetate to propylparaben from the *Sample solution*
R_S = peak response ratio of megestrol acetate to propylparaben from the *Standard solution*
C_S = concentration of USP Megestrol Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of megestrol acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩
Sample: Tablets labeled solely for veterinary use; proceed as directed for plain-coated Tablets, but use film-coated Tablets instead.
Time: 30 min
Acceptance criteria: Meet the requirements
• **DISSOLUTION** ⟨711⟩
Medium: 1% sodium lauryl sulfate; 900 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: USP Megestrol Acetate RS in *Medium*
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
Analytical wavelength: UV 292 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of megestrol acetate ($C_{24}H_{32}O_4$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Standard solution: 10 µg/mL of USP Megestrol Acetate RS in methanol
Sample solution: Nominally 10 µg/mL of megestrol acetate prepared as follows. Place 1 Tablet in a volumetric flask of suitable size so that the final expected solution concentration is between 0.2 and 1.0 mg of megestrol acetate per mL. Add 1 mL of water, and gently shake until the Tablet has disintegrated. Fill the flask to three-quarters of its nominal capacity with methanol, and shake by mechanical means for 20 min. Dilute with methanol to volume, mix, and filter, discarding the first 15 mL of the filtrate. Dilute 5.0 mL of the subsequent filtrate with methanol.
Instrumental conditions
Mode: UV-Vis
Wavelength range: 260–350 nm
Analytical wavelength: Absorption maximum at about 288 nm
Cell: 1 cm
Blank: Methanol
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Record the absorbances of the *Standard solution* and the *Sample solution* against the *Blank*, scanning from 260 to 350 nm.
Calculate the percentage of megestrol acetate ($C_{24}H_{32}O_4$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

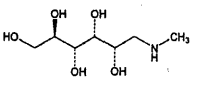
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Megestrol Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of megestrol acetate in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Tablets intended solely for veterinary use are so labeled.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Megestrol Acetate RS

Meglumine



$C_7H_{17}NO_5$ 195.21
D-Glucitol, 1-deoxy-1-(methylamino)-;
1-Deoxy-1-(methylamino)-D-glucitol [6284-40-8].

DEFINITION

Meglumine contains NLT 99.0% and NMT 100.5% of meglumine ($C_7H_{17}NO_5$), calculated on the dried basis.

IDENTIFICATION

• **A.**
Sample solution: Transfer 250 mg to a dry, 50-mL centrifuge tube, add 500 mg of sodium metaperiodate, then add 5 mL of water rapidly in one portion. Allow to stand undisturbed.
Analysis: The solution instantly turns yellow, and heat is produced. The color then changes from deep yellow to orange-brown (rust), and after 20 min, the rust-colored solution is cloudy. Then add 2 mL of 2.5 N sodium hydroxide.
Acceptance criteria: The mixture turns bright yellow and becomes clear.

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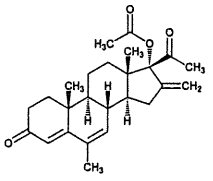
ASSAY
• **PROCEDURE**
Sample: 500 mg
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N hydrochloric acid
Endpoint detection: Visual
Analysis: Transfer the *Sample* into a conical flask, dissolve in 40 mL of water, and add 2 drops of methyl red TS. Titrate with *Titrant*. Each mL of *Titrant* is equivalent to 19.52 mg of meglumine ($C_7H_{17}NO_5$).
Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS
• **ABSENCE OF REDUCING SUBSTANCES**
Sample solution: 50 mg/mL
Analysis: To 5 mL of *Sample solution* add 5 mL of alkaline cupric tartrate TS, and heat to boiling.
Acceptance criteria: The color of the solution does not change.
• **COMPLETENESS AND COLOR OF SOLUTION**
Sample solution: 200 mg/mL
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Analytical wavelength: 420 nm
Cell: 1 cm
Blank: Water
Analysis
Sample: *Sample solution*
Acceptance criteria: Absorbance is NMT 0.030.
• **LOSS ON DRYING** ⟨731⟩
Sample: 1 g
Analysis: Dry the *Sample* at 105° to constant weight.
Acceptance criteria: NMT 1.0%
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 128°–132°
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 100 mg/mL, undried, in water
Acceptance criteria: –15.7° to –17.3°

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Melengestrol Acetate



$C_{25}H_{32}O_4$ 396.52
Pregna-4,6-diene-3,20-dione, 17-(acetyloxy)-6-methyl-16-methylene-.
17-Hydroxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione acetate [2919-66-6].

» Melengestrol Acetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{25}H_{32}O_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Melengestrol Acetate RS
USP Melengestrol Acetate Related Compound A RS
16-Methylene-17α-hydroxy-4-pregnene-3,20-dione 17-acetate.
USP Melengestrol Acetate Related Compound B RS
17α-Hydroxy-6,16-dimethyleneprogna-4-ene-3,20-dione 17-acetate.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: alcohol.
C: The retention time of the melengestrol acetate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Melting temperature ⟨741⟩: between 219° and 226°.
Specific rotation ⟨781S⟩: between –132.0° and –122.0°, at 20°.
Test solution: 10.0 mg per mL, in chloroform.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Related compounds—
Mobile phase—Prepare a mixture of acetonitrile and water (50:50).
Standard solution—Dissolve an accurately weighed quantity of USP Melengestrol Acetate RS, USP Melengestrol Acetate Related Compound A RS, and USP Melengestrol Acetate Related Compound B RS in methanol to obtain a solution having known concentrations of about 0.005 mg of each per mL.
Test solution—Use the *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a multiwavelength detector set at 240 and 262 nm and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure* [NOTE—Melengestrol acetate and melengestrol related compound B will generate larger peak areas at 262 nm than at 240 nm; melengestrol acetate related compound A will generate a larger peak area at 240 nm than at 262 nm] : the relative retention times are about 0.78, 1.0, and 1.05 for melengestrol acetate related compound A, melengestrol acetate, and melengestrol acetate related compound B, respectively; the resolution, *R,* between melengestrol acetate related compound A and melengestrol acetate related compound B is not less than 5.0; the column efficiency for the melengestrol acetate related compound A peak is greater than 1500 theoretical plates; the tailing factor is less than 2.0; and the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, identify the peaks, and determine which detector wavelength generates

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the larger peak area for each impurity. Using the larger peak area, calculate the percentage of each impurity in the portion of Melengestrol Acetate taken by the formula:

$$100(C_S/C_U)(r_i/r_S)$$

in which C_S is the concentration, in mg per mL, of either melengestrol related compound A or melengestrol related compound B in the *Standard solution* [NOTE—If using the impurity peak area generated at 240 nm, C_S is the concentration of melengestrol related compound A; if using the impurity peak area generated at 262 nm, C_S is the concentration of melengestrol related compound B] ; C_U is the concentration, in mg per mL, of melengestrol acetate in the *Test solution;* r_i is the peak area of each impurity obtained from the *Test solution;* and r_S is the peak area of either melengestrol related compound A or melengestrol related compound B obtained from the *Standard solution* [NOTE—If using the impurity peak area generated at 240 nm, r_S is the peak area of melengestrol related compound A; if using the impurity peak area generated at 262 nm, C_S is the peak area of melengestrol related compound B] : not more than 0.5% of any identified impurity is found; not more than 0.2% of any unidentified impurity is found; and not more than 1.0% of total impurities is found.

Assay—

Mobile phase—Prepare a mixture of acetonitrile and water (50:50).

Standard preparation—Dissolve an accurately weighed quantity of USP Melengestrol Acetate RS in methanol to obtain a solution having a known concentration of about 0.5 mg per mL.

Assay preparation—Transfer about 50 mg of Melengestrol Acetate, accurately weighed, to a 100-mL volumetric flask, and dissolve in and dilute with methanol to volume.

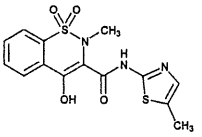
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 287-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure:* the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* in duplicate into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{25}H_{32}O_4$ in the portion of Melengestrol Acetate taken by the formula:

$$2CW(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the *Standard preparation;* W is the weight, in mg, of Melengestrol Acetate used to prepare the *Assay preparation;* r_U is the average peak area of the melengestrol acetate peak obtained from the *Assay preparation;* and r_S is the average peak area of the melengestrol acetate peak obtained from the *Standard preparation.*

Meloxicam



$C_{14}H_{13}N_3O_4S_2$ 351.40

4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide [71125-38-7].

DEFINITION

Meloxicam contains NLT 98.0% and NMT 102.0% of meloxicam ($C_{14}H_{13}N_3O_4S_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K** (CN 1-May-2020)
- **B.** The retention time of the meloxicam peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Solution A: Mixture of a 0.1% (w/v) solution of ammonium acetate adjusted with 10% ammonia solution to a pH of 9.1

Mobile phase: Methanol and *Solution A* (21:29)

Diluent: Methanol and 1 N sodium hydroxide (250:1)

System suitability solution: 0.08 mg/mL each of USP Meloxicam RS and USP Meloxicam Related Compound A RS prepared as follows. Dissolve USP Meloxicam RS and USP Meloxicam Related Compound A RS in 50% of the flask volume of *Diluent,* and dilute with water to volume.

Standard solution: 0.2 mg/mL of USP Meloxicam RS prepared as follows. Dissolve USP Meloxicam RS in 50% of the flask volume of *Diluent,* and dilute with water to volume.

Sample solution: 0.2 mg/mL of Meloxicam prepared as follows. Dissolve Meloxicam in 50% of the flask volume of *Diluent,* and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 360 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for meloxicam related compound A and meloxicam are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between meloxicam related compound A and meloxicam, *System suitability solution*

Tailing factor: NMT 2.0 for the meloxicam peak, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of meloxicam ($C_{14}H_{13}N_3O_4S_2$) in the portion of Meloxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of meloxicam from the *Sample solution*
r_S = peak response of meloxicam from the *Standard solution*
C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES, PROCEDURE 1**

Perform either *Procedure 1* or *Procedure 2*, depending on the manufacturing process used.

Solution A: 0.1% (w/v) solution of monobasic potassium phosphate adjusted with 1 N sodium hydroxide to a pH of 6.0

Solution B: Methanol

Diluent: Methanol and 1 N sodium hydroxide (50:3)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
2	60	40
10	30	70
15	30	70
15.1	60	40
18	60	40

System suitability solution: 0.08 mg/mL each of USP Meloxicam RS, USP Meloxicam Related Compound A RS, and USP Meloxicam Related Compound B RS prepared as follows. Dissolve USP Meloxicam RS, USP Meloxicam Related Compound A RS, and USP Meloxicam Related Compound B RS in 10% of the flask volume of *Diluent*, and dilute with water to volume.

Standard stock solution: 0.6 mg/mL of USP Meloxicam RS prepared as follows. Dissolve USP Meloxicam RS in 25% of the flask volume of *Diluent*, and dilute with methanol to volume.

Standard solution: 0.012 mg/mL of USP Meloxicam RS in methanol from the *Standard stock solution*

Sample solution: 4 mg/mL of Meloxicam prepared as follows. Dissolve Meloxicam in 25% of the flask volume of *Diluent*, and dilute with methanol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 and 350 nm (variable wavelength or multi-wavelength detector)
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 5 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times are listed in *Table 2*.]
Suitability requirements
Resolution: NLT 3.0 between meloxicam related compound A and meloxicam at 350 nm; NLT 3.0 between meloxicam related compound B and meloxicam at 260 nm, *System suitability solution*
Relative standard deviation: NMT 10%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Meloxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of meloxicam at 350 nm from the *Standard solution*
C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

[NOTE—For the specified impurities, calculate the percentage content of each impurity, using the peak responses from the *Sample solution* recorded at the detection wavelength given in *Table 2*. For an unknown impurity, calculate the percentage content, using peak responses recorded at the wavelength that gives the greater response.]

Acceptance criteria: See *Table 2*.

Table 2 (Meloxicam)

Name	Relative Retention Time	Wavelength (nm)	Relative Response Factor	Acceptance Criteria, NMT (%)
Meloxicam related compound B[a]	0.4	260	1.0	0.1
Meloxicam	1.0	—	—	—
Meloxicam related compound A[b]	1.4	350	0.5	0.1
Methyl-meloxicam[c]	1.7	350	1.0	0.05
Ethyl-meloxicam[d]	1.9	350	1.0	0.05
Individual unknown impurity	—	260/350	1.0	0.1
Total impurities	—	—	—	0.3

[a] 5-Methylthiazol-2-amine.
[b] Ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide.
[c] *N*-[3,5-Dimethylthiazol-2(3*H*]-ylidene)-4-hydroxy-2-methyl-2*H*-benzo[*e*][1,2]thiazine-3-carboxamide 1,1-dioxide.
[d] *N*-[3-Ethyl-5-methylthiazol-2(3*H*)-ylidene]-4-hydroxy-2-methyl-2*H*-benzo[*e*][1,2]thiazine-3-carboxamide 1,1-dioxide.

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• **ORGANIC IMPURITIES, PROCEDURE 2**

If an article complies with this test, the labeling indicates that it meets the requirements under *Organic Impurities, Procedure 2*.

Solution A and **Solution B:** Proceed as directed in *Procedure 1*.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	45	55
25	45	55
30	30	70
40	30	70
45	45	55
50	45	55

Diluent A: *Diluent B* and 0.4 N sodium hydroxide (50:3)

Diluent B: Methanol and water (2:3)

Standard stock solution A: 0.01 mg/mL of USP Meloxicam RS prepared as follows. Dilute a solution of 0.05 mg/mL of USP Meloxicam RS in *Diluent A* with *Diluent B*.

Standard stock solution B: 0.05 mg/mL each of USP Meloxicam Related Compound B RS and USP Meloxicam Related Compound C RS prepared as follows. Transfer suitable amounts of USP Meloxicam Related Compound B RS and USP Meloxicam Related Compound C RS to an adequate volumetric flask. Add 0.4 N sodium hydroxide to 6% of the flask volume, and sonicate for 2 min. Add an additional 40% of the flask volume of methanol, sonicate for 2 min, and dilute with water to volume.

Standard solution: 0.001 mg/mL of USP Meloxicam RS and 0.0015 mg/mL each of USP Meloxicam Related Compound B RS and USP Meloxicam Related Compound C RS prepared as follows. Transfer suitable volumes of *Standard stock solution A* and *Standard stock solution B* to an adequate volumetric flask, and dilute with *Diluent B* to volume.

Sample solution: 1 mg/mL of Meloxicam prepared as follows. Dissolve a suitable amount of Meloxicam with 50% of the flask volume of *Diluent A*, and dilute with *Diluent B* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV variable wavelength or multi-wavelength detector at 260 and 350 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times are listed in *Table 4*.]

Suitability requirements

Relative standard deviation: NMT 5.0% for meloxicam, meloxicam related compound B, and meloxicam related compound C

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Meloxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of the corresponding related compound from the *Standard solution*

C_S = concentration of the corresponding USP Related Compound RS in the *Standard solution* (mg/mL). [NOTE—Use the concentration of USP Meloxicam RS for unknown impurities.]

C_U = concentration of the *Sample solution* (mg/mL)

[NOTE—Use the peak response and concentration of USP Meloxicam RS for unknown impurities; for the specified impurities, calculate the percentage content of each impurity using the *Sample solution* peak responses recorded at the detection wavelength given in *Table 4*. For an unknown impurity, calculate the percentage content using peak responses recorded at the wavelength that gives the greater response.]

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Meloxicam	1.0	350	—
Meloxicam related compound B[a]	0.8	260	0.1
Meloxicam related compound C[b]	3.2	350	0.1
Individual unknown impurity	—	260/350	0.1
Total impurities	—	—	0.3

[a] 5-Methylthiazol-2-amine.

[b] Isopropyl-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate-1,1-dioxide.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

• **LABELING:** The labeling states with which *Procedure* under *Organic Impurities* the article complies if a test other than *Procedure 1* is used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Meloxicam RS

USP Meloxicam Related Compound A RS

Ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide.

$C_{12}H_{13}NO_5S$ 283.30

USP Meloxicam Related Compound B RS

5-Methylthiazol-2-amine.

$C_4H_6N_2S$ 114.175

USP Meloxicam Related Compound C RS

Isopropyl-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate-1,1-dioxide.

$C_{13}H_{15}NO_5S$ 297.33

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Meloxicam Oral Suspension

DEFINITION

Meloxicam Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of meloxicam ($C_{14}H_{13}N_3O_4S_2$).

IDENTIFICATION

- **A.** The UV absorption spectrum of the meloxicam peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 2 g of *monohydrate citric acid* and 2 g of boric acid in 1000 mL of *water*, and adjust with dihydrate trisodium citrate to a pH of 2.9.

Solution A: *Acetonitrile*, *methanol*, and *Buffer* (200:260:565)

Mobile phase: Dissolve 200 mg of sodium dodecyl sulfate in 1000 mL of *Solution A*.

Diluent: Dissolve 3 g of boric acid and 1.5 g of dihydrate trisodium citrate in 1000 mL of *water*, and adjust with 2 M sodium hydroxide to a pH of 8.3. Mix 420 mL of the resulting buffer with 420 mL of *methanol* and 160 mL of *acetonitrile*.

Related compound standard stock solution: 8.4 µg/mL of USP Meloxicam Related Compound B RS prepared as follows. Transfer 21 mg of USP Meloxicam Related Compound B RS into a 100-mL volumetric flask. Add 3.0 mL of dimethylformamide, 15 mL of *methanol*, and about 60 mL of *Diluent*. Sonicate, and mix until dissolved. Cool to room temperature. Dilute with *Diluent* to volume. Dilute further with *Diluent* to a concentration of 8.4 µg/mL.

System suitability solution: Transfer a volume of Oral Suspension, nominally equivalent to 15 mg of meloxicam, to a 50-mL volumetric flask. Add 3.0 mL of *Related compound standard stock solution*. Add 3.0 mL of dimethylformamide. Swirl the flask, and allow to stand for 5 min. Add 15 mL of *methanol*. Dilute with *Diluent* to just below volume. Sonicate for 30 min, mixing the flask vigorously about every 5 min. Cool to room temperature. Dilute with *Diluent* to volume. Mix, and allow particulates to settle. Pass through a 0.45-µm membrane filter with a fiberglass prefilter.

Standard stock solution: Transfer about 67 mg of USP Meloxicam RS into a 100-mL volumetric flask. Add 3.0 mL of dimethylformamide. Swirl the flask, and allow to stand for 5 min. Add 15 mL of *methanol*. Dilute with *Diluent* to just below volume. Sonicate for 30 min, and mix until dissolved. Cool to room temperature. Dilute with *Diluent* to volume.

Standard solution: 0.3 mg/mL of USP Meloxicam RS in *Diluent* from *Standard stock solution*

Sample solution: Nominally 0.3 mg/mL of meloxicam prepared as follows. Transfer a volume of Oral Suspension, nominally equivalent to 15 mg of meloxicam, to a 50-mL volumetric flask. Add 3.0 mL of dimethylformamide. Swirl the flask, and allow to stand for about 5 min. Add 15 mL of methanol. Dilute with *Diluent* to just below volume. Sonicate for 30 min, mixing the flask vigorously about every 5 min. Cool to room temperature. Dilute with *Diluent* to volume. Mix, and allow particulates to settle. Pass through a 0.45-µm membrane filter with a fiberglass prefilter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 360 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 4-mm × 12.5-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Run time: NLT 2 times the retention time of meloxicam

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between meloxicam and any other adjacent peak, *System suitability solution*

Tailing factor: NMT 2.0 for the meloxicam peak, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of meloxicam ($C_{14}H_{13}N_3O_4S_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of meloxicam from the *Sample solution*

r_S = peak area of meloxicam from the *Standard solution*

C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of meloxicam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: pH 7.5 *phosphate buffer*; 900 mL

Apparatus 2: 25 rpm

Time: 15 min

Standard solution: Transfer about 20.83 mg of USP Meloxicam RS into a 100-mL volumetric flask. Dissolve in 5 mL of *methanol* and 1 mL of 0.1 M sodium hydroxide, and dilute with *Medium* to volume. Dilute with *Medium* to a final concentration of 8.3 µg/mL of meloxicam.

Sample solution: Shake each sample for 15 min. Weigh six portions, equivalent to 7.5 mg of the Oral Suspension, into separate tared 10-mL beakers, and record each weight. Introduce each of the samples to the middle of the dissolution vessels, and rinse each beaker with 20 mL of the *Medium* withdrawn from the vessel. Carefully lower the paddle to the appropriate height and start the rotation. After completion of the dissolution, pass a 20-mL aliquot through a nylon filter having 0.45-µm porosity, discarding the first 3 mL of the filtrate.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: At about 362 nm (wavelength of maximum absorbance)

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of meloxicam ($C_{14}H_{13}N_3O_4S_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (1/W_U) \times (1/L) \times d \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)
W_U = weight of the Oral Suspension taken (mg)
L = label claim (mg/mL)
d = density of the Oral Suspension (g/mL)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of meloxicam ($C_{14}H_{13}N_3O_4S_2$) is dissolved.

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Solution A, Mobile phase, Diluent, Related compound standard stock solution, and Sample solution: Proceed as directed in the *Assay*.

Sensitivity solution: 0.08 µg/mL of USP Meloxicam Related Compound B RS in *Diluent* from *Related compound standard stock solution*

Standard solution: 0.5 µg/mL of USP Meloxicam Related Compound B RS in *Diluent* from *Related compound standard stock solution*

Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.

Detector: UV 260 and 360 nm

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the meloxicam related compound B peak at 260 nm, *Standard solution*

Relative standard deviation: NMT 10% for meloxicam related compound B at 260 nm, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of meloxicam related compound B in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of meloxicam related compound B in the *Sample solution* at 260 nm
r_S = peak area of meloxicam related compound B in the *Standard solution* at 260 nm
C_S = concentration of USP Meloxicam Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of meloxicam in the *Sample solution* (mg/mL)

Calculate the percentage of each unknown degradation product in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of any unknown degradation product in the *Sample solution* at 360 nm
r_T = sum of peak areas of meloxicam and all impurities in the *Sample solution* at 360 nm

Acceptance criteria

Meloxicam related compound B: NMT 0.15%

Any individual unknown degradation product: NMT 0.2%

Total degradation products: NMT 0.5%

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g or 10^2 cfu/mL. The total yeasts and molds count does not exceed 5×10^1 cfu/g or 5×10^1 cfu/mL. It meets the requirements of the test for the absence of *Escherichia coli*.

• **pH** ⟨791⟩**:** 3.5–4.5

• **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩

Analysis: Determine at 20° by using a shear rate programmable rotational viscometer.

Acceptance criteria: 40–100 centipoises

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Meloxicam RS
USP Meloxicam Related Compound B RS
5-Methylthiazol-2-amine.
$C_4H_6N_2S$ 114.175

Meloxicam Tablets

DEFINITION

Meloxicam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of meloxicam ($C_{14}H_{13}N_3O_4S_2$).

IDENTIFICATION

Change to read:

• **A:** ▲The UV absorption spectrum of the meloxicam peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

• **B:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

Solution A: Dissolve 2.0 g of dibasic ammonium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 7.0 ± 0.1.

Solution B: Methanol and isopropyl alcohol (65:10)

Mobile phase: *Solution B* and *Solution A* (37:63)

▲Standard stock solution 1

For Tablets labeled to contain 7.5 mg/Tablet: 0.75 mg/mL of USP Meloxicam RS, prepared as follows. Transfer a quantity of USP Meloxicam RS to a suitable volumetric flask, dissolve with 2% of the final volume of 1 N sodium hydroxide and 60% of the final volume of methanol, and dilute with methanol to volume.

For Tablets labeled to contain 15 mg/Tablet: 1.5 mg/mL of USP Meloxicam RS, prepared as follows. Transfer a quantity of USP Meloxicam RS to a suitable volumetric flask, dissolve with 2% of the final volume of 1 N sodium hydroxide and 60% of the final volume of methanol, and dilute with methanol to volume.

Standard stock solution 2

For Tablets labeled to contain 7.5 mg/Tablet: 0.075 mg/mL of USP Meloxicam RS, prepared as follows. Transfer a suitable volume of *Standard stock solution 1* to an adequate volumetric flask, add 10% of the final volume of 1 N sodium hydroxide, and dilute with methanol to volume.

USP Monographs

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For Tablets labeled to contain 15 mg/Tablet: 0.15 mg/mL of USP Meloxicam RS, prepared as follows. Transfer a suitable volume of *Standard stock solution 1* to a suitable volumetric flask, add 10% of the final volume of 1 N sodium hydroxide, and dilute with methanol to volume. ▲2S (USP41)

Standard solution

▲For Tablets labeled to contain 7.5 mg/Tablet: 0.045 mg/mL of USP Meloxicam RS in water from *Standard stock solution 2*

For Tablets labeled to contain 15 mg/Tablet: 0.09 mg/mL of USP Meloxicam RS in water from *Standard stock solution 2* ▲2S (USP41)

Sample stock solution: Transfer 10 Tablets to a 1000-mL volumetric flask, add about 100 mL of 1 N sodium hydroxide, shake to disperse the Tablets, and add 800 mL of methanol. Sonicate the solution for 15 min, then stir for 30 min. Dilute with methanol to volume. Filter the resulting solution, and use the filtrate. ▲The nominal concentration of this solution is 0.075 mg/mL of meloxicam for Tablets labeled to contain 7.5 mg/Tablet and 0.15 mg/mL of meloxicam for Tablets labeled to contain 15 mg/Tablet. ▲2S (USP41)

Sample solution

▲For Tablets labeled to contain 7.5 mg/Tablet: Nominally 0.045 mg/mL of meloxicam in water from the *Sample stock solution*

For Tablets labeled to contain 15 mg/Tablet: Nominally 0.09 mg/mL of meloxicam in water from the *Sample stock solution* ▲2S (USP41)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm. ▲2S (USP41)

Columns

Guard: ▲4-mm × 1-cm; 10-µm ▲2S (USP41) packing L1

Analytical: 4-mm × 10-cm; ▲10-µm ▲2S (USP41) packing L1

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of meloxicam ($C_{14}H_{13}N_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of meloxicam from the *Sample solution*

r_S = peak response of meloxicam from the *Standard solution*

C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of meloxicam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Medium: pH 7.5 phosphate buffer (dissolve 6.81 g of monobasic potassium phosphate in 800 mL of water, adjust with 0.5 N sodium hydroxide to a pH of 7.5, and dilute with water to 1 L); 900 mL

Time: 30 min

Apparatus 2: 75 rpm

▲Standard stock solution: 0.333 mg/mL of USP Meloxicam RS, prepared as follows. Transfer a quantity of USP Meloxicam RS to a suitable volumetric flask. Add 5% of the total volume of methanol, 1% of the total volume of 0.1 N sodium hydroxide, and dilute with *Medium* to volume.

Standard solution

For Tablets labeled to contain 7.5 mg/Tablet: 0.008325 mg/mL of USP Meloxicam RS in *Medium* from the *Standard stock solution*

For Tablets labeled to contain 15 mg/Tablet: 0.01665 mg/mL of USP Meloxicam RS in *Medium* from the *Standard stock solution* ▲2S (USP41)

Sample solution: Pass portions of the solution under test through a suitable filter of 10-µm pore size. Discard the first few milliliters.

Instrumental conditions

Mode: UV

Analytical wavelength: 362 nm (maximum absorbance)

Cell: 1 cm

Blank: *Medium*

Analysis

Samples: ▲*Standard solution* ▲2S (USP41) and *Sample solution*

Calculate the percentage of the labeled amount of meloxicam dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from the ▲*Standard solution* ▲2S (USP41)

C_S = concentration of USP Meloxicam RS in the ▲*Standard solution* ▲2S (USP41) (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of meloxicam is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, ▲Standard stock solution 1, Standard stock solution 2, ▲2S (USP41) **Standard solution, ▲Sample stock solution,** ▲2S (USP41) **Sample solution,** and **Chromatographic system:** *Assay*

▲Diluent: Methanol, water, and 1 N sodium hydroxide (54:40:6)

Sensitivity solution: 0.000045 mg/mL of USP Meloxicam RS in *Diluent* from the *Standard solution* ▲2S (USP41)

System suitability

Samples: *Standard solution* and ▲*Sensitivity solution* ▲2S (USP41)

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Suitability requirements
Tailing factor: NMT 2.0, ▲*Standard solution*▲ 2S (USP41)
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, ▲*Sensitivity solution*▲ 2S (USP41)
Analysis
Samples: *Standard solution* and *Sample solution* ▲▲ 2S (USP41)
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of meloxicam from the *Standard solution*
C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of meloxicam in the *Sample solution* (mg/mL)
F = relative response factor ▲(see *Table 1*)▲ 2S (USP41)

Acceptance criteria: ▲See *Table 1*.

Table 1

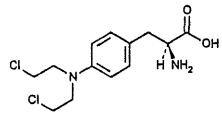
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Methylthiazol-2-amine (meloxicam related compound B)	0.5	2.7	0.15
Meloxicam	1.0	—	—
Individual unknown impurity	—	1.0	0.2
Total impurities	—	—	0.5▲ 2S (USP41)

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in well-closed containers ▲in a dry place.▲ 2S (USP41) Store at ▲controlled room temperature.▲ 2S (USP41)
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Meloxicam RS

Melphalan



$C_{13}H_{18}Cl_2N_2O_2$ 305.20
L-Phenylalanine, 4-bis(2-chloroethyl)amino]-.
L-3-[*p*-[Bis(2-chloroethyl)amino]phenyl]alanine [148-82-3].

» Melphalan contains not less than 93.0 percent and not more than 100.5 percent of $C_{13}H_{18}Cl_2N_2O_2$, calculated on the dried and ionizable chlorine-free basis.
[**CAUTION**—Handle Melphalan with exceptional care because it is a highly potent agent.]

Packaging and storage—Preserve in tight, light-resistant, glass containers.
USP Reference standards ⟨11⟩—
USP Melphalan Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 5 µg per mL.
Medium: methanol.
B: To 1 mL of 1 in 10,000 solution in alcohol in a glass-stoppered test tube add 1 mL of pH 4.0 acid phthalate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*), 1 mL of a 1 in 20 solution of 4-(*p*-nitrobenzyl) pyridine in acetone, and 1 mL of saline TS. Heat on a water bath at 80° for 20 minutes, and cool quickly. Add 10 mL of alcohol and 1 mL of 1 N potassium hydroxide: a violet to red-violet color is produced.
C: Heat 100 mg with 10 mL of 0.1 N sodium hydroxide on a water bath for 10 minutes: the resulting solution, after acidification with 2 N nitric acid, responds to the tests for *Chloride* ⟨191⟩.
Specific rotation ⟨781S⟩: between −30° and −36°.
Test solution: 7 mg per mL, in methanol, prepared with the aid of gentle heating.
Loss on drying ⟨731⟩: Dry it in vacuum at 105° to constant weight: it loses not more than 7.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.3%.
Ionizable chlorine—Dissolve about 500 mg of Melphalan, accurately weighed, in a mixture of 75 mL of water and 2 mL of nitric acid, allow to stand for 2 minutes, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically: not more than 1.0 mL of 0.1 N silver nitrate is required for each 500 mg of test specimen.
Nitrogen Determination ⟨461⟩—Determine the nitrogen content as directed under *Method II*, using about 325 mg of Melphalan, accurately weighed, and 0.1 N sulfuric acid VS for the titration: not less than 8.90% and not more than 9.45% of N is found, calculated on the dried basis.
Assay—Transfer to a beaker about 200 mg of Melphalan, accurately weighed, and dissolve in 20 mL of 0.5 N sodium hydroxide. Cover the beaker with a watch glass, and boil the solution for 30 minutes, adding water as necessary to maintain the volume. Cool, neutralize to phenolphthalein TS with acetic acid, and add 1 mL of acetic acid in excess. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using silver and calomel electrodes, the latter modified to contain saturated potassium sulfate solution. From the results obtained in the test for *Ionizable chlorine*, calculate the volume, in mL, of 0.1 N silver nitrate that is equivalent to the ionizable chlorine in the quantity of Melphalan taken for the *Assay*, and subtract it from the *Assay* titration volume. Each mL of 0.1 N silver nitrate is equivalent to 15.26 mg of $C_{13}H_{18}Cl_2N_2O_2$.

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Melphalan Tablets

» Melphalan Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of melphalan ($C_{13}H_{18}Cl_2N_2O_2$).

Packaging and storage—Preserve in well-closed, light-resistant, glass containers.
USP Reference standards ⟨11⟩—
USP Melphalan Hydrochloride RS

Identification—
A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
B: Shake a portion of finely powdered Tablets, equivalent to about 2 mg of melphalan, with 20 mL of alcohol, and filter: a 1-mL portion of the solution so obtained responds to *Identification* test *B* under *Melphalan.*

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Determine the amount of $C_{13}H_{18}Cl_2N_2O_2$ dissolved by employing the following method.
Mobile phase—Transfer 2 grams of ammonium acetate, 2 mL of glacial acetic acid, and 0.4 mL of triethylamine to a suitable flask containing 1500 mL of water and 500 mL of acetonitrile. Stir until all solids are dissolved and well mixed, then filter and degas.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 5-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the Standard solution, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 3.0%.
Procedure—Inject a volume (about 50 µL) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of $C_{13}H_{18}Cl_2N_2O_2$ dissolved in comparison with a Standard solution having a known concentration of USP Melphalan Hydrochloride RS in the same *Medium* and similarly chromatographed.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{13}H_{18}Cl_2N_2O_2$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Place 1 Tablet in a 200-mL volumetric flask, add 10 mL of water and 10 mL of alcohol, sonicate to dissolve the soluble components in the mixture, dilute with alcohol to volume, mix, and filter to obtain a clear solution. Dissolve an accurately weighed quantity of USP Melphalan Hydrochloride RS in alcohol to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of the solution from the Tablet and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 260 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of melphalan ($C_{13}H_{18}Cl_2N_2O_2$) in the Tablet taken by the formula:

$$(305.20/341.66)(T/D)C(A_U/A_S)$$

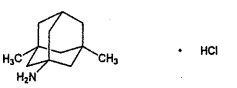
in which 305.20 and 341.66 are the molecular weights of melphalan and melphalan hydrochloride, respectively; T is the labeled quantity, in mg, of melphalan in the Tablet; D is the concentration, in µg per mL, of melphalan in the solution from the Tablet, on the basis of the labeled quantity per Tablet and the extent of dilution; C is the concentration, in µg per mL, of USP Melphalan Hydrochloride RS in the Standard solution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—
Mobile phase—Prepare a solution of 0.025 M diethylamine in a mixture of methanol and water (1:1), adjust with 3.5 N hydrochloric acid to a pH of 5.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Melphalan Hydrochloride RS in alcohol, and quantitatively dilute with alcohol to obtain a solution having a known concentration of about 0.9 mg of melphalan hydrochloride per mL. Pipet 10 mL of this solution into a 100-mL volumetric flask containing 75 mL of alcohol and 2.0 mL of glacial acetic acid, dilute with alcohol to volume, and mix to obtain a *Standard preparation* having a known concentration of about 90 µg of USP Melphalan Hydrochloride RS per mL (equivalent to about 80 µg of melphalan per mL).
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 8 mg of anhydrous melphalan, to a 100-mL volumetric flask. Add about 75 mL of alcohol and 2.0 mL of glacial acetic acid to the flask, and sonicate for 15 minutes. Cool, dilute with alcohol to volume, and mix. Filter through a medium-porosity, sintered-glass funnel, discarding the first few mL of the filtrate, and use the remainder of the filtrate as the *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.2-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (between 10 and 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of melphalan ($C_{13}H_{18}Cl_2N_2O_2$) in the portion of Tablets taken by the formula:

$$(305.20/341.67)(0.1C)(r_U/r_S)$$

in which 305.20 and 341.67 are the molecular weights of melphalan and melphalan hydrochloride, respectively; C is the concentration, in µg per mL, of melphalan hydrochloride in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Memantine Hydrochloride



$C_{12}H_{21}N \cdot HCl$ 215.76
Tricyclo[3.3.1.1^{3,7}]decan-1-amine, 3,5-dimethyl-, hydrochloride;

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1-Amino-3,5-dimethyladamantane hydrochloride [41100-52-1].

DEFINITION

Memantine Hydrochloride contains NLT 98.0% and NMT 102.0% of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Internal standard solution: 4.0 mg/mL of adamantane in *n*-hexane

Standard solution: 4.0 mg/mL of USP Memantine Hydrochloride RS in *Internal standard solution* prepared as follows. Transfer 100 mg of USP Memantine Hydrochloride RS to a 50-mL centrifuge tube. Add 15 mL of 1 N sodium hydroxide, and mix. Add 25 mL of *Internal standard solution*, and shake for 15 min. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear filtrate.

Sample solution: 4.0 mg/mL of Memantine Hydrochloride in *Internal standard solution* prepared as follows. Transfer 100 mg of Memantine Hydrochloride to a 50-mL centrifuge tube. Add 15 mL of 1 N sodium hydroxide, and mix. Add 25 mL of *Internal standard solution*, and shake for 15 min. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 50-m × 0.32-mm; 0.52-µm packing G27

Temperatures

Injection port: 220°

Detector: 300°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	5	145	0
145	10	250	20

Carrier gas: Helium

Flow rate: 4.0 ± 0.4 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 1:50

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 each for memantine and adamantane

Relative standard deviation: NMT 2.0% for the ratio of the peak areas of adamantane and memantine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$) in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of memantine to the internal standard from the *Sample solution*

R_S = peak response ratio of memantine to the internal standard from the *Standard solution*

C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

Standard stock solution A: 2.5 mg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, and USP Memantine Related Compound E RS in *n*-hexane

Standard stock solution B: 2.5 mg/mL of USP Memantine Hydrochloride RS prepared as follows. To the flask containing a weighed amount of USP Memantine Hydrochloride RS, add 5.0 N sodium hydroxide to fill 20% of the final volume and *n*-hexane to fill 20% of the final volume. Shake for 10 min, and transfer the contents to a separator. Allow the layers to separate, filter a portion of the top hexane layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure all the remaining water has been removed. Use the clear filtrate.

System suitability solution: 25 µg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, and USP Memantine Related Compound E RS, from *Standard stock solution A* in *Standard stock solution B*. The concentration of USP Memantine Hydrochloride RS is 2.5 mg/mL.

Standard solution: 25 µg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, USP Memantine Related Compound E RS, and USP Memantine Hydrochloride RS, from *Standard stock solution A* and *Standard stock solution B*, respectively, in *n*-hexane

Sample solution: 25 mg/mL of Memantine Hydrochloride prepared as follows. Transfer the weighed amount of Memantine Hydrochloride to a suitable volumetric flask. Add 5.0 N sodium hydroxide to fill 30% of the final volume and *n*-hexane to fill 40% of the final volume. Shake for 10 min, and transfer the contents to a separator. Allow the layers to separate, filter a portion of the top hexane layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure all the remaining water has been removed. Use the clear filtrate.

Chromatographic system: Proceed as directed in the *Assay*.

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System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 6.0 between memantine and memantine related compound B; NLT 2.0 between memantine related compound B and memantine related compound C, *System suitability solution*
Tailing factor: NMT 2.0 for memantine, *Standard solution*
Relative standard deviation: NMT 10.0% for memantine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Ignore the peaks at the relative retention times 0.11, 0.12, 0.13, 0.18, and 0.26 with respect to the memantine peak, as they correspond to residual solvents.]
Calculate the percentage of each of memantine related compounds A, B, C, D, and E in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of memantine related compounds A, B, C, D, or E from the *Sample solution*
r_S = peak response of the corresponding USP Memantine Related Compound RS from the *Standard solution*
C_S = concentration of the corresponding USP Memantine Related Compound RS in the *Standard solution* (mg/mL)
C_U = concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other impurity from the *Sample solution*
r_S = peak response of memantine hydrochloride from the *Standard solution*
C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Memantine related compound A	0.77	0.15
Memantine	1.0	—
Memantine related compound B	1.03	0.15
Memantine related compound C	1.07	0.15
Memantine related compound D	1.19	0.15
Memantine related compound E	1.44	0.15

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Memantine Hydrochloride RS
USP Memantine Related Compound A RS
1,3-Dimethyladamantane.
$C_{12}H_{20}$ 164.29
USP Memantine Related Compound B RS
3,5-Dimethyladamantane-1-ol.
$C_{12}H_{20}O$ 180.29
USP Memantine Related Compound C RS
1-Chloro-3,5-dimethyladamantane.
$C_{12}H_{19}Cl$ 198.73
USP Memantine Related Compound D RS
1-Bromo-3,5-dimethyladamantane.
$C_{12}H_{19}Br$ 243.18
USP Memantine Related Compound E RS
N-3,5-Dimethyladamantan-1-yl formamide.
$C_{13}H_{21}NO$ 207.31

Memantine Hydrochloride Tablets

DEFINITION
Memantine Hydrochloride Tablets contain an amount of memantine hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
Analytical range: 4000–400 cm^{-1}
Standard: 6.7 mg/mL of USP Memantine Hydrochloride RS in dichloromethane. Shake for 10 min, and pass through a suitable filter. Evaporate the solvent at room temperature. Collect the residue powder, and dry at 60° for 15 min. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.
Sample: 6.7 mg/mL of memantine hydrochloride in dichloromethane from NLT 20 crushed Tablets. Shake for 10 min, and centrifuge for 10 min. Pass the supernatant through a suitable filter. Evaporate the solvent at room temperature. Collect the residue powder, and dry at 60° for 15 min. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.
Acceptance criteria: Fingerprint region of the *Standard* and *Sample* spectrum exhibit maxima at the same wave numbers.
• **B.** The retention time of the memantine peak of the *Sample solution* corresponds to that of the memantine peak of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

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ASSAY

• **PROCEDURE**

Solution A: 200 mg/mL of sodium hydroxide in water

Internal standard solution: 25 µg/mL of USP Amantadine Hydrochloride RS in water

Standard stock solution: 25 µg/mL of USP Memantine Hydrochloride RS prepared as follows. Weigh a suitable quantity of the Standard into a volumetric flask. Add methanol to fill 40% of the final flask volume, and sonicate. Dilute with water to volume.

Standard solution: Pipet 4.0 mL each of the *Internal standard solution* and the *Standard stock solution* into a test tube. Add 2 mL of *Solution A*, and mix on a vortex mixer for 1 min. Add 4.0 mL of toluene, and mix on a vortex mixer for 3 min. Allow the two layers to separate. Inject the toluene layer.

Sample stock solution: Nominally 20 µg/mL of memantine hydrochloride prepared as follows. Transfer a suitable number of Tablets to a volumetric flask to obtain a 0.1 mg/mL memantine hydrochloride solution. Add methanol to fill 40% of the final flask volume, and sonicate for 30 min with intermittent shaking. Add water to fill 40% of the final flask volume, and sonicate for 30 min with intermittent shaking. Dilute with water to volume, and centrifuge a portion for 10 min. Pipet a suitable volume of the clear centrifugate into a volumetric flask, and dilute with water to volume.

Sample solution: Pipet 5.0 mL of the *Sample stock solution*, 4.0 mL of the *Internal standard solution*, and 2 mL of *Solution A* into a test tube, and mix on a vortex mixer for 1 min. Add 4.0 mL of toluene, and mix on a vortex mixer for 5 min. Allow the two layers to separate. Inject the toluene layer.

Blank: To 5.0 mL of 80 µL/mL of methanol in water add 2 mL of *Solution A*, and mix on a vortex mixer for 1 min. Add 4.0 mL of toluene, and mix on a vortex mixer for 5 min. Allow the two layers to separate. Inject the toluene layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.32-mm; 0.25-µm packing G27

Temperatures

Injection port: 210°

Detector: 300°

Oven: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	2
50	20	140	0
140	30	200	5

Carrier gas: Helium

Flow rate: 34.8 psi

Injection volume: 4 µL

Injection type: Split ratio, 1:10

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for amantadine and memantine are 0.97 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between amantadine and memantine

Tailing factor: NMT 2.5 for amantadine; NMT 2.0 for memantine

Relative standard deviation: NMT 2.0% for the ratio of the peak areas of amantadine and memantine

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of memantine to amantadine from the *Sample solution*

R_S = peak area ratio of memantine to amantadine from the *Standard solution*

C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of memantine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid with sodium chloride (2 g/L of sodium chloride in water), adjusted with hydrochloric acid to a pH of 1.2; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard stock solution: (*L*/900) mg/mL of USP Memantine Hydrochloride RS in *Medium*, where *L* is the label claim in mg/Tablet

Internal standard solution: 28 µg/mL of USP Amantadine Hydrochloride RS in *Medium*

Standard solution

For Tablets labeled to contain 5 mg: Transfer 5 mL of the *Standard stock solution* to a test tube, add 1 mL of the *Internal standard solution* and 2 mL of 5 N sodium hydroxide, and mix for 1 min. Add 3 mL of toluene, and mix for 2 min. Use the toluene layer.

For Tablets labeled to contain 10 mg: Transfer 5 mL of the *Standard stock solution* to a test tube, add 2 mL of the *Internal standard solution* and 2 mL of 5 N sodium hydroxide, and mix for 1 min. Add 3 mL of toluene, and mix for 2 min. Use the toluene layer.

Sample solution: Pass a portion of the solution under test through a suitable filter.

For Tablets labeled to contain 5 mg: Transfer 5 mL of the filtrate to a test tube, add 1 mL of the *Internal standard solution* and 2 mL of 5 N sodium hydroxide, and mix for 1 min. Add 3 mL of toluene, and mix for 2 min. Use the toluene layer.

For Tablets labeled to contain 10 mg: Transfer 5 mL of the filtrate to a test tube, add 2 mL of the *Internal standard solution* and 2 mL of 5 N sodium hydroxide, and mix for 1 min. Add 3 mL of toluene, and mix for 2 min. Use the toluene layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC, splitless

Detector: Flame ionization

Column: 30-m × 0.32-mm; 0.25-µm packing G27

Flow rate: 34.8 psi

Temperatures

Injection port: 210°

Detector: 300°

Oven: See *Table 2*.

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Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	2
50	20	140	0
140	30	200	5

Carrier gas: Helium
Injection volume: 4 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between amantadine and memantine
Tailing factor: NMT 2.0 each for amantadine and memantine
Relative standard deviation: NMT 2.0% for the ratio of memantine to amantadine peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$) dissolved:

$$\text{Result} = (R_U/R_S) \times (C_S/L) \times V \times 100$$

R_U = peak area ratio of memantine to amantadine from the *Sample solution*
R_S = peak area ratio of memantine to amantadine from the *Standard solution*
C_S = concentration of USP Memantine Hydrochloride RS in the *Standard stock solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Limit of Memantine–Lactose Adduct**
[Note—Perform this test if lactose is present in the formulation.]
Solution A: 40 mg/mL of sodium hydroxide in water
Buffer: Dissolve 3.3 g of monobasic potassium phosphate and 2.3 g of sodium 1-octane sulfonate in 1 L of water. Adjust with *Solution A* to a pH of 6.1.
Mobile phase: Acetonitrile, methanol, and *Buffer* (26:4:70)
Standard solution: 0.2 mg/mL of USP Memantine Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 10 mg/mL of memantine hydrochloride from NLT 25 crushed Tablets, prepared as follows. Transfer an amount of powder equivalent to 100 mg of memantine hydrochloride to a 20-mL volumetric flask. Add 10 mL of *Mobile phase*, and sonicate for 30 min. Centrifuge, and pass a portion of the centrifugate through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 40°
Detector: 35°
Flow rate: 1.3 mL/min
Injection volume: 50 µL
Run time: 1.3 times the retention time of the memantine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 3.5
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the memantine–lactose adduct in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the memantine–lactose adduct from the *Sample solution*
r_S = peak response of memantine from the *Standard solution*
C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of memantine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor of the memantine–lactose adduct (see *Table 3*)

Acceptance criteria: See *Table 3*.
Disregard all peaks other than the memantine–lactose adduct peak.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Memantine–lactose adduct	0.41	0.53	1.4
Memantine	1.0	1.0	—

• **Organic Impurities**
Solution A: 1 N sodium hydroxide
System suitability stock solution A: 0.5 mg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, and USP Memantine Related Compound E RS in *n*-hexane
System suitability stock solution B: Transfer 75 mg of USP Memantine Hydrochloride RS into a suitable container, add 9 mL of 1.0 N sodium hydroxide and 6 mL of *n*-hexane, and mix for 10 min.
System suitability solution: Pipet 4.0 mL of the *n*-hexane layer from *System suitability stock solution B* into a 10-mL volumetric flask. Add 0.5 mL of *System suitability stock solution A*, and dilute with *n*-hexane to volume.
Standard stock solution: 1.3 mg/mL of USP Memantine Hydrochloride RS in *n*-hexane prepared as follows. Weigh a suitable quantity of the Standard into a volumetric flask. Add *Solution A* to fill 30% of the final flask volume, and mix for 5 min. Add *n*-hexane to fill 40% of the final flask volume, and shake for 10 min. Transfer the contents of the flask into a separator. Allow the layers to separate, and filter a portion of the top *n*-hexane layer through anhydrous sodium sulfate. Use the clear solution.
Standard solution: Pipet 2.0 mL of the clear solution from the *Standard stock solution* into a 100-mL volumetric flask, and dilute with *n*-hexane to volume.

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Sample solution: Nominally 5 mg/mL of memantine hydrochloride in *n*-hexane from NLT 20 crushed Tablets, prepared as follows. Transfer a weighed amount of powder equivalent to 100 mg of memantine hydrochloride to a suitable volumetric flask. Add *Solution A* to fill 15% of the final flask volume. Shake to disperse the material, and then shake for 5 min. Sonicate for 5 min with intermittent shaking. Add *n*-hexane to fill 20% of the final flask volume, and shake for 10 min. Transfer the contents into a separator. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear solution.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 50-m × 0.32-mm; 0.52-µm packing G27
Temperatures
Injection port: 220°
Detector: 300°
Oven: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	2
50	5	145	0
145	10	250	20

Carrier gas: Helium
Flow rate: 4.0 ± 0.2 mL/min
Injection volume: 3 µL
Injection type: Split ratio, 1:20

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 5* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between memantine and memantine related compound B; NLT 2.0 between memantine related compound B and memantine related compound C, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of memantine related compound E or any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of memantine related compound E or any individual degradation product from the *Sample solution*
r_S = peak response of memantine hydrochloride from the *Standard solution*
C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of memantine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 5*.

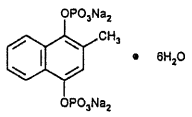
Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Memantine related compound A[a]	0.77	—
Memantine	1.0	—
Memantine related compound B[a]	1.03	—
Memantine related compound C[a]	1.1	—
Memantine related compound D[a]	1.2	—
Memantine related compound E	1.4	0.3
Any individual unspecified degradation product	—	0.20
Total impurities[b]	—	0.5

[a] Process impurities controlled in the drug substance and are included for identification only. Not reported for the drug product and not included in the total impurities.
[b] Excludes memantine–lactose adduct monitored in the test for *Limit of Memantine–Lactose Adduct*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amantadine Hydrochloride RS
  USP Memantine Hydrochloride RS
  USP Memantine Related Compound A RS
  1,3-Dimethyladamantane.
  $C_{12}H_{20}$ 164.29
  USP Memantine Related Compound B RS
  3,5-Dimethyladamantane-1-ol.
  $C_{12}H_{20}O$ 180.29
  USP Memantine Related Compound C RS
  1-Chloro-3,5-dimethyladamantane.
  $C_{12}H_{19}Cl$ 198.73
  USP Memantine Related Compound D RS
  1-Bromo-3,5-dimethyladamantane.
  $C_{12}H_{19}Br$ 243.18
  USP Memantine Related Compound E RS
  N-3,5-Dimethyladamantan-1-yl formamide.
  $C_{13}H_{21}NO$ 207.31

Menadiol Sodium Diphosphate



$C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$ 530.17
1,4-Naphthalenediol, 2-methyl-, bis(dihydrogen phosphate), tetrasodium salt, hexahydrate.
2-Methyl-1,4-naphthalenediol bis(dihydrogen phosphate) tetrasodium salt, hexahydrate [6700-42-1].
Anhydrous 422.09
[131-13-5].

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» Menadiol Sodium Diphosphate contains not less than 97.5 percent and not more than 102.0 percent of $C_{11}H_8Na_4O_8P_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a cold place.

Identification—

A: Dissolve about 200 mg of Menadiol Sodium Diphosphate in 10 mL of water, add 10 mL of 2 N sulfuric acid, 10 mL of 0.1 N ceric sulfate, and 1 mL of 30 percent hydrogen peroxide previously diluted with 5 mL of water, and extract the solution with two 10-mL portions of chloroform. Gently evaporate the clear chloroform solution on a steam bath to dryness, and dry the residue at 80° for 1 hour: the menadione so obtained melts between 104° and 107°.

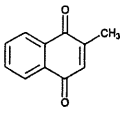
B: To 50 mg of the dried residue obtained in *Identification* test *A* add 5 mL of water, then add 75 mg of sodium bisulfite, and heat on a steam bath, shaking vigorously until the substance is dissolved and the solution is practically colorless. Dilute with water to 50 mL, and mix. To 2 mL of the solution add 2 mL of alcoholic ammonia (prepared by mixing equal volumes of alcohol and ammonium hydroxide), shake, and add 3 drops of ethyl cyanoacetate: a deep purplish blue color is produced, and on the addition of 1 mL of sodium hydroxide solution (1 in 3), it changes to green and then to yellow.

C: To about 20 mg contained in a small beaker add 1 mL of water, 2 drops of nitric acid, and 1 mL of sulfuric acid, and heat slowly to the evolution of white fumes. Cool, cautiously dilute with water to about 10 mL, and filter if not clear. Render the filtrate slightly alkaline to litmus with 6 N ammonium hydroxide, then render it acid with nitric acid, and add to the warm solution 3 mL of ammonium molybdate TS: a yellow precipitate is formed within a few minutes.

Water Determination, *Method I* ⟨921⟩: between 19.0% and 21.5%.

Assay—Dissolve about 100 mg of Menadiol Sodium Diphosphate, accurately weighed, in 25 mL of water, and add 25 mL of glacial acetic acid and 25 mL of 3 N hydrochloric acid. Titrate the solution with 0.02 N ceric sulfate VS, determining the endpoint potentiometrically using a calomel-platinum electrode system. Each mL of 0.02 N ceric sulfate is equivalent to 4.221 mg of $C_{11}H_8Na_4O_8P_2$.

Menadione



$C_{11}H_8O_2$ 172.18

1,4-Naphthalenedione, 2-methyl-;
2-Methyl-1,4-naphthoquinone [58-27-5].

DEFINITION

Menadione contains NLT 98.5% and NMT 101.0% of menadione ($C_{11}H_8O_2$), calculated on the dried basis.

[**CAUTION**—Menadione powder is irritating to the respiratory tract and to the skin, and a solution of it in alcohol is a vesicant.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Standard solution: 5 µg/mL of USP Menadione RS in alcohol
Sample solution: 5 µg/mL in alcohol
Analytical wavelength: 250 nm
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

- **PROCEDURE**

Sample solution: Transfer about 150 mg of Menadione into a 150-mL volumetric flask. Add 15 mL each of glacial acetic acid and 3 N hydrochloric acid, and rotate the flask until Menadione is dissolved. Add about 3 g of zinc dust, and close the flask with a stopper bearing a Bunsen valve. Shake, and allow to stand in the dark for 1 h, with frequent shaking. Rapidly decant the solution through a pledget of cotton into another flask, immediately wash the reduction flask with three 10-mL portions of freshly boiled and cooled water, and add 0.1 mL of orthophenanthroline TS.

Titrimetric system
Mode: Direct titration
Titrant: 0.1 N ceric sulfate VS
Endpoint detection: Potentiometric

Analysis: Immediately titrate the combined filtrate and washings with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N ceric sulfate is equivalent to 8.609 mg of menadione ($C_{11}H_8O_2$).

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORDINARY IMPURITIES** ⟨466⟩

Standard solution and **Sample solution:** Methanol
Eluant: Chloroform
Visualization: 1
Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩: 105°–107°
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over silica gel for 4 h.
Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

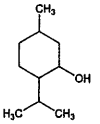
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Menadione RS

USP Monographs

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Menthol



$C_{10}H_{20}O$ 156.27
Cyclohexanol, 5-methyl-2-(1-methylethyl)- [1490-04-6].

DEFINITION
Menthol is an alcohol obtained from oils derived from a variety of mints or prepared synthetically. Menthol may be levorotatory (*l*-menthol) from natural or synthetic sources, or racemic (*dl*-menthol). It contains NLT 98.0% and NMT 102.0% of menthol ($C_{10}H_{20}O$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the menthol peak of the *Standard solution*, as obtained in the *Assay*.
- **B.** It meets the requirements in *Specific Tests* for *Optical Rotation* ⟨781S⟩, *Procedures, Specific Rotation*.

ASSAY
- **PROCEDURE**

Standard solution: 10 mg/mL of USP Menthol RS in hexanes
Sample solution: 10 mg/mL of Menthol in hexanes
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.18-mm × 20-m fused silica; coated with a 0.18-µm layer of G16 stationary phase
Temperatures
Injection port: 250°
Detector: 260°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	20	110	10

Carrier gas: Hydrogen
Flow rate: 0.9 mL/min
Injection volume: 0.5 µL
Injection type: Split ratio, 50:1
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for the menthol peak in replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of menthol ($C_{10}H_{20}O$) in the portion of Menthol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of menthol from the *Sample solution*
r_S = peak area of menthol from the *Standard solution*
C_S = concentration of USP Menthol RS in the *Standard solution* (mg/mL)
C_U = concentration of Menthol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES
- **LIMIT OF NONVOLATILE RESIDUE**

Analysis: Evaporate 2 g, accurately weighed, in a tared open porcelain dish on a steam bath, and dry the residue at 105° for 1 h.
Acceptance criteria: NMT 0.05%
- **RELATED COMPOUNDS**

Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Menthol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_T = sum of the peak areas from the *Sample solution*

Acceptance criteria
Individual impurities: NMT 0.5% for isomenthol (relative retention time is 1.08) from synthetic racemic menthol and 0.3% for all other impurities from natural and synthetic menthol
Total impurities: NMT 2.0%
- **READILY OXIDIZABLE SUBSTANCES IN *dl*-MENTHOL**

Sample solution: Place 500 mg of *dl*-menthol in a clean, dry test tube, and add 10 mL of a solution of potassium permanganate, prepared by diluting 3 mL of 0.1 N potassium permanganate with water to 100 mL.
Analysis: Place the test tube in a beaker with water at a temperature between 45° and 50°. Remove the tube from the bath at intervals of 30 s, and mix quickly by shaking.
Acceptance criteria: The purple color of potassium permanganate is still apparent after 5 min.

SPECIFIC TESTS
- **CONGEALING RANGE OF *dl*-MENTHOL**

(See *Congealing Temperature* ⟨651⟩.)
[NOTE—Perform this test preferably in a room having a temperature below 30° and a relative humidity below 50%.]
Sample: 10 g of *dl*-menthol, previously dried in a desiccator over silica gel for 24 h
Analysis: Place the *Sample* in a dry test tube having an internal diameter of 18–20 mm, and melt the contents at a temperature of about 40°. Suspend the test tube in water having a temperature of 23°–25°, and stir the contents of the tube continually with a thermometer, keeping the bulb of the thermometer immersed in the liquid.
Acceptance criteria: *dl*-Menthol congeals at a temperature between 27° and 28°. Shortly after the temperature has stabilized at the congealing point, add a few milligrams of dried *dl*-menthol to the congealed mass, and continue stirring. After a few minutes, the temperature of the mass quickly rises to 30.5°–32.0°.
- **MELTING RANGE OF *l*-MENTHOL**

(See *Melting Range or Temperature* ⟨741⟩.)
Acceptance criteria: 41°–44°
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 100 mg/mL in alcohol
Acceptance criteria
***l*-Menthol:** −45° to −51°
***dl*-Menthol:** −2° to +2°

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at controlled room temperature.
- **LABELING:** Label it to indicate whether it is levorotatory or racemic.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Menthol RS

Menthol Lozenges

DEFINITION
Menthol Lozenges contain NLT 90.0% and NMT 125.0% of the labeled amount of menthol ($C_{10}H_{20}O$), in a suitable molded base.

IDENTIFICATION
- **A.** The retention time of the menthol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 250 mg/mL of sodium chloride in water
Internal standard solution: 2 mg/mL of anethole in hexanes
Standard solution: 0.20*L* mg/mL of USP Menthol RS in *Internal standard solution*, where *L* is the labeled quantity, in mg, of menthol in each Lozenge
Sample solution: Transfer 20 Lozenges to a 1-L screw-capped conical flask. [NOTE—Use caps with inert white rubber liners.] Add 200 mL of water, 260 mL of *Solution A*, and 100.0 mL of the *Internal standard solution*, and shake by mechanical means for 30 min. Allow the phases to separate, and transfer a portion of the hexanes phase to a suitable container.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused silica; coated with a 1-µm layer of G16 stationary phase
Temperatures
Column: 125° (isothermally)
Injection port: 250°
Detector: 250°
Carrier gas: Helium
Flow rate: 10 mL/min
Injection volume: 1 µL
Injection type: Split ratio of 10:1
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for menthol and anethole are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 15 between menthol and anethole
Tailing factor: NMT 2.0 for menthol and anethole
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of menthol ($C_{10}H_{20}O$) in the portion of Lozenges taken:

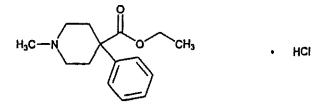
$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the menthol to the anethole from the *Sample solution*
R_S = peak response ratio of the menthol to the anethole from the *Standard solution*
C_S = concentration of USP Menthol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of menthol in the hexanes phase of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–125.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Menthol RS

Meperidine Hydrochloride



$C_{15}H_{21}NO_2 \cdot HCl$ 283.79
4-Piperidinecarboxylic acid, 1-methyl-4-phenyl-, ethyl ester, hydrochloride;
Ethyl 1-methyl-4-phenylisonipecotate hydrochloride [50-13-5].

DEFINITION
Meperidine Hydrochloride contains NLT 98.0% and NMT 102.0% of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩**:** Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
  Sample solution: 10 mg/mL
  Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: Transfer about 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Add 10 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 7.0, and filter.
Mobile phase: Acetonitrile and *Solution A* (550:450), filtered and degassed
Standard stock solution: 0.6 mg/mL of USP Meperidine Hydrochloride RS in water
Standard solution: 0.12 mg/mL of USP Meperidine Hydrochloride RS from the *Standard stock solution* in *Mobile phase*
Sample stock solution: 0.6 mg/mL of Meperidine Hydrochloride in water
Sample solution: 0.12 mg/mL of Meperidine Hydrochloride from the *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2 for the meperidine peak
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) in the portion of Meperidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of meperidine from the *Sample solution*
r_S = peak response of meperidine from the *Standard solution*
C_S = concentration of USP Meperidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Meperidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS

• **CONTENT OF CHLORIDE**
Sample solution: Transfer about 500 mg of Meperidine Hydrochloride, previously dried, to a 250-mL conical flask. Add 15 mL of water, 5 mL of glacial acetic acid, 50 mL of methanol, and 0.2 mL of eosin Y TS.
Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS to a rose-colored endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.
Acceptance criteria: 12.2%–12.7% of chloride is found.

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
Sample solution: 10 mg/mL in water
Chromatographic system
Mode: GC
Detector: Flame ionization
Column: 2-mm × 2-m glass; 10% phase G3 on support S1A
Temperatures
Column: 190°
Injection port: 255°
Detector: 280°
Carrier gas: Helium
Flow rate: 28 mL/min
Injection volume: 2.0 µL
Analysis
Sample: *Sample solution*
Calculate the area percentage of each peak.
Acceptance criteria: No peak other than the principal peak (except for the solvent peak) constitutes more than 1.0% of the total area.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at a pressure between 20 and 40 mm of mercury at 80° for 4 h.
Acceptance criteria: NMT 1.0%
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩
Sample: Dried under vacuum at 80° for 4 h
Acceptance criteria: 186°–189°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Meperidine Hydrochloride RS

Meperidine Hydrochloride Injection

DEFINITION

Meperidine Hydrochloride Injection is a sterile solution of Meperidine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).

IDENTIFICATION

• **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩**:** Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: Transfer about 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Add 10 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 7.0, and filter.
Mobile phase: Acetonitrile and *Buffer* (550:450), filtered and degassed
Standard stock solution: 0.6 mg/mL of USP Meperidine Hydrochloride RS in water
Standard solution: 0.12 mg/mL of USP Meperidine Hydrochloride RS from the *Standard stock solution* in *Mobile phase*
Sample stock solution: Transfer a measured volume of Injection equivalent to about 300 mg to a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 1.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Meperidine Hydrochloride RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of meperidine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.5–6.0
- **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 2.4 USP Endotoxin Units/mg of meperidine hydrochloride.
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **USP Reference Standards** ⟨11⟩
  USP Meperidine Hydrochloride RS

Meperidine Hydrochloride Oral Solution

DEFINITION
Meperidine Hydrochloride Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).

IDENTIFICATION
- **A.**
  Sample solution: Transfer a volume of Oral Solution nominally equivalent to about 100 mg of meperidine hydrochloride to a 125-mL separator. Add 40 mL of water and 3 mL of 1 N sodium hydroxide, and extract with three 25-mL portions of *n*-hexane. Wash the combined extracts with two 20-mL portions of water, discard the water, and then extract with three 25-mL portions of 0.1 N hydrochloric acid. Transfer the extracts to a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.
  Standard solution: Prepare in a way similar to that for the *Sample solution*, using USP Meperidine Hydrochloride RS.
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*.

ASSAY
- **Procedure**
  Sample solution: Transfer a suitable volume of Oral Solution nominally equivalent to about 250 mg of meperidine hydrochloride to a separator, and add 3 mL of 1 N sodium hydroxide. Extract with five 20-mL portions of chloroform, and filter the extracts through a pledget of cotton into a 250-mL conical flask. Wash the cotton with 5 mL of chloroform, and add the washing to the combined filtrates. Add 10 mL of glacial acetic acid and 2 drops of crystal violet TS.
  Analysis: Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 28.38 mg of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).
  Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **Deliverable Volume** ⟨698⟩: Meets the requirements for oral solution packaged in multiple-unit containers
- **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements for oral solution packaged in single-unit containers

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.5–4.1

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Meperidine Hydrochloride RS

Meperidine Hydrochloride Tablets

DEFINITION
Meperidine Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).

IDENTIFICATION
- **A. Identification—Organic Nitrogenous Bases** ⟨181⟩
  Sample solution: Transfer an amount nominally equivalent to about 50 mg of meperidine hydrochloride from powdered Tablets to a separator, add 10 mL of water, and shake. Add 5 mL of saturated sodium chloride solution and 1 mL of sodium hydroxide solution (1 in 25). Extract with three 20-mL portions of chloroform, filtering the extracts through cotton overlaid with anhydrous sodium sulfate. Evaporate the chloroform on a steam bath, and dissolve the residue in 4 mL of carbon disulfide.
  Standard solution: In a second separator, proceed as directed in the *Sample solution*, using 50 mg of USP Meperidine Hydrochloride RS.
  Analysis: Proceed as directed in the chapter, beginning with "Determine the absorption spectra".
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
  Solution A: Transfer about 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Add 10 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 7.0, and filter.
  Mobile phase: Acetonitrile and *Solution A* (550:450), filtered and degassed
  Standard stock solution: 0.6 mg/mL of USP Meperidine Hydrochloride RS in water
  Standard solution: 0.12 mg/mL of USP Meperidine Hydrochloride RS from the *Standard stock solution* in *Mobile phase*
  Sample stock solution: Transfer an amount nominally equivalent to about 60 mg of meperidine hydrochloride from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add about 70 mL of *Mobile phase*, and sonicate for 10 min with occasional shaking. Shake by mechanical means for about 30 min, dilute with *Mobile phase* to volume, mix, and filter.
  Sample solution: Nominally equivalent to 0.12 mg/mL of meperidine hydrochloride in *Mobile phase* from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2 for the meperidine peak
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

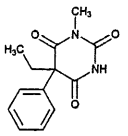
r_U = peak response of meperidine from the *Sample solution*
r_S = peak response of meperidine from the *Standard solution*
C_S = concentration of USP Meperidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of meperidine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: A known concentration of USP Meperidine Hydrochloride RS in *Medium*
Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium*, if necessary.
Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.
Analysis: Determine the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) dissolved by comparing the peak response of meperidine from the *Sample solution* with that from the *Standard solution*.
Tolerances: NLT 75% (*Q*) of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Meperidine Hydrochloride RS

Mephobarbital



$C_{13}H_{14}N_2O_3$ 246.26
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-1-methyl-5-phenyl-;
5-Ethyl-1-methyl-5-phenylbarbituric acid [115-38-8].

DEFINITION
Mephobarbital contains NLT 98.0% and NMT 100.5% of mephobarbital ($C_{13}H_{14}N_2O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
• **B.**
Sample: 200 mg of Mephobarbital
Analysis: Boil the *Sample* with 10 mL of 1 N sodium hydroxide.
Acceptance criteria: Ammonia is evolved.
• **C.**
Diluent: Sodium hydroxide solution (1 in 500)
Sample solution: 12 mg/mL of Mephobarbital prepared as follows. Shake about 60 mg of Mephobarbital with 5 mL of *Diluent*, and filter. Use the filtrate.
Analysis 1: Add 3 drops of mercuric nitrate TS to 1 mL of the *Sample solution*.
Acceptance criteria 1: A white precipitate is formed, and it is soluble in 6 N ammonium hydroxide.
Analysis 2: Add silver nitrate TS to 1 mL of the *Sample solution*.
Acceptance criteria 2: A white precipitate is formed, and it dissolves readily in 6 N ammonium hydroxide.

ASSAY
• **Procedure**
Sample solution: 10 mg/mL of Mephobarbital in dimethylformamide
Analysis: Transfer 50 mL of *Sample solution* to a 200-mL flask. Add 4 drops of thymolphthalein TS. Titrate with 0.1 N lithium methoxide in toluene VS using a magnetic stirrer and a cover for the flask to protect against atmospheric carbon dioxide. Perform a blank determination. Each mL of 0.1 N lithium methoxide is equivalent to 24.63 mg of mephobarbital ($C_{13}H_{14}N_2O_3$).
Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩**:** NMT 0.1%

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 105° for 4 h.
Acceptance criteria: NMT 1.0%
• **Melting Range or Temperature,** *Class I* ⟨741⟩**:** 176°–181°

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Mephobarbital RS

Mephobarbital Tablets

DEFINITION
Mephobarbital Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of mephobarbital ($C_{13}H_{14}N_2O_3$).

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: Residue obtained in the *Assay*
Acceptance criteria: The infrared absorption spectrum of *Sample* is consistent with that of USP Mephobarbital RS.

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• **B. MELTING RANGE OR TEMPERATURE** ⟨741⟩
Sample: Residue obtained in the *Assay*
Acceptance criteria: 174°–181°

ASSAY
• **PROCEDURE**
Sample: Weigh a suitable number of powdered Tablets (NLT 20), and transfer an accurately weighed portion of powder, equivalent to 300 mg of mephobarbital, to a suitable extraction thimble. Extract with 15 mL of hexane, allow the thimble to drain, transfer to a continuous-extraction apparatus provided with a tared flask, and extract the mephobarbital with chloroform for 2 h. Evaporate the chloroform on a steam bath with the aid of a current of air, and cool.
Analysis: Dissolve the residue in 10 mL of alcohol, and evaporate. Dry the residue at 105°; for 1 h, cool, and weigh. The weight of the residue represents the weight of mephobarbital ($C_{13}H_{14}N_2O_3$) in the portion of Tablets taken.
Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Buffer: 1 L of 1% of 3-(dodecyldimethylammonio) propanesulfonate in pH 8.0 phosphate buffer prepared as follows. Transfer 10.0 g of 3-(dodecyldimethylammonio) propanesulfonate in 400 mL of warm water, and add 250 mL of 0.2 M monobasic potassium phosphate and about 220 mL of 0.2 M sodium hydroxide. Cool to room temperature, adjust with 0.2 M sodium hydroxide to a pH of 8.0, dilute with water to 1000 mL, mix, and degas.
Medium: *Buffer*; 900 mL
Apparatus 2: 75 rpm
Time: 75 min
Standard solution: Known concentration of USP Mephobarbital RS in *Medium*
Sample solution: Pass a portion of the solution through a nylon filter of 0.45-µm pore size, and, if necessary, suitably dilute with *Medium*.
Instrumental conditions
Mode: UV
Analytical wavelength: 244 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 70% (*Q*) of the labeled amount of mephobarbital ($C_{13}H_{14}N_2O_3$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS,** *Content Uniformity* ⟨905⟩
Prepare all of the solutions concomitantly.
Diluent: 6.2 g of boric acid and 7.45 g of potassium chloride in 500 mL of water. Add 210 mL of sodium hydroxide solution (1 in 25). Add water to make 2000 mL of *Diluent*.
Standard stock solution: 10 mg/mL of USP Mephobarbital RS in *Diluent*
Standard solution: 1.5 mg/mL of USP Mephobarbital RS in water from *Standard stock solution*
Sample solution: Nominally 1.5 mg/mL of mephobarbital prepared as follows. Transfer 1 Tablet to a glass-stoppered centrifuge tube, crush the Tablet, and add 25.0 mL of *Diluent*. Insert the stopper, shake for 10 min, and, if necessary, centrifuge until clear, filtering the supernatant. Dilute a portion of the subsequent liquid with water.
Instrumental conditions
Mode: UV
Analytical wavelength: 245 nm
Cell: 1 cm
Blank: *Diluent* and water (1:3)
Analysis
Samples: *Standard solution* and *Sample solution*
Transfer 3.0 mL each of the *Standard solution* and the *Sample solution* to separate 200-mL volumetric flasks, and dilute each with *Blank* to volume.
Determine the percentage of the labeled amount of mephobarbital ($C_{13}H_{14}N_2O_3$) in the Tablet taken:

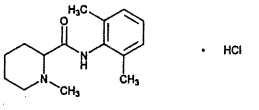
$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the USP Mephobarbital RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mephobarbital in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mephobarbital RS

Mepivacaine Hydrochloride



$C_{15}H_{22}N_2O \cdot HCl$ 282.81
2-Piperidinecarboxamide, *N*-(2,6-dimethylphenyl)-1-methyl-, monohydrochloride, (±)-;
(±)-1-Methyl-2′,6′-pipecoloxylidide monohydrochloride [1722-62-9].

DEFINITION
Mepivacaine Hydrochloride contains NLT 98.0% and NMT 102.0% of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: 10 mg/mL
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Buffer: 2.25 g/L solution of phosphoric acid, adjusted with 50% sodium hydroxide to a pH of 7.6
Mobile phase: Acetonitrile and *Buffer* (35:65)
System suitability solution: 2 µg/mL of USP Mepivacaine Hydrochloride RS and 3 µg/mL of USP Bupivacaine Related Compound B RS in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Mepivacaine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

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Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between bupivacaine related compound B and mepivacaine, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) in the portion of Mepivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mepivacaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Mepivacaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*. The run time is three times the retention time of the mepivacaine peak.
Standard solution: 2 µg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*
Sample solution: 2 mg/mL of Mepivacaine Hydrochloride in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between bupivacaine related compound B and mepivacaine, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any impurity in the portion of Mepivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of mepivacaine from the *Standard solution*
C_S = concentration of USP Mepivacaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Mepivacaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bupivacaine related compound B (desmethyl mepivacaine)[a]	0.4	0.15
Mepivacaine	1.0	—
Picolinamide analog[b]	2.1	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.4

[a] *N*-(2,6-Dimethylphenyl)piperidine-2-carboxamide.
[b] *N*-(2,6-Dimethylphenyl)picolinamide.

• **2,6-DIMETHYLANILINE**

Prepare the *Standard solution* and *Sample solution* fresh, just before use.
Standard stock solution: 0.6 µg/mL of USP Ropivacaine Related Compound A RS in 1 N hydrochloric acid. [NOTE—Ropivacaine related compound A is 2,6-dimethylaniline hydrochloride.]
Standard solution: Transfer 2.0 mL of the *Standard stock solution* and 1.0 mL of 3 N sodium hydroxide to a 20-mL headspace vial, and immediately close the vial with a cap.
Sample stock solution: 30 mg/mL of Mepivacaine Hydrochloride in 1 N hydrochloric acid
Sample solution: Transfer 2.0 mL of the *Sample stock solution* and 1.0 mL of 3 N sodium hydroxide to a 20-mL headspace vial, and immediately close the vial with a cap.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m capillary; coated with 3-µm film of G43
Temperatures
Injection port: 225°
Detector: 250°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	10	230	5

Carrier gas: Helium
Flow rate: 4 mL/min
Injection type: Split ratio, 1:1
Headspace sampler
Equilibration time: 15 min
Equilibration temperature: 90°
Loop temperature: 215°
Transfer line temperature: 220°
Vial pressure: About 15 psi
Loop size: 3 mL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 15%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in ppm, of 2,6-dimethylaniline in the portion of Mepivacaine Hydrochloride taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 10^6$$

r_U = peak response of 2,6-dimethylaniline from the *Sample solution*
r_S = peak response of 2,6-dimethylaniline from the *Standard solution*
C_S = concentration of USP Ropivacaine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Mepivacaine Hydrochloride in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of 2,6-dimethylaniline, 121.18
M_{r2} = molecular weight of 2,6-dimethylaniline hydrochloride (ropivacaine related compound A), 157.64

Acceptance criteria: NMT 20 ppm

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 4 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bupivacaine Related Compound B RS
  ▲*N*-(2,6-Dimethylphenyl)piperidine-2-carboxamide hydrochloride.
  $C_{14}H_{20}N_2O \cdot HCl$ 268.79▲ (ERR 1-Oct-2018)
  USP Mepivacaine Hydrochloride RS
  USP Ropivacaine Related Compound A RS
  2,6-Dimethylaniline hydrochloride.
  $C_8H_{11}N \cdot HCl$ 157.64

Mepivacaine Hydrochloride Injection

DEFINITION

Mepivacaine Hydrochloride Injection is a sterile solution of Mepivacaine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$).

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩: Meets the requirements
- **B.**
  Analysis: Extract a volume of Injection, equivalent to 200 mg of mepivacaine, with two 10-mL portions of ether, and discard the ether extracts. Render the remaining solution slightly alkaline with sodium carbonate TS, and extract the precipitate with ether. Evaporate the ether extract on a steam bath to dryness, and dry the residue under vacuum at 60° for 1 h.
  Acceptance criteria: The mepivacaine obtained melts between 149° and 153°.

ASSAY

- **PROCEDURE**
  Buffer: 3.40 g/L of monobasic potassium phosphate and 4.35 g/L of dibasic potassium phosphate in water. Adjust with potassium hydroxide or phosphoric acid to a pH of 6.3.
  Mobile phase: Acetonitrile and *Buffer* (35:65)
  System suitability solution: 0.05 mg/mL of methylparaben and 1.0 mg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*
  Standard solution: 1.0 mg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*
  Sample solution: Nominally 1 mg/mL of mepivacaine hydrochloride from Injection in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 263 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1[1]
  Column temperature: 40°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for mepivacaine and methylparaben are 1.0 and 1.4, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between methylparaben and mepivacaine, *System suitability solution*
  Capacity factor: NLT 1.0 for the mepivacaine peak, *System suitability solution*
  Tailing factor: NMT 2.0 for the mepivacaine peak, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mepivacaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.5–6.8
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.8 USP Endotoxin Unit/mg of mepivacaine hydrochloride.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Injection labeled to contain 2% or less of mepivacaine hydrochloride may be packaged in 50-mL multiple-dose containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mepivacaine Hydrochloride RS

[1] A Whatman Partisphere RTF C18 brand of L1 column has been shown to be an appropriate column.

USP Monographs

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Mepivacaine Hydrochloride and Levonordefrin Injection

DEFINITION

Mepivacaine Hydrochloride and Levonordefrin Injection is a sterile solution of Mepivacaine Hydrochloride and Levonordefrin in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) and NLT 90.0% and NMT 110.0% of the labeled amount of levonordefrin ($C_9H_{13}NO_3$).

IDENTIFICATION

• **A.**

Analysis: Extract a volume of Injection, equivalent to 200 mg of mepivacaine, with two 10-mL portions of ether, and discard the ether extracts. Render slightly alkaline with sodium carbonate TS, extract the precipitate with ether, evaporate the ether extract on a steam bath to dryness, and dry the residue under vacuum at 60° for 1 h.

Acceptance criteria: The mepivacaine obtained melts between 149° and 153°.

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY

• **MEPIVACAINE HYDROCHLORIDE**

Buffer: 3.40 g/L of monobasic potassium phosphate and 4.35 g/L of dibasic potassium phosphate in water. Adjust with potassium hydroxide or phosphoric acid to a pH of 6.3.

Mobile phase: Acetonitrile and *Buffer* (35:65)

System suitability solution: 0.05 mg/mL of methylparaben and 1.0 mg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*

Standard solution: 1.0 mg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of mepivacaine hydrochloride from Injection in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 263 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1[1]

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mepivacaine and methylparaben are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylparaben and mepivacaine, *System suitability solution*

Capacity factor: NLT 1.0 for the mepivacaine peak, *System suitability solution*

Tailing factor: NMT 2.0 for the mepivacaine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mepivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mepivacaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

• **LEVONORDEFRIN**

Ferro-citrate solution and **Buffer solution:** Prepare as directed in *Epinephrine Assay* ⟨391⟩.

Standard stock solution: With the aid of 20 mL of sodium bisulfite solution (1 in 50), transfer 25 mg of USP Levonordefrin RS to a 50-mL volumetric flask, and dilute with water to volume.

Standard solution: 50 µg/mL of USP Levonordefrin RS in sodium bisulfite solution (1 in 500) from the *Standard stock solution*. Make the final dilution at the time the *Assay* is to be carried out.

Sample solution: Nominally 50 µg/mL of levonordefrin from Injection, diluting if necessary

Analysis: Proceed as directed in *Epinephrine Assay* ⟨391⟩, except use levonordefrin wherever epinephrine [base] is called for. When the *Ferro-citrate solution* and the *Buffer solution* are mixed with the *Sample solution*, a fine precipitate may be formed. Remove this precipitate by centrifugation or by passing through dry filter paper before the colorimetric measurements are taken.

Calculate the percentage of the labeled amount of levonordefrin ($C_9H_{13}NO_3$) in the volume of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Levonordefrin RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of levonordefrin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **COLOR AND CLARITY**

Standard solution: Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, and dilute with water to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Visually examine a portion of the Injection (*Sample solution*) in a suitable clear glass test tube against a white background: it is not pinkish, and it contains no precipitate. If any yellow color is observed in the *Sample solution*, concomitantly determine the absorbances of the *Sample solution* and *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm.

Acceptance criteria: The absorbance of the *Sample solution* does not exceed that of the *Standard solution*.

• **pH** ⟨791⟩ **:** 3.3–5.5

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩ **:** It contains NMT 0.8 USP Endotoxin Unit/mg of mepivacaine hydrochloride.

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

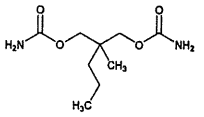
[1] A Whatman Partisphere RTF C18 brand of L1 column has been shown to be an appropriate column.

USP Monographs

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- **LABELING:** The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Levonordefrin RS
  USP Mepivacaine Hydrochloride RS

Meprobamate



$C_9H_{18}N_2O_4$ 218.25

1,3-Propanediol, 2-methyl-2-propyl-, dicarbamate;
2-Methyl-2-propyl-1,3-propanediol dicarbamate [57-53-4].

DEFINITION

Meprobamate contains NLT 97.0% and NMT 101.0% of meprobamate ($C_9H_{18}N_2O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: 1 mg in 200 mg
  Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the *Sample*, previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Meprobamate RS. If a difference appears, dissolve portions of both the *Sample* and the Reference Standard in acetone at a concentration of 8 mg/mL. Dilute 0.1-mL portions of the acetone solutions with 1 mL of *n*-heptane, and remove the solvents by evaporation under nitrogen at a temperature of 30°. Dry the residues under vacuum at room temperature for 30 min, and repeat the test on the residues.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile and water (30:70)
  Standard solution: 5 mg/mL of USP Meprobamate RS prepared as follows. Dissolve the Standard first in acetonitrile using 30% of final volume. Sonicate if necessary to dissolve, and cool to room temperature. Dilute with water to volume.
  Sample solution: 5 mg/mL of Meprobamate prepared as follows. Dissolve the sample first in acetonitrile using 30% of final volume. Sonicate if necessary to dissolve, and cool to room temperature. Dilute with water to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 200 nm
  Column: 4.6-mm × 25-cm; 4-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  Run time: 2 times the retention time of meprobamate
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of meprobamate ($C_9H_{18}N_2O_4$) in the portion of Meprobamate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Meprobamate RS in the *Standard solution* (mg/mL)
C_U = concentration of Meprobamate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES: PROCEDURE 1**
  Standard solutions: Dissolve USP Meprobamate RS in alcohol, and mix to obtain *Standard solution A* with a known concentration of 1.0 mg/mL. Dilute quantitatively with alcohol to the obtain the *Standard solutions* with the compositions given in *Table 1*.

Table 1

Standard Solution	Dilution	Concentration (mg RS/mL)	Percentage (%, for Comparison with Sample)
A	(Undiluted)	1.0	1.0
B	(4 in 5)	0.8	0.8
C	(3 in 5)	0.6	0.6
D	(2 in 5)	0.4	0.4
E	(1 in 5)	0.2	0.2

  Sample solution: 100 mg/mL of Meprobamate in alcohol
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: Thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel
  Application volume: 2 µL
  Developing solvent system: Hexane, acetone, and pyridine (70:30:10)
  Spray reagent: 5 mg/mL of vanillin in a cooled mixture of sulfuric acid and alcohol (80:20)
  Analysis
  Samples: *Standard solutions* and *Sample solution*
  Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and air-dry the plate for 15 min. Heat the plate at 100° for 15 min, cool, and spray with *Spray reagent*. Heat the plate at 110° for 15–20 min, cool, and allow the plate to develop blue-purple spots at room temperature. [NOTE—Color development requires about 30–60 min.] Examine the plate, and compare the intensities of any secondary spots of the *Sample solution* with those of the principal spots of the *Standard solutions*.
  Acceptance criteria: No secondary spot of the *Sample solution* is larger or more intense than the principal spot of *Standard solution A* (1.0%), and the sum of the intensities

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of all secondary spots of the *Sample solution* corresponds to NMT 2.0%.

• **ORGANIC IMPURITIES, PROCEDURE 2: LIMIT OF METHYL CARBAMATE**

Standard solution: 1.0 mg/mL of methyl carbamate

Sample solution: Transfer 1.0 g of finely powdered Meprobamate to a beaker, add 5.0 mL of water, and stir to wet the powder completely. Filter the slurry through a small plug of glass wool in the stem of a glass funnel. Use the clear filtrate.

Mobile phase: Water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 3.9–4.6-mm × 25–30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The peak response of the *Sample solution* is not greater than that of the *Standard solution*, corresponding to NMT 0.5% of methyl carbamate.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample under vacuum at 60° for 3 h.

Acceptance criteria: NMT 0.5%

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 103°–107°, but the range between the beginning and end of melting is NMT 2°.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Meprobamate RS

Meprobamate Tablets

DEFINITION

Meprobamate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: A portion of finely powdered Tablets, equivalent to 800 mg of meprobamate

Analysis: To the *Sample* add 5 mL of dehydrated alcohol, and heat to just below boiling for about 5 min, with occasional swirling. Cool, and filter into 15 mL of solvent hexane. With the aid of suction, filter the crystals that form, and dry at 60°.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion (about 1 mg in 200 mg) from a portion of crystals obtained from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Meprobamate RS. If a difference appears, dissolve portions of both the *Sample* and the Reference Standard in acetone at a concentration of 8 mg/mL. Dilute 0.1-mL portions of the acetone solutions with 1 mL of *n*-heptane, and remove the solvents by evaporation under nitrogen at a temperature of about 30°. Dry the residues under vacuum at room temperature for 30 min, and repeat the test on the residues.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (30:70)

Phenacetin stock solution: 125 µg/mL of phenacetin in acetonitrile

Phenacetin solution: 25 µg/mL of phenacetin prepared as follows from the *Phenacetin stock solution*. Pipet a suitable volume of *Phenacetin stock solution* into a volumetric flask. Add acetonitrile to fill 30% of the final flask volume, and dilute with water to volume.

Standard solution: 5 mg/mL of USP Meprobamate RS prepared as follows. Transfer a suitable amount of the Reference Standard to a suitable volumetric flask. Dissolve in 30% of the final flask volume of acetonitrile, and dilute with water to volume.

System suitability solution: 5 mg/mL of USP Meprobamate RS and 5 µg/mL of phenacetin prepared as follows. Dissolve a weighed amount of USP Meprobamate RS, first in acetonitrile, using 20% final volume. Shake to dissolve. Add a suitable volume of *Phenacetin solution*, and dilute with water to volume.

Sample solution: Nominally equivalent to 5 mg/mL of meprobamate prepared as follows. Transfer an amount of meprobamate from a portion of finely powdered Tablets (NLT 20) to a suitable volumetric flask. Add acetonitrile to fill 30% of final volume, and shake to dissolve. Dilute with water to volume, and filter, discarding the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 3.9–4.6-mm × 25–30-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for meprobamate and phenacetin are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the meprobamate and the phenacetin peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of meprobamate from the *Sample solution*

r_S = peak response of meprobamate from the *Standard solution*

C_S = concentration of USP Meprobamate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of meprobamate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

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PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Procedure for a pooled sample

Medium: Deaerated water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Calculate the percentage of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Meprobamate RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

Acceptance criteria: NLT 75% (Q) of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

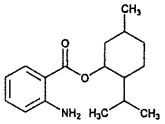
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Meprobamate RS

Meradimate



$C_{17}H_{25}NO_2$ 275.39

Cyclohexanol, 5-methyl-2-(1-methylethyl)-, 2-aminobenzoate;

Anthranilic acid, *p*-menth-3-yl ester [134-09-8].

DEFINITION

Meradimate contains NLT 95.0% and NMT 105.0% of meradimate ($C_{17}H_{25}NO_2$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 5 µg/mL in alcohol

Acceptance criteria: Meets the requirements

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Standard solution: 20.0 mg/mL of USP Meradimate RS in *tert*-butyl methyl ether

Sample solution: 20 mg/mL of Meradimate in *tert*-butyl methyl ether

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 25-m; coated with a 0.1-µm film of G1

Temperatures

Injector: 240°

Detector: 260°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	8	240	10

Carrier gas: Helium

Flow rate: 6 mL/min

Injection type: Split ratio, 30:1

[NOTE—The split ratio can be modified to optimize performance.]

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of meradimate ($C_{17}H_{25}NO_2$) in the portion of Meradimate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Meradimate RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Meradimate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity

r_T = sum of all the peak responses

Acceptance criteria

Any individual impurity: NMT 0.5%

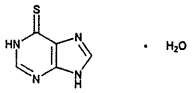
Total impurities: NMT 2.0%

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SPECIFIC TESTS
• **ACIDITY**
Sample: 5.0 mL of Meradimate
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Transfer 50 mL of alcohol to a suitable container, add 1 mL of phenolphthalein TS, and add sufficient volume of *Titrant* to obtain a persistent pink color. Transfer 50 mL of this solution to a suitable container, add the *Sample*, and titrate with *Titrant*.
Acceptance criteria: NMT 0.2 mL of *Titrant* per mL of Meradimate is necessary.
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩**:** −4° to +4°
Sample solution: 10 mg/mL in alcohol
• **REFRACTIVE INDEX** ⟨831⟩**:** 1.540–1.544 at 20°

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Meradimate RS

Mercaptopurine



$C_5H_4N_4S \cdot H_2O$ 170.19
$C_5H_4N_4S$ 152.18
6H-Purine-6-thione, 1,7-dihydro-, monohydrate; Purine-6-thiol monohydrate [6112-76-1].
Anhydrous [50-44-2].

DEFINITION
Mercaptopurine contains NLT 97.0% and NMT 102.0% of mercaptopurine ($C_5H_4N_4S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 0.77 g/L of ammonium acetate in water
Mobile phase: Methanol and *Solution A* (25:75)
Standard stock solution: 0.2 mg/mL of USP Mercaptopurine RS in a mixture of methanol and water (1:1). Transfer USP Mercaptopurine RS into a suitable volumetric flask, and add methanol equivalent to 50% of the final volume. Shake mechanically to dissolve, and dilute with water to volume.
Standard solution: 0.02 mg/mL of USP Mercaptopurine RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: Transfer 25 mg of Mercaptopurine into a 50-mL volumetric flask. Add 25 mL of methanol, shake mechanically for at least 45 min, and dilute with water to volume. Transfer 20 mL of this solution into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: 0.02 mg/mL of Mercaptopurine in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 325 nm
Column: 4.6-mm × 15-cm; 5-µm packing L68
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mercaptopurine ($C_5H_4N_4S$) in the portion of Mercaptopurine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mercaptopurine RS in the *Standard solution* (mg/mL)
C_U = concentration of Mercaptopurine in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**
Solution A: 0.1% (v/v) Formic acid in water
Solution B: Methanol and *Solution A* (2:98)
Solution C: Methanol and *Solution A* (1:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
8	100	0
20	0	100
25	0	100
27	100	0
30	100	0

Standard stock solution: 0.06 mg/mL of USP Mercaptopurine RS in *Solution A*. [NOTE—Use methanol equivalent to 2.5% of the final volume to help dissolve.]
Standard solution: 1.2 µg/mL of USP Mercaptopurine RS in *Solution B* from the *Standard stock solution*
Sensitivity solution: 0.06 µg/mL of USP Mercaptopurine RS in *Solution B* from the *Standard solution*
▲**Sample stock solution:** 0.5 mg/mL of mercaptopurine in a mixture of methanol and *Solution A* (1:9) prepared as follows. Transfer a suitable quantity of Mercaptopurine to an appropriate volumetric flask, add methanol equivalent

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to 10% of the final volume, and shake to dissolve. Dilute with *Solution A* to volume. ▲ (ERR 1-Apr-2019)

Sample solution: 0.12 mg/mL of mercaptopurine in *Solution A* ▲from the *Sample stock solution*. ▲ (ERR 1-Apr-2019) [NOTE—Inject the *Sample solution* within 1 h of preparation.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Temperatures
Column: 30°
Sample: 4°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Mercaptopurine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of mercaptopurine from the *Standard solution*
C_S = concentration of USP Mercaptopurine RS in the *Standard solution* (mg/mL)
C_U = concentration of Mercaptopurine in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Didanosine related compound A[a]	0.54	6.3	0.15
Mercaptopurine	1.00	—	—
Mercaptopurine disulfide[b]	2.90	4.4	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] Hypoxanthine.
[b] 1,2-Di(9*H*-purin-6-yl)disulfane.

SPECIFIC TESTS
• **PHOSPHORUS**
Standard phosphate solution: 43.96 µg/mL of dried monobasic potassium phosphate (equivalent to 10 µg of phosphorus)
Standard solution: Transfer 2 mL of *Standard phosphate solution* to a 25-mL volumetric flask. Add 1 mL of 15 N sulfuric acid, 0.5 mL of nitric acid, 0.75 mL of ammonium molybdate TS, and 1 mL of aminonaphtholsulfonic acid TS, then dilute with water to volume, and mix. Allow to stand for 5 min.
Sample solution: Digest 200 mg with 2 mL of 15 N sulfuric acid in a large test tube, periodically adding nitric acid, dropwise and with caution. Continue heating until practically all of the liquid has evaporated and the residue is colorless. Transfer the residue, with the aid of small portions of water, to a 25-mL volumetric flask. Add 1 mL of 15 N sulfuric acid, 0.5 mL of nitric acid, 0.75 mL of ammonium molybdate TS, and 1 mL of aminonaphtholsulfonic acid TS, then dilute with water to volume. Allow to stand for 5 min.
Blank: Transfer 2 mL of 15 N sulfuric acid to a large test tube, periodically adding nitric acid, dropwise and with caution. Continue heating until practically all of the liquid has evaporated and the residue is colorless. Transfer the residue, with the aid of small portions of water, to a 25-mL volumetric flask. Add 1 mL of 15 N sulfuric acid, 0.5 mL of nitric acid, 0.75 mL of ammonium molybdate TS, and 1 mL of aminonaphtholsulfonic acid TS, then dilute with water to volume. Allow to stand for 5 min.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 750 nm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 100 ppm).
• **WATER DETERMINATION,** *Method I* ⟨921⟩
Medium: 30 mL of methanol and 5 g of salicylic acid in the titration vessel
Sample: 0.3 g of Mercaptopurine
Acceptance criteria: NMT 12.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mercaptopurine RS

Mercaptopurine Tablets

DEFINITION
Mercaptopurine Tablets contain NLT 93.0% and NMT 110.0% of the labeled amount of mercaptopurine ($C_5H_4N_4S \cdot H_2O$).

IDENTIFICATION
• **A.** The UV absorption spectrum exhibits a maximum at 325 ± 2 nm, and the ratio A_{255}/A_{325} does not exceed 0.09.
Sample: 5 µg/mL of mercaptopurine in a mixture of methanol and water (1:1), from the *Sample solution* in the *Assay*
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 0.77 g/L of ammonium acetate in water
Solution B: Methanol and *Solution A* (5:95)
Solution C: Methanol and *Solution A* (30:70)
Mobile phase: *Solution B* and *Solution C* (80:20)
Diluent: Methanol and water (1:1)
Standard solution: 0.25 mg/mL of USP Mercaptopurine RS in a mixture of methanol and water (1:1). Transfer USP

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Mercaptopurine RS into a suitable volumetric flask, and add methanol equivalent to 50% of the final volume. Shake mechanically to dissolve, and dilute with water to volume.
Sample stock solution: 0.5 mg/mL of mercaptopurine in *Diluent* from NLT 5 Tablets. Place the Tablets into a suitable volumetric flask, add methanol equivalent to 50% of the final volume, and shake mechanically for a minimum of 30 min. Dilute with water to volume. Pass through a PVDF filter of 0.45-µm pore size, and discard the first 3 mL of filtrate.
Sample solution: 0.25 mg/mL of mercaptopurine in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mercaptopurine ($C_5H_4N_4S \cdot H_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mercaptopurine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mercaptopurine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of mercaptopurine, 170.19
M_{r2} = molecular weight of anhydrous mercaptopurine, 152.18

Acceptance criteria: 93.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Mobile phase: 0.1% acetic acid in water
Standard solution: USP Mercaptopurine RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a concentration that is similar to the *Standard solution*, if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.9-mm × 15-cm; packing L1
Flow rate: 2.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for mercaptopurine is NLT 4 min.]
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (*Q*) of the labeled amount of $C_5H_4N_4S \cdot H_2O$ is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Apparatus 2, Chromatographic system, and **Analysis:** Proceed as directed for *Test 1*.
Time: 120 min
Tolerances NLT 80% (*Q*) of the labeled amount of $C_5H_4N_4S \cdot H_2O$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**
Solution A: 0.1% (v/v) formic acid in water
Solution B: Methanol and *Solution A* (2:98)
Solution C: Methanol and *Solution A* (1:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
8	100	0
20	0	100
25	0	100
27	100	0
30	100	0

Standard stock solution: 0.06 mg/mL of USP Mercaptopurine RS in *Solution A*. [NOTE—Use methanol equivalent to 2.5% of the final volume to help dissolve.]
Standard solution: 1.2 µg/mL of USP Mercaptopurine RS in *Solution B* from the *Standard stock solution*
Sensitivity solution: 0.06 µg/mL of USP Mercaptopurine RS in *Solution B* from the *Standard solution*
Sample stock solution: 0.5 mg/mL of mercaptopurine in a mixture of methanol and *Solution A* (1:9) from NLT 5 Tablets. Place the Tablets into a suitable volumetric flask, add methanol equivalent to 10% of the final volume, and shake mechanically for a minimum of 30 min. Dilute with *Solution A* to volume. Pass through a PVDF filter of 0.45-µm pore size, and discard the first 3 mL of filtrate.
Sample solution: 0.12 mg/mL of mercaptopurine in *Solution A*. Transfer 6.0 mL of the *Sample stock solution* into a 25-mL volumetric flask, and dilute with *Solution A* to volume. Pass through a PVDF filter of 0.45-µm pore size, and discard the first 5 mL of filtrate. [NOTE—Inject the *Sample solution* within 1 h of preparation.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Temperature
Column: 30°
Sample: 4°
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*

USP Monographs

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Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of mercaptopurine from the *Standard solution*
C_S = concentration of USP Mercaptopurine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mercaptopurine in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria
Individual impurities: See *Table 2*. [NOTE—Disregard any impurity peak less than 0.05%.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Didanosine related compound A[a]	0.54	6.3	0.3
Mercaptopurine	1.00	—	—
Mercaptopurine disulfide[b]	2.90	4.4	0.4
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.6

[a] Hypoxanthine.
[b] 1,2-Di(9*H*-purin-6-yl)disulfane.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mercaptopurine RS

Ammoniated Mercury

$Hg(NH_2)Cl$ 252.07
Mercury amide chloride [10124-48-8].

DEFINITION
Ammoniated Mercury contains NLT 98.0% and NMT 100.5% of ammoniated mercury [$Hg(NH_2)Cl$].

IDENTIFICATION
- **A.**
  Sample: 0.1 g
  Analysis: Place the *Sample* in a cold solution of 1 g of sodium thiosulfate in 2 mL of water.
  Acceptance criteria: The *Sample* is soluble, with the evolution of ammonia. When this solution is heated gently, a rust-colored mixture is formed, from which a red precipitate is obtained on centrifugation. If the solution is strongly heated, a black mixture forms.
- **B.**
  Sample: A suitable quantity
  Analysis: Heat the *Sample* with 1 N sodium hydroxide.
  Acceptance criteria: The solution becomes yellow, and ammonia is evolved.
- **C.**
  Sample solution: A suitable quantity in warm acetic acid
  Analysis: *Sample solution* with potassium iodide TS
  Acceptance criteria: The solution yields a red precipitate that is soluble in an excess of the reagent. The solution yields a white precipitate with silver nitrate TS.

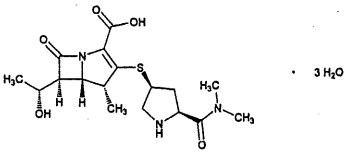
ASSAY
- **PROCEDURE**
  Sample solution: Mix 0.25 g of Ammoniated Mercury with 10 mL of water. Add 3 g of potassium iodide, mix occasionally until dissolved, add about 40 mL of water, and add methyl red TS.
  Analysis: Titrate with 0.1 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N hydrochloric acid is equivalent to 12.60 mg of ammoniated mercury [$Hg(NH_2)Cl$].
  Acceptance criteria: 98.0%–100.5%

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **MERCUROUS COMPOUNDS**
  Sample: 2.5 g
  Analysis: Dissolve the *Sample* in 25 mL of warm hydrochloric acid. Pass through a tared filtering crucible, wash with water, and dry at 60° to constant weight.
  Acceptance criteria: NMT 0.2%; the weight of the residue does not exceed 5 mg.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

Meropenem

Change to read:



$C_{17}H_{25}N_3O_5S \cdot 3H_2O$ 437.51

$C_{17}H_{25}N_3O_5S$ ▲383.46▲ (USP 1-Dec-2019)
1-Azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, 3-[[5-[(dimethylamino)carbonyl]-3-pyrrolidinyl]thio]-6-(1-hydroxyethyl)-4-methyl-7-oxo, trihydrate, [4*R*-[3(3*S**,5*S**),4α,5β,6β(*R**)]]-;
(4*R*,5*S*,6*S*)-3-{[(3*S*,5*S*)-5-(Dimethylcarbamoyl)pyrrolidin-3-yl]thio}-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, trihydrate [119478-56-7].
Anhydrous [96036-03-2].

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DEFINITION

Meropenem contains NLT 98.0% and NMT 101.0% of meropenem ($C_{17}H_{25}N_3O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Change to read:

- **B.** ▲The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

- **PROCEDURE**

▲Solution A: Dilute 15 mL of tetrabutylammonium hydroxide solution (25% in methanol) with water to 750 mL. Adjust with 10% phosphoric acid TS to a pH of 7.5 ± 0.1.

Mobile phase: Acetonitrile, methanol, and *Solution A* (150:100:750)

Standard solution: 0.1 mg/mL of USP Meropenem RS in *Mobile phase*. Immediately after preparation, store this solution in a refrigerator, and use within 24 h.

Sample solution: 0.1 mg/mL of Meropenem in *Mobile phase*. Immediately after preparation, store this solution in a refrigerator, and use within 8 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 300 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of meropenem ($C_{17}H_{25}N_3O_5S$) in the portion of Meropenem taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of meropenem from the *Sample solution*

r_S = peak response of meropenem from the *Standard solution*

C_S = concentration of USP Meropenem RS in the *Standard solution* (mg/mL)

C_U = concentration of Meropenem in the *Sample solution* (mg/mL)

P = designated potency of USP Meropenem RS (mg/mg)▲ (USP 1-Dec-2019)

Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

Change to read:

- **RESIDUE ON IGNITION ⟨281⟩**

Analysis: Ignite at 500 ± 50°▲▲ (USP 1-Dec-2019)

Acceptance criteria: NMT 0.1%

Change to read:

- **ORGANIC IMPURITIES**

Solution A: Mix 1.0 mL of triethylamine and 900 mL of water. Adjust with 10% phosphoric acid TS to a pH of 5.0 ± 0.1, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Solution A* (70:1000)

▲Peak identification solution: 5 mg/mL of USP Meropenem RS in *Mobile phase*. Use this solution between 1 and 24 h from preparation.▲ (USP 1-Dec-2019)

Standard solution: 0.025 mg/mL of USP Meropenem RS in *Solution A*. ▲Store this solution in a refrigerator and use within 24 h.▲ (USP 1-Dec-2019)

Sample solution: 5 mg/mL of Meropenem in *Solution A*. Use this *Sample solution* immediately.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.6 mL/min

▲▲ (USP 1-Dec-2019)

Injection volume: 10 µL

Run time: NLT 3 times the retention time of meropenem

System suitability

Sample: *Standard solution*

Suitability requirements

▲▲ (USP 1-Dec-2019)

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: ▲*Peak identification solution*, *Standard solution*, and *Sample solution*

Chromatograph the *Peak identification solution* and identify the components on the basis of their relative retention times, as shown in *Table 1*.▲ (USP 1-Dec-2019)

Calculate the percentage of each individual impurity in the portion of Meropenem taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P▲\times 100▲ \text{ (USP 1-Dec-2019)}$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of meropenem from the *Standard solution*

C_S = concentration of USP Meropenem RS in the *Standard solution* (mg/mL)

C_U = concentration of Meropenem in the *Sample solution* (mg/mL)

P = ▲potency of meropenem in USP Meropenem RS (mg/mg)▲ (USP 1-Dec-2019)

Acceptance criteria: ▲See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Meropenem open ring[a]	0.45	0.3

USP Monographs

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Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Meropenem	1.0	—
Meropenem dimer[b]	1.9	0.3
Any individual unspecified impurity	—	0.05
Total impurities, excluding meropenem open ring and meropenem dimer	—	0.3

[a] (4*R*,5*S*)-5-[(1*S*,2*R*)-1-Carboxy-2-hydroxypropyl]-3-{[(3*S*,5*S*)-5-(dimethylcarbamoyl)pyrrolidin-3-yl]thio}-4-methyl-4,5-dihydro-1*H*-pyrrole-2-carboxylic acid.

[b] (4*R*,5*S*,6*S*)-3-{[(3*S*,5*S*)-1-{(2*S*,3*R*)-2-[(2*S*,3*R*)-5-Carboxy-4-{[(3*S*,5*S*)-5-(dimethylcarbamoyl)pyrrolidin-3-yl]thio}-3-methyl-2,3-dihydro-1*H*-pyrrol-2-yl]-3-hydroxybutanoyl}-5-(dimethylcarbamoyl)pyrrolidin-3-yl]thio}-6-[(*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid. ▲ (USP 1-Dec-2019)

Delete the following:

▲• **LIMIT OF ACETONE**
Internal standard solution: 0.05 µL/mL of ethyl acetate in dimethylformamide
Standard stock solution: 0.5 mg/mL of acetone in dimethylformamide
Standard solution: Combine 1.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution*.
Sample solution: Dissolve 100 mg of Meropenem in 0.2 mL of dimethylformamide and 2.0 mL of *Internal standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 3-mm × 2-m column; support S2
Temperature
Column: 150°
Injection port: 170°
Carrier gas: Nitrogen
Flow rate: Adjusted so that the retention time for acetone is about 3 min
Injection volume: 2 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetone in the portion of Meropenem taken:

$$\text{Result} = (W_A/5 \times W_U) \times (r_U/r_S)$$

W_A = weight of acetone in the *Standard solution* (mg)
W_U = weight of Meropenem in the *Sample solution* (mg)
r_U = peak area ratio of acetone to the internal standard from the *Sample solution*
r_S = peak area ratio of acetone to the internal standard from the *Standard solution*

Acceptance criteria: NMT 0.05% ▲ (USP 1-Dec-2019)

SPECIFIC TESTS

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Meropenem is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, ▲the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Meropenem is used can be met.▲ (USP 1-Dec-2019)

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 5 mg/mL in water
Acceptance criteria: −17° to −21°, at 20°

Change to read:

• **PH** ⟨791⟩
Sample solution: ▲10 mg/mL in water▲ (USP 1-Dec-2019)
Acceptance criteria: 4.0–6.0

Change to read:

• **STERILITY TESTS** ⟨71⟩**:** ▲Where the label states that Meropenem is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements.▲ (USP 1-Dec-2019)

Change to read:

• **WATER DETERMINATION** ⟨921⟩, *Method I*, ▲*Method Ia*▲ (USP 1-Dec-2019) or *Method Ic*: 11.4%–13.4%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store the dry powder at controlled room temperature.

Change to read:

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, ▲it is so labeled. Where Meropenem must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.▲ (USP 1-Dec-2019)

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Meropenem RS

Meropenem for Injection

DEFINITION

Meropenem for Injection is a sterile dry mixture of Meropenem and Sodium Carbonate. It contains NLT 90.0% and NMT 120.0% of the labeled amount of meropenem ($C_{17}H_{25}N_3O_5S$).

IDENTIFICATION

• **A.** The retention time of the meropenem peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

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ASSAY

Change to read:

• **PROCEDURE**

▲▲ (USP 1-Dec-2019)

Buffer: Dilute 15 mL of tetrabutylammonium hydroxide solution (25% in water) with water to 750 mL. Adjust with ▲10% phosphoric acid TS▲ (USP 1-Dec-2019) to a pH of 7.5 ± 0.1.

Mobile phase: Acetonitrile, methanol, and *Solution A* (150:100:750)

Standard solution: 0.11 mg/mL of USP Meropenem RS in *Mobile phase*. Immediately after preparation, store this solution in a refrigerator, and use within 24 h.

Sample stock solution 1 (where it is represented as being a single-dose container): Nominally 1 mg/mL of meropenem, prepared as follows. Constitute a container of Meropenem for Injection with a volume of water, corresponding to the quantity of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and transfer to a suitable volumetric flask. Dilute with water to volume, and mix.

Sample solution 1: Nominally 0.1 mg/mL of meropenem in *Mobile phase* from *Sample stock solution 1*. Hold this *Sample solution 1* for 2 h at 25 ± 1° before testing.

Sample stock solution 2 (where the label states the quantity of meropenem in a given volume of constituted solution): Nominally 1 mg/mL of meropenem, prepared as follows. Constitute a container of Meropenem for Injection with a volume of water corresponding to the quantity of solvent specified in the labeling, and dilute with water.

Sample solution 2: Nominally 0.1 mg/mL of meropenem in *Mobile phase* from *Sample stock solution 2*. Hold this *Sample solution 2* for 2 h at 25 ± 1° before testing.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*)

Mode: LC

Detector: UV 300 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Dec-2019)

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min▲▲ (USP 1-Dec-2019)

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of meropenem ($C_{17}H_{25}N_3O_5S$) in the portion of Meropenem for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of meropenem from *Sample solution 1* or *Sample solution 2*

r_S = peak response of meropenem from the *Standard solution*

C_S = concentration of USP Meropenem RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of meropenem in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of meropenem in USP Meropenem RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

OTHER COMPONENTS

• **CONTENT OF SODIUM**

Solution A: 38.1 g/L of potassium chloride in water

Standard stock solution: 25.42 µg/mL of sodium chloride (previously dried at 105° for 2 h) in water

Standard solution: 2.5 µg/mL of sodium chloride from the *Standard stock solution* mixed first with *Solution A* to 10% of the final volume and diluted with water to volume

Sample stock solution 1 (where it is represented as being a single-dose container): Nominally 0.125 mg/mL of meropenem, prepared as follows. Constitute a container of Meropenem for Injection with a volume of water corresponding to the quantity of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and transfer to a suitable volumetric flask. Dilute with water to volume.

Sample stock solution 2 (where the label states the quantity of meropenem in a given volume of constituted solution): Nominally 0.125 mg/mL of meropenem, prepared as follows. Constitute a container of Meropenem for Injection with a volume of water, corresponding to the quantity of solvent specified in the labeling. Transfer the constituted solution to a suitable volumetric flask, and dilute with water to volume.

Sample solution: Nominally 0.0125 mg/mL of meropenem from *Sample stock solution 1* or *Sample stock solution 2* mixed first with *Solution A* to 10% of the final volume, and dilute with water to volume

Blank: *Solution A* and water (1:10)

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectroscopy

Analytical wavelength: 589.6 nm sodium emission line

Burner: Single-slot

Flame: Air–acetylene

Lamp: Sodium hollow-cathode

Analysis

Samples: *Standard solution, Sample solution,* and *Blank*

Calculate the percentage of sodium (Na) in the portion of Meropenem for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of sodium chloride in the *Standard solution* (µg/mL)

C_U = nominal concentration of meropenem in the *Sample solution* (µg/mL)

M_{r1} = atomic weight of sodium, 22.99

M_{r2} = molecular weight of sodium chloride, 58.44

Acceptance criteria: 80%–120% of the labeled amount of sodium

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

▲▲ (USP 1-Dec-2019)

Buffer: Mix ▲1.0 mL▲ (USP 1-Dec-2019) of triethylamine and 900 mL of water. Adjust with 10% phosphoric acid TS to a pH of 5.0 ± 0.1, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (70:1000)

USP Monographs

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▲Peak identification solution: 5 mg/mL of USP Meropenem RS in *Mobile phase*. Use this solution between 1 and 24 h from preparation.▲ (USP 1-Dec-2019)

Standard solution: 0.029 mg/mL of USP Meropenem RS in *Buffer*. Store this solution in a refrigerator immediately after preparation, and use within 24 h.

Sample solution: Nominally prepare 5 mg/mL of meropenem in *Buffer* from Meropenem for Injection. This solution has to be prepared fresh and used immediately.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.6 mL/min▲▲ (USP 1-Dec-2019)
Injection volume: 10 µL
Run time: ▲NLT▲ (USP 1-Dec-2019) 3 times the retention time of meropenem

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: ▲*Peak identification solution*, *Standard solution*, and *Sample solution*
Chromatograph the *Peak identification solution* and identify the components on the basis of their relative retention times, as shown in *Table 1*.▲ (USP 1-Dec-2019)
Calculate the percentage of each individual impurity in the portion of Meropenem for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of meropenem from the *Standard solution*
C_S = concentration of USP Meropenem RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of meropenem in the *Sample solution* (mg/mL)
P = potency of meropenem in USP Meropenem RS (mg/mg)

Acceptance criteria: See *Table 1*.

▲Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Meropenem open ring[a]	0.45	0.8
Meropenem	1.0	—
Meropenem dimer[b]	1.9	0.6
Any individual unspecified impurity	—	0.10
Total unspecified impurities	—	1.0
Total impurities	—	2.0

[a] (4*R*,5*S*)-5-[(1*S*,2*R*)-1-Carboxy-2-hydroxypropyl]-3-{[(3*S*,5*S*)-5-(dimethylcarbamoyl]pyrrolidin-3-yl]thio)-4-methyl-4,5-dihydro-1*H*-pyrrole-2-carboxylic acid.
[b] (4*R*,5*S*,6*S*)-3-{[(3*S*,5*S*)-1-{(2*S*,3*R*)-2-[(2*S*,3*R*)-5-Carboxy-4-{[(3*S*,5*S*)-5-(dimethylcarbamoyl]pyrrolidin-3-yl]thio)-3-methyl-2,3-dihydro-1*H*-pyrrol-2-yl]-3-hydroxybutanoyl}-5-(dimethylcarbamoyl)pyrrolidin-3-yl]thio)-6-[(*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.▲ (USP 1-Dec-2019)

Table 1 *(continued)*

SPECIFIC TESTS

Change to read:

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements▲ (USP 1-Dec-2019)
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions*
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at 65° for 6 h.
  Acceptance criteria: 9.0%–12.0%
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **pH** ⟨791⟩
  Sample solution: 50 mg/mL
  Acceptance criteria: 7.3–8.3

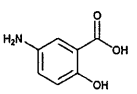
Change to read:

- **STERILITY TESTS** ⟨71⟩: Meets the requirements▲▲ (USP 1-Dec-2019)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for Constitution*. Store at controlled room temperature.
- **LABELING:** Meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. Label it to state the quantity, in mg, of sodium (Na) in a given dosage of meropenem.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Meropenem RS

Mesalamine



$C_7H_7NO_3$ 153.14
Benzoic acid, 5-amino-2-hydroxy-;
5-Aminosalicylic acid [89-57-6].

DEFINITION
Mesalamine contains NLT 98.5% and NMT 101.5% of mesalamine ($C_7H_7NO_3$), calculated on the dried basis.

USP Monographs

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the mesalamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Transfer 7.1 g of anhydrous dibasic sodium phosphate and 6.9 g of monobasic sodium phosphate to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 7.5 mL of a solution of tetrabutylammonium hydroxide 30-hydrate in methanol (1 in 4), dilute with water to volume, and mix.

Mobile phase: Methanol and *Buffer* (15:85)

System suitability solution: 0.25 mg/mL of 4-aminosalicylic acid and 0.4 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Mesalamine RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: 1 mg/mL of Mesalamine in *Mobile phase*

Sample solution: 0.4 mg/mL of Mesalamine in *Mobile phase* from the *Sample stock solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 15 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between 4-aminosalicylic acid and mesalamine, *System suitability solution*
Tailing factor: NMT 2.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mesalamine ($C_7H_7NO_3$) in the portion of Mesalamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesalamine from the *Sample solution*
r_S = peak response of mesalamine from the *Standard solution*
C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL)
C_U = concentration of Mesalamine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample solution: Disperse 500 mg in 40 mL of water, sonicate for 5 min, and filter the dispersion. Use the filtrate.
Analysis: Add 1 mL of nitric acid to the *Sample solution*.
Acceptance criteria: No more chloride than corresponds to 0.7 mL of 0.020 N hydrochloric acid (0.1%)

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample solution: Dissolve 500 mg in water. Filter if necessary. Use the filtrate.
Acceptance criteria: The *Sample solution* shows no more sulfate than corresponds to 1.0 mL of 0.02 N sulfuric acid (0.2%).

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
- **HYDROGEN SULFIDE AND SULFUR DIOXIDE**

Analysis: Dissolve about 500 mg in 5 mL of 1 N sodium hydroxide, add 6 mL of 3 N hydrochloric acid, and stir vigorously. Hold a piece of moistened lead acetate test paper over the mixture.
Acceptance criteria: The test paper so obtained does not become discolored.

- **CONTENT OF 3-AMINOSALICYLIC ACID AND OTHER RELATED IMPURITIES**

[NOTE—Use this test to measure 3-aminosalicylic acid and other related impurities not measured in the test for *Content of Aniline, 2-Aminophenol, and 4-Aminophenol*.]

Mobile phase: Dissolve 1.36 g of monobasic potassium phosphate and 2.2 g of sodium 1-octanesulfonate in 890 mL of water, and adjust with phosphoric acid to a pH of 2.2. Pass through a filter of 0.5-µm or finer pore size. To the filtrate add 80 mL of methanol and 30 mL of acetonitrile.

Standard solution: 1 µg/mL each of USP Mesalamine RS and 3-aminosalicylic acid in *Mobile phase*

Sample solution: 0.5 mg/mL of Mesalamine in *Mobile phase*. Initially add about 75% of the final volume of *Mobile phase*, and sonicate briefly to dissolve. Dilute with *Mobile phase* to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: 3 times the retention time of mesalamine

System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 2 between mesalamine and 3-aminosalicylic acid
Relative standard deviation: NMT 5.0% for both mesalamine and 3-aminosalicylic acid

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 3-aminosalicylic acid:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 3-aminosalicylic acid from the *Sample solution*
r_S = peak response of 3-aminosalicylic acid from the *Standard solution*
C_S = concentration of 3-aminosalicylic acid in the *Standard solution* (µg/mL)
C_U = concentration of Mesalamine in the *Sample solution* (µg/mL)

Calculate the percentage of any other impurity:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

USP Monographs

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r_S = peak response of mesalamine from the *Standard solution*
C_S = concentration of USP Mesalamine RS in the *Standard solution* (µg/mL)
C_U = concentration of Mesalamine in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mesalamine	1.0	—
3-Aminosalicylic acid	1.3	0.2
Any other impurity	—	0.2
Total impurities	—	1.0

• **CONTENT OF ANILINE, 2-AMINOPHENOL, AND 4-AMINOPHENOL**

Standard stock solution: 0.05 mg/mL of aniline, 2 mg/mL of 2-aminophenol, and 2 mg/mL of USP 4-Aminophenol RS in methanol
Standard solution: 0.5 µg/mL of aniline, 20 µg/mL of 2-aminophenol, and 20 µg/mL of USP 4-Aminophenol RS from the *Standard stock solution* in methylene chloride
Sample solution: 100 mg/mL of Mesalamine in methylene chloride. Allow to settle, and use the clear methylene chloride solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 10-m fused-silica capillary; 2.65-µm film of G27
Temperatures
Injection port: 280°
Detector: 300°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	—	70	2
70	30	150	1

Carrier gas: Helium
Flow rate: 15 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between aniline and 2-aminophenol; NLT 2.0 between 2-aminophenol and 4-aminophenol
Relative standard deviation: NMT 10.0% for aniline, 2-aminophenol, and 4-aminophenol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aniline, 2-aminophenol, and 4-aminophenol in the portion of Mesalamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aniline, 2-aminophenol, or 4-aminophenol from the *Sample solution*
r_S = peak response of aniline, 2-aminophenol, or 4-aminophenol from the *Standard solution*
C_S = concentration of aniline, 2-aminophenol, or USP 4-Aminophenol RS in the *Standard solution* (µg/mL)
C_U = concentration of Mesalamine in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aniline	0.5	0.0005
2-Aminophenol	0.9	0.02
4-Aminophenol	1.0	0.02

SPECIFIC TESTS

• **CLARITY OF SOLUTION**
Sample solution: Freshly prepare a solution of 0.25 g of Mesalamine in 10 mL of 1 N hydrochloric acid.
Acceptance criteria: The *Sample solution* is clear.
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 105° for 3 h.
Acceptance criteria: NMT 0.5%
• **pH** ⟨791⟩
Sample: A suspension (1 in 40)
Acceptance criteria: 3.5–4.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP 4-Aminophenol RS
4-Aminophenol.
C_6H_7NO 109.13
USP Mesalamine RS

Mesalamine Extended-Release Capsules

DEFINITION

Mesalamine Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of mesalamine ($C_7H_7NO_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Use the powdered, undried Capsule contents.
Analysis: Record the spectra in the range between 2000 cm^{-1} and 1240 cm^{-1}.
Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**
Buffer: Dissolve 6.8 g of monobasic potassium phosphate and 1.65 g of sodium hydroxide in 800 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5, dilute with water to 1000 mL, and mix.

USP Monographs

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Solution A: Dissolve 3.4 g of tetrabutylammonium hydrogen sulfate and 1.4 g of sodium acetate trihydrate in 1000 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.6. Add 200 mL of acetonitrile, mix, and pass through a filter of 0.5-µm or finer pore size. [NOTE—Increasing the proportion of acetonitrile decreases the retention times. Prepare fresh daily.]
Solution B: Dissolve 4.6 g of tetrabutylammonium hydrogen sulfate and 1.9 g of sodium acetate trihydrate in 1000 mL of water, and adjust with 1 N sodium hydroxide to a pH of 6.6. Add 650 mL of acetonitrile, mix, and pass through a filter of 0.5-µm or finer pore size. [NOTE—Prepare fresh daily.]
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
7	0	100
15	0	100
17	100	0
20	100	0

Internal standard solution: 35 mg/mL of sodium benzoate in *Buffer*
Standard solution: Transfer about 50 mg of USP Mesalamine RS to a 100-mL volumetric flask. Add 4.0 mL of the *Internal standard solution*, dilute with *Buffer* to volume, and mix. Dilute 5.0 mL of this solution with *Buffer* to 25 mL.
Sample solution: Transfer, as completely as possible, the contents of NLT 20 Capsules to a suitable tared container, and determine the average weight of the contents of a Capsule. Finely powder the Capsule contents so that the powder thus obtained passes through a No. 40 sieve (see *Powder Fineness* ⟨811⟩). Transfer a portion of the powder, nominally equivalent to about 250 mg of mesalamine, to a 500-mL volumetric flask. Add 20.0 mL of the *Internal standard solution* and about 300 mL of *Buffer*, and shake by mechanical means for 1 h. Dilute with *Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask. Dilute with *Buffer* to volume, mix, and pass about 10 mL of this solution through a filter of 0.5-µm or finer pore size. Use the filtrate as the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for mesalamine and sodium benzoate are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.5 between mesalamine and sodium benzoate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) in the portion of Capsule contents taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of mesalamine to sodium benzoate from the *Sample solution*
R_S = peak response ratio of mesalamine to sodium benzoate from the *Standard solution*
C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mesalamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Buffer: 0.05 M pH 7.5 phosphate buffer prepared as follows. Dissolve 6.8 g of monobasic potassium phosphate and 1 g of sodium hydroxide in water to make 1000 mL of solution, and adjust with 10 N sodium hydroxide to a pH of 7.50 ± 0.05.
Medium: *Buffer*; 900 mL
Apparatus 2: 100 rpm
Times: 1, 2, 4, and 8 h
Standard solution: A known concentration of USP Mesalamine RS in *Medium*
Sample solution: Filter portions of the solution under test suitably diluted with *Medium*, if necessary.
Analysis: Calculate the percentages of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved at the wavelength of maximum absorbance at about 330 nm by comparing the UV absorbances of the *Sample solution* with that of the *Standard solution*.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved
1	5%–25%
2	30%–50%
4	60%–90%
8	NLT 85%

The percentages of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mesalamine RS

Mesalamine Rectal Suspension

DEFINITION
Mesalamine Rectal Suspension is a suspension of Mesalamine in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mesalamine ($C_7H_7NO_3$). It contains one or more suitable preservatives.

USP Monographs

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IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Buffer: Transfer 7.1 g of anhydrous dibasic sodium phosphate and 6.9 g of monobasic sodium phosphate to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 7.5 mL of a solution of tetrabutylammonium hydroxide in methanol (1 in 4), dilute with water to volume, and mix.

Mobile phase: Methanol and *Buffer* (15:85)

System suitability solution: 0.25 mg/mL of 4-aminosalicylic acid and 0.4 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Mesalamine RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Transfer an accurately measured, well-shaken quantity of Rectal Suspension, nominally equivalent to about 100 mg of mesalamine, to a 100-mL volumetric flask. Add 55 mL of *Mobile phase,* and dissolve by shaking for about 10 min. Dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass this solution through a suitable filter of 0.5-µm or finer pore size, and use the filtrate as the *Sample solution.*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 15 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between 4-aminosalicylic acid and mesalamine, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) in the portion of Rectal Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesalamine from the *Sample solution*

r_S = peak response of mesalamine from the *Standard solution*

C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mesalamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF SODIUM BENZOATE** (if present)

Mobile phase: Transfer 390 mg of ammonium acetate to a 1000-mL volumetric flask, add 100 mL of water, and dissolve by swirling. Add 6 mL of glacial acetic acid and 300 mL of methanol, dilute with water to volume, and mix. Pass this solution through a filter of 0.5-µm or finer pore size.

Standard solution: 1 mg/mL of sodium benzoate in water. To 5.0 mL of this solution add 40 mL of methanol, and dilute with water to 100 mL. Pass this solution through a filter of 0.5-µm or finer pore size.

Sample solution: Transfer about 5 g of well-shaken Rectal Suspension to a 100-mL volumetric flask. Add 40 mL of methanol, dilute with water to volume, and mix. Pass the solution through a filter of 0.5-µm or finer pore size.

Chromatographic system

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25.0-cm; packing L7

Flow rate: 1.5 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage (w/w) of sodium benzoate in the Rectal Suspension taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (10/W)$$

r_U = peak response of sodium benzoate from the *Sample solution*

r_S = peak response of sodium benzoate from the *Standard solution*

C_S = concentration of sodium benzoate in the *Standard solution* (mg/mL)

W = weight of Rectal Suspension taken (g)

Acceptance criteria: 0.05%–0.125%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Buffer: Transfer 7.1 g of anhydrous dibasic sodium phosphate and 6.9 g of monobasic sodium phosphate to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 7.5 mL of a solution of tetrabutylammonium hydroxide in methanol (1 in 4), dilute with water to volume, and mix.

Mobile phase: Methanol and *Buffer* (15:85)

System suitability solution: 0.25 mg/mL of 4-aminosalicylic acid and 0.4 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard stock solution: Transfer about 100 mg of USP Mesalamine RS to a 50-mL volumetric flask, add 15 mL of 2 N hydrochloric acid, and dissolve by swirling. Dilute with 2 N hydrochloric acid to volume, and mix.

Standard solution: Add 5 mL of 2 N sodium hydroxide to 5.0 mL of the *Standard stock solution,* and dilute with *Mobile phase* to 25 mL. Pass this solution through a filter of 0.5-µm or finer pore size.

Sample stock solution: Transfer, with the aid of 2 N hydrochloric acid, the contents of a container of Rectal Suspension to a 200-mL volumetric flask. Add 2 N hydrochloric acid to obtain about 160 mL of solution, and shake for 10 min. Dilute with 2 N hydrochloric acid to volume, and mix.

Sample solution: Transfer a suitable volume of the *Sample stock solution,* nominally equivalent to 40 mg of mesalamine, to a 100-mL volumetric flask. Add a volume of 2 N hydrochloric acid, equal to the added volume of

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the *Sample stock solution*, dilute with *Mobile phase* to volume, and mix. Pass this solution through a suitable filter of 0.5-µm or finer pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 15 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between 4-aminosalicylic acid and mesalamine, *System suitability solution*
Tailing factor: NMT 2.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) in the container of Rectal Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesalamine from the *Sample solution*
r_S = peak response of mesalamine from the *Standard solution*
C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mesalamine in the *Sample solution* (mg/mL)

Acceptance criteria: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase: Dissolve 1.36 g of monobasic potassium phosphate and 2.2 g of sodium 1-octanesulfonate in 890 mL of water, and adjust with phosphoric acid to a pH of 2.2. Pass through a filter of 0.5-µm or finer pore size. To the filtrate add 80 mL of methanol and 30 mL of acetonitrile.

Standard solution: 1 µg/mL each of USP Mesalamine RS and 3-aminosalicylic acid in *Mobile phase*

Sample solution: Transfer a volume of Rectal Suspension, previously well shaken, nominally equivalent to 100 mg of mesalamine, to a beaker. Add water to give a volume of about 80 mL, and adjust with phosphoric acid to a pH of 2.0. Sonicate briefly to dissolve, transfer to a 100-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: 3 times the retention time of mesalamine

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for mesalamine and 3-aminosalicylic acid are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 2 between mesalamine and 3-aminosalicylic acid

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Rectal Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of mesalamine from the *Standard solution*
C_S = concentration of USP Mesalamine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of mesalamine in the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurities: NMT 0.2%
Total impurities: NMT 1.0%

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: Dilute the Rectal Suspension 1 to 10 with water.
Acceptance criteria: 3.5–5.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mesalamine RS

Mesalamine Delayed-Release Tablets

DEFINITION
Mesalamine Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mesalamine ($C_7H_7NO_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
Sample solution: To about 50 mL of water add a quantity of finely powdered Tablets, nominally equivalent to about 800 mg of mesalamine. Boil the mixture for about 5 min, with constant stirring. Filter the hot solution, and allow the filtrate to cool. Collect the precipitated crystals, and dry at about 110°.
Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**
Mobile phase: Dissolve 4.3 g of sodium 1-octanesulfonate in 1 L of water. Adjust with phosphoric acid to a pH of 2.15, pass through a filter of 0.45-µm or finer pore size, and degas.
System suitability stock solution: Transfer about 20 mg each of 3-aminosalicylic acid and USP Salicylic Acid RS to a 200-mL volumetric flask. Dissolve in 50 mL of 1 N hydrochloric acid, sonicate to dissolve, dilute with water to volume, and mix.
System suitability solution: 0.01 mg/mL each of 3-aminosalicylic acid and salicylic acid in water from the *System suitability stock solution*

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Standard stock solution: Transfer about 25 mg of USP Mesalamine RS to a 25-mL volumetric flask. Dissolve in 5 mL of 0.25 N hydrochloric acid, sonicate to dissolve, dilute with water to volume, and mix.
Standard solution: Transfer 10.0 mL of the *Standard stock solution* and 5.0 mL of the *System suitability solution* to a 50-mL volumetric flask. Dilute with water to volume, mix, and pass through a filter of 0.5-µm or finer pore size.
Sample solution: Pipet a 25.0-mL aliquot of the *Sample solution*, obtained as directed in *Organic Impurities*, into a 100-mL volumetric flask. Dilute with water to volume, and pass through a filter of 0.5-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Columns
Precolumns: Two 4.6-mm × 3.0-cm; each containing 10-µm packing L1 and located between the pump and the injector
Analytical: 4.6-mm × 3.3-cm; 3-µm base-deactivated packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2 between mesalamine and salicylic acid or 3-aminosalicylic acid
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesalamine from the *Sample solution*
r_S = peak response of mesalamine from the *Standard solution*
C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mesalamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Solution A: Transfer about 43.35 g of monobasic potassium phosphate and 1.65 g of sodium hydroxide to a 2-L volumetric flask. Dissolve in and dilute with water to volume, and mix. Adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 6.0, and mix.
Solution B: Transfer 133.6 g of sodium hydroxide to a 2-L volumetric flask, dissolve in and dilute with water to volume, and mix.
Medium
Acid stage: 500 mL of 0.1 N hydrochloric acid
Buffer stages: 900 mL of *Solution A*
Apparatus 2
Acid stage: 100 rpm
Buffer stage 1: 100 rpm
Buffer stage 2: 50 rpm
Times
Acid stage: 2 h
Buffer stage 1: 1 h
Buffer stage 2: 90 min

Acid stage
After 2 h of operation, withdraw an aliquot of the fluid, discard the remaining solution, and retain the Tablets in proper order so that each will be returned later to its respective vessel. Blot the Tablets with a paper towel to dry, and proceed immediately as directed in *Buffer stage 1*.
Standard solution: A known concentration of USP Mesalamine RS in *Medium*, equivalent to about 1% of the labeled amount of mesalamine ($C_7H_7NO_3$)
Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium*, if necessary.
Analysis: Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved by comparing the UV maximum absorbance at about 302 nm of the *Sample solution* with that of the *Standard solution*.
Tolerances: See *Table 1*. Continue testing through all levels unless the results conform at an earlier level.
Buffer stage 1
[NOTE—Use *Solution A* that has been equilibrated to a temperature of 37 ± 0.5°.]
Transfer *Solution A* to each of the dissolution vessels, and place each Tablet from the *Acid stage* into its respective vessel. After 1 h, remove a 50-mL aliquot, and proceed immediately as directed in *Buffer stage 2*.
Standard solution: A known concentration of USP Mesalamine RS in *Medium*, equivalent to about 1% of the labeled amount of mesalamine ($C_7H_7NO_3$)
Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium*, if necessary.
Analysis: Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved by comparing the UV maximum absorbance at about 330 nm of the *Sample solution* with that of the *Standard solution*.
Tolerances: See *Table 1*. Continue testing through all levels unless the results conform at an earlier level.

Table 1

Level	Number Tested	Acceptance Criteria
L1	6	No individual value exceeds 1% dissolved.
L2	6	Average of the 12 units ($L_1 + L_2$) is NMT 1% dissolved, and no individual unit is greater than 10% dissolved.
L3	12	Average of the 24 units ($L_1 + L_2 + L_3$) is NMT 1% dissolved, and NMT one individual unit is greater than 10% dissolved.

Buffer stage 2
Add 50 mL of *Solution B* to each dissolution vessel to adjust to a pH of 7.2, and continue the run.
Standard solution: A known concentration of USP Mesalamine RS in *Medium*
Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium*, if necessary.
Analysis: Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved by comparing the UV maximum absorbance at about 332 nm of the *Sample solution* with that of the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of mesalamine ($C_7H_7NO_3$) is dissolved. The requirements are met if the quantities dissolved from the product conform to *Acceptance Table 4* in ⟨711⟩. Continue testing through all levels unless the results conform at an earlier level.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements for *Weight Variation*

USP Monographs

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IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, System suitability stock solution, System suitability solution, Standard stock solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Sample solution: Transfer a portion nominally equivalent to about 400 mg of mesalamine, from NLT 20 finely powdered Tablets, to a 500-mL volumetric flask. Add 50 mL of 1 N hydrochloric acid, and sonicate to dissolve. Shake by mechanical means for 10 min, dilute with water to volume, mix, and pass through a filter of 0.5-µm or finer pore size. [NOTE—Use an aliquot of this solution for the preparation of the *Sample solution* in the *Assay*.]

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of all the peak responses

Acceptance criteria

Individual impurity: The largest secondary peak is NMT 1.0% of the total area.

Any other individual impurity: NMT 0.5%

Total impurities: NMT 2.0%

ADDITIONAL REQUIREMENTS

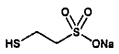
• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Mesalamine RS

USP Salicylic Acid RS

Mesna



$C_2H_5NaO_3S_2$ 164.18

Ethanesulfonic acid, 2-mercapto-, monosodium salt; Sodium 2-mercaptoethanesulfonate [19767-45-4].

DEFINITION

Mesna contains NLT 96.0% and NMT 102.0% of mesna ($C_2H_5NaO_3S_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Add the following:

▲• **B.** The retention time of the mesna peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

Change to read:

• **▲C.**▲ 2S (USP41) **IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements

ASSAY

Change to read:

• **PROCEDURE**

▲Mobile phase: In a 1000-mL volumetric flask, dissolve 2.94 g of potassium dihydrogen phosphate, 2.94 g of dipotassium hydrogen phosphate, and 2.6 g of tetrabutylammonium hydrogen sulfate in about 600 mL of water. Adjust with phosphoric acid to a pH of 2.3, add 335 mL of methanol, and dilute with water to volume.

Standard solution: 4 mg/mL of USP Mesna RS in *Mobile phase*

Sample solution: 4 mg/mL of Mesna in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 15-cm; 4-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Run time: NLT 4 times the retention time of mesna

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—If peak splitting is observed for the mesna peak, the peak response of mesna is the sum of split peaks.]

Calculate the percentage of mesna ($C_2H_5NaO_3S_2$) in the portion of Mesna taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesna from the *Sample solution*

r_S = peak response of mesna from the *Standard solution*

C_S = concentration of USP Mesna RS in the *Standard solution* (mg/mL)

C_U = concentration of Mesna in the *Sample solution* (mg/mL)▲ 2S (USP41)

Acceptance criteria: 96.0%–102.0% on the dried basis

IMPURITIES

Change to read:

• **LIMIT OF CHLORIDE**

Chloride standard solution: 8.24 µg/mL of sodium chloride in water

Sample solution: 200 mg/mL of Mesna in carbon dioxide-free water

Analysis: To 1 mL of the *Sample solution* and 15 mL of water add 1 mL of 2 M nitric acid. Add the resulting solution to 1 mL of silver nitrate solution (17 g in 1000 mL), and allow to stand for 5 min, protected from light. To 10 mL of the *Chloride standard solution* add 5 mL of water and 1 mL of 2 M nitric acid. To this solution add 1 mL of silver nitrate solution (17 g in 1000 mL), and allow to stand for 5 min, protected from light. ▲▲ 2S (USP41)

Acceptance criteria: ▲When viewed against a dark background, the *Sample solution* is not more turbid than the *Chloride standard solution* (NMT 250 ppm).▲ 2S (USP41)

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Change to read:

• **LIMIT OF SULFATE**

Diluent: 30% (v/v) ethanol in water

Sulfate standard stock solution: 1.81 mg/mL of potassium sulfate in *Diluent*

Sulfate standard solution: 0.0181 mg/mL of potassium sulfate in *Diluent*, prepared immediately before use from the *Sulfate standard stock solution*

Sample solution: Add 5.0 mL of the *Sample solution*, prepared as directed in the test for *Limit of Chloride* to a 30-mL volumetric flask, and dilute with water to volume.

Analysis: Add 3 mL of a 250-g/L solution of barium chloride to 4.5 mL of the *Sulfate standard solution*. Shake and allow to stand for 1 min. To 2.5 mL of this solution, add 15 mL of the *Sample solution* and 0.5 mL of acetic acid. Use 15 mL of this mixture for comparison with 15 mL of the *Sulfate standard solution*, prepared in the same manner, but using the *Sulfate standard solution* instead of the *Sample solution*. After 5 min, ▲measure the opalescences in the *Sample solution* and in the *Sulfate standard solution*.▲ 2S (USP41)

Acceptance criteria: ▲Any opalescence in the *Sample solution* is not more intense than that in the *Sulfate standard solution* (NMT 300 ppm).▲ 2S (USP41)

Change to read:

• **ORGANIC IMPURITIES**

Mobile phase: In a 1000-mL volumetric flask, dissolve 2.94 g of potassium dihydrogen phosphate, 2.94 g of dipotassium hydrogen phosphate, and 2.6 g of tetrabutylammonium hydrogen sulfate in about 600 mL of water. Adjust with phosphoric acid to a pH of 2.3, add 335 mL of methanol, and dilute with water to volume.

System suitability solution: 0.18 mg/mL of USP Mesna RS and 0.004 mg/mL of USP Mesna Related Compound A RS in *Mobile phase*

Standard solution 1: 8 µg/mL of USP Mesna Related Compound A RS and 120 µg/mL of USP Mesna Related Compound B RS in *Mobile phase*

Standard solution 2: 12 µg/mL of USP Mesna RS in *Mobile phase*

Sample solution: 4 mg/mL of Mesna in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1 mL/min

Run time: ▲NLT▲ 2S (USP41) 4 times the retention time of mesna

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for mesna and mesna related compound A are about 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 3.0 between mesna and mesna related compound A

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

[NOTE—Identify the peaks using the relative retention times provided in *Table 1*.]

Calculate the percentage of mesna related compound A in the portion of Mesna taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesna related compound A from the *Sample solution*

r_S = peak response of mesna related compound A from *Standard solution 1*

C_S = concentration of USP Mesna Related Compound A RS in *Standard solution 1* (mg/mL)

C_U = concentration of Mesna in the *Sample solution* (mg/mL)

Calculate the percentage of mesna related compound B in the portion of Mesna taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesna related compound B from the *Sample solution*

r_S = peak response of mesna related compound B from *Standard solution 1*

C_S = concentration of USP Mesna Related Compound B RS in *Standard solution 1* (mg/mL)

C_U = concentration of Mesna in the *Sample solution* (mg/mL)

Calculate the percentage of any specified impurities (thiouronium ethanesulfonic acid, guanidinethiouronium ethanesulfonic acid, and mesna triazine analog) and any unspecified impurities in the portion of Mesna taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any specified or unspecified, individual impurity from the *Sample solution*

r_S = peak response of mesna from *Standard solution 2*

C_S = concentration of USP Mesna RS in *Standard solution 2* (mg/mL)

C_U = concentration of Mesna in the *Sample solution* (mg/mL)

F = relative response factors (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Thiouronium ethanesulfonic acid[a]	0.6	100	0.3
Guanidinethiouronium ethanesulfonic acid[b]	0.6	100	0.3
Mesna triazine analog[c]	0.8	100	0.3
Mesna	1.0	—	—
Mesna related compound A	1.4	—	0.2
Mesna related compound B	2.3	—	3.0
Individual, unspecified impurities	—	1.0	0.1
Total unspecified impurities	—	—	0.3

[a] 2-(Carbamimidoylthio)ethane-1-sulfonic acid.

[b] 2-[(*N*-Carbamimidoylcarbamimidoyl)thio]ethane-1-sulfonic acid.

[c] 2-((4,6-Diamino-1,3,5-triazin-2-yl)thio)ethane-1-sulfonic acid.

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SPECIFIC TESTS

Change to read:

- **▲Loss on Drying** ⟨731⟩▲ 2S (USP41)
  Sample: 1 g
  Analysis: Dry the *Sample* under vacuum at a pressure not exceeding 1 mm of mercury at 60° over phosphorus pentoxide for 2 h.
  Acceptance criteria: NMT 1.0%
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL of Mesna in carbon dioxide-free water
  Acceptance criteria: 4.5–6.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in a tight container, and store at room temperature.

Change to read:

- **USP Reference Standards** ⟨11⟩
  USP Mesna RS
  USP Mesna Related Compound A RS
  2-(Acetylthio)ethane-1-sulfonic acid, ▲potassium salt, crystal adduct with potassium chloride.
  $C_4H_7KO_4S_2 \cdot KCl$ 296.86▲ (ERR 1-Mar-2019)
  USP Mesna Related Compound B RS
  2,2′-Disulfanediylbis(ethane-1-sulfonic acid), ▲dipotassium salt, crystal adduct with sodium chloride.
  $C_4H_8K_2O_6S_4 \cdot NaCl$ 416.98▲ (ERR 1-Mar-2019)

Add the following:

▲Mesna Tablets

DEFINITION

Mesna Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of mesna ($C_2H_5NaO_3S_2$).

IDENTIFICATION

- **A.** The retention time of the mesna peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Standard: Mix 1.2 mg of USP Mesna RS with potassium bromide.
  Sample: Grind 1 Tablet in a mortar heated to 30°–50°. Mix a nominal amount of 1.2 mg of mesna of this powder with potassium bromide.
  Acceptance criteria: The IR spectra of the *Sample* exhibit the absorption bands at about 1180, 1120, and 1060 cm^{-1}, similar to the *Standard*.

ASSAY

- **Procedure**
  Solution A: Dissolve 2.72 g of monobasic potassium phosphate and 6.79 g of tetrabutylammonium hydrogen sulfate in 700 mL of water.
  Mobile phase: Methanol and *Solution A* (30:70). [Note—The pH of the *Mobile phase* is about 2.8.]
  System suitability solution: 4 mg/mL of USP Mesna RS and 0.02 mg/mL of USP Mesna Related Compound A RS in *Mobile phase*
  Standard solution: 4 mg/mL of USP Mesna RS in *Mobile phase*
  Sample solution: Nominally 4 mg/mL of mesna in *Mobile phase* prepared as follows. Transfer an adequate amount of mesna from NLT 10 finely powdered Tablets to a suitable volumetric flask. Add about 70% of the total volume of *Mobile phase* and sonicate for about 20 min. Dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size and discard the first 5 mL of the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 2.1-mm × 20-cm; 5-µm packing L7
  Column temperature: 40°
  Flow rate: 0.325 mL/min
  Injection volume: 5 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 1.5 between mesna and mesna related compound A, *System suitability solution*
  Relative standard deviation: NMT 2%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of mesna ($C_2H_5NaO_3S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesna from the *Sample solution*
r_S = peak response of mesna from the *Standard solution*
C_S = concentration of USP Mesna RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mesna in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

- **Dissolution** ⟨711⟩
  Medium: 0.06 N hydrochloric acid; 500 mL
  Apparatus 2: 50 rpm
  Time: 15 min
  Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
  Standard solution: 0.8 mg/mL of USP Mesna RS in *Medium*
  Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first 5 mL of the filtrate.
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 1.5 between mesna and mesna related compound A, *System suitability solution*
  Relative standard deviation: NMT 2%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of mesna ($C_2H_5NaO_3S_2$) dissolved:

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$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of mesna from the *Sample solution*
r_S = peak response of mesna from the *Standard solution*
C_S = concentration of USP Mesna RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of mesna ($C_2H_5NaO_3S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Mobile phase, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.

Standard solution: 0.02 mg/mL of USP Mesna RS and 0.1 mg/mL of USP Mesna Related Compound B RS in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Detector

For *System suitability solution*: UV 230 nm

For *Standard solution* and ***Sample solution*:** UV 202 nm

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between mesna and mesna related compound A, *System suitability solution*

Relative standard deviation: NMT 2% for both mesna and mesna related compound B, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of mesna related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesna related compound B from the *Sample solution*
r_S = peak response of mesna related compound B from the *Standard solution*
C_S = concentration of USP Mesna Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mesna in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of mesna from the *Standard solution*
C_S = concentration of USP Mesna RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mesna in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiouronium ethanesulfonic acid[a, b]	0.6	—
Guanidinethiouronium ethanesulfonic acid[a, c]	0.6	—
Mesna	1.0	—
Mesna related compound A[a]	1.3	—
Mesna related compound B[a]	2.5	3
Individual unspecified degradation product	—	0.1
Total degradation products (except mesna related compound B)	—	0.5

[a] Not included in the total degradation products.
[b] 2-(Carbamimidoylthio)ethane-1-sulfonic acid.
[c] 2-[(*N*-Carbamimidoylcarbamimidoyl)thio]ethane-1-sulfonic acid.

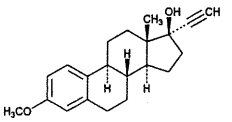
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mesna RS
USP Mesna Related Compound A RS
2-(Acetylthio)ethane-1-sulfonic acid, ▲potassium salt, crystal adduct with potassium chloride.
$C_4H_7KO_4S_2 \cdot KCl$ 296.86▲ (ERR 1-Mar-2019)
USP Mesna Related Compound B RS
2,2′-Disulfanediylbis(ethane-1-sulfonic acid), ▲dipotassium salt, crystal adduct with sodium chloride.
$C_4H_8K_2O_6S_4 \cdot NaCl$ 416.98▲ (ERR 1-Mar-2019)▲ 2S (USP41)

Mestranol



$C_{21}H_{26}O_2$ 310.43

19-Norpregna-1,3,5(10)-trien-20-yn-17-ol, 3-methoxy-, (17α)-;
3-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol [72-33-3].

DEFINITION

Mestranol contains NLT 97.0% and NMT 102.0% of mestranol ($C_{21}H_{26}O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

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Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Sample solution: 100 µg/mL of Mestranol in methanol
Acceptance criteria: Meets the requirements
• **C.**
Standard solution: 1 mg/mL of USP Mestranol RS in chloroform
Sample solution: 1 mg/mL of Mestranol in chloroform
Chromatographic system
(See *Chromatography ⟨621⟩, Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform and dehydrated alcohol (29:1)
Spray reagent: Prepare as directed in *Solution A* in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Apply both solutions to a line parallel to and 2.5 cm from the bottom edge of a thin-layer chromatographic plate. Place the plate in the *Developing solvent system*. Develop the chromatogram until the solvent front has moved about 15 cm from the origin. Remove the plate from the chamber, allow the solvent to evaporate, and spray with *Spray reagent*. Heat the plate in an oven at 105° for 5 min, and observe under long-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**
Solution A: Pipet 30 mL of methanol into a 100-mL volumetric flask contained in an ice bath. Add slowly and cautiously and with continuous stirring about 65 mL of sulfuric acid, taking care that the temperature remains below 15°. Allow the solution to warm to room temperature, and dilute with sulfuric acid to volume.
Standard solution: 5 µg/mL of USP Mestranol RS in chloroform
Sample solution: 5 µg/mL of Mestranol in chloroform
Analysis: Pipet 4 mL each of the *Standard solution* and the *Sample solution* into separate glass-stoppered, 25-mL conical flasks. Evaporate the solutions under a slow current of air, without the aid of heat, to dryness. Dissolve the residue in 0.3 mL of methanol. Place the flasks in a water bath maintained at a temperature of 25°, and pipet into each, with constant swirling, 10 mL of *Solution A*. Insert the stoppers in the flasks. At 6 min, accurately timed after the addition of *Solution A*, concomitantly determine the absorbances of the solutions.
Instrumental conditions
Mode: Vis
Analytical wavelength: Maximum at about 545 nm
Cell: 1 cm
Blank: *Solution A*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of mestranol ($C_{21}H_{26}O_2$) in the portion of Mestranol taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Mestranol RS in the *Standard solution* (µg/mL)
C_U = concentration of Mestranol in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

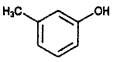
SPECIFIC TESTS

• **LOSS ON DRYING ⟨731⟩**
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%
• **MELTING RANGE OR TEMPERATURE ⟨741⟩:** 146°–154°, but the range between beginning and end of melting does not exceed 4°.
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg, previously dried, per mL, in dioxane
Acceptance criteria: +2° to +8°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Mestranol RS

Metacresol



C_7H_8O 108.14
3-Methylphenol;
3-Hydroxytoluene [108-39-4].

DEFINITION

Metacresol contains NLT 98.0% and NMT 102.0% of metacresol (C_7H_8O).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)

Change to read:

• **B.** The retention time of the ▲metacresol▲ (ERR 1-Jan-2019) peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Internal standard solution: 1 mg/mL of USP Phenol RS in methanol
System suitability solution: 5 mg/mL each of metacresol, orthocresol, and paracresol in methanol
Standard solution: 1 mg/mL of USP Metacresol RS in the *Internal standard solution*
Sample solution: 1 mg/mL of Metacresol in the *Internal standard solution*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability.*)
Mode: GC
Detector: Flame-ionization
Column: 0.25-mm × 30-m; 0.25-µm coating of G7
Temperatures
Injection port: 200°

USP Monographs

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Detector: 200°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
90	0	90	10
90	2	120	0
120	10	150	3

Carrier gas: Helium
Flow rate: 1.8 mL/min
Injection type: Split ratio of 1:40
Injection volume: 1 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for phenol, orthocresol, paracresol, and metacresol are about 0.77, 0.85, 0.99, and 1.00, respectively.]
Suitability requirements
Resolution: NLT 1.4 between the paracresol and metacresol peaks, *System suitability solution*
Relative standard deviation: NMT 1.0% for the peak ratio of metacresol to phenol, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of metacresol (C_7H_8O) in the portion of Metacresol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the metacresol peak response to the phenol peak response from the *Sample solution*
R_S = ratio of the metacresol peak response to the phenol peak response from the *Standard solution*
C_S = concentration of USP Metacresol RS in the *Standard solution* (mg/mL)
C_U = concentration of Metacresol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **ORGANIC IMPURITIES**
System suitability solution: 5 mg/mL each of metacresol, orthocresol, and paracresol in methanol
Sensitivity solution: 5 µg/mL of USP Metacresol RS in methanol
Sample solution: 10 mg/mL of Metacresol in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame-ionization
Column: 0.25-mm × 30-m; 0.25-µm coating of G7
Temperatures
Injection port: 200°
Detector: 200°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
90	0	90	10
90	2	150	15

Carrier gas: Helium
Flow rate: 1.8 mL/min
Injection type: Split ratio of 1:40
Injection volume: 1 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.4 between the paracresol and metacresol peaks, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Metacresol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_T = sum of all the peak areas from the *Sample solution*

Acceptance criteria: See *Table 3*. Disregard any peak less than 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Orthocresol	0.85	0.5
Paracresol	0.99	0.5
Metacresol	1.00	—
Any unspecified impurity	—	0.1
Total impurities	—	1.0

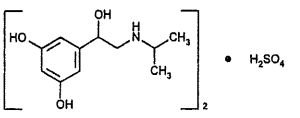
SPECIFIC TESTS

• **CLARITY OF SOLUTION**
A.
Analysis: Add 10 mL of Metacresol to 10 mL of hexane, and mix.
Acceptance criteria: A clear solution is obtained.
B.
Analysis: Add 1.0 mL of Metacresol to 20 mL of 1 N sodium hydroxide, and mix.
Acceptance criteria: A clear solution is obtained.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Metacresol RS
USP Phenol RS
Phenol.
C_6H_6O 94.11

Metaproterenol Sulfate



$(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ 520.59

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1,3-Benzenediol, 5-[1-hydroxy-2-(1-methylethyl)amino] ethyl-, (±)-, sulfate (2:1) (salt).
(±)-3,5-Dihydroxy-α-[(isopropylamino)methyl]benzyl alcohol sulfate (2:1) [5874-97-5].

» Metaproterenol Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$, calculated on the anhydrous, isopropyl alcohol-free, and methanol-free basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Metaproterenol Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: To a solution of 10 mg in 1 mL of water add 1 drop of ferric chloride TS: a violet color is produced.

C: It responds to the tests for *Sulfate* ⟨191⟩.

D: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for metaproterenol, the retention time of which corresponds with that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

pH ⟨791⟩: between 4.0 and 5.5, in a solution containing 100 mg per mL.
Water Determination, *Method I* ⟨921⟩: not more than 2.0%.
Residue on ignition ⟨281⟩: not more than 0.1%.
Iron ⟨241⟩—Dissolve 2.0 g in 45 mL of water, add 2 mL of hydrochloric acid, and mix: the limit is 5 ppm.
Limit of metaproterenone sulfate—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 328 nm, determined in an aqueous solution containing 9.0 mg per mL, is not more than 0.009 (0.1%).

Isopropyl alcohol and methanol—

Isopropyl alcohol standard solution—Transfer about 0.3 g of isopropyl alcohol, accurately weighed, to a 100-mL volumetric flask containing about 10 mL of water, dilute with water to volume, and mix. Pipet 10 mL of the resulting solution into a 100-mL volumetric flask, add about 85 mL of pyridine, mix, and allow to stand for 1 hour. Dilute with pyridine to volume, and mix. Pipet 5 mL of this solution to a 50-mL volumetric flask, dilute with pyridine to volume, and mix. The solution so obtained contains about 30 µg of isopropyl alcohol per mL.

Methanol standard solution—Prepare as directed for *Isopropyl alcohol standard solution*, using about 0.1 g of methanol, accurately weighed. The resulting solution contains about 10 µg of methanol per mL.

Test preparation—Transfer about 1 g of Metaproterenol Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in about 2 mL of water, dilute with pyridine to volume, and mix.

Chromatographic system—The gas chromatograph is equipped with a flame-ionization detector and contains a 2-m × 2-mm column packed with 0.1% liquid phase G25 on 80- to 100-mesh support S7. The injection port is maintained at a temperature of about 150°; the column is programmed for 2 minutes at 40°, to increase at a rate of about 15° per minute to 200°, and for 10 minutes at 200°; the detector is maintained at about 250°; and helium is used as the carrier gas at a flow rate of about 15 mL per minute.

Procedure—Inject equal volumes (about 2 µL) of the *Test preparation*, the *Isopropyl alcohol standard solution*, and the *Methanol standard solution* successively into the gas chromatograph. Measure the responses of the isopropyl alcohol peak and the methanol peak in each chromatogram. Determine the quantities, in mg, of isopropyl alcohol and methanol in the portion of Metaproterenol Sulfate taken by the formula:

$$0.1C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of isopropyl alcohol or methanol in the *Isopropyl alcohol standard solution* or the *Methanol standard solution*; and r_U and r_S are the responses of the respective analytes in the *Test preparation* and of the corresponding *Isopropyl alcohol standard solution* or *Methanol standard solution:* not more than 0.3% of isopropyl alcohol and not more than 0.1% of methanol are found.

Assay—

Mobile phase—Dissolve 11.9 g of anhydrous dibasic sodium phosphate in water to make 1000 mL of solution, and mix (*Solution A*). Dissolve 9.1 g of monobasic potassium phosphate in water to make 1000 mL of solution, and mix (*Solution B*). Mix 735 mL of *Solution A* and 140 mL of *Solution B*, add 125 mL of methanol, and mix. Filter and degas this solution before use.

Standard preparation—Dissolve an accurately weighed quantity of USP Metaproterenol Sulfate RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 2 mg per mL.

Assay preparation—Transfer about 100 mg of Metaproterenol Sulfate, accurately weighed, to a 50-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 5-cm guard column that contains packing L7 and a 4.6-mm × 25-cm analytical column that contains 10-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 500 theoretical plates, the tailing factor for the analyte peak is not more than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ in the portion of Metaproterenol Sulfate taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Metaproterenol Sulfate RS in the *Standard preparation*, and r_U and r_S are the peak responses from the *Assay preparation* and the *Standard preparation*, respectively.

Metaproterenol Sulfate Oral Solution

» Metaproterenol Sulfate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$.

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Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Metaproterenol Sulfate RS

Identification—
A: Transfer a portion of Oral Solution, equivalent to about 10 mg of metaproterenol sulfate, to a separator, and extract with four 30-mL portions of ether, discarding the ether extracts. Apply 10 µL of the extracted portion of Oral Solution to the lower right corner of a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Develop the chromatogram in a solvent system consisting of the lower layer of a well-shaken mixture of dioxane, methylene chloride, alcohol, and ammonium hydroxide (4:4:1:1). Allow the solvent front to move about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry in vacuum at 35° to 40° for 30 minutes. Rotate the plate 90°. At a point about four-fifths of the distance between the initial application of the Oral Solution extract and the solvent front, apply 10 µL of a Standard solution of USP Metaproterenol Sulfate RS in water containing about 2 mg per mL. Proceed as directed in *Identification* test *A* under *Metaproterenol Sulfate Inhalation Solution*, beginning with "Allow the spots to dry": the R_F value of the principal spot obtained from the Oral Solution corresponds to that obtained from the Standard solution.
B: The retention time of the major peak for metaproterenol in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
pH ⟨791⟩: between 2.5 and 4.0, in a solution obtained by mixing 1 volume of Oral Solution and 4 volumes of water.

Assay—
Mobile phase—Mix 10 mL of formic acid and water to make 1000 mL of solution. Filter and degas this solution before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Metaproterenol Sulfate RS in water to obtain a solution having a known concentration of about 0.2 mg per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 20 mg of metaproterenol sulfate, to a 100-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector, a 4.6-mm × 5-cm guard column that contains packing L2, and a 3.9-mm × 30-cm analytical column that contains packing L1. [NOTE—After use, rinse the analytical column with water and store with water in it.] The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the analyte peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Metaproterenol Sulfate RS in the *Standard preparation; V* is the volume, in mL, of Oral Solution taken; and r_U and r_S are the peak responses from the *Assay preparation* and the *Standard preparation,* respectively.

Metaproterenol Sulfate Tablets

» Metaproterenol Sulfate Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Metaproterenol Sulfate RS

Identification—
A: Powder a number of Tablets, equivalent to about 100 mg of metaproterenol sulfate, add 10 mL of water, stir for about 3 minutes, and centrifuge. Use the clear solution so obtained as the *Test solution.* Dissolve a suitable quantity of *USP Metaproterenol Sulfate RS* in water to obtain a Standard solution having a concentration of 10 mg per mL. Apply separate 10-µL portions of the *Test solution* and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Proceed as directed in *Identification* test *A* under *Metaproterenol Sulfate Inhalation Solution,* beginning with "Allow the spots to dry": the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the Standard solution.
B: Mix a quantity of powdered Tablets, equivalent to about 20 mg of metaproterenol sulfate, with 5 mL of water, and filter: the filtrate responds to the tests for *Sulfate* ⟨191⟩.
C: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for metaproterenol, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay.*

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Metaproterenol Sulfate RS in the same *Medium.*
Tolerances—Not less than 70% *(Q)* of the labeled amount of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Metaproterenol Sulfate.*
Assay preparation—Transfer 20 Tablets to a 500-mL conical flask. Add an accurately measured volume of 0.01 N hydrochloric acid sufficient to yield a solution containing about 2 mg of metaproterenol sulfate per mL, shake by mechanical means for 30 minutes, and filter. Use the filtrate so obtained as the *Assay preparation.*

USP Monographs

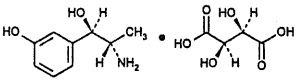
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Procedure—Proceed as directed for *Procedure* in the *Assay* under *Metaproterenol Sulfate*. Calculate the quantity, in mg, of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ in each Tablet taken by the formula:

$$(CV/20)(r_U/r_S)$$

in which V is the volume, in mL, of 0.01 N hydrochloric acid added; and C, r_U, and r_S are as defined therein.

Metaraminol Bitartrate



$C_9H_{13}NO_2 \cdot C_4H_6O_6$ 317.29

Benzenemethanol, α-(1-aminoethyl)-3-hydroxy-, [*R*-(*R**,*S**)]-, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1) (salt); (–)-α-(1-Aminoethyl)-*m*-hydroxybenzyl alcohol tartrate (1:1) (salt) [33402-03-8].

DEFINITION

Metaraminol Bitartrate contains NLT 98.0% and NMT 102.0% of metaraminol bitartrate $(C_9H_{13}NO_2 \cdot C_4H_6O_6)$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 3.24 g of anhydrous octanesulphonic acid sodium salt in 1 L of water.
Solution B: Acetonitrile and water (90:10)
Diluent: Acetonitrile and water (20:80)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
20	5	95
30	5	95
30.1	80	20
40	80	20

Standard solution: 0.75 mg/mL of USP Metaraminol Bitartrate RS in *Diluent*
Sample solution: 0.75 mg/mL of Metaraminol Bitartrate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of metaraminol bitartrate $(C_9H_{13}NO_2 \cdot C_4H_6O_6)$ in the portion of Metaraminol Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metaraminol from the *Sample solution*
r_S = peak response of metaraminol from the *Standard solution*
C_S = concentration of USP Metaraminol Bitartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Metaraminol Bitartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, Mobile phase, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Peak identification solution: 0.1 mg/mL each of USP Metaraminol Bitartrate RS, USP Metaraminol Related Compound A RS, and USP Metaraminol Related Compound B RS in *Diluent*
Sensitivity solution: 0.76 µg/mL of USP Metaraminol Bitartrate RS in *Diluent* from the *Standard solution*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Signal-to-noise ratio: NLT 15, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of metaraminol related compound A and any unspecified impurity in the portion of Metaraminol Bitartrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of metaraminol related compound A or any unspecified impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard peaks due to tartaric acid and metaraminol related compound B.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tartaric acid	0.13 and 0.14	—
Metaraminol	1.00	—
Metaraminol related compound B	1.52	—

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Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metaraminol related compound A	1.82	0.15
Any unspecified impurity	—	0.10
Total impurities	—	1.0

• **LIMIT OF METARAMINOL RELATED COMPOUND B**

Solution A: Dissolve 1.88 g of anhydrous 1-hexane sulfonic acid sodium salt and 1.36 g of monobasic potassium phosphate in 1 L of water. Adjust with dilute phosphoric acid to a pH of 2.6.

Solution B: Acetonitrile and water (85:15)

Diluent: Acetonitrile and water (20:80)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
10	55	45
15	50	50
18	50	50
21	10	90
30	10	90
31	70	30
35	70	30

Standard solution: 0.0012 mg/mL of USP Metaraminol Related Compound B RS in *Diluent*

Sample solution: 0.75 mg/mL of Metaraminol Bitartrate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 15.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metaraminol related compound B in the portion of Metaraminol Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metaraminol related compound B from the *Sample solution*

r_S = peak response of metaraminol related compound B from the *Standard solution*

C_S = concentration of USP Metaraminol Related Compound B RS in the *Standard solution* (mg/mL)

C_U = concentration of Metaraminol Bitartrate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.15%

• **ENANTIOMERIC PURITY**

Mobile phase: Heptane, methanol, isopropyl alcohol, and methane sulfonic acid (80: 10: 10: 0.2)

Metaraminol enantiomer stock solution: 0.05 mg/mL of USP Metaraminol Enantiomer RS in *Mobile phase*

System suitability solution: 1 mg/mL of USP Metaraminol Bitartrate RS and 0.005 mg/mL of USP Metaraminol Enantiomer RS from *Metaraminol enantiomer stock solution* in *Mobile phase*

Sensitivity solution: 1 µg/mL of USP Metaraminol Bitartrate RS in *Mobile phase*

Sample solution: 1 mg/mL of Metaraminol Bitartrate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 3-µm packing L51

Column temperature: 40°

Flow rate: 0.5 mL/min

Injection volume: 10 µL

Run time: NLT 3 times the retention time of metaraminol

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times for metaraminol and the metaraminol enantiomer are about 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the metaraminol and the metaraminol enantiomer peaks, *System suitability solution*

Signal-to-noise ratio: NLT 15, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of metaraminol enantiomer in the portion of Metaraminol Bitartrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of metaraminol enantiomer from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: NMT 0.15%

SPECIFIC TESTS

• **CONTENT OF TARTARIC ACID**

Sample solution: To 0.1 g of Metaraminol Bitartrate, add 60 mL of dimethylformamide, and sonicate for 2 min.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N tetrabutylammonium hydroxide VS

Endpoint detection: Potentiometric

Blank: Dimethylformamide

Analysis: Titrate with *Titrant* potentiometrically using a suitable electrode. [NOTE—A suitable electrode may be 6.0229.100, 0–14 pH, 70°, lithium chloride saturated in alcohol.] Perform a blank determination, and make any necessary correction.

Calculate the percentage of tartaric acid in the portion of Metaraminol Bitartrate taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/2 \times W\} \times 100$$

USP Monographs

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V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 150.09 mg/mEq
W = weight of Metaraminol Bitartrate in the *Sample solution* (mg)

Acceptance criteria: 44.9%–49.7% on the dried basis

• **pH** ⟨791⟩
Sample solution: 50 mg/mL of Metaraminol Bitartrate in water
Acceptance criteria: 3.2–3.5

• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩
USP Metaraminol Bitartrate RS
USP Metaraminol Related Compound A RS
(*E*)-1-[3-(Benzyloxy)phenyl]-2-(hydroxyimino)propan-1-one.
$C_{16}H_{15}NO_3$ 269.30
USP Metaraminol Related Compound B RS
2-Amino-1-[3-(benzyloxy)phenyl]propan-1-ol.
$C_{16}H_{19}NO_2$ 257.33
USP Metaraminol Enantiomer RS
3-[(1*S*,2*R*)-2-Amino-1-hydroxypropyl]phenol.
$C_9H_{13}NO_2$ 167.21

Metaraminol Bitartrate Injection

» Metaraminol Bitartrate Injection is a sterile solution of Metaraminol Bitartrate in Water for Injection. It contains, in each mL, an amount of metaraminol bitartrate equivalent to not less than 9.0 mg and not more than 11.0 mg of metaraminol ($C_9H_{13}NO_2$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Metaraminol Bitartrate RS

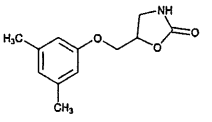
Identification—
A: Evaporate a 1-mL portion to dryness: the residue so obtained meets the requirements for *Identification* test *A* under *Metaraminol Bitartrate*.
B: It meets the requirements for *Identification* tests *B* and *C* under *Metaraminol Bitartrate*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.5 USP Endotoxin Units per mg of metaraminol.
pH ⟨791⟩: between 3.2 and 4.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
0.0032 M Hexanesulfonate buffer—Mix 600 mg of sodium 1-hexanesulfonate with water to obtain 1000 mL of solution, adjust with phosphoric acid to a pH of 3.0 ± 0.05, and filter.
Mobile phase—Prepare a suitable degassed and filtered mixture of methanol and *0.0032 M Hexanesulfonate buffer* (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Metaraminol Bitartrate RS in water to obtain a solution having a known concentration of about 0.2 mg of metaraminol per mL.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 20 mg of metaraminol, to a 100-mL volumetric flask, dilute with water to volume, and mix.
System suitability preparation—Prepare a solution of propylparaben in alcohol containing 0.4 mg per mL. Mix 1 volume of this solution with 99 volumes of the *Standard preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 264-nm detector and a 4-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability preparation* and the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2600 theoretical plates, the resolution, *R*, between the metaraminol bitartrate and propylparaben peaks is not less than 3.0 with propylparaben eluting first, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of metaraminol ($C_9H_{13}NO_2$) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of metaraminol represented by the USP Metaraminol Bitartrate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Metaxalone



$C_{12}H_{15}NO_3$ 221.25
2-Oxazolidinone, 5-[(3,5-dimethylphenoxy)methyl]-; 5-[(3,5-Xylyloxy)methyl]-2-oxazolidinone; 5-[(3,5-Dimethylphenoxy)methyl]-1,3-oxazolidin-2-one [1665-48-1].

DEFINITION
Metaxalone contains NLT 98.0% and NMT 102.0% of metaxalone ($C_{12}H_{15}NO_3$), calculated on dried basis.

USP Monographs

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A** or **197K**▲ (CN 1-May-2020)

Change to read:

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the ▲*Standard solution*,▲ (ERR 1-Apr-2019) as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 0.68 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (50:50)

Standard stock solution: 0.5 mg/mL of USP Metaxalone RS prepared as follows. Transfer a suitable quantity of USP Metaxalone RS to a suitable volumetric flask. Add 50% of the flask volume of methanol. Sonicate for 5 min to dissolve. Add 40% of the flask volume of *Buffer*, and mix. Cool to room temperature. Dilute with *Buffer* to volume.

Standard solution: 0.05 mg/mL of USP Metaxalone RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: 0.5 mg/mL of Metaxalone prepared as follows. Transfer a suitable quantity of Metaxalone to a suitable volumetric flask. Add 50% of the flask volume of methanol. Sonicate for 5 min to dissolve. Add 40% of the flask volume of *Buffer*, and mix. Cool to room temperature. Dilute with *Buffer* to volume.

Sample solution: 0.05 mg/mL of Metaxalone from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of metaxalone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metaxalone ($C_{12}H_{15}NO_3$) in the portion of Metaxalone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)

C_U = concentration of Metaxalone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.30%

• **ORGANIC IMPURITIES, PROCEDURE 1**

If metaxalone related compound B, metaxalone related compound C, or *N*-benzyl metaxalone is a known process impurity, *Organic Impurities, Procedure 2* is recommended.

Solution A: 0.1% Trifluoroacetic acid in water

Solution B: 0.1% Trifluoroacetic acid in acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10.0	65	35
11.0	75	25
15.0	75	25

Diluent: Acetonitrile and water (75:25)

System suitability solution: 4 mg/mL of USP Metaxalone RS and 0.01 mg/mL of 3,5-dimethylphenol in *Diluent*

Sensitivity solution: 0.002 mg/mL of USP Metaxalone RS in *Diluent*

Sample solution: 4 mg/mL of Metaxalone in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 273 nm

Column: 4.6-mm × 25-cm; 5-µm packing L68

Flow rate: 2.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between metaxalone and 3,5-dimethylphenol, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Metaxalone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the peak responses of metaxalone and impurities from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metaxalone	1.0	—
3,5-Dimethylphenol	1.1	0.05
Any individual unspecified impurity	—	0.05
Total impurities	—	0.50

• **ORGANIC IMPURITIES, PROCEDURE 2**

If metaxalone related compound B, metaxalone related compound C, or *N*-benzyl metaxalone is a known process impurity, *Organic Impurities, Procedure 2* is recommended. If the article complies with *Procedure 2*, the labeling indicates that it meets *Organic Impurities, Procedure 2*.

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Buffer, Mobile phase, and **Standard stock solution:** Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL of USP Metaxalone RS from the *Standard stock solution* in *Mobile phase*
Impurity stock solution: 0.2 mg/mL each of USP Metaxalone Related Compound B RS and USP Metaxalone Related Compound C RS in methanol. Sonicate to dissolve if necessary.
Peak identification solution: 1 mg/mL of USP Metaxalone RS and 0.02 mg/mL each of USP Metaxalone Related Compound B RS and USP Metaxalone Related Compound C RS prepared as follows. Transfer a suitable quantity of USP Metaxalone RS to a suitable volumetric flask. Add 50% of the flask volume of methanol, and sonicate to dissolve. Transfer suitable volumes of *Impurity stock solution* to the flask. Dilute with *Buffer* to volume.
Sample solution: 2.0 mg/mL of Metaxalone prepared as follows. Transfer a suitable quantity of Metaxalone to a suitable volumetric flask. Add 50% of the flask volume of methanol. Sonicate for 5 min to dissolve. Add 40% of the flask volume of *Buffer*, and mix. Cool to room temperature. Dilute with *Buffer* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 8 times the retention time of metaxalone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 10.0%
Signal-to-noise ratio: NLT 25
Analysis
Samples: *Standard solution* and *Sample solution*
Use the *Peak identification solution* to identify the peaks.
Calculate the percentage of each impurity in the portion of Metaxalone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of metaxalone from the *Standard solution*
C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)
C_U = concentration of Metaxalone in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.03%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Metaxalone related compound B	0.35	1.0	0.05
Metaxalone	1.0	—	—
Metaxalone related compound C	3.6	1.0	0.05
N-Benzylmetaxalone[a]	6.9	0.64	0.05
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	0.3

[a] 3-Benzyl-5-[(3,5-dimethylphenoxy)methyl]oxazolidin-2-one.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 90° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **Labeling:** The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1* is not used.
• **USP Reference Standards** ⟨11⟩
USP Metaxalone RS
USP Metaxalone Related Compound B RS
1-Amino-3-(3,5-dimethylphenoxy)propan-2-ol.
$C_{11}H_{17}NO_2$ 195.26
USP Metaxalone Related Compound C RS
Bis[2-hydroxy-3-(3,5-dimethylphenoxy)propyl]amine.
$C_{22}H_{31}NO_4$ 373.49

Metaxalone Tablets

DEFINITION
Metaxalone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metaxalone ($C_{12}H_{15}NO_3$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: 0.68 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 4.5.
Mobile phase: Methanol and *Buffer* (50:50)
Standard stock solution: 0.5 mg/mL of USP Metaxalone RS prepared as follows. Transfer a suitable amount of USP Metaxalone RS to a suitable volumetric flask. Add 50% of the flask volume of methanol and sonicate to dissolve. Dilute with *Buffer* to volume.
Standard solution: 0.05 mg/mL of USP Metaxalone RS from *Standard stock solution* in *Mobile phase*
Sample stock solution: Nominally 1.0 mg/mL of metaxalone from NLT 20 Tablets prepared as follows. Transfer a portion of finely powdered Tablets equivalent to NLT 500 mg of metaxalone to a suitable volumetric flask. Add 50% of the flask volume of methanol and sonicate for 10 min with occasional swirling. Shake on a mechanical shaker for 15 min. Add 40% of the flask volume of *Buffer* and allow the solution to cool to room temperature. Dilute with *Buffer* to volume. Pass a portion of the solution through a PVDF filter of 0.45-µm pore size. Discard the first 5 mL. Use the filtrate.
Sample solution: Nominally 0.05 mg/mL of metaxalone from *Sample stock solution* and *Mobile phase*

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of metaxalone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metaxalone ($C_{12}H_{15}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metaxalone from the *Sample solution*
r_S = peak response of metaxalone from the *Standard solution*
C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metaxalone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.5% sodium lauryl sulfate; 900 mL
Apparatus 2: 100 rpm
Time: 60 min
Buffer, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*, except use 270 nm for analysis.
Standard solution: (*L*/900) mg/mL of USP Metaxalone RS, where *L* is the label claim of metaxalone, in mg/Tablet, prepared as follows. Transfer a suitable quantity of USP Metaxalone RS to a suitable volumetric flask. Add 4% of the flask volume of methanol, sonicate to dissolve, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable PVDF membrane filter of 0.45-µm pore size. Discard the first 5 mL of the filtrate and use the remaining amount for analysis.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metaxalone ($C_{12}H_{15}NO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim of metaxalone (mg/Tablet)

Tolerances: NLT 60% (*Q*) of the labeled amount of metaxalone ($C_{12}H_{15}NO_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

• **Organic Impurities**
Buffer, Mobile phase, Standard solution, and Chromatographic system: Proceed as directed in the *Assay*.
Impurity stock solution: 0.2 mg/mL each of USP Metaxalone Related Compound B RS and USP Metaxalone Related Compound C RS in methanol. Sonicate to dissolve if necessary.
Peak identification solution: 1 mg/mL of USP Metaxalone RS and 0.02 mg/mL each of USP Metaxalone Related Compound B RS and USP Metaxalone Related Compound C RS prepared as follows. Transfer a suitable quantity of USP Metaxalone RS to a suitable volumetric flask. Add 50% of the flask volume of methanol and sonicate to dissolve. Transfer suitable volumes of *Impurity stock solution* to the flask. Dilute with *Buffer* to volume.
Sensitivity solution: 0.5 µg/mL of USP Metaxalone RS from *Standard solution* and *Mobile phase*
Sample solution: Nominally 1.0 mg/mL of metaxalone prepared from NLT 20 Tablets as follows. Transfer a portion of NLT 20 finely powdered Tablets equivalent to NLT 500 mg of metaxalone to a suitable volumetric flask. Add 50% of the flask volume of methanol and sonicate for 10 min with occasional swirling. Shake on a mechanical shaker for 15 min. Add 40% of the flask volume of *Buffer* and cool to room temperature. Dilute with *Buffer* to volume. Pass a portion of the solution through a PVDF filter of 0.45-µm pore size. Discard the first 5 mL.
System suitability
Samples: *Peak identification solution* and *Sensitivity solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Tailing factor: NMT 2.0, *Sensitivity solution*
Relative standard deviation: NMT 10.0% for the metaxalone peak, *Sensitivity solution*
Signal-to-noise ratio: NLT 25 for the metaxalone peak, *Sensitivity solution*
Analysis
Samples: *Standard solution*, *Peak identification solution*, and *Sample solution*
Use the *Peak identification solution* to identify the peaks.
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of metaxalone from the *Standard solution*
C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metaxalone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metaxalone related compound B	0.35	0.15
Metaxalone	1.0	—
Metaxalone related compound C[a]	3.6	—
N-Benzylmetaxalone[b]	6.9	—

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Table 1 *(continued)*

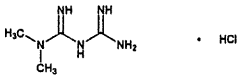
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified degradation product	—	0.10
Total degradation products	—	0.5

[a] Process impurity, included for peak identification only; monitored in the drug substance.
[b] 3-Benzyl-5-[(3,5-dimethylphenoxy)methyl]oxazolidin-2-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metaxalone RS
  USP Metaxalone Related Compound B RS
  1-Amino-3-(3,5-dimethylphenoxy)propan-2-ol.
  $C_{11}H_{17}NO_2$ 195.26
  USP Metaxalone Related Compound C RS
  Bis[2-hydroxy-3-(3,5-dimethylphenoxy)propyl]amine.
  $C_{22}H_{31}NO_4$ 373.49

Metformin Hydrochloride



$C_4H_{11}N_5 \cdot HCl$ 165.62
Imidodicarbonimidic diamide, *N,N*-dimethyl-, monohydrochloride;
1,1-Dimethylbiguanide monohydrochloride [1115-70-4].

DEFINITION

Change to read:

Metformin Hydrochloride contains ▲NLT 98.0% and NMT 102.0%▲ (USP 1-May-2020) of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **A. SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K or 197A▲ (USP 1-May-2020)
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

Add the following:

▲• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**
▲**Buffer:** 9.8 g/L of monobasic ammonium phosphate in water
Mobile phase: *Buffer* and acetonitrile (95:5). Adjust with phosphoric acid to a pH of 3.0.
Standard solution: 0.05 mg/mL of USP Metformin Hydrochloride RS in *Mobile phase*. Sonication may be used to promote dissolution. Allow the solution to equilibrate to ambient temperature.
Sample solution: 0.05 mg/mL of Metformin Hydrochloride in *Mobile phase*. Sonication may be used to promote dissolution. Allow the solution to equilibrate to ambient temperature.
[NOTE—Silanized HPLC vials may be used.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 10-µm packing L9
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the portion of Metformin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metformin from the *Sample solution*
r_S = peak response of metformin from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Metformin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis▲ (USP 1-May-2020)

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**
Mobile phase: 17 g/L of monobasic ammonium phosphate in water; adjusted with phosphoric acid to a pH of 3.0
System suitability stock solution: 0.25 mg/mL of ▲USP Metformin Hydrochloride RS▲ (USP 1-May-2020) and 0.1 mg/mL of ▲USP Melamine RS▲ (USP 1-May-2020) in water
System suitability solution: ▲5 µg/mL of USP Metformin Hydrochloride RS and 2 µg/mL of USP Melamine RS from *System suitability stock solution* in *Mobile phase*▲ (USP 1-May-2020)
Standard stock solution: 0.2 mg/mL of USP Metformin Related Compound A RS in water
Standard solution: 0.001 mg/mL of USP Metformin Related Compound A RS from *Standard stock solution* in *Mobile phase*
Sample solution: 5 mg/mL of Metformin Hydrochloride in *Mobile phase*
Diluted sample solution: 0.005 mg/mL of Metformin Hydrochloride in *Mobile phase* from the *Sample solution*

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 218 nm
Column: 4.6-mm × 25-cm; packing L9
Flow rate: 1.0–1.7 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of metformin
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 10 between melamine and metformin
Analysis
Samples: *Standard solution*, *Sample solution*, and *Diluted sample solution*
Calculate the percentage of metformin related compound A in the portion of Metformin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metformin related compound A from the *Sample solution*
r_S = peak response of metformin related compound A from the *Standard solution*
C_S = concentration of USP Metformin Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Metformin Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Metformin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of metformin from the *Diluted sample solution*
D = dilution factor for the preparation of the *Diluted sample solution*, 0.001

Acceptance criteria
Individual impurities: NMT 0.02% for metformin related compound A; NMT 0.1% for any other impurity
Total impurities: NMT 0.5%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 5 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Melamine RS
2,4,6-Triamino-1,3,5-triazine.
$C_3H_6N_6$ 126.12▲ (USP 1-May-2020)
USP Metformin Hydrochloride RS
USP Metformin Related Compound A RS
1-Cyanoguanidine.
$C_2H_4N_4$ 84.08

Metformin Hydrochloride Tablets

DEFINITION
Metformin Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer an amount of powdered Tablets, equivalent to 20 mg of metformin hydrochloride, to a suitable flask. Add 20 mL of dehydrated alcohol, and shake. Filter, evaporate the filtrate on a water bath to dryness, and dry the residue at 105° for 2 h.
Acceptance criteria: Meet the requirements
• **B.**
Solution A: Dissolve 1 g of 1-naphthol in a solution containing 6 g of sodium hydroxide and 16 g of anhydrous sodium carbonate in 100 mL of water.
Sample solution: Triturate an amount of powdered Tablets, equivalent of 50 mg of metformin hydrochloride, with 10 mL of water, filter, and use the filtrate.
Analysis: To 5 mL of the *Sample solution* add 1.5 mL of 5 N sodium hydroxide solution and 1 mL of *Solution A*. Add 0.5 mL of sodium hypochlorite TS, dropwise, and with shaking.
Acceptance criteria: An orange-red color is produced that darkens on standing.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: Prepare as directed for the *Sample solution* in *Identification* test *B*.
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Standard solution: 10 µg/mL of USP Metformin Hydrochloride RS in water
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the amount of powder, equivalent to 100 mg of metformin hydrochloride, to a 100-mL volumetric flask. Add 70 mL of water, shake by mechanical means for 15 min, dilute with water to volume, and filter, discarding the first 20 mL of the filtrate. Dilute 10.0 mL of the filtrate with water to 100.0 mL, and dilute 10.0 mL of the resulting solution with water to 100.0 mL. The nominal concentration of this solution is 10 µg/mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Wavelength of maximum absorbance at about 232 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the portion of the Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*

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C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1
Medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Wavelength of maximum absorbance at about 233 nm
Analysis: Determine the amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved by using UV absorption of filtered portions of the *Sample solution*, suitably diluted with *Medium*, if necessary, in comparison with the *Standard solution*.
Tolerances: NLT 70% (*Q*) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
For products labeled to contain 500 mg of metformin hydrochloride
Medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1*.
Tolerances: NLT 80% (*Q*) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.
For products labeled to contain 850 or 1000 mg of metformin hydrochloride
Medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 2: 75 rpm
Time: 30 min
Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1*.
Tolerances: NLT 75% (*Q*) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 1: 100 rpm
Time: 60 min
Buffer: Dissolve 1.38 g of monobasic sodium phosphate in about 1800 mL of water. Add 3.484 g of 1-pentanesulfonic acid sodium salt. Adjust with diluted phosphoric acid to a pH of 3.00 ± 0.05. Dilute with water to 2000 mL.
Mobile phase: Acetonitrile and *Buffer* (1:19)
Standard stock solution: 0.25 mg/mL of USP Metformin Hydrochloride RS in *Medium*. Use sonication to dissolve.
Standard solution: 0.05 mg/mL of USP Metformin Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a nylon filter of 0.45-µm pore size. Dilute with *Medium*, if necessary, to obtain a solution with a concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 1500 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (V/D) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL
D = dilution factor of the *Sample solution*

Tolerances: NLT 70% (*Q*) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase: 17 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 3.0
System suitability stock solution: 0.25 mg/mL of metformin hydrochloride and 0.1 mg/mL of melamine in water
System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the amount of powder, equivalent to 500 mg of metformin hydrochloride, to a 100-mL volumetric flask. Dissolve in *Mobile phase* with shaking, dilute with *Mobile phase* to volume, and filter.
Diluted sample solution: Nominally 0.005 mg/mL of metformin hydrochloride in *Mobile phase* from the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 218 nm
Column: 4.6-mm × 25-cm; packing L9
Flow rate: 1.0–1.7 mL/min
Run time: NLT twice the retention time of metformin
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 10 between melamine and metformin
Analysis
Samples: *Sample solution* and *Diluted sample solution*
Calculate the percentage of any individual impurity in the portion of the Tablets taken:

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$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of metformin from the *Diluted sample solution*
D = dilution factor for the preparation of the *Diluted sample solution*, 0.001

Acceptance criteria
Any individual impurity: NMT 0.1%
Total impurities: NMT 0.6%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Metformin Hydrochloride RS

Metformin Hydrochloride Extended-Release Tablets

DEFINITION
Metformin Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION
- **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer solution: 0.5 g/L of sodium 1-heptanesulfonate and 0.5 g/L of sodium chloride in water. Before final dilution, adjust with 0.06 M phosphoric acid to a pH of 3.85.
Mobile phase: Acetonitrile and *Buffer solution* (1:9). [NOTE—To improve the separation, the composition of acetonitrile and *Buffer solution* may be changed to 1:19, if necessary.]
Diluent: 1.25% solution of acetonitrile in water
Standard solution: (L/4000) mg/mL of USP Metformin Hydrochloride RS in *Diluent*, where L is the labeled quantity, in mg, of metformin hydrochloride in each Tablet
System suitability stock solution: 12.5 µg/mL each of USP Metformin Related Compound B RS and USP Metformin Related Compound C RS in *Diluent*
System suitability solution: Dilute 0.5 mL of the *System suitability stock solution* with the *Standard solution* to 50 mL.
Sample stock solution: Finely powder NLT 10 Tablets. Transfer powder, equivalent to the average Tablet weight, to a homogenization vessel, and add 500 mL of a 10% acetonitrile solution. Alternately, homogenize and allow to soak until the sample is fully homogenized. [NOTE—A suggested homogenization sequence is as follows. Homogenize the sample using five pulses, each of 5 s, at about 20,000 rpm, and allow to soak for 2 min. Repeat these steps two additional times.]
Sample solution: Pass a portion of the *Sample stock solution* through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate. Transfer 25 mL of the filtrate to a 200-mL volumetric flask, and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 218 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: Until after the elution locus of metformin related compound C

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for metformin related compound B, metformin, and metformin related compound C are 0.86, 1.0, and 2.1–2.3, respectively. Metformin related compound C can have a variable retention time. The composition of the *Mobile phase* may be changed to 1:19, if it elutes at a relative retention time of less than 2.1.]
Suitability requirements
Resolution: NLT 1.5 between the peaks due to metformin related compound B and metformin
Tailing factor: NLT 0.8 and NMT 2.0 for the metformin peak
Relative standard deviation: NMT 1.5% for the metformin peak and NMT 10% for each of the peaks due to metformin related compound B and metformin related compound C

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metformin hydrochloride in the *Sample solution*

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: pH 6.8 phosphate buffer solution; 1000 mL
Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg
Apparatus 2: 100 rpm for Tablets labeled to contain 500 mg
Times: 1, 3, and 10 h
Detector: UV 232 nm
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable hydrophilic polyethylene filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.
Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

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$$\text{Result} = [(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{180} \times V_S)] \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = initial volume of *Medium* in the vessel (mL)
V_S = volume withdrawn from the vessel for previous samplings (mL)
C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)
C_{180} = concentration of metformin hydrochloride in *Medium* determined at 3 h (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 750-mg Tablet (%)
1	20–40	22–42
3	45–65	49–69
10	NLT 85	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Prepare as directed for *Test 1*; 1000 mL.
Apparatus 2: 100 rpm
Times: 1, 2, 6, and 10 h
Detector: UV 232 nm
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable polyethylene filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration that is similar to that of the *Standard solution*.
Analysis: Calculate, in mg/mL, the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) (C_t), in *Medium* at each time point (t):

$$\text{Result} = (A_U \times C_S \times D_U)/A_S$$

A_U = absorbance of the *Sample solution*
C_S = concentration of metformin hydrochloride in the *Standard solution* (mg/mL)
D_U = dilution factor of the solution under test
A_S = absorbance of the *Standard solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at each time point by the following formulas.
Percentage dissolved at the first time point (1 h):

$$\text{Result} = (C_1 \times V \times 100)/L$$

C_1 = content of metformin hydrochloride in *Medium* at the first time interval (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)

Percentage dissolved at the second time point (2 h):

$$\text{Result} = [C_2 \times (V - SV_1) + C_1 \times SV_1] \times (100/L)$$

C_2 = content of metformin hydrochloride in *Medium* at the second time interval (mg/mL)
V = volume of *Medium*, 1000 mL
SV_1 = volume of the sample withdrawn at 1 h (mL)
C_1 = content of metformin hydrochloride in *Medium* at 1 h (mg/mL)
L = label claim (mg/Tablet)

Percentage dissolved at the nth time point:

$$\text{Result} = \{C_n \times [V - (n-1)V_S] + (C_1 + C_2 + \ldots + C_{n-1}) \times V_S\} \times (100/L)$$

C_n = content of metformin hydrochloride in *Medium* at the nth time interval (mg/mL)
V = volume of *Medium*, 1000 mL
n = time interval of interest
V_S = volume of sample withdrawn at each time interval (mL)
C = as C_1, C_2, C_3, … C_{n-1}, the content of metformin hydrochloride in *Medium* at each time interval (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	20–40
2	35–55
6	65–85
10	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium, Apparatus 1, and **Apparatus 2:** Proceed as directed in *Test 1*.
Times: 1, 2, 5, and 12 h for Tablets labeled to contain 500 mg; and 1, 3, and 10 h for Tablets labeled to contain 750 mg
Detector: UV 232 nm
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable hydrophilic polyethylene filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.
Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \{[(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{120} \times V_S) + (C_{300} \times V_S) + (C_{720} \times V_S)] \times 100\}/L$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = initial volume of *Medium* in the vessel (mL)

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V_S = volume withdrawn from the vessel for previous samplings (mL)
C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)
C_{120} = concentration of metformin hydrochloride in *Medium* determined at 2 h (mg/mL)
C_{300} = concentration of metformin hydrochloride in *Medium* determined at 5 h (mg/mL)
C_{720} = concentration of metformin hydrochloride in *Medium* determined at 12 h (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Tables 3* and *4*.

Table 3. For Tablets Labeled to Contain 500 mg

Time (h)	Amount Dissolved (%)
1	20–40
2	35–55
5	60–80
12	NLT 85

Table 4. For Tablets Labeled to Contain 750 mg

Time (h)	Amount Dissolved (%)
1	22–42
3	49–69
10	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: Prepare as directed for *Test 1*; 1000 mL.
Apparatus 2: 100 rpm
Times: 1, 3, 6, and 10 h
Detector: UV 250 nm (shoulder)
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.
Analysis: Calculate, in mg/mL, the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) (C_t), in *Medium* at each time point (t), by the formulas specified in *Test 2*.
Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	20–40
3	45–65
6	65–85
10	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: pH 6.8 phosphate buffer solution; 900 mL, deaerated
Apparatus 1: 100 rpm, with the vertical holder described in *Figure 1* and *Figure 2*
Times: 2, 8, and 16 h
Detector: UV 250 nm
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.
Analysis: Place a vertical sample holder into each basket (see *Figures 1* and *2*). Place 1 Tablet inside the sample holder, making sure that the Tablets are vertical at the bottom of the baskets.
Calculate, in mg/mL, the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) (C_t), in *Medium* at each time point (t), by the formulas specified in *Test 2*.
Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 1000-mg Tablet (%)
2	NMT 30	NMT 30
8	60–85	65–90
16	NLT 90	NLT 90

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

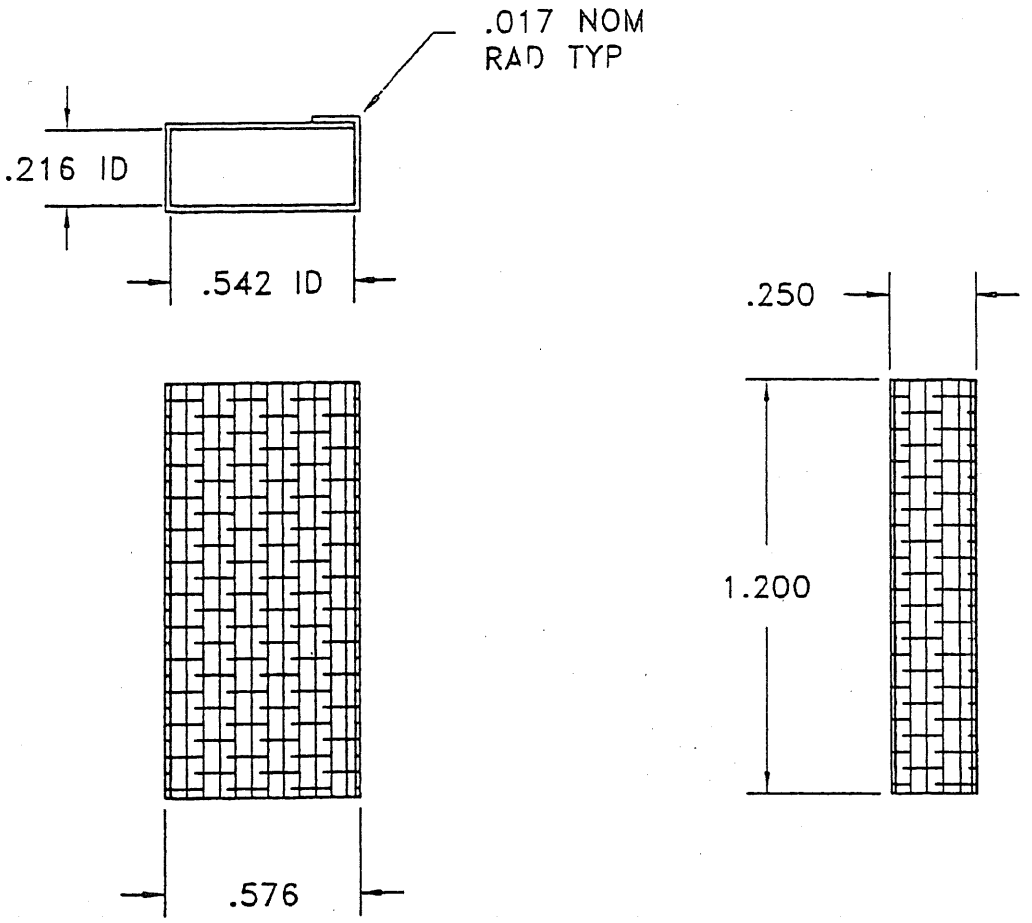
Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: pH 6.8 phosphate buffer solution; 1000 mL, deaerated
Apparatus 2: 100 rpm, with USP sinker, if necessary
Detector: UV 233 nm
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable hydrophilic polyethylene filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.
Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \{[(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{180} \times V_S) + (C_{600} \times V_S)] \times 100\}/L$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = initial volume of *Medium* in the vessel (mL)
V_S = volume withdrawn from the vessel for previous samplings (mL)
C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)
C_{180} = concentration of metformin hydrochloride in *Medium* determined at 3 h (mg/mL)

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NOTES:
1. MATERIAL: 316SS OR EQUIVALENT .017 WIRE VERTICAL MEAS SQUARE WEAVE WITH .039 SQUARE OPENINGS.
2. ALL DIMENSIONS ARE IN INCHES. TOLERANCES TO BE +/-.010

Figure 1 (Metformin Hydrochloride Extended-Release Tablets)

C_{600} = concentration of metformin hydrochloride in *Medium* determined at 10 h (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 7*.

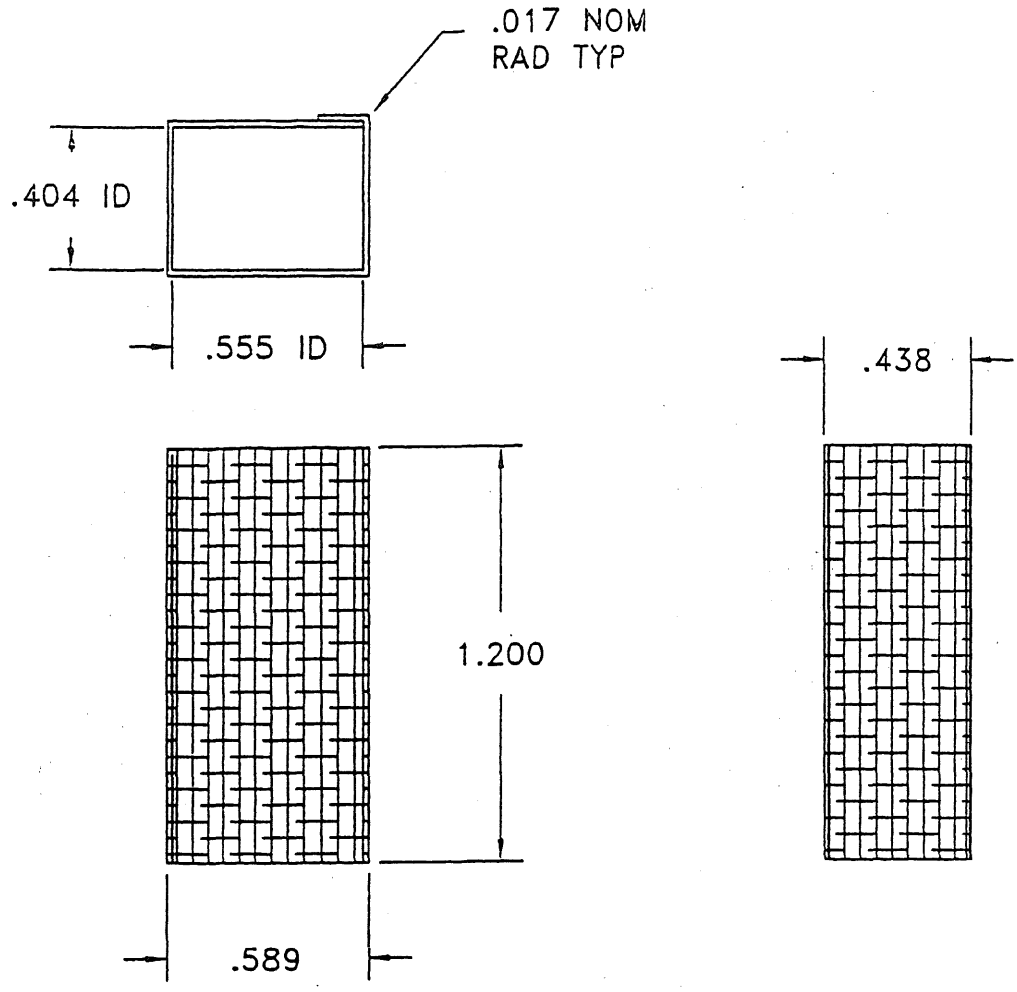
Table 7

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 750-mg Tablet (%)
1	20–40	20–40
3	45–65	45–65
10	NLT 85	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

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NOTES:

1. MATERIAL: 316SS OR EQUIVALENT .017 WIRE VERTICAL MEAS SQUARE WEAVE WITH .039 SQUARE OPENINGS.
2. ALL DIMENSIONS ARE IN INCHES. TOLERANCES TO BE +/-.010

Figure 2 (Metformin Hydrochloride Extended-Release Tablets)

Medium: Prepare as directed in *Test 1*; 1000 mL.
Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg
Apparatus 2: 50 rpm, with USP sinker, for Tablets labeled to contain 500 mg
Times: 1, 3, and 10 h
Detector: UV 232 nm
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \{[(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{180} \times V_S) + (C_{600} \times V_S)] \times 100\}/L$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = initial volume of *Medium* in the vessel (mL)
V_S = volume withdrawn from the vessel for previous samplings (mL)
C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)

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C_{180} = concentration of metformin hydrochloride in *Medium* determined at 3 h (mg/mL)
C_{600} = concentration of metformin hydrochloride in *Medium* determined at 10 h (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 750-mg Tablet (%)
1	20–40	20–40
3	45–65	40–60
10	NLT 85	NLT 80

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.
Medium: Prepare as directed in *Test 1*; 1000 mL.
Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg
Apparatus 2: 100 rpm, with sinker, for Tablets labeled to contain 500 mg
Times: 1, 2, 6, and 10 h
Detector: UV 232 nm
Standard solution: USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.
Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \{[(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{120} \times V_S) + (C_{360} \times V_S) + (C_{600} \times V_S)] \times 100\}/L$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = initial volume of *Medium* in the vessel (mL)
V_S = volume withdrawn from the vessel for previous samplings (mL)
C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)
C_{120} = concentration of metformin hydrochloride in *Medium* determined at 2 h (mg/mL)
C_{360} = concentration of metformin hydrochloride in *Medium* determined at 6 h (mg/mL)
C_{600} = concentration of metformin hydrochloride in *Medium* determined at 10 h (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 9*.

Table 9

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 750-mg Tablet (%)
1	20–40	20–40
2	30–50	35–55
6	65–85	75–95
10	NLT 85	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.
Medium: 0.05 M phosphate buffer, pH 6.8; 1000 mL
Apparatus 1: 100 rpm, for Tablets labeled to contain 750 mg
Apparatus 2: 100 rpm, for Tablets labeled to contain 500 mg
Times: 1, 5, 12, and 20 h for Tablets labeled to contain 500 mg; and 1, 4, 10, and 24 h for Tablets labeled to contain 750 mg
Standard solution: 0.5 mg/mL of USP Metformin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Detector: UV 232 nm
Path length: 0.01 cm, flow cell
Blank: *Medium*
Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \{[(A_U/A_S) \times C_S \times (V - V_S) + (C_1 \times V_S) + (C_2 \times V_S) + (C_3 \times V_S) + (C_4 \times V_S)] \times 100\}/L$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = initial volume of *Medium* in the vessel (mL)
V_S = volume withdrawn from the vessel for previous samplings (mL)
C_1 = concentration of metformin hydrochloride in *Medium* determined at the first time point (mg/mL)
C_2 = concentration of metformin hydrochloride in *Medium* determined at the second time point (mg/mL)
C_3 = concentration of metformin hydrochloride in *Medium* determined at the third time point (mg/mL)
C_4 = concentration of metformin hydrochloride in *Medium* determined at the fourth time point (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Tables 10* and *11*.

Table 10. For Tablets Labeled to Contain 500 mg

Time (h)	Amount Dissolved (%)
1	20–40
5	45–65
12	70–90
20	NLT 85

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Table 11. For Tablets Labeled to Contain 750 mg

Time (h)	Amount Dissolved (%)
1	20–45
4	45–70
10	70–95
24	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 10: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*.
Medium: 0.05 M phosphate buffer (prepared by dissolving 6.8 g of monobasic potassium phosphate in 250 mL of water, adding 77 mL of 0.2 N sodium hydroxide and 500 mL of water, adjusting with 2 N sodium hydroxide or 2 N hydrochloric acid to pH 6.8, and diluting with water to 1000 mL)
Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg
Apparatus 2: 100 rpm for Tablets labeled to contain 500 mg
Times: 1, 3, and 10 h
Standard solution: (L/100,000) mg/mL of USP Metformin Hydrochloride RS in *Medium*, where L is the label claim, in mg/Tablet. This solution is stable for 72 h at room temperature.
Sample solution: At the times specified, withdraw 10 mL of the solution under test and replace with 10 mL of *Medium* previously equilibrated at 37.0 ± 0.5°. Centrifuge at 2500 rpm for 10 min. Dilute a portion of the supernatant with *Medium* to obtain a theoretical concentration of (L/100,000) mg/mL, where L is the label claim, in mg/Tablet.
Detector: UV 233 nm
Path length: 1 cm
Blank: *Medium*
Analysis: Calculate the concentration, in mg/mL, of metformin hydrochloride (C_i) at each time point:

$$C_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved (Q_i) at each time point (i):
At $i = 1$:

$$Q_1 = (C_1 \times V/L) \times 100$$

At $i = 3$:

$$Q_3 = [C_3(V - V_S) + (C_1 \times V_S)] \times 100/L$$

At $i = 10$:

$$Q_{10} = [C_{10}(V - 2V_S) + (C_1 + C_3)V_S] \times 100/L$$

V = initial volume of *Medium*, 1000 mL
V_S = sampling volume, 10 mL
L = label claim (mg/Tablet)

Tolerances: See *Table 12*.

Table 12

Time (h)	Amount Dissolved (%)
1	25–45
3	50–70
10	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 11: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 11*.
Medium: pH 6.8 phosphate buffer solution; 1000 mL
Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg
Apparatus 2: 100 rpm for Tablets labeled to contain 500 mg
Times: 1, 3, and 10 h
Standard solution: 7.5 µg/mL of USP Metformin Hydrochloride RS in *Medium*
Sample solution: At the times specified, withdraw 10 mL of the solution under test, and pass it through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate. Dilute 3.0 mL of the filtrate with *Medium* to 200 mL. For Tablets labeled to contain 750 mg, dilute 2.0 mL of the filtrate with *Medium* to 200 mL. Replace the volume of *Medium* taken with the same volume of *Medium* preheated at 37.0 ± 0.5°.
Detector: UV 232 nm
Path length: 1 cm
Blank: *Medium*
Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at each time point:

$$Q_i = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

At 1 h:

$$\text{Result} = Q_1$$

At 3 h:

$$\text{Result} = Q_3 + [(Q_1 \times 10)/V]$$

At 10 h:

$$\text{Result} = Q_{10} + \{[(Q_1 \times 10)/V] + [(Q_3 \times 10)/V]\}$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL
D = dilution factor of the *Sample solution*

Tolerances: See *Table 13*.

Table 13

Time (h)	Amount Dissolved (%)
1	25–45

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Table 13 *(continued)*

Time (h)	Amount Dissolved (%)
3	50–70
10	NLT 80

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 12: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 12*.
Medium: pH 6.8 phosphate buffer solution; 1000 mL
Apparatus 1: 100 rpm
Times: 1, 4, and 12 h
Standard stock solution: 0.2 mg/mL of USP Metformin Hydrochloride RS in *Medium*
Standard solution: 0.01 mg/mL of USP Metformin Hydrochloride RS in water, from the *Standard stock solution*
Sample solution: At the times specified, withdraw 10 mL of the solution under test, and replace with 10 mL of *Medium* previously equilibrated at 37.0 ± 0.5°. Pass it through a suitable filter, discarding the first few mL of the filtrate.
For Tablets labeled to contain 500 mg: Dilute 2.0 mL of the filtrate with water to 100 mL.
For Tablets labeled to contain 1000 mg: Dilute 1.0 mL of the filtrate with water to 100 mL.
Detector: UV 232 nm
Blank: Dilute 1 mL of *Medium* with water to 100 mL.
Analysis: Calculate the concentration (C_i), in mg/mL, of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the sample withdrawn at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved (Q_i) at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times V] + [C_1 \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of metformin hydrochloride in the portion of sample withdrawn at time point i (mg/mL)
V = initial volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn, 10 mL

Tolerances: See *Table 14*.

Table 14

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 15
2	4	35–65
3	12	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 13: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 13*.
Medium: pH 6.8 phosphate buffer solution; 1000 mL
Apparatus 1: 100 rpm
Times: 1, 4, 6, and 14 h
Standard stock solution: 0.2 mg/mL of USP Metformin Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Metformin Hydrochloride RS into an appropriate volumetric flask. Dissolve by adding *Medium* to fill 50% of the flask volume and dilute with *Medium* to volume.
Standard solution: 0.01 mg/mL of USP Metformin Hydrochloride RS from *Standard stock solution* in water
Sample stock solution: At the times specified, withdraw 10 mL of the solution under test, and replace with the same volume of *Medium* preheated at 37.0 ± 0.5°. Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discard the first few mL, and use the filtrate.
Sample solution
For Tablets labeled to contain 500 mg: Dilute 2 mL of *Sample stock solution* with water to 100 mL.
For Tablets labeled to contain 1000 mg: Dilute 1 mL of *Sample stock solution* with water to 100 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 232 nm
Blank
For Tablets labeled to contain 500 mg: Dilute 2 mL of *Medium* with water to 100 mL.
For Tablets labeled to contain 1000 mg: Dilute 1 mL of *Medium* with water to 100 mL.
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the concentration (C_i), in mg/mL, of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

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C_i = concentration of metformin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 15*.

Table 15

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	4	45–65
3	6	65–85
4	14	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 14: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 14*.
Medium: pH 6.8 phosphate buffer solution; 1000 mL
Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg
Apparatus 2: 100 rpm for Tablets labeled to contain 500 mg
Times: 1, 3, and 10 h
Standard solution: 7.5 µg/mL of USP Metformin Hydrochloride RS in *Medium*
Sample solution: At the times specified, withdraw 10 mL of the solution under test and replace with the same volume of *Medium*. Pass the solution under test through a suitable filter of 10-µm pore size. Pass a portion of the filtered solution through a suitable filter of 0.45-µm pore size, discarding the first few milliliters. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 232 nm
Blank: *Medium*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the concentration (C_i), in mg/mL, of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
D = dilution factor of the *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of metformin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 16*.

Table 16

Time Point (*i*)	Time (h)	Amount Dissolved (%)	
		500 mg Tablets	750 mg Tablets
1	1	30–50	25–45
2	3	55–75	50–70
3	10	NLT 85	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 16:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 16*.
Medium: pH 6.8 phosphate buffer solution; 1000 mL
Apparatus 1: 100 rpm
Times: 1, 3, and 10 h
Standard solution: 0.015 mg/mL of USP Metformin Hydrochloride RS in *Medium*. Sonicate as needed.
Sample stock solution: At the times specified, withdraw 10 mL of the solution under test and pass it through a suitable filter.
Sample solution
For Tablets labeled to contain 500 mg: Dilute 3 mL of the *Sample stock solution* with *Medium* to 100 mL.
For Tablets labeled to contain 750 mg: Dilute 2 mL of the *Sample stock solution* with *Medium* to 100 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: UV 232 nm
Path length: 1 cm
Blank: *Medium*
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the specified time point:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

USP Monographs

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$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of metformin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 18*.

Table 18

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	25–45
2	3	50–70
3	10	NLT 80

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Mar-2019)

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis: From the chromatogram of the *Sample solution* obtained in the *Assay*, calculate the percentage of each impurity in the portion of Tablets taken:

$$Result = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

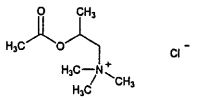
Acceptance criteria
Individual impurities: NMT 0.1%
Total impurities: NMT 0.6%
[NOTE—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one dissolution test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Metformin Hydrochloride RS
USP Metformin Related Compound B RS
1-Methylbiguanide hydrochloride.
$C_3H_9N_5HCl$ 151.60
USP Metformin Related Compound C RS
N,N-Dimethyl-[1,3,5]triazine-2,4,6-triamine.
$C_5H_{10}N_6$ 154.17

Methacholine Chloride



$C_8H_{18}ClNO_2$ 195.69
1-Propanaminium, 2-(acetyloxy)-*N,N,N*-trimethyl-, chloride, (±)-;
(±)-(2-Hydroxypropyl)trimethylammonium chloride acetate [62-51-1].

DEFINITION

Change to read:

Methacholine Chloride contains NLT 98.0% and NMT 101.0% of methacholine chloride ($C_8H_{18}ClNO_2$), calculated on the ▲anhydrous▲ (USP 1-May-2020) basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197M or 197A▲ (USP 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
  Sample solution: 20 mg/mL
  Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: 0.5 g/L of methanesulfonic acid in water
Standard solution: 50 µg/mL of USP Methacholine Chloride RS in water
System suitability solution: 10 µg/mL of USP Acetylcholine Chloride RS in *Standard solution*
Sample solution: 50 µg/mL of Methacholine Chloride in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: IC
Detector: Conductivity
Columns
Guard: 4.0-mm × 50-mm; ▲7-µm▲ (USP 1-May-2020) packing L77
Analytical: 4.0-mm × 25-cm; ▲7-µm▲ (USP 1-May-2020) packing L77
▲Autosampler temperature: 4°▲ (USP 1-May-2020)
Suppressor: Ion-exchange membrane autosuppressor[1] or a suitable chemical suppression system
Suppressant: Autosuppression
Flow rate: 1 mL/min
Injection volume: 20 µL
▲Run time: NLT 2 times the retention time of methacholine▲ (USP 1-May-2020)
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]

[1] Available as Cation Self-Regenerating Suppressor (CSRS) from Dionex Inc., or equivalent.

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Suitability requirements
Resolution: NLT ▲1.5▲ (USP 1-May-2020) between acetylcholine and methacholine, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methacholine chloride ($C_8H_{18}ClNO_2$) in the portion of Methacholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methacholine Chloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Methacholine Chloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–101.0% on the ▲anhydrous▲ (USP 1-May-2020) basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**
Mobile phase: 0.5 g/L of methanesulfonic acid in water
System suitability solution: 1 mg/mL of USP Methacholine Chloride RS, 1 µg/mL of USP Acetylcholine Chloride RS, ▲and 1 µg/mL of USP Beta-Methylcholine Chloride RS▲ (USP 1-May-2020) in water
▲**Sensitivity solution:** 0.5 µg/mL each of USP Methacholine Chloride RS, USP Acetylcholine Chloride RS, and USP Beta-Methylcholine Chloride RS in water▲ (USP 1-May-2020)
Standard solution: 1 µg/mL each of USP Methacholine Chloride RS, ▲USP Acetylcholine Chloride RS, and USP Beta-Methylcholine Chloride RS▲ (USP 1-May-2020) in water
Sample solution: 1 mg/mL of Methacholine Chloride in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: IC
Detector: Conductivity
Columns
Guard: 4.0-mm × 50-mm; ▲7-µm▲ (USP 1-May-2020) packing L77▲▲ (USP 1-May-2020)
Analytical: 4.0-mm × 25-cm; ▲7-µm▲ (USP 1-May-2020) packing L77
▲**Autosampler temperature:** 4°▲ (USP 1-May-2020)
Suppressor: Ion-exchange membrane autosuppressor[1] or a suitable chemical suppression system
Suppressant: Autosuppression
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: ▲NLT▲ (USP 1-May-2020) 2 times the retention time of methacholine
System suitability
Samples: *System suitability solution* ▲and *Sensitivity solution*▲ (USP 1-May-2020)
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT ▲1.5▲ (USP 1-May-2020) between acetylcholine and methacholine; ▲NLT 2 between acetylcholine and beta-methylcholine, *System suitability solution*
Tailing factor: NMT 1.5 each for acetylcholine, beta-methylcholine, and methacholine, *Sensitivity solution*
Relative standard deviation: NMT 5.0% each for acetylcholine, beta-methylcholine, and methacholine, *Sensitivity solution*▲ (USP 1-May-2020)
Signal-to-noise ratio: NLT ▲10 each for acetylcholine, beta-methylcholine, and methacholine, *Sensitivity solution*▲ (USP 1-May-2020)
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of beta-methylcholine in the portion of Methacholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of beta-methylcholine from the *Sample solution*
r_S = peak response of beta-methylcholine from the *Standard solution*
C_S = concentration of USP Beta-Methylcholine Chloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Methacholine Chloride in the *Sample solution* (µg/mL)▲ (USP 1-May-2020)

Calculate the percentage of ▲▲ (USP 1-May-2020) acetylcholine in the portion of Methacholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ▲▲ (USP 1-May-2020) acetylcholine from the *Sample solution*
r_S = peak response of methacholine chloride from the *Standard solution*
C_S = concentration of USP Methacholine Chloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Methacholine Chloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*. ▲The reporting threshold is 0.05%.▲ (USP 1-May-2020)

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Beta-methylcholine	0.6	0.10
Acetylcholine	0.8	0.10
Methacholine	1.0	—

SPECIFIC TESTS

Delete the following:

▲• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 4 h.
Acceptance criteria: NMT 1.5%▲ (USP 1-May-2020)

Add the following:

▲• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: NMT 0.5%▲ (USP 1-May-2020)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

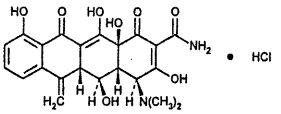
USP Monographs

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Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetylcholine Chloride RS
▲USP Beta-Methylcholine Chloride RS
2-Hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium chloride.
$C_6H_{16}ClNO$ 153.65▲ (USP 1-May-2020)
USP Methacholine Chloride RS

Methacycline Hydrochloride



$C_{22}H_{22}N_2O_8 \cdot HCl$ 478.88
2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,lum12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-, monohydrochloride, [4*S*-(4α,4aα,5α,5aα,12aα)]-.
4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [3963-95-9].

» Methacycline Hydrochloride has a potency equivalent to not less than 832 µg and not more than 970 µg of methacycline ($C_{22}H_{22}N_2O_8$) per mg.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Doxycycline Hyclate RS
USP Methacycline Hydrochloride RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* ***197U***▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: hydrochloric acid in methanol (1 in 1200).
Absorptivity at 345 nm, calculated on the dried basis, is between 88.4% and 96.4% of the USP Methacycline Hydrochloride RS, the potency of the Reference Standard being taken into account.
Crystallinity ⟨695⟩**:** meets the requirements.
pH ⟨791⟩**:** between 2.0 and 3.0, in a solution containing 10 mg of methacycline per mL.
Water Determination, *Method I* ⟨921⟩ **:** not more than 2.0%.

Assay—
Mobile phase—Prepare a mixture of 0.2 M ammonium oxalate, dimethylformamide, and 0.1 M edetate disodium (11:5:4), adjust with tetrabutylammonium hydroxide, 40 percent in water, to a pH of 7.0, and filter. Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability preparation—Prepare a solution of USP Methacycline Hydrochloride RS and USP Doxycycline Hyclate RS in *Mobile phase* containing about 0.5 mg of each per mL.
Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Methacycline Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.
Assay preparation—Transfer about 50 mg of Methacycline Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 354-nm detector and a 4.6-mm × 15-cm column that contains 3.5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.75 for methacycline and 1.0 for doxycycline; and the resolution, *R*, between methacycline and doxycycline is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of methacycline ($C_{22}H_{22}N_2O_8$) in each mg of Methacycline Hydrochloride taken by the formula:

$$100(CE/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Methacycline Hydrochloride RS in the *Standard preparation*; E is the methacycline content, in µg per mg, of *USP Methacycline Hydrochloride RS*; W is the quantity, in mg, of Methacycline Hydrochloride taken to prepare the *Assay preparation;* and r_U and r_S are the methacycline peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methacycline Hydrochloride Oral Suspension

» Methacycline Hydrochloride Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of methacycline ($C_{22}H_{22}N_2O_8$). It contains one or more suitable and harmless buffers, colors, diluents, dispersants, flavors, and preservatives.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Doxycycline Hyclate RS
USP Methacycline Hydrochloride RS
Identification—To an accurately measured volume of Oral Suspension, equivalent to about 50 mg of methacycline, add 50 mL of methanol, shake, and allow the mixture to settle. Using the clear supernatant as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩.

Uniformity of dosage units ⟨905⟩—
FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 6.5 and 8.0.

USP Monographs

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Assay—

Mobile phase, System suitability preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Methacycline Hydrochloride.*

Standard preparation—Transfer about 28 mg of USP Methacycline Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of water, dilute with *Mobile phase* to volume, and mix.

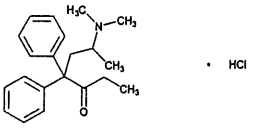
Assay preparation—Transfer an accurately measured quantity of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 50 mg of methacycline ($C_{22}H_{22}N_2O_8$), to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter.

Procedure—Proceed as directed in the *Assay* under *Methacycline Hydrochloride.* Calculate the quantity, in mg, of methacycline ($C_{22}H_{22}N_2O_8$) in each mL of the Oral Suspension taken by the formula:

$$100(CE/1000V)(r_U/r_S)$$

in which V is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation;* and the other terms are as defined therein.

Methadone Hydrochloride



$C_{21}H_{27}NO \cdot HCl$ 345.91

3-Heptanone, 6-(dimethylamino)-4,4-diphenyl-, hydrochloride;

6-(Dimethylamino)-4,4-diphenyl-3-heptanone hydrochloride [1095-90-5].

DEFINITION

Methadone Hydrochloride contains NLT 98.5% and NMT 100.5% of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: A solution meets the requirements of the tests.

ASSAY

- **PROCEDURE**

Sample: 500 mg

Mode: Direct titration

Titrimetric system

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of 10 mL of glacial acetic acid and 10 mL of mercuric acetate TS, warming slightly if necessary to dissolve. Cool the solution to room temperature, add 10 mL of dioxane, then add crystal violet TS, and titrate rapidly with *Titrant.* Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of *Titrant* is equivalent to 34.59 mg of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

Acceptance criteria: 98.5%–100.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORDINARY IMPURITIES** ⟨466⟩

Standard solution: Alcohol

Sample solution: Alcohol

Eluant: Methanol and ammonium hydroxide (100: 1.5)

Visualization: 3

Acceptance criteria: The sum of the intensities of all secondary spots from the *Sample solution* corresponds to NMT 1.0%.

SPECIFIC TESTS

- **PH** ⟨791⟩

Sample solution: 10 mg/mL

Acceptance criteria: 4.5–6.5

- **LOSS ON DRYING** ⟨731⟩

Sample: 500 mg

Analysis: Dry the *Sample* at 105° for 1 h.

Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Methadone Hydrochloride RS

Methadone Hydrochloride Oral Concentrate

DEFINITION

Methadone Hydrochloride Oral Concentrate contains, in each mL, NLT 9.0 mg and NMT 11.0 mg of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$). It contains a suitable preservative and may contain suitable coloring, flavoring, and surface-active agents.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: A volume of Oral Concentrate equivalent to 5 mg of methadone hydrochloride

Chromatographic system

Developing solvent system: Alcohol, glacial acetic acid, and water (5:3:2)

Analysis: Shake the *Sample solution* with 5 mL of sodium carbonate TS, and extract with 5 mL of chloroform. Proceed as directed, using iodoplatinate TS to visualize the spots.

Acceptance criteria: Meets the requirements

- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 0.033 M monobasic potassium phosphate (40:60). Adjust with phosphoric acid to a pH of 4.0, filter, and degas.

Standard solution: 0.4 mg/mL of USP Methadone Hydrochloride RS in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of methadone hydrochloride from Oral Concentrate in *Mobile phase*

Sample solution: 0.4 mg/mL of methadone hydrochloride in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L11

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Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in the portion of Oral Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times L$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methadone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
L = label claim (mg/mL)

Acceptance criteria: 9.0–11.0 mg/mL

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 1.0–6.0

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers, protected from light, and store at controlled room temperature.
- **Labeling:** Label it to indicate that it is to be diluted with water or other liquid to 30 mL or more before administration.
- **USP Reference Standards** ⟨11⟩
USP Methadone Hydrochloride RS

Methadone Hydrochloride Injection

DEFINITION
Methadone Hydrochloride Injection is a sterile solution of Methadone Hydrochloride in Water for Injection. It contains, in each mL, NLT 9.5 mg and NMT 10.5 mg of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

IDENTIFICATION
- **A. Identification—Organic Nitrogenous Bases** ⟨181⟩**:** Meets the requirements

ASSAY

Change to read:

- **Procedure**
Internal standard solution: 5 mg/mL of procaine in methylene chloride
Standard solution: Transfer 10 mg of USP Methadone Hydrochloride RS to a 60-mL separator. Add 1 mL of water and 2 mL of 0.5 N sodium hydroxide, and extract with three 10-mL portions of methylene chloride, combining the extracts in a vessel containing about 3 g of anhydrous sodium sulfate. Transfer 2.0 mL of *Internal standard solution* to the vessel containing the extracts, insert the stopper, and mix. Decant 15 mL of the methylene chloride solution to a test tube, and evaporate to a volume of 2–3 mL using vacuum or a stream of nitrogen.
Sample solution: Transfer 1.0 mL of Injection, equivalent to 10 mg of methadone hydrochloride, to a 60-mL separator. Add 2 mL of 0.5 N sodium hydroxide, and extract with three 10-mL portions of methylene chloride, combining the extracts in a vessel containing about 3 g of anhydrous sodium sulfate. Transfer 2.0 mL of *Internal standard solution* to the vessel containing the extracts, insert the stopper, and mix. Decant 15 mL of the methylene chloride solution to a test tube, and evaporate to a volume of 2–3 mL using vacuum or a stream of nitrogen.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: Glass column, 1.2-m long and 4-mm in diameter; packed with 3% phase G2 on 100- to 200-mesh support S1A
Temperatures
Column: 170°
Injection port: 225°
Detector: 240°
Carrier gas: Dry helium
Flow rate: 55 mL/min
Injection volume: Containing 5 µg of methadone
System suitability
Sample: *Standard solution* (six replicate injections)
Suitability requirements
Resolution: NLT 5.0 between methadone and procaine
Coefficient of variation: NMT 1% in the ratios of the peak areas of methadone to the peak area of procaine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in mg, of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in each mL of Injection taken:

$$\text{Result} = (R_U/R_S) \times W$$ ▲(ERR 1-Oct-2018)▲

R_U = peak area ratio of methadone to procaine from the *Sample solution*
R_S = peak area ratio of methadone to procaine from the *Standard solution*
W = weight, in mg, of USP Methadone Hydrochloride RS in the *Standard solution*

Acceptance criteria: 9.5–10.5 mg/mL

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.0–6.5
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 8.8 USP Endotoxin Units/mg of methadone hydrochloride
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose, light-resistant containers, preferably of Type I glass.

Change to read:

- **USP Reference Standards** ⟨11⟩
▲(CN 1-May-2018)▲
USP Methadone Hydrochloride RS

USP Monographs

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Methadone Hydrochloride Oral Solution

DEFINITION
Methadone Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Sample solution: A volume of Oral Solution equivalent to 5 mg of methadone hydrochloride
Developing solvent system: Alcohol, glacial acetic acid, and water (5:3:2)
Analysis: Shake the *Sample solution* with 5 mL of sodium carbonate TS, and extract with 5 mL of chloroform. Proceed as directed using iodoplatinate TS to visualize the spots.
Acceptance criteria: Meets the requirements
• **B. Identification Tests—General,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY
• **Procedure**
Mobile phase: Acetonitrile and 0.033 M monobasic potassium phosphate (40:60). Adjust dropwise with phosphoric acid to a pH of 4.0.
Internal standard solution: 250 µg/mL of pyrilamine maleate
Standard solution: Transfer 20 mg of USP Methadone Hydrochloride RS to a 25-mL volumetric flask. Add 2.0 mL of *Internal standard solution*, and dilute with water to volume.
Sample solution: Transfer a volume of Oral Solution equivalent to 20 mg of methadone hydrochloride to a 125-mL separator. Extract the specimen with two 50-mL portions of ether, collecting the ether extracts in a second separator. Wash the combined ether extracts with 2 mL of water, and discard the ether extract. Transfer the aqueous wash and the aqueous specimen to a 25-mL volumetric flask. Add 2.0 mL of *Internal standard solution*, and dilute with water to volume. Pass the solution through a filter of 5-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L11
Flow rate: 1.3 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
The retention times for the internal standard and methadone hydrochloride are about 5.5 and 9 min, respectively.
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in the Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methadone hydrochloride to the internal standard from the *Sample solution*
R_S = peak response ratio of methadone hydrochloride to the internal standard from the *Standard solution*
C_S = concentration of USP Methadone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methadone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS
• **Alcohol Determination,** *Method II* ⟨611⟩ (if present)**:** 90.0%–115.0% of the labeled amount of C_2H_5OH, determined by the gas chromatographic procedure using acetone as the internal standard

PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements for Oral Solution packaged in single-unit containers
• **Deliverable Volume** ⟨698⟩**:** Meets the requirements for Oral Solution packaged in multiple-unit containers

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 1.0–4.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, protected from light, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Methadone Hydrochloride RS

Methadone Hydrochloride Tablets

DEFINITION
Methadone Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Sample: Equivalent to 5 mg of methadone hydrochloride from a quantity of finely powered Tablets
Chromatographic system
Developing solvent system: Alcohol, glacial acetic acid, and water (5:3:2)
Analysis: Shake the *Sample* with 5 mL of sodium carbonate TS, and extract with 5 mL of chloroform. Proceed as directed using iodoplatinate TS to visualize the spots.
Acceptance criteria: Meet the requirements

ASSAY
• **Procedure**
Mobile phase: Acetonitrile and 0.03 M monobasic potassium phosphate (40:60). Adjust with phosphoric acid to a pH of 3.2.
Standard solution: 0.4 mg/mL of USP Methadone Hydrochloride RS in *Mobile phase*
Sample solution: 0.4 mg/mL of methadone hydrochloride in *Mobile phase*. Prepare by transferring an amount of finely powdered Tablets (NLT 20) equivalent to 10 mg of methadone hydrochloride to a 25-mL volumetric flask, add 10 mL of *Mobile phase*, and sonicate briefly. Shake by mechanical means for 15 min, dilute with *Mobile phase* to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm

USP Monographs

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Column: 3.9-mm × 30-cm; packing L11
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methadone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methadone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Methadone Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩.
Instrumental conditions
Mode: Vis
Analytical wavelength: Maximum at about 405 nm
Analysis: Filter a portion of the *Sample solution*, and pipet a volume of the filtrate equivalent to about 400 µg of methadone hydrochloride into a suitable separator. Add 1 mL of glacial acetic acid and 20 mL of a solution of bromocresol purple prepared by dissolving 200 mg of bromocresol purple in 1000 mL of dilute glacial acetic acid (1 in 50). Mix, and extract with 20.0 mL of chloroform. Determine the amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) dissolved from visible absorbances at the wavelength of maximum absorbance of the chloroform extract so obtained in comparison with the chloroform extract similarly prepared from the *Standard solution*.
Tolerances: NLT 75% (*Q*) of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Methadone Hydrochloride RS

Methadone Hydrochloride Tablets for Oral Suspension

DEFINITION
Methadone Hydrochloride Tablets for Oral Suspension contain NLT 93.0% and NMT 107.0% of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Sample solution: Shake a quantity of powdered Tablets for Oral Suspension equivalent to 5 mg of methadone hydrochloride with 5 mL of sodium carbonate TS, and extract with 5 mL of chloroform.
Developing solvent system: Alcohol, glacial acetic acid, and water (5:3:2)
Analysis: Proceed as directed, using iodoplatinate TS to visualize the spots.
Acceptance criteria: Meet the requirements

ASSAY
• **Procedure**
Mobile phase: Acetonitrile and 0.03 M monobasic potassium phosphate (40:60). Adjust with phosphoric acid to a pH of 3.2.
Standard solution: 0.4 mg/mL of USP Methadone Hydrochloride RS in *Mobile phase*
Sample solution: 0.4 mg/mL of methadone hydrochloride in *Mobile phase*. Prepare by transferring an amount of finely powdered Tablets for Oral Suspension (NLT 20) equivalent to 10 mg of methadone hydrochloride to a 25-mL volumetric flask, adding 10 mL of *Mobile phase*, and sonicating briefly. Shake by mechanical means for 15 min, dilute with *Mobile phase* to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L11
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methadone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methadone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

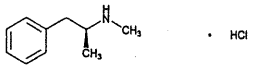
PERFORMANCE TESTS
• **Disintegration** ⟨701⟩
Time: 15 min
Acceptance criteria: Meet the requirements

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- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label the Tablets for Oral Suspension to indicate that they are intended for dispersion in a liquid before oral administration of the prescribed dose.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methadone Hydrochloride RS

Methamphetamine Hydrochloride



$C_{10}H_{15}N \cdot HCl$ 185.69

Benzeneethanamine, *N*,α-dimethyl-, hydrochloride, (*S*)-; (+)-(*S*)-*N*,α-Dimethylphenethylamine hydrochloride [51-57-0].

DEFINITION

Methamphetamine Hydrochloride contains NLT 98.5% and NMT 100.5% of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY
- **PROCEDURE**

Mobile phase: Prepare a degassed solution of 1.1 g of sodium 1-heptanesulfonate in a mixture of water, methanol, and diluted glacial acetic acid (7 in 50) (575:400:25). Adjust with acetic acid to a pH of 3.3 ± 0.1, if necessary. Filter through a 0.5-µm disk.

Standard solution: 0.2 mg/mL of USP Methamphetamine Hydrochloride RS in 0.12 M phosphoric acid. Sonicate if necessary.

Sample solution: 0.2 mg/mL of Methamphetamine Hydrochloride in 0.12 M phosphoric acid

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 3.9-mm × 30.0-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Methamphetamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methamphetamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Methamphetamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–100.5% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORDINARY IMPURITIES** ⟨466⟩

Standard solution and **Sample solution:** Chloroform
Eluant: Chloroform, cyclohexane, and diethylamine (5:4:1)
Visualization: 1
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 171°–175°
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 20 mg/mL in water
Acceptance criteria: Between +16° and +19°
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methamphetamine Hydrochloride RS

Methamphetamine Hydrochloride Tablets

DEFINITION

Methamphetamine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

IDENTIFICATION
- **A.** The UV absorption spectrum of the *Sample solution*, prepared as described in *Procedure for content uniformity* in *Uniformity of Dosage Units*, exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, concomitantly measured.

ASSAY
- **PROCEDURE**

Mobile phase: Prepare a degassed solution of 1.1 g of sodium 1-heptanesulfonate in a mixture of water, methanol, and diluted glacial acetic acid (7 in 50) (575:400:25). Adjust with acetic acid to a pH of 3.3 ± 0.1, if necessary. Filter through a 0.5-µm disk.

Standard solution: 0.2 mg/mL of USP Methamphetamine Hydrochloride RS in 0.12 M phosphoric acid. Sonicate if necessary.

Sample solution: Transfer a portion of fine powder from NLT 20 Tablets, nominally equivalent to about 10 mg of methamphetamine hydrochloride, to a 50-mL volumetric flask. Add 20 mL of 0.12 M phosphoric acid, and sonicate for 5 min. Dilute with 0.12 M phosphoric acid to volume, and filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

USP Monographs

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Detector: UV 257 nm
Column: 3.9-mm × 30.0-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methamphetamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methamphetamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Acetonitrile and dilute perchloric acid (1 in 20) (300:700). Filter, and degas.
Sample solution: Filter aliquots of the solution in the test, and dilute 2:1 with 0.15 M perchloric acid.
Standard solution: Dissolve USP Methamphetamine Hydrochloride RS in water to obtain a concentration similar to the one expected in the *Sample solution*. Dilute 2:1 with 0.15 M perchloric acid.
Chromatographic system
Mode: LC
Detector: UV 211 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) dissolved by comparing the major peak response of the *Sample solution* with that of the *Standard solution*.
Tolerances: NLT 75% (*Q*) of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Solution A: Shake 250 mL of 0.1 N sulfuric acid with 25 mL of chloroform for 10 min. Allow to stand for 1 h with occasional shaking. Drain off the chloroform, and retain the chloroform-saturated sulfuric acid in a stoppered flask.
Standard solution: 0.5 mg/mL of USP Methamphetamine Hydrochloride RS in *Solution A*
Sample solution: Place 1 Tablet in a 125-mL separator. Add 15 mL of water, and shake by mechanical means for 15 min to dissolve. Add 2.5 mL of 1 N sodium hydroxide, and shake. Extract the liberated methamphetamine with four 10-mL portions of chloroform, collecting the chloroform extracts in a second 125-mL separator. Transfer 10.0 mL of *Solution A* to the second separator, and shake by mechanical means for 10 min. Allow the layers to separate, and collect the aqueous layer.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum at about 257 nm
Cell: 1 cm
Blank: *Solution A*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Tablets taken:

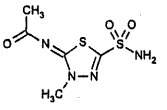
$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Methamphetamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methamphetamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methamphetamine Hydrochloride RS

Methazolamide



$C_5H_8N_4O_3S_2$ 236.27
Acetamide, *N*-[5-(aminosulfonyl)-3-methyl-1,3,4-thiadiazol-2(3*H*)-ylidene]-.
N-(4-Methyl-2-sulfamoyl-Δ²-1,3,4-thiadiazolin-5-ylidene)-acetamide [554-57-4].

» Methazolamide contains not less than 98.0 percent and not more than 102.0 percent of $C_5H_8N_4O_3S_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Methazolamide RS

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: sodium hydroxide solution in water (1 in 250).
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Selenium ⟨291⟩: 0.003%, a 200-mg specimen being used.

Assay—
Buffer solution—Dissolve 1.80 g of anhydrous sodium acetate in 1 L of water. Adjust, if necessary, with glacial acetic acid to a pH of 4.5 ± 0.2.
Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (86:14). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve about 20 mg of USP Methazolamide RS, accurately weighed, in 20 mL of acetonitrile contained in a 200-mL volumetric flask. Dilute with *Buffer solution* to volume, and mix. Quantitatively dilute an accurately measured volume of this solution with *Buffer solution* to obtain a solution having a known concentration of about 50 µg of USP Methazolamide RS per mL.
Resolution solution—Prepare a solution of acetaminophen and USP Methazolamide RS in acetonitrile containing 0.3 mg of acetaminophen and 0.5 mg of methazolamide per mL. Quantitatively dilute an accurately measured volume of this solution with *Buffer solution* to obtain a solution containing 30 µg of acetaminophen and 50 µg of methazolamide per mL.
Assay preparation—Transfer about 100 mg of Methazolamide, accurately weighed, to a 200-mL volumetric flask, dissolve in 20 mL of acetonitrile, dilute with *Buffer solution* to volume, and mix. Quantitatively dilute an accurately measured volume of this solution with *Buffer solution* to obtain a solution having a known concentration of about 50 µg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 265-nm detector and a 3.9-mm × 15.0-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for acetaminophen and 1.0 for methazolamide, the resolution, *R*, between the acetaminophen peak and the methazolamide peak is not less than 4.0, and the tailing factor is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in mg, of $C_5H_8N_4O_3S_2$ in the portion of Methazolamide taken by the formula:

$$2C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Methazolamide RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methazolamide Tablets

» Methazolamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methazolamide ($C_5H_8N_4O_3S_2$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Methazolamide RS

Identification—
A: Extract a quantity of finely powdered Tablets, equivalent to about 250 mg of methazolamide, with about 50 mL of acetone. Filter, and add solvent hexane until a heavy white precipitate is formed. Collect the solid on a filter, and dry: the IR absorption spectrum of a potassium bromide dispersion of the methazolamide so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Methazolamide RS.
B: Dissolve about 100 mg of the dried solid obtained in *Identification* test *A* in 5 mL of 1 N sodium hydroxide, and add 5 mL of a mixture of 1 g of hydroxylamine hydrochloride and 500 mg of cupric sulfate in 100 mL of water. Heat the solution on a steam bath for 15 minutes: the solution turns dark amber, then a black precipitate is formed.
C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: pH 4.5 acetate buffer, prepared by mixing 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of a solution having a pH of 4.5; 900 mL.
Apparatus 2: 75 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_5H_8N_4O_3S_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 252 nm of filtered portions of the solution under test, suitably diluted with pH 4.5 acetate buffer, in comparison with a Standard solution having a known concentration of USP Methazolamide RS in the same *Medium*.
Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_5H_8N_4O_3S_2$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
pH 2.5 Buffer—Transfer 16.8 mL of dibutylamine to a beaker containing 70 mL of water. Adjust with phosphoric acid to a pH of 2.5, dilute with water to 100 mL, and mix.
Mobile phase—Prepare a mixture of water, methanol, and *pH 2.5 Buffer* (375:15:6). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
pH 4.5 Acetate buffer—Dissolve 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid in water, dilute with water to 1000 mL, and mix. Adjust, if necessary, with glacial acetic acid or sodium hydroxide to a pH of 4.5.
Standard preparation—Dissolve an accurately weighed quantity of USP Methazolamide RS in methanol to obtain a solution having a concentration of about 0.5 mg per mL. Dilute this solution quantitatively, and stepwise if necessary,

USP Monographs

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with *pH 4.5 Acetate buffer* to obtain a solution having a known concentration of about 50 µg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of methazolamide, to a 250-mL volumetric flask, add 65 mL of *pH 4.5 Acetate buffer*, and sonicate to dissolve. Add 65 mL of methanol, and sonicate again until dissolved. Dilute with *pH 4.5 Acetate buffer* to volume, mix, and filter. Dilute an accurately measured volume of the filtrate with *pH 4.5 Acetate buffer* to obtain a solution having a concentration of about 50 µg of methazolamide per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 252-nm detector and an 8-mm × 10-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of methazolamide ($C_5H_8N_4O_3S_2$) in the portion of Tablets taken by the formula:

$$C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Methazolamide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methenamine



$C_6H_{12}N_4$ 140.19

1,3,5,7-Tetraazatricyclo[3.3.1.1^{3,7}]decane; Hexamethylenetetramine [100-97-0].

DEFINITION

Methenamine, dried over phosphorus pentoxide for 4 h, contains NLT 99.0% and NMT 100.5% of methenamine ($C_6H_{12}N_4$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**

Analysis: Heat a solution (1 in 10) with 2 N sulfuric acid.

Acceptance criteria: Formaldehyde is liberated, recognizable by its odor and by its darkening of paper moistened with silver ammonium nitrate TS. On the subsequent addition of an excess of 1 N sodium hydroxide to the solution, ammonia is evolved.

ASSAY

- **PROCEDURE**

Chromotropic acid spot test solution: Suspend 100 mg of chromotropic acid in 2 mL of water, and cautiously add 3 mL of sulfuric acid. Allow to cool, and add 25 mL of sulfuric acid. If excessive heat generated during mixing causes a violet color to appear in the solution, discard the solution and prepare another, taking precautions to avoid excessive heat.

Sample solution: Transfer 1 g of Methenamine, previously dried, to a beaker. Add 40.0 mL of 1 N sulfuric acid VS, and heat to a gentle boil, adding water from time to time if necessary, until the formaldehyde has been expelled. Test for the absence of formaldehyde by adding a drop of the assay solution to a glass fiber filter disk, on a watch glass, on which has previously been placed 3 or 4 drops of *Chromotropic acid spot test solution*. Formaldehyde produces a violet color with this reagent. Repeat the test until no violet color is obtained on the warmed test filter disk upon comparison with a blank filter disk to which no assay specimen is added. Cool, add 20 mL of water, then add methyl red TS.

Titrimetric system

Mode: Residual titration

Titrant: 1 N sulfuric acid VS

Back-titrant: 1 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Titrate the excess acid in the *Sample solution* with 1 N sodium hydroxide VS. Perform a blank determination (see *Titrimetry* ⟨541⟩, *Residual Titrations*). Each mL of 1 N sulfuric acid is equivalent to 35.05 mg of methenamine ($C_6H_{12}N_4$).

Acceptance criteria: 99.0%–100.5%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample: 1.0 g

Acceptance criteria: 0.014%; shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid.

- **SULFATE**

Sample solution: 20 mg/mL

Analysis: 10 mL of the *Sample solution*, acidified with 5 drops of hydrochloric acid. Add 5 drops of barium chloride TS.

Acceptance criteria: No turbidity is produced within 1 min.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over phosphorus pentoxide for 4 h.

Acceptance criteria: NMT 2.0%

- **AMMONIUM SALTS**

Sample solution: 50 mg/mL

Analysis: Add to 10 mL of the *Sample solution* 1 mL of alkaline mercuric–potassium iodide TS.

Acceptance criteria: The mixture is not darker in color than a mixture of 1 mL of the reagent and 10 mL of water.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Methenamine RS

Methenamine Tablets

DEFINITION

Methenamine Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methenamine ($C_6H_{12}N_4$).

IDENTIFICATION

- **A.**

Sample: 500 mg of powdered Tablets

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Analysis: Dissolve the *Sample* in 10 mL of water, add 10 mL of 2 N sulfuric acid, and heat.
Acceptance criteria: Formaldehyde is liberated, recognizable by its odor and by its darkening of paper moistened with silver ammonium nitrate TS. On the subsequent addition of an excess of 1 N sodium hydroxide to the solution, ammonia is evolved.

ASSAY

• **PROCEDURE**

Chromotropic acid solution: Mix 100 mg of chromotropic acid with 50 mL of water in a 100-mL volumetric flask. Cool in an ice bath and, while cooling, cautiously and slowly add 50 mL of sulfuric acid. Allow the solution to reach room temperature, and add dilute sulfuric acid (1 in 2) to volume. If excessive heat generated during mixing causes a violet color to appear in the solution, discard the solution and prepare another, taking precautions to avoid excessive heat.

Standard stock solution: 50 µg/mL of USP Methenamine RS

Standard solution: 1 µg/mL of USP Methenamine RS prepared as follows. Transfer 2.0 mL of *Standard stock solution* to a 100-mL volumetric flask, add 25 mL of *Chromotropic acid solution* and 50 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Standard blank: Transfer 2.0 mL of *Standard stock solution* to a 100-mL volumetric flask, then add 75 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Sample stock solution: Nominally 50 µg/mL of methenamine prepared as follows. Transfer an equivalent to 500 mg of methenamine from powdered Tablets (NLT 20) to a 250-mL volumetric flask. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Transfer 25.0 mL of the subsequent filtrate to a 1000-mL volumetric flask. Dilute with water to volume.

Sample solution: Nominally 1 µg/mL of methenamine prepared as follows. Transfer 2.0-mL of *Sample stock solution* to a 100-mL volumetric flask, then add 25 mL of *Chromotropic acid solution* and 50 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Sample blank: Transfer 2.0 mL of *Sample stock solution* to a 100-mL volumetric flask, then add 75 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Instrumental conditions

Mode: Vis
Analytical wavelength: Maxima at about 570 nm
Cell: 1 cm
Blank: Dilute sulfuric acid (1 in 2)

Analysis

Samples: *Standard solution*, *Standard blank*, *Sample solution*, *Sample blank*, and *Blank*

Calculate the percentage of the labeled amount of methenamine ($C_6H_{12}N_4$) in the portion of Tablets taken:

$$\text{Result} = [(A_U - B_U)/(A_S - B_S)] \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
B_U = absorbance of the *Sample blank*
A_S = absorbance of the *Standard solution*
B_S = absorbance of the *Standard Blank*
C_S = concentration of USP Methenamine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methenamine in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Procedure for a pooled sample

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Analysis: Determine the amount of methenamine ($C_6H_{12}N_4$) dissolved by using the procedure set forth in the *Assay*, making any necessary modifications.
Tolerances: NLT 75% (*Q*) of the labeled amount of methenamine ($C_6H_{12}N_4$) is dissolved.

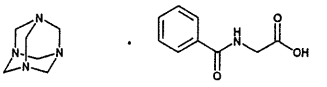
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methenamine RS

Methenamine Hippurate



$C_6H_{12}N_4 \cdot C_9H_9NO_3$ 319.36

Glycine, *N*-benzoyl, compd. with 1,3,5,7-tetraazatricyclo[3.3.1.1^{3,7}]decane (1:1);
Hexamethylenetetramine monohippurate [5714-73-8].

DEFINITION

Methenamine Hippurate, dried under vacuum at 60° for 1 h, contains NLT 95.5% and NMT 102.0% of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$), and contains NLT 54.0% and NMT 58.0% of hippuric acid ($C_9H_9NO_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample solution: Dissolve 700 mg of Methenamine Hippurate in 50 mL of glacial acetic acid.

Titrimetric system

Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric

Analysis: Titrate with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.94 mg of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$).

Acceptance criteria: 95.5%–102.0% on the previously dried basis

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OTHER COMPONENTS

• **CONTENT OF HIPPURIC ACID**
Sample solution: Transfer 1 g to a 250-mL conical flask, and add 50 mL of water. When the solution is complete, add phenolphthalein TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Titrate with *Titrant*. Each mL of 0.1 N sodium hydroxide is equivalent to 17.92 mg of hippuric acid ($C_9H_9NO_3$).
Acceptance criteria: 54.0%–58.0%

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **SULFATE**
Sample solution: 200 mg in 10 mL of water
Analysis: Add 5 drops of 3 N hydrochloric acid and 5 drops of barium chloride TS.
Acceptance criteria: No turbidity appears within 1 min.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 60° for 1 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methenamine Hippurate RS

Methenamine Hippurate Tablets

DEFINITION

Methenamine Hippurate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: A portion of finely powdered Tablets
Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**
Sample solution: Transfer an equivalent to 700 mg of methenamine hippurate from finely powdered Tablets (NLT 20) to a 250-mL conical flask. Add 50 mL of alcohol, then add thymolphthalein TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Titrate the *Sample solution* with *Titrant*. Perform a blank determination on a mixture of 50 mL of alcohol and 20 mL of water, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 31.94 mg of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$).
Acceptance criteria: 95.0%–105.0%

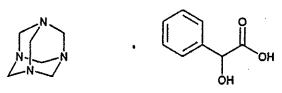
PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 2: 100 rpm
Time: 30 min
Standard solution: 22 µg/mL of USP Methenamine Hippurate RS
Instrumental conditions
Mode: UV
Analytical wavelength: Maxima at about 227 nm
Analysis: Determine the quantity of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$) dissolved of filtered portions of the solution under test, suitably diluted with water if necessary, in comparison with the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$) is dissolved.
Test 2
[NOTE—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.]
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Standard solution: 22 µg/mL of USP Methenamine Hippurate RS
Instrumental conditions
Mode: UV
Analytical wavelength: Maxima at about 227 nm
Analysis: Determine the quantity of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$) dissolved of filtered portions of the solution under test, suitably diluted with water if necessary, in comparison with the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methenamine Hippurate RS

Methenamine Mandelate



$C_6H_{12}N_4 \cdot C_8H_8O_3$ 292.33
Benzeneacetic acid, α-hydroxy-, (±)-, compd. with 1,3,5,7-tetraazatricyclo[3.3.1.1^{3,7}]decane (1:1);
Hexamethylenetetramine mono-(±)-mandelate [587-23-5].

DEFINITION

Methenamine Mandelate contains NLT 95.5% and NMT 102.0% of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$), and contains NLT 50.0% and NMT 53.0% of mandelic acid ($C_8H_8O_3$), calculated on the dried basis.

USP Monographs

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample solution: Transfer 60 mg of Methenamine Mandelate to a 250-mL conical flask. Add 15 mL of dehydrated alcohol, stir to dissolve, and add 40 mL of chloroform.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N silver nitrate in dehydrated alcohol prepared as follows. Dissolve by stirring 8.5 g of silver nitrate in 1000 mL of dehydrated alcohol. Transfer 100 mg of sodium chloride, previously dried at 110° for 2 h, to a 100-mL beaker, and dissolve in 50 mL of water. Titrate with the silver nitrate solution to the potentiometric endpoint, using a silver billet indicator electrode and a silver–silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Calculate the normality of the titrant.

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically, using a silver billet indicator electrode and a silver–silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 7.308 mg of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

Acceptance criteria: 95.5%–102.0% on the dried basis

OTHER COMPONENTS

• **CONTENT OF MANDELIC ACID**

Sample solution: Transfer 90 mg to a 250-mL conical flask containing 50 mL of water. When the solution is complete, titrate the magnetically stirred solution.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N ceric ammonium nitrate VS

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically. Each mL of 0.05 N ceric ammonium nitrate is equivalent to 3.804 mg of mandelic acid ($C_8H_8O_3$).

Acceptance criteria: 50.0%–53.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample: 1.0 g

Analysis: Dissolve the *Sample* in 10 mL of water, and add gradually 500 mg of anhydrous sodium carbonate. Evaporate to dryness, and ignite the residue at a dull-red heat. Add 20 mL of 2 N nitric acid, stir gently, and filter.

Acceptance criteria: 0.01%; the filtrate shows no more chloride than corresponds to 0.15 mL of 0.020 N hydrochloric acid.

• **SULFATE**

Sample: 0.20 g

Analysis: Dissolve the *Sample* in 10 mL of water. Add 5 drops of 3 N hydrochloric acid and 5 drops of barium chloride TS.

Acceptance criteria: No turbidity appears within 1 min.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over silica gel for 18 h.

Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Methenamine Mandelate RS

Methenamine Mandelate Tablets

DEFINITION

Methenamine Mandelate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Triturate a portion of finely powdered Tablets, equivalent to 5.0 mg of methenamine mandelate, with 5 mL of chloroform, and pass through a 0.45-µm membrane filter. Evaporate the solvent, and allow the residue to air-dry.

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Sample solution: Transfer an equivalent to 60 mg of methenamine mandelate, from NLT 20 finely powdered Tablets, to a 250-mL conical flask. Add 15 mL of dehydrated alcohol, stir to dissolve, and add 40 mL of chloroform.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N silver nitrate in dehydrated alcohol prepared as follows. Dissolve by stirring 8.5 g of silver nitrate in 1000 mL of dehydrated alcohol. Transfer 100 mg of sodium chloride, previously dried at 110° for 2 h, to a 100-mL beaker, and dissolve in 50 mL of water. Titrate with the silver nitrate solution to the potentiometric endpoint, using a silver billet indicator electrode and a silver–silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Calculate the normality of the titrant.

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically, using a silver billet indicator electrode and a silver–silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 7.308 mg of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

For uncoated or plain coated Tablets

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: USP Methenamine Mandelate RS in *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: UV maximum at about 257 nm

Analysis: Determine the amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$) dissolved in the portion of the solution under test, passed through a filter of 0.45-µm

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pore size and suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Methenamine Mandelate RS in the same *Medium*.

Tolerances: NLT 75% (*Q*) of the labeled amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methenamine Mandelate RS

Methimazole



$C_4H_6N_2S$ 114.17

2*H*-Imidazole-2-thione, 1,3-dihydro-1-methyl-;
1-Methylimidazole-2-thiol [60-56-0].

DEFINITION

Methimazole contains NLT 98.0% and NMT 101.0% of methimazole ($C_4H_6N_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample: 250 mg of Methimazole

Analysis: Dissolve the *Sample* in 75 mL of water. Add 15.0 mL of 0.1 N sodium hydroxide VS, mix, and add, with stirring, 30 mL of 0.1 N silver nitrate. Continue the titration with the 0.1 N sodium hydroxide VS, determining the end-point potentiometrically. Each mL of 0.1 N sodium hydroxide is equivalent to 11.42 mg of methimazole ($C_4H_6N_2S$).

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **SELENIUM** ⟨291⟩

Sample: 200 mg of Methimazole

Acceptance criteria: NMT 30 ppm

- **ORGANIC IMPURITIES**

Standard solution: 0.01 mg/mL each of USP Methimazole RS, USP Methimazole Related Compound A RS, 1-methylimidazole, and USP Methimazole Related Compound C RS in chloroform

Sample solution: 10 mg/mL of Methimazole in chloroform

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.25-mm; 0.5-µm coating of G27

Temperatures

Injection port: 150°

Detector: 250°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	2
100	30	250	15

Carrier gas: Helium

Flow rate: 1.5 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 3:20

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methimazole related compound A and 1-methylimidazole

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methimazole related compound A, 1-methylimidazole, and methimazole related compound C in the portion of Methimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each related compound from the *Sample solution*

r_S = peak response of the corresponding related compound from the *Standard solution*

C_S = concentration of the corresponding related compound in the *Standard solution* (mg/mL)

C_U = concentration of Methimazole in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Methimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of methimazole from the *Standard solution*

C_S = concentration of USP Methimazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Methimazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.02%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methimazole related compound A	0.3	0.1
1-Methylimidazole	0.4	0.1
Methimazole related compound C	0.7	0.1
Methimazole	1.0	—
Any other individual impurity	—	0.1
Total impurities	—	0.5

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 2 h.

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Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methimazole RS
  USP Methimazole Related Compound A RS
  2,2-Dimethoxy-*N*-methylethanamine.
  $C_5H_{13}NO_2$ 119.16
  USP Methimazole Related Compound C RS
  1-Methyl-2-(methylthio)-1*H*-imidazole.
  $C_5H_8N_2S$ 128.20

Methimazole Tablets

DEFINITION
Methimazole Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of methimazole ($C_4H_6N_2S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Digest a quantity of powdered Tablets, equivalent to 10 mg of methimazole, with 10 mL of warm chloroform for 20 min, filter, and evaporate the filtrate on a steam bath to dryness.
  Acceptance criteria: Meet the requirements

ASSAY
- **PROCEDURE**
  Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 120 mg of methimazole, to a 100-mL volumetric flask. Add about 80 mL of water, insert the stopper, and shake by mechanical means or occasionally by hand for 30 min. Dilute with water to volume, and filter.
  Analysis: Add 3.5 mL of 0.1 N sodium hydroxide VS to 50.0 mL of *Sample solution*, mix, and add, with stirring, 7 mL of 0.1 N silver nitrate. Continue the titration with the 0.1 N sodium hydroxide VS, determining the end-point potentiometrically. Each mL of 0.1 N sodium hydroxide is equivalent to 11.42 mg of methimazole ($C_4H_6N_2S$).
  Acceptance criteria: 94.0%–106.0%

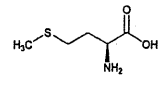
PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Water; 500 mL
  Apparatus 1: 100 rpm
  Time: 30 min
  Standard solution: USP Methimazole RS at a known concentration in *Medium*
  Sample solutions: Filtered solution under test, suitably diluted with *Medium*
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum absorbance at about 252 nm
  Tolerances: NLT 80% (*Q*) of the labeled amount of methimazole ($C_4H_6N_2S$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
  Procedure for content uniformity
  Standard solution: 5 µg/mL of USP Methimazole RS in water
  Sample stock solution: Place 1 Tablet, previously crushed or finely powdered, in a 100-mL volumetric flask. Add 50 mL of water, and shake by mechanical means for 30 min. Dilute with water to volume, mix, and filter, discarding the first 20 mL of filtrate.
  Sample solution: Nominally 5 µg/mL of methimazole in water from *Sample stock solution*
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum absorbance at about 252 nm
  Cell: 1 cm
  Blank: Water
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*
  Calculate the percentage of the labeled amount of methimazole ($C_4H_6N_2S$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Methimazole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methimazole in the *Sample solution* (µg/mL)

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methimazole RS

Methionine



$C_5H_{11}NO_2S$ 149.21
L-Methionine [63-68-3].

DEFINITION
Methionine contains NLT 98.5% and NMT 101.5% of L-methionine ($C_5H_{11}NO_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample: 140 mg of Methionine
  Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Endpoint detection: Potentiometric
  Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with the *Titrant*.
  Calculate the percentage of L-methionine ($C_5H_{11}NO_2S$) in the portion of the *Sample* taken:

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$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 149.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.4%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*
  Standard solution: 0.50 mL of 0.020 N hydrochloric acid
  Sample: 0.73 g of Methionine
  Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*
  Standard solution: 0.10 mL of 0.020 N sulfuric acid
  Sample: 0.33 g of Methionine
  Acceptance criteria: NMT 0.03%
- **IRON** ⟨241⟩: NMT 30 ppm
- **RELATED COMPOUNDS**
  Phosphoric acid solution: 23 g/L of phosphoric acid
  Solution A: Acetonitrile, *Phosphoric acid solution*, and water (0.5: 12.5: 87)
  Solution B: Acetonitrile, *Phosphoric acid solution*, and water (47.5: 12.5: 40)
  Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6	100	0
50	0	100
60	0	100
70	100	0

System suitability solution: Transfer 5 mg each of USP L-Methionine RS and L-methionine sulfoxide to a 50-mL volumetric flask, and dissolve in and dilute with *Solution A* to volume.
Standard solution: 30 µg/mL of USP L-Methionine RS in *Solution A*
***N*-Acetyl- D,L-methionine standard solution:** 0.06 mg/mL of USP *N*-Acetyl-D,L-methionine RS in *Solution A*
Sample solution: 30 mg/mL of Methionine in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for L-methionine and L-methionine sulfoxide are 1.0 and 0.5, respectively.]
Suitability requirements
Resolution: NLT 5.0 between L-methionine and L-methionine sulfoxide peaks
Analysis
Samples: *Standard solution*, *N-Acetyl- D,L-methionine standard solution*, and *Sample solution*

Calculate the percentage of *N*-acetyl-D,L-methionine in the portion of Methionine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *N*-acetyl-D,L-methionine from the *Sample solution*
r_S = peak response of *N*-acetyl-D,L-methionine from the *N-Acetyl- D,L-methionine standard solution*
C_S = concentration of USP *N*-Acetyl-D,L-methionine RS in the *N-Acetyl- D,L-methionine standard solution* (mg/mL)
C_U = concentration of Methionine in the *Sample solution* (mg/mL)

Separately calculate the percentage of methionine sulfoxide and any unspecified impurity in the portion of Methionine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of methionine sulfoxide or any unspecified impurity from the *Sample solution*
r_S = peak response of methionine from the *Standard solution*
C_S = concentration of USP L-Methionine RS in the *Standard solution* (mg/mL)
C_U = concentration of Methionine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurities less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methionine sulfoxide	0.5	0.53	0.1
Methionine	1.00	—	—
N-Acetyl-D,L-methionine	3.4	—	0.2
Any unspecified impurity	—	1.0	0.1
Total unspecified impurities	—	—	0.30

SPECIFIC TESTS

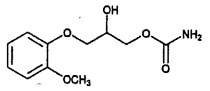
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures*, *Specific Rotation*
  Sample solution: 20 mg/mL in 6 N hydrochloric acid
  Acceptance criteria: +22.4° to +24.7°
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL of solution
  Acceptance criteria: 5.6–6.1
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP *N*-Acetyl-D,L-methionine RS
  USP L-Methionine RS

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Methocarbamol



$C_{11}H_{15}NO_5$ 241.24

1,2-Propanediol, 3-(2-methoxyphenoxy)-, 1-carbamate, (±)-; (±)-3-(*o*-Methoxyphenoxy)-1,2-propanediol 1-carbamate [532-03-6].

DEFINITION

Methocarbamol contains NLT 98.5% and NMT 101.5% of methocarbamol ($C_{11}H_{15}NO_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (30:70)

System suitability solution: 1.0 mg/mL of USP Methocarbamol RS and 0.005 mg/mL of USP Guaifenesin RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Methocarbamol in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 274 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

Run time: 1.5 times the retention time of methocarbamol

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methocarbamol ($C_{11}H_{15}NO_5$) in the portion of Methocarbamol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methocarbamol from the *Sample solution*

r_S = peak response of methocarbamol from the *Standard solution*

C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U = concentration of Methocarbamol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.005 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: 1 mg/mL of Methocarbamol in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Methocarbamol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of methocarbamol from the *Standard solution*

C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U = concentration of Methocarbamol in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guaifenesin	0.84	1.2	0.15
Methocarbamol isomer[a]	0.90	1.0	0.05
Methocarbamol	1.0	—	—
Methocarbamol dioxolone[b]	1.3	1.0	0.05
Any individual unspecified impurity	—	—	0.05
Total impurities	—	—	1.0

[a] 1-Hydroxy-3-(2-methoxyphenoxy)propan-2-yl carbamate.

[b] 4-[(2-Methoxyphenoxy)methyl]-1,3-dioxolan-2-one.

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 60° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Guaifenesin RS
USP Methocarbamol RS

Methocarbamol Injection

DEFINITION
Methocarbamol Injection is a sterile solution of Methocarbamol in an aqueous solution of Polyethylene Glycol 300. It contains NLT 95.0% and NMT 105.0% of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Mix a volume with 40 mL of water equivalent to 500 mg of methocarbamol from Injection in a small separator. Extract with 10 mL of ethyl acetate, and dry the ethyl acetate layer over anhydrous sodium sulfate. Evaporate the ethyl acetate with the use of a water bath maintained at 40° under a stream of nitrogen to dryness.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 4.5.
Mobile phase: Methanol and *Buffer* (30:70)
Standard solution: 1 mg/mL of USP Methocarbamol RS in *Mobile phase*
Sample solution: Nominally 1 mg/mL of methocarbamol from a suitable volume of Injection containing NLT 100 mg of methocarbamol in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 274 nm
Column: 4.6-mm × 10.0-cm; 3-µm or 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methocarbamol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES
• **LIMIT OF ALDEHYDES**
Diluent: Alcohol and water (20:80)
Solution A: 10 mg/mL of phenylhydrazine hydrochloride in *Diluent*
Solution B: 10 mg/mL of potassium ferricyanide in water
Solution C: 10 µg/mL of formaldehyde in water prepared as follows. Dissolve 1.37 g of formaldehyde solution in 1 L of water. Dilute 10 mL of the resulting solution with water to 500 mL.
Standard solution: Transfer 4 mL of *Solution C* to a 25-mL volumetric flask. Add 2.0 mL of filtered *Solution A*. Allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.
Sample solution: Empty the contents of NLT 10 vials of Injection to a suitable container. Transfer 4.0 mL of the composite sample of Injection to a 25-mL volumetric flask. Add 2.0 mL of filtered *Solution A*, and allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.
Blank: Transfer 4 mL of water to a 25-mL volumetric flask. Add 2.0 mL of filtered *Solution A*, and allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.
Instrumental conditions
Mode: Vis
Analytical wavelength: 515 nm
Cell: 1 cm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Determine the absorbances of the *Samples*.
Acceptance criteria: The absorbance of the *Sample solution* is NMT the absorbance of the *Standard solution* (NMT 10 µg of formaldehyde in each mL of Injection).

SPECIFIC TESTS
• **PH** ⟨791⟩: 3.5–6.0
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.2 USP Endotoxin Units/mg of methocarbamol
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methocarbamol RS

Methocarbamol Tablets

DEFINITION
Methocarbamol Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Mix a portion of finely powdered Tablets equivalent to 1 g of methocarbamol with 25 mL of water

USP Monographs

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in a separator, and extract with 25 mL of chloroform. Filter the extract, and evaporate to dryness.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide to a pH of 4.5.
Mobile phase: Methanol and *Buffer* (30:70)
System suitability solution: 1.0 mg/mL of USP Methocarbamol RS and 0.005 mg/mL of USP Guaifenesin RS in *Mobile phase*
Standard solution: 0.1 mg/mL of USP Methocarbamol RS in *Mobile phase*
Sample stock solution: Nominally 1 mg/mL of methocarbamol solution prepared as follows. Transfer a portion of finely powdered Tablets (NLT 10) to a volumetric flask of suitable size. Add 60% of the volume of the flask with *Mobile phase*. Sonicate for 30 min with intermittent shaking. Dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally 0.1 mg/mL of methocarbamol from the *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 274 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
Run time: 1.5 times the retention time of methocarbamol
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methocarbamol from the *Sample solution*
r_S = peak response of methocarbamol from the *Standard solution*
C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methocarbamol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: USP Methocarbamol RS in *Medium*
Sample solution: Filtered portion of the solution under test, diluted with *Medium* if necessary
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of methocarbamol from the *Sample solution*
r_S = peak response of methocarbamol from the *Standard solution*
C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim for methocarbamol (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.005 mg/mL of USP Methocarbamol RS in *Mobile phase*
Sample solution: Use the *Sample stock solution* from the *Assay*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of methocarbamol from the *Standard solution*
C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methocarbamol in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guaifenesin	0.84	1.2	0.15

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Table 1 *(continued)*

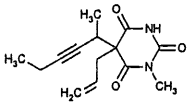
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methocarbamol isomer[a]	0.90	1.0	0.05
Methocarbamol	1.0	—	—
Methocarbamol dioxolone[b]	1.3	1.0	0.05
Any individual unspecified degradation product	—	—	0.10
Total impurities	—	—	1.0

[a] 1-Hydroxy-3-(2-methoxyphenoxy)propan-2-yl carbamate.
[b] 4-[(2-Methoxyphenoxy)methyl]-1,3-dioxolan-2-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Guaifenesin RS
  USP Methocarbamol RS

Methohexital



$C_{14}H_{18}N_2O_3$ 262.30
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 1-methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-, (±)-.
(±)-5-Allyl-1-methyl-5-(1-methyl-2-pentynyl)barbituric acid [151-83-7].

» Methohexital contains not less than 98.0 percent and not more than 101.0 percent of $C_{14}H_{18}N_2O_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Methohexital RS

Change to read:

▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197S***▲ (CN 1-May-2020)—
Solution: 1 in 100.
Medium: chloroform.
Melting range ⟨741⟩: between 92° and 96°, but the range between beginning and end of melting does not exceed 3°.
Water Determination, *Method I* ⟨921⟩: not more than 2.0%.
Chloride ⟨221⟩—Dissolve 200 mg in a mixture of 75 mL of ether and 25 mL of water, agitate, and allow to separate: the water solution shows no more chloride than corresponds to 0.17 mL of 0.010 N hydrochloric acid (0.03%).

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Eluant: a mixture of chloroform and acetone (7:3).

Visualization—Expose the plate to chlorine gas for 1 minute, and air-dry the plate at room temperature for 2 minutes. Prepare a solution of 0.5 g of potassium iodide in 50 mL of water, and prepare a solution of 1.5 g of soluble starch in 50 mL of hot water. Mix 10 mL of each solution with 4 mL of alcohol to obtain the *Detection reagent.* [NOTE—The *Detection reagent* so obtained may be used for up to 3 or 4 days.] Spray the plate with the *Detection reagent.*
Assay—Dissolve about 100 mg of Methohexital, accurately weighed, in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a solution having a concentration of about 10 mg per mL. Dissolve an accurately weighed quantity of USP Methohexital RS in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a Standard solution having a known concentration of about 10 mg per mL. Concomitantly determine the absorbances of both solutions in 0.1-mm cells at the wavelength of maximum absorbance at about 5.93 µm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of $C_{14}H_{18}N_2O_3$ in the portion of Methohexital taken by the formula:

$$10C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Methohexital RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Methohexital and the *Standard solution,* respectively.

Methohexital Sodium for Injection

$C_{14}H_{17}N_2NaO_3$ 284.29
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 1-methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-, (±)-, monosodium salt.
Sodium 5-allyl-1-methyl-5-(1-methyl-2-pentynyl)barbiturate [309-36-4; 22151-68-4].

» Methohexital Sodium for Injection is a freeze-dried, sterile mixture of methohexital sodium and anhydrous Sodium Carbonate as a buffer, prepared from an aqueous solution of Methohexital, Sodium Hydroxide, and Sodium Carbonate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methohexital sodium ($C_{14}H_{17}N_2NaO_3$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging.* Store at controlled room temperature. Injection may be packaged in 50-mL multiple-dose containers.
USP Reference standards ⟨11⟩—
USP Methohexital RS
Completeness of solution—Mix 1 g with 20 mL of carbon dioxide–free water: after 1 minute, the solution is clear and free from undissolved solid.
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Identification—
A: Dissolve about 500 mg in 10 mL of water in a separator, add 10 mL of 3 N hydrochloric acid, and extract the liberated

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methohexital with two 25-mL portions of chloroform. Evaporate the combined chloroform extracts to dryness, add 10 mL of ether, evaporate again, and dry the residue in vacuum at 80° for 4 hours. Dissolve 50 mg of the residue so obtained in 5 mL of chloroform: the solution exhibits IR absorption maxima at the same wavelengths as that of a similar preparation of USP Methohexital RS.

B: The methohexital obtained and dried as directed for *Identification* test *A* melts between 92° and 96°.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.5 USP Endotoxin Unit per mg of methohexital sodium.

Uniformity of dosage units ⟨905⟩: meets the requirements.

Procedure for content uniformity—

Standard solution—Transfer about 23 mg of USP Methohexital RS, accurately weighed, to a 250-mL volumetric flask, add 50 mL of water, 0.5 mL of sodium hydroxide solution (1 in 10), and 1.5 mL of sodium carbonate solution (1 in 1000), and mix. Dilute with water to volume, and mix. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Transfer the contents of 1 vial of Methohexital Sodium for Injection with the aid of water to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer an accurately measured volume of this solution, equivalent to about 100 mg of methohexital sodium, to a 1000-mL volumetric flask, add about 200 mL of water and 2.0 mL of sodium hydroxide solution (1 in 10), mix, dilute with water to volume, and again mix. Transfer 20.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* in 1-cm cells at the wavelength of maximum absorbance at about 247 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{14}H_{17}N_2NaO_3$ in the Methohexital Sodium for Injection taken by the formula:

$$(284.29/262.30)(TC/D)(A_U/A_S)$$

in which 284.29 and 262.30 are the molecular weights of methohexital sodium and methohexital, respectively; *T* is the labeled quantity, in mg, of methohexital sodium in the Methohexital Sodium for Injection; *C* is the concentration, in µg per mL, of USP Methohexital RS in the *Standard solution*; *D* is the concentration, in µg per mL, of methohexital sodium in the *Test solution* based on the labeled quantity per container and the extent of dilution; and A_U and A_S are the absorbances of the *Test solution* and the *Standard solution*, respectively.

pH ⟨791⟩: between 10.6 and 11.6 in the solution prepared in the test for *Completeness of solution*.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 2.0% of its weight.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Internal standard solution—Dissolve aprobarbital in chloroform to obtain a solution having a concentration of about 1.35 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Methohexital RS in chloroform to obtain a solution having a known concentration of about 0.46 mg per mL. Transfer 5.0 mL of the resulting solution to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix to obtain a *Standard preparation* having a known concentration of about 230 µg per mL.

Assay preparation—Combine and mix the constituted solutions prepared from the contents of 5 vials of Methohexital Sodium for Injection. Transfer an accurately measured volume of the resulting solution, equivalent to about 50 mg of methohexital sodium, to a 125-mL separator containing 25 mL of water, and mix. Add 0.2 mL of dilute hydrochloric acid (1 in 2), and mix. Extract with three 25-mL portions of chloroform, shaking each extraction for 2 minutes and filtering the extracts through about 15 g of anhydrous sodium sulfate, that previously has been washed with about 5 mL of chloroform, into a 100-mL volumetric flask. Wash the sodium sulfate with several small portions of chloroform, collecting the washings in the 100-mL volumetric flask. Dilute with chloroform to volume, and mix. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix.

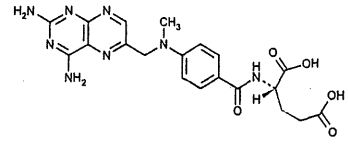
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and contains a 1.2-m × 4-mm column packed with 3% phase G10 on support S1AB. The column is maintained at about 230°, the injection port at about 265°, and the detector block at about 265°. Dry helium is used as the carrier gas at a flow rate of about 60 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between methohexital and aprobarbital is not less than 4.0, and the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Assay preparation* and the *Standard preparation* into the gas chromatograph, and measure the peak responses for the major peak. The relative retention times are about 0.6 for methohexital and 1.0 for aprobarbital. Calculate the quantity, in mg, of methohexital sodium ($C_{14}H_{17}N_2NaO_3$) in the portion of Methohexital Sodium for Injection taken by the formula:

$$(284.29/262.30)(0.2C)(R_U/R_S)$$

in which 284.29 and 262.30 are the molecular weights of methohexital sodium and methohexital, respectively; *C* is the concentration, in µg per mL, of USP Methohexital RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methotrexate



$C_{20}H_{22}N_8O_5$ 454.44

L-Glutamic acid, *N*-[4-[[(2,4-diamino-6-pteridinyl)methyl] methylamino]benzoyl]-;

(*S*)-2-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino} benzamido)pentanedioic acid;

4-Amino-10-methylfolic acid [59-05-2].

DEFINITION

Methotrexate contains NLT 98.0% and NMT 102.0% of methotrexate ($C_{20}H_{22}N_8O_5$), calculated on the anhydrous basis.

[**CAUTION**—Great care should be taken to prevent inhaling particles of methotrexate and exposing the skin to it.]

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*, 197A or 197K▲ (CN 1-May-2020)**:** Do not dry specimens.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲ (CN 1-May-2020)
Sample solution: 10 µg/mL in 0.1 N hydrochloric acid VS
Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**
Buffer: 3.4 g/L of anhydrous monobasic sodium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 5.8.
Solution A: Acetonitrile and *Buffer* (1:19)
Solution B: Acetonitrile and *Buffer* (1:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	50	50
34	50	50
35	100	0
40	100	0

Standard stock solution: 1.0 mg/mL of USP Methotrexate RS prepared as follows. Transfer a known amount of USP Methotrexate RS to a suitable volumetric flask, dissolve in dimethyl sulfoxide equivalent to 5% of the final volume, and dilute with *Solution A* to volume.
Standard solution: 0.2 mg/mL of USP Methotrexate RS in *Solution A*, from the *Standard stock solution*
Sample stock solution: 1.0 mg/mL of Methotrexate prepared as follows. Transfer a known amount of Methotrexate to a suitable volumetric flask, dissolve in dimethyl sulfoxide equivalent to 5% of the final volume, and dilute with *Solution A* to volume.
Sample solution: 0.2 mg/mL of Methotrexate in *Solution A*, from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 15°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.6
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Methotrexate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methotrexate RS in the *Standard solution* (mg/mL)
C_U = concentration of Methotrexate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES, PROCEDURE 1: RELATED COMPOUNDS**
Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard stock solution A: Use the *Standard solution* from the *Assay*.
Standard solution A: 0.1 µg/mL of USP Methotrexate RS in *Solution A*, from *Standard stock solution A*
Standard stock solution B: Transfer known quantities of USP Methotrexate Related Compound B RS, USP Methotrexate Related Compound C RS, and USP Methotrexate Related Compound E RS to a suitable volumetric flask, dissolve in dimethyl sulfoxide equivalent to 5% of the final volume, and dilute with *Solution A* to volume to obtain 0.1 mg/mL of USP Methotrexate Related Compound B RS, 0.2 mg/mL of USP Methotrexate Related Compound C RS, and 0.1 mg/mL of USP Methotrexate Related Compound E RS.
Standard solution B: 0.4 µg/mL of USP Methotrexate Related Compound C RS, 0.2 µg/mL of USP Methotrexate Related Compound B RS, and 0.2 µg/mL of USP Methotrexate Related Compound E RS in *Solution A*, from *Standard stock solution B*
System suitability solution: Transfer a known quantity of USP Methotrexate System Suitability Mixture RS to a suitable volumetric flask, and dissolve in dimethyl sulfoxide equivalent to about 2% of the final volume. Add *Standard stock solution B* equivalent to 0.2% of the final volume, and dilute with *Solution A* to volume to prepare 0.1 mg/mL of USP Methotrexate System Suitability Mixture RS, 0.2 µg/mL of USP Methotrexate Related Compound B RS, 0.4 µg/mL of USP Methotrexate Related Compound C RS, and 0.2 µg/mL of USP Methotrexate Related Compound E RS.
System suitability
Samples: *Standard solution A* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.7 between methotrexate related compound B and methotrexate related compound C; NLT 10.0 between methotrexate related compound C and methotrexate; and NLT 5.0 between methotrexate related compound I and methotrexate related compound H, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution A*
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Calculate the percentage of methotrexate related compound B and methotrexate related compound C in the portion of Methotrexate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution B*
C_S = concentration of the corresponding methotrexate related compound in *Standard solution B* (mg/mL)

USP Monographs

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C_U = concentration of Methotrexate in the *Sample solution* (mg/mL)

Calculate the percentage of methotrexate related compound E free base in the portion of Methotrexate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution B*
C_S = concentration of USP Methotrexate Related Compound E RS in *Standard solution B* (mg/mL)
C_U = concentration of Methotrexate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of methotrexate related compound E free base, 325.33
M_{r2} = molecular weight of methotrexate related compound E, 343.56

Calculate the percentage of methotrexate related compound H, methotrexate related compound I, and any unspecified impurity in the portion of Methotrexate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response of methotrexate from *Standard solution A*
C_S = concentration of USP Methotrexate RS in *Standard solution A* (mg/mL)
C_U = concentration of Methotrexate in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methotrexate related compound B	0.72	—	0.3
Methotrexate related compound C	0.77	—	0.5
Methotrexate	1.00	—	—
Methotrexate related compound E free base	1.46	—	0.3
Methotrexate dimethylamide[a] and methotrexate related compound I[b]	1.54	0.71	0.2[c]
Methotrexate related compound H[d]	1.65	1.0	0.2
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] (*S*)-2-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzamido)-5-(dimethylamino)-5-oxopentanoic acid.
[b] (*S*)-4-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzamido)-5-methoxy-5-oxopentanoic acid.
[c] If present, methotrexate dimethylamide and methotrexate related compound I may not be completely resolved by the method. These peaks are integrated together to determine conformance. If present and resolved, the relative retention time of methotrexate dimethylamide is 1.51.
[d] (*S*)-2-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzamido)-5-methoxy-5-oxopentanoic acid.

• **ORGANIC IMPURITIES, PROCEDURE 2: ENANTIOMERIC PURITY**
Solution A: 7.1 g/L of anhydrous dibasic sodium phosphate in water
Solution B: 6.9 g/L of monobasic sodium phosphate in water
Solution C: *Solution A* and *Solution B* (5:6). Adjust with 2 N sodium hydroxide TS to a pH of 6.9.
Mobile phase: *n*-Propanol and *Solution C* (2:23)
System suitability solution: 0.02 mg/mL each of USP Methotrexate RS and USP *R*-Methotrexate RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Methotrexate in *Mobile phase*
Diluted sample solution: 2 µg/mL of Methotrexate in *Mobile phase*, from the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 302 nm
Column: 4.0-mm × 15-cm; 7-µm packing L75
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for methotrexate and *R*-methotrexate are 1.0 and 1.95, respectively.]
Suitability requirements
Resolution: NLT 1.3 between methotrexate and *R*-methotrexate
Relative standard deviation: NMT 5.0% for the methotrexate peak
Analysis
Samples: *Sample solution* and *Diluted sample solution*
Calculate the percentage of *R*-methotrexate in the portion of Methotrexate taken:

$$\text{Result} = [r_U/(r_S \times 100)] \times 100$$

r_U = peak area of *R*-methotrexate from the *Sample solution*
r_S = peak area of methotrexate from the *Diluted sample solution*

Acceptance criteria: NMT 3.0%

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP *R*-Methotrexate RS
(*R*)-2-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzamido)pentanedioic acid.
$C_{20}H_{22}N_8O_5$ 454.44
USP Methotrexate System Suitability Mixture RS
This mixture contains:
Methotrexate
Methotrexate dimethylester hydrochloride;
(*S*)-Dimethyl-2-(4-{[(2,4-diaminopteridin-6-yl)methyl](methyl)amino}benzamido) pentanedioate hydrochloride.
$C_{22}H_{26}N_8O_5 \cdot HCl$ 518.95
Methotrexate related compound I;
(*S*)-4-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzamido)-5-methoxy-5-oxopentanoic acid.
$C_{21}H_{24}N_8O_5$ 468.47
Methotrexate related compound H;

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(*S*)-2-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl) amino}benzamido)-5-methoxy-5-oxopentanoic acid.
$C_{21}H_{24}N_8O_5$ 468.47
USP Methotrexate RS
USP Methotrexate Related Compound B RS
(*S*)-2-{4-[(2,4-Diaminopteridin-6-yl)methylamino] benzamido}pentanedioic acid.
$C_{19}H_{20}N_8O_5$ 440.41
USP Methotrexate Related Compound C RS
(*S*)-2-(4-{[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl) methyl](methyl)amino}benzamido)pentanedioic acid.
$C_{20}H_{21}N_7O_6$ 455.42
USP Methotrexate Related Compound E RS
4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino} benzoic acid, hemihydrochloride.
$C_{15}H_{15}N_7O_2 \cdot ½HCl$ 343.56

Methotrexate Injection

DEFINITION

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injection prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: ⟨197K⟩▲ (CN 1-May-2020)

Sample: Dilute, if necessary, a volume of Injection, equivalent to about 25 mg of methotrexate, with water to obtain a solution with a concentration of about 2.5 mg/mL. Adjust with 0.1 N hydrochloric acid to a pH of 4.0. Place the slurry in a 50-mL centrifuge tube, and centrifuge. Decant the supernatant, add 25 mL of acetone, shake, and pass through a solvent-resistant membrane filter of 0.45-µm pore size. Air-dry the filtered precipitate.
Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 3.4 mg/mL of anhydrous monobasic sodium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 6.0.
Solution A: Acetonitrile and *Buffer* (5:95)
Solution B: Acetonitrile and *Buffer* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
30	50	50
34	50	50
35	100	0
40	100	0

Standard solution: 0.2 mg/mL of USP Methotrexate RS in *Solution A* prepared as follows. Add a sufficient amount of USP Methotrexate RS to a suitable volumetric flask and add dimethyl sulfoxide equivalent to 5% of the flask volume. Sonicate to achieve dissolution, then dilute with *Solution A* to volume.
Sample solution: Nominally 0.2 mg/mL of methotrexate from Injection prepared as follows. Transfer a sufficient amount of Injection to an appropriate volumetric flask. Add about 5% of the flask volume of dimethyl sulfoxide and sonicate for 2 min at ambient temperature, then add 30% of the flask volume of *Solution A* and sonicate. Dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methotrexate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.2 µg/mL each of USP Methotrexate RS, USP Methotrexate Related Compound B RS, USP Methotrexate Related Compound C RS, and USP Methotrexate Related Compound E RS in *Solution A* prepared as follows. Add a sufficient amount of each Reference Standard to a suitable volumetric flask and add dimethyl sulfoxide equivalent to 5% of the flask volume. Sonicate to achieve dissolution, then dilute with *Solution A* to volume. Sonicate if necessary to aid dissolution.
Sample solution: Nominally 0.2 mg/mL of methotrexate from Injection prepared as directed in the *Assay*
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between methotrexate related compound B and methotrexate related compound C
Relative standard deviation: NMT 5.0% each for methotrexate, methotrexate related compound B, methotrexate related compound C, and methotrexate related compound E

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Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methotrexate related compound B and methotrexate related compound C in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each corresponding impurity from the *Sample solution*

r_S = peak response of each corresponding Reference Standard from the *Standard solution*

C_S = concentration of each corresponding Reference Standard in the *Standard solution* (µg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Calculate the percentage of methotrexate related compound E free acid in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methotrexate Related Compound E RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of methotrexate related compound E free acid, 325.33

M_{r2} = molecular weight of USP Methotrexate Related Compound E RS, 343.56

[NOTE—USP Methotrexate Related Compound E RS is 4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino} benzoic acid, hemihydrochloride.]

Calculate the percentage of any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of methotrexate from the *Standard solution*

C_S = concentration of USP Methotrexate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methotrexate related compound B	0.67	0.3
Methotrexate related compound C	0.73	▲4.0▲ (RB 1-May-2019)
Methotrexate	1.0	—
Methotrexate related compound E free acid[a]	1.41	0.3
Any individual unspecified degradation product	—	0.2
Total unspecified degradation products	—	1.0

[a] 4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzoic acid.

SPECIFIC TESTS

- **pH** ⟨791⟩: 7.0–9.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.4 USP Endotoxin Units/mg of methotrexate sodium
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Methotrexate RS

USP Methotrexate Related Compound B RS
(*S*)-2-{4-[(2,4-Diaminopteridin-6-yl)methylamino] benzamido}pentanedioic acid.
$C_{19}H_{20}N_8O_5$ 440.41

USP Methotrexate Related Compound C RS
(*S*)-2-(4-{[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl) methyl](methyl)amino}benzamido)pentanedioic acid.
$C_{20}H_{21}N_7O_6$ 455.42

USP Methotrexate Related Compound E RS
4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino} benzoic acid, hemihydrochloride.
$C_{15}H_{15}N_7O_2 \cdot ½HCl$ 343.56 (anhydrous)
$C_{15}H_{15}N_7O_2$ 325.33 (free acid)

Methotrexate for Injection

DEFINITION

Methotrexate for Injection is a sterile, freeze-dried preparation of methotrexate sodium with or without suitable added substances, buffers, and/or diluents. It contains NLT 95.0% and NMT 115.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

[CAUTION—Great care should be taken to prevent inhaling particles of methotrexate sodium and exposure to skin.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: 2.5 mg/mL of methotrexate in water from Methotrexate for Injection. Adjust with 0.1 N hydrochloric acid to a pH of 4.0. Place the slurry in a 50-mL centrifuge tube, and centrifuge. Decant the supernatant, add 25 mL of acetone, shake, and pass through a solvent-resistant membrane filter of 0.45-µm pore size. Air-dry the filtered precipitate.

Acceptance criteria: Meets the requirements

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

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ASSAY

Change to read:

• **PROCEDURE**

Buffer: 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37); adjusted, if necessary, with 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0

Mobile phase: Acetonitrile and *Buffer* ▲(8:92)▲ (USP 1-Aug-2019)

Standard solution: 0.1 mg/mL of USP Methotrexate RS in *Mobile phase*

Sample solution: Nominally equivalent to 0.1 mg/mL of methotrexate from 1 container of Methotrexate for Injection in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 302 nm

Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L1

▲Autosampler temperature: 5 ± 3°▲ (USP 1-Aug-2019)

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: ▲*Standard solution*▲ (USP 1-Aug-2019)

Suitability requirements

▲Tailing factor: NMT 2.0▲ (USP 1-Aug-2019)

Relative standard deviation: NMT ▲1.0%▲ (USP 1-Aug-2019)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Methotrexate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methotrexate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–115.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution: 0.05 mg/mL each of USP Methotrexate Related Compound B RS and USP Methotrexate Related Compound C RS prepared as follows. Transfer USP Methotrexate Related Compound B RS and USP Methotrexate Related Compound C RS into a suitable volumetric flask, add ammonium hydroxide equivalent to 2.5% of the final volume, and sonicate for 5 min. Add *Mobile phase* equivalent to 20% of the final volume and continue to sonicate until completely dissolved. Dilute with *Mobile phase* to volume.

System suitability solution: 2.5 µg/mL each of USP Methotrexate Related Compound B RS and USP Methotrexate Related Compound C RS in *Mobile phase* from *System suitability stock solution*

Sensitivity solution: 0.25 µg/mL of USP Methotrexate RS in *Mobile phase* from *Standard solution*

Sample solution: Nominally equivalent to 0.5 mg/mL of methotrexate from 1 container of Methotrexate for Injection in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.2 between the methotrexate related compound B and methotrexate related compound C peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Methotrexate for Injection taken:

$$\text{Result} = r_U/\{\Sigma[r_U \times (1/F)] + r_M\} \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*

F = relative response factor for each individual impurity (see *Table 1*)

r_M = peak area of methotrexate from the *Sample solution*

Table 1

Name	Relative Retention Time	Relative Response Factor
Methotrexate related compound B	0.36	0.72
Methotrexate related compound C	0.42	1.0
Methotrexate	1.0	—
Any other individual impurity	—	1.0

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Acceptance Criteria, NMT (%)
Each individual impurity	0.5
Total impurities	2.0▲ (USP 1-Aug-2019)

SPECIFIC TESTS

• **PH** ⟨791⟩

Sample: Constitute as directed in the labeling, except that water is used as the diluent.

Acceptance criteria: 7.0–9.0

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions*.

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Change to read:

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements▲ (USP 1-Aug-2019)
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **OTHER REQUIREMENTS:** Meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, protected from light.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methotrexate RS
  ▲USP Methotrexate Related Compound B RS
  (*S*)-2-{4-[(2,4-Diaminopteridin-6-yl)methylamino] benzamido}pentanedioic acid.
  $C_{19}H_{20}N_8O_5$ 440.41
  USP Methotrexate Related Compound C RS
  (*S*)-2-(4-{[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl) methyl](methyl)amino}benzamido)pentanedioic acid.
  $C_{20}H_{21}N_7O_6$ 455.42▲ (USP 1-Aug-2019)

Methotrexate Tablets

DEFINITION

Methotrexate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Standard solution: 25 µg/mL of USP Methotrexate RS in dilute hydrochloric acid (1 in 100)
  Sample solution: Dissolve 1 Tablet in 100 mL of dilute hydrochloric acid (1 in 100), and filter the solution.
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.

ASSAY

- **PROCEDURE**
  Buffer: 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37), adjusted if necessary with 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0
  Mobile phase: Acetonitrile and *Buffer* (10:90)
  System suitability solution: 0.1 mg/mL each of USP Methotrexate RS and folic acid in *Mobile phase*
  Standard solution: 100 µg/mL of USP Methotrexate RS in *Mobile phase*
  Sample solution: Equivalent to 100 µg/mL of methotrexate from powdered Tablets (NLT 20 Tablets) in *Mobile phase*. Dissolve the methotrexate using a mechanical shaker or ultrasonic bath.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 302 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 1.2 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for folic acid and methotrexate are 0.35 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 8.0 between the folic acid and methotrexate peaks
  Relative standard deviation: NMT 2.5% for the methotrexate peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methotrexate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

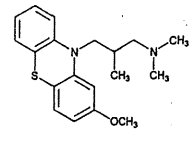
PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 2: 50 rpm
  Time: 45 min
  Standard solution: USP Methotrexate RS in *Medium*
  Detector: UV 306 nm (maximum absorbance)
  Analysis: Determine the amount of $C_{20}H_{22}N_8O_5$ dissolved from UV absorbances on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a *Standard solution*.
  Tolerances: NLT 75% (*Q*) of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. A unit-of-use container contains a quantity of Tablets sufficient to provide one week's therapy as indicated in the labeling.
- **LABELING:** When packaged in a unit-of-use container, the label indicates the total amount of methotrexate present as one week's supply.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methotrexate RS

Methotrimeprazine



$C_{19}H_{24}N_2OS$ 328.47
10*H*-Phenothiazine-10-propanamine, 2-methoxy-*N*,*N*,β-trimethyl-, (–)-;
(–)-10-[3-(Dimethylamino)-2-methylpropyl]-2-methoxyphenothiazine [60-99-1].

USP Monographs

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DEFINITION

Methotrimeprazine contains NLT 98.0% and NMT 101.0% of methotrimeprazine ($C_{19}H_{24}N_2OS$), calculated on the dried basis.

[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and the solutions containing them by conducting the procedures without delay under subdued light or by using low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲** (CN 1-May-2020)

Solution: 7 µg/mL
Medium: Alcohol
Acceptance criteria: Absorptivities at 255 nm, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

- **PROCEDURE**

Sample solution: Dissolve about 700 mg of Methotrimeprazine in 100 mL of chloroform. Add 1 drop of a solution (1 in 500) of crystal violet in chloroform.
Analysis: Titrate the *Sample solution* with 0.1 N perchloric acid VS to the first disappearance of the violet tinge. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.85 mg of methotrimeprazine ($C_{19}H_{24}N_2OS$).
Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

- **SELENIUM ⟨291⟩**

Test solution: Prepare with 100 mg of Methotrimeprazine and 100 mg of magnesium oxide.
Acceptance criteria: The absorbance of the *Test solution* is NMT one-half that of the *Standard Solution* (0.003%).

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 50 mg/mL in chloroform
Acceptance criteria: –15° to –18°

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 100° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Methotrimeprazine RS

Methotrimeprazine Injection

DEFINITION

Methotrimeprazine Injection is a sterile solution of Methotrimeprazine in Water for Injection, prepared with the aid of hydrochloric acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methotrimeprazine ($C_{19}H_{24}N_2OS$), as the hydrochloride.

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay under subdued light or by using low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Sample: Place 1 mL of Injection in a 125-mL separator, and add 1 N sodium hydroxide dropwise until the solution becomes opaque white. Extract with 50 mL of ether, wash the ether extract with 25 mL of water, and discard the washing. Filter the ether extract through a layer of anhydrous sodium sulfate into a beaker, and evaporate the filtrate by means of a stream of nitrogen to complete dryness. Dry at 100° for 3 h.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: Transfer 23.5 mL of 85% phosphoric acid into a 100-mL volumetric flask containing water, and dilute with water to volume.
Mobile phase: Add 20 mL of *Solution A* to 450 mL of water. To this solution add 5 mL of triethylamine, and adjust with 1 N sodium hydroxide to a pH of 3.0. Add 500 mL of acetonitrile, and dilute with water to 1000 mL. Filter, and degas.
System suitability solution: 2.0 mg/mL of benzyl alcohol, using appropriate amounts of 1% benzyl alcohol, and 0.1 mg/mL of USP Methotrimeprazine RS in *Mobile phase*
Standard solution: 0.1 mg/mL of USP Methotrimeprazine RS in *Mobile phase*
Sample solution: Nominally equivalent to 0.1 mg/mL of methotrimeprazine in *Mobile phase* from an appropriate amount of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between benzyl alcohol and methotrimeprazine, *System suitability solution*
Tailing factor: NMT 1.2, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methotrimeprazine ($C_{19}H_{24}N_2OS$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methotrimeprazine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methotrimeprazine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

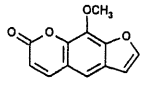
USP Monographs

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SPECIFIC TESTS
- **pH** ⟨791⟩: 3.0–5.0
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 17.9 USP Endotoxin Units/mg of methotrimeprazine
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.
- **USP Reference Standards** ⟨11⟩
  USP Methotrimeprazine RS

Methoxsalen



$C_{12}H_8O_4$ 216.19

7H-Furo[3,2-*g*][1]benzopyran-7-one, 9-methoxy-;
9-Methoxy-*7H*-furo[3,2-*g*][1]benzopyran-7-one;
9-Methoxy-*7H*-furo[3,2-*g*]chromen-7-one [298-81-7].

DEFINITION
Methoxsalen contains NLT 98.0% and NMT 102.0% of methoxsalen ($C_{12}H_8O_4$), calculated on the anhydrous basis. [**Caution**—Avoid contact with the skin.]

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Mobile phase: Acetonitrile and water (35:65)
Standard stock solution: 0.2 mg/mL of USP Methoxsalen RS in alcohol
Standard solution: 4 µg/mL of USP Methoxsalen RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 0.2 mg/mL of Methoxsalen in alcohol
Sample solution: 4 µg/mL of Methoxsalen in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1; or 3.9-mm × 30-cm; 10-µm packing L1; or 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methoxsalen ($C_{12}H_8O_4$) in the portion of Methoxsalen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methoxsalen from the *Sample solution*
r_S = peak response of methoxsalen from the *Standard solution*
C_S = concentration of USP Methoxsalen RS in the *Standard solution* (µg/mL)
C_U = concentration of Methoxsalen in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩

Sample: 1 g
Acceptance criteria: NMT 0.1%
- **Organic Impurities**

Solution A: 6% tetrahydrofuran in water
Solution B: Methanol
Solution C: *Solution A* and methanol (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	0	0	100
21.00	0	0	100
21.01	60	40	0
40.00	35	65	0
45.00	60	40	0
45.01	0	0	100
50.00	0	0	100

Diluent: Acetonitrile and water (1:1)
System suitability solution: 0.05 mg/mL each of USP Methoxsalen Related Compound A RS and USP Methoxsalen Related Compound B RS in *Diluent*
Standard stock solution: 1.0 mg/mL of USP Methoxsalen RS in *Diluent*
Standard solution: 1.0 µg/mL of USP Methoxsalen RS in *Diluent* from the *Standard stock solution*
Sensitivity solution: 0.5 µg/mL of USP Methoxsalen RS in *Diluent* from the *Standard solution*
Sample solution: 1.0 mg/mL of Methoxsalen in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 247 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.0 between methoxsalen related compound A and methoxsalen related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of each impurity in the portion of Methoxsalen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of methoxsalen from the *Standard solution*
C_S = concentration of USP Methoxsalen RS in the *Standard solution* (mg/mL)
C_U = concentration of Methoxsalen in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methoxsalen	1.00	1.0	—
Methoxsalen related compound B	1.50	0.51	0.15
Methoxsalen related compound A	1.57	0.68	0.15
Any individual unspecified impurity	—	—	0.10
Total impurities	—	—	0.5

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methoxsalen RS
  USP Methoxsalen Related Compound A RS
  4-Methoxy-7*H*-furo[3,2-*g*]chromen-7-one.
  $C_{12}H_8O_4$ 216.19
  USP Methoxsalen Related Compound B RS
  4,9-Dimethoxy-7*H*-furo[3,2-*g*]chromen-7-one.
  $C_{13}H_{10}O_5$ 246.22

Methoxsalen Capsules

DEFINITION

Methoxsalen Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of methoxsalen ($C_{12}H_8O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: Nominally 4 µg/mL of methoxsalen in alcohol prepared as follows. Place 1 Capsule in 50 mL of alcohol contained in a high-speed glass blender jar, blend thoroughly until the shell is completely dispersed, and dilute a portion with alcohol.
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of a similar solution of USP Methoxsalen RS, concomitantly measured.
  ▲[NOTE—The UV spectra of the major peaks of the *Sample solution* and the *Standard solution* as obtained in the *Assay* may also be used to meet the *Acceptance criteria*.]▲ (USP 1-May-2019)

ASSAY

Change to read:

- **PROCEDURE**
  Mobile phase: Acetonitrile and water (65:35)
  Standard stock solution: 0.2 mg/mL of USP Methoxsalen RS in alcohol
  Standard solution: 4 µg/mL of USP Methoxsalen RS in *Mobile phase*, from the *Standard stock solution*
  Sample stock solution 1 (for hard gelatin Capsules): Nominally equivalent to 0.04 mg/mL of methoxsalen in alcohol prepared as follows. Place NLT 10 Capsules in a high-speed glass blender jar containing 100.0 mL of alcohol, and blend thoroughly. Transfer a portion of the aliquot, nominally equivalent to 2 mg of methoxsalen, from the blender jar into a 50-mL volumetric flask, dilute with alcohol to volume, and filter.
  Sample stock solution 2 (for soft gelatin Capsules): Nominally equivalent to 0.04 mg/mL of methoxsalen in alcohol prepared as follows. Place the end of a long-stem glass funnel on a 250-mL volumetric flask, punch a hole at each end of a Capsule with a syringe containing 15 mL of alcohol, and rinse the contents into the flask. Cut the Capsule shell with a scalpel, and wash the inside of the shell with 15 mL of alcohol into the same flask. Repeat these steps for NLT 4 additional Capsules, and collect the rinsings. Wash the funnel, and collect the rinsings in the same flask. Dilute with alcohol to volume, and mix. Transfer a portion of this solution, nominally equivalent to 2 mg of methoxsalen, into a 50-mL volumetric flask. Dilute with alcohol to volume, and filter.
  Sample solution: Nominally equivalent to 4 µg/mL of methoxsalen in *Mobile phase*, from *Sample stock solution 1* or *Sample stock solution 2*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector set at 200–400 nm.▲ (USP 1-May-2019)
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of methoxsalen ($C_{12}H_8O_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methoxsalen from the *Sample solution*
r_S = peak response of methoxsalen from the *Standard solution*

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C_S = concentration of USP Methoxsalen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methoxsalen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
For soft gelatin Capsules
Medium: Water; 900 mL
Apparatus 2: 50 rpm ▲with sinkers, if necessary[1]▲ (USP 1-May-2019)
Time: 45 min
Standard solution: USP Methoxsalen RS in *Medium*. An amount of alcohol NMT 1% of the total volume of the *Standard solution* may be used to bring the Reference Standard into solution before diluting with *Medium*.
Sample solution: Dilute a filtered portion of the solution under test with *Medium* to a concentration similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 300 nm
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of the labeled amount of methoxsalen ($C_{12}H_8O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration USP Methoxsalen RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, if needed
L = label claim (mg/Capsule)▲ (USP 1-May-2019)

Tolerances: NLT 75% (Q) of the labeled amount of methoxsalen ($C_{12}H_8O_4$) is dissolved.
For hard gelatin Capsules
Medium: Water; 900 mL
Apparatus 1: 150 rpm
Time: 90 min
Sample solution: Filter a portion of the solution under test.
Standard solution: USP Methoxsalen RS in alcohol and diluted with *Medium* to a concentration similar to that of the *Sample solution*
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 252 nm
Analysis
Samples: *Sample solution* and *Standard solution*
▲Calculate the percentage of the labeled amount of methoxsalen ($C_{12}H_8O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Methoxsalen RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)▲ (USP 1-May-2019)

Tolerances: NLT 75% (Q) of the labeled amount of methoxsalen ($C_{12}H_8O_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**
Solution A: Water
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
35	35	65
40	35	65
42	70	30
50	70	30

Diluent: Methanol and water (7:3)
Standard stock solution: 1.0 mg/mL of USP Methoxsalen RS in methanol
Sensitivity solution: 0.1 µg/mL of USP Methoxsalen RS in *Diluent*, from the *Standard stock solution*
Standard solution: 0.5 µg/mL of USP Methoxsalen RS in *Diluent*, from the *Standard stock solution*
System suitability stock solution A: 0.1 mg/mL of USP Methoxsalen Related Compound A RS in methanol
System suitability stock solution B: 0.1 mg/mL of USP Methoxsalen Related Compound B RS in methanol
System suitability solution: 1.0 µg/mL each of USP Methoxsalen RS, USP Methoxsalen Related Compound A RS, and USP Methoxsalen Related Compound B RS in *Diluent*, from the *Standard stock solution*, *System suitability stock solution A*, and *System suitability stock solution B*, respectively
Sample stock solution: Nominally equivalent to 0.2 mg/mL of methoxsalen in methanol prepared as follows. Transfer the contents of 5 Capsules into a 250-mL volumetric flask. Use methanol to rinse the shells and collect the rinsings in the same volumetric flask. Dilute with methanol to volume.
Sample solution: Nominally equivalent to 0.1 mg/mL of methoxsalen prepared as follows. Transfer 5.0 mL of the *Sample stock solution* into a 10-mL volumetric flask, and dilute with *Diluent* to volume. Pass this solution through a suitable filter of 0.45-µm pore size, discard the first few milliliters, and use the filtrate for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 20 µL

[1] A suitable sinker is available as catalog number CAPWST-23 from www.qla-llc.com.

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System suitability
Samples: *Sensitivity solution, Standard solution,* and *System suitability solution*
Suitability requirements
Resolution: NLT 6.0 between methoxsalen and methoxsalen related compound B; NLT 1.5 between methoxsalen related compound B and methoxsalen related compound A, *System suitability solution*
Relative standard deviation: NMT 10%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of methoxsalen from the *Standard solution*
C_S = concentration of USP Methoxsalen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methoxsalen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methoxsalen	1.00	—
Methoxsalen related compound B[a]	1.24	—
Methoxsalen related compound A[a]	1.25	—
Any individual unspecified impurity	—	0.2
Total impurities	—	0.5

[a] This is a process impurity and is listed for identification only. It is controlled in the drug substance. It is not reported for the drug product and should not be included in the total impurities. ▲ (USP 1-May-2019)

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store at controlled room temperature.▲ (USP 1-May-2019)
- **LABELING:** Label the Capsules to state that Methoxsalen Hard Gelatin Capsules may not be interchangeable with Methoxsalen Soft Gelatin Capsules without retitration of the patient.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Methoxsalen RS
▲USP Methoxsalen Related Compound A RS
4-Methoxy-7*H*-furo[3,2-*g*]chromen-7-one.
$C_{12}H_8O_4$ 216.19
USP Methoxsalen Related Compound B RS
4,9-Dimethoxy-7*H*-furo[3,2-*g*]chromen-7-one.
$C_{13}H_{10}O_5$ 246.22▲ (USP 1-May-2019)

Methoxsalen Topical Solution

DEFINITION

Change to read:

Methoxsalen Topical Solution is a solution of methoxsalen in a suitable vehicle. ▲It contains NLT 92.0% and NMT 108.0% of the labeled amount of methoxsalen ($C_{12}H_8O_4$).▲ (USP 1-May-2019)

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: Nominally equivalent to 8 µg/mL of methoxsalen in alcohol, from Topical Solution
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of a similar solution of USP Methoxsalen RS, concomitantly measured.
▲[NOTE—The UV spectra of the major peaks of the *Sample solution* and the *Standard solution* as obtained in the *Assay* may also be used to meet the *Acceptance criteria*.]▲ (USP 1-May-2019)

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

- **PROCEDURE**
Mobile phase: Acetonitrile and water (35:65)
▲▲ (USP 1-May-2019)
Standard stock solution: 0.2 mg/mL of USP Methoxsalen RS in alcohol
Standard solution: 4 µg/mL of USP Methoxsalen RS in *Mobile phase* ▲from the *Standard stock solution*▲ (USP 1-May-2019)
Sample stock solution: Nominally 0.2 mg/mL of methoxsalen in alcohol prepared as follows. Transfer a volume of Topical Solution nominally equivalent to 20 mg of methoxsalen to a 100-mL volumetric flask and dilute with alcohol to volume.
Sample solution: Nominally equivalent to 4 µg/mL of methoxsalen as follows. Transfer 2.0 mL of *Sample stock solution* ▲▲ (USP 1-May-2019) to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification A*, use a diode array detector set at 200–400 nm.▲ (USP 1-May-2019)
Column: 4-mm × 30-cm; packing L1; ▲or 3.9-mm × 30-cm; 10-µm packing L1; or 4.6-mm × 25-cm; 5-µm packing L1▲ (USP 1-May-2019)
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*

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▲▲ (USP 1-May-2019)

Suitability requirements

▲Tailing factor: NMT 2.0▲ (USP 1-May-2019)

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

▲Calculate the percentage of the labeled amount of methoxsalen ($C_{12}H_8O_4$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methoxsalen RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methoxsalen in the *Sample solution* (µg/mL)▲ (USP 1-May-2019)

Acceptance criteria: ▲92.0%–108.0%▲ (USP 1-May-2019)

OTHER COMPONENTS

• **ALCOHOL DETERMINATION** ⟨611⟩ (if present)**:** 66.5%–77.0%

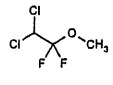
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Methoxsalen RS

Methoxyflurane



$C_3H_4Cl_2F_2O$ 164.97

Ethane, 2,2-dichloro-1,1-difluoro-1-methoxy-;

2,2-Dichloro-1,1-difluoroethyl methyl ether [76-38-0].

DEFINITION

Methoxyflurane contains NLT 99.9% and NMT 100.0% of methoxyflurane ($C_3H_4Cl_2F_2O$). It may contain a suitable stabilizer.

IDENTIFICATION

• **A. INFRARED ABSORPTION**

Standard solution: USP Methoxyflurane RS (1 in 20) in chloroform

Sample solution: Methoxyflurane (1 in 20) in chloroform

Acceptance criteria: The IR absorption spectrum of the *Sample solution* exhibits maxima only at the same wavelengths as those of the *Standard solution*.

• **B.**

Sample: Methoxyflurane

Analysis: To 1 mL of the *Sample* in a test tube add 1 mL of sulfuric acid.

Acceptance criteria: The *Sample* forms a layer over the acid (distinction from halothane).

• **C.**

Analysis: Cautiously heat the contents of the test tube from *Identification* test *B* with agitation.

Acceptance criteria: The interface disappears, and hydrofluoric acid is evolved (distinction from chloroform, trichloroethylene, and halothane).

ASSAY

• **PROCEDURE**

Sample: Methoxyflurane

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Thermal conductivity

Column: 4-mm × 3-m; stainless steel packed with liquid phase G11 on support S1A

Temperatures

Injection port: 150°

Column: 100°–110°

Carrier gas: Dry helium

Flow rate: 60 mL/min

Injection volume: NMT 30 µL

Analysis

Sample: *Sample*

Calculate the percentage of methoxyflurane ($C_3H_4Cl_2F_2O$) in the portion of Methoxyflurane taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of methoxyflurane

r_T = sum of all the peak areas

Acceptance criteria: 99.9%–100.0%

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩**:** 1.420–1.425

• **ACIDITY**

Sample: 25 mL of Methoxyflurane

Titrimetric system

Mode: Direct titration

Titrant: 0.010 N sodium hydroxide

Endpoint detection: Visual

Analysis: Shake the *Sample* with 25 mL of carbon dioxide-free water for 2 min, and allow the layers to separate. Add 1 drop of methyl red TS to the water extract, boil for 1 min, and titrate with *Titrant*.

Acceptance criteria: NMT 0.50 mL of *Titrant* is required to produce a distinct yellow color.

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.1%

• **LIMIT OF NONVOLATILE RESIDUE**

Sample: 50 mL of Methoxyflurane

Analysis: Evaporate the *Sample* at room temperature in a tared evaporating dish, and dry the residue at 105° for 1 h.

Acceptance criteria: The weight of the residue does not exceed 1 mg.

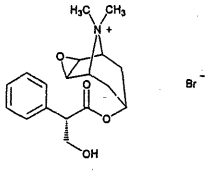
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Methoxyflurane RS

Methscopolamine Bromide



$C_{18}H_{24}BrNO_4$ 398.29

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3-Oxa-9-azoniatricyclo[3.3.1.0^{2,4}]nonane, 7-(3-hydroxy-1-oxo-2-phenylpropoxy)-9,9-dimethyl-, bromide, [7(*S*)-(1α, 2β,4β,5α,7β]-;
6β,7β-Epoxy-3α-hydroxy-8-methyl-1α*H*,5α*H*-tropanium bromide (–)-tropate;
(1*R*,2*R*,4*S*,5*S*,7*s*)-7-{[(*S*)-3-Hydroxy-2-phenylpropanoyl]oxy}-9,9-dimethyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-9-ium bromide [155-41-9].

DEFINITION

Methscopolamine Bromide contains NLT 97.0% and NMT 103.0% of methscopolamine bromide ($C_{18}H_{24}BrNO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*,** 197A or 197K▲ (CN 1-May-2020)**:** Meets the requirements
- **B. Identification Tests—General ⟨191⟩, *Chemical Identification Tests, Bromide***

Sample solution: 50 mg/mL in water
Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**

Buffer: A solution containing 5.16 g/L of sodium 1-hexanesulfonate monohydrate and 3.40 g/L of monobasic potassium phosphate in water, adjusted with 1 M phosphoric acid to a pH of 2.8
Solution A: Acetonitrile and *Buffer* (15:85)
Solution B: Acetonitrile and *Buffer* (50:50)
Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
10	85	15

Standard solution: 1.0 mg/mL of USP Methscopolamine Bromide RS in *Solution A*
Sample solution: 1.0 mg/mL of Methscopolamine Bromide in *Solution A*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10.0-cm; monolithic packing L1
Column temperature: 50°
Flow rate: 3 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1% for 6 replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methscopolamine bromide ($C_{18}H_{24}BrNO_4$) in the portion of Methscopolamine Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methscopolamine Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Methscopolamine Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Organic Impurities**

Buffer, Solution A, Solution B, Mobile phase, and **Sample solution:** Prepare as directed in the *Assay*.
Scopolamine hydrobromide solution: 0.05 mg/mL of USP Scopolamine Hydrobromide RS in *Solution A*
System suitability solution: 1.0 mg/mL of USP Methscopolamine Bromide RS and 1.0 µg/mL of USP Scopolamine Hydrobromide RS in *Solution A*, from the *Scopolamine hydrobromide solution*. [NOTE—This solution contains 0.1% of scopolamine hydrobromide.]
Standard stock solution: Prepare as directed for the *Standard solution* in the *Assay*.
Diluted standard solution: 0.5 µg/mL of USP Methscopolamine Bromide RS in *Solution A*, from the *Standard stock solution*
Chromatographic system: Proceed as directed in the *Assay*.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between methscopolamine and scopolamine
Tailing factor: NMT 2.0 for the methscopolamine peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Methscopolamine Bromide taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak area of any impurity from the *Sample solution*
r_S = peak area of methscopolamine from the *Sample solution*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak with an area less than that of the methscopolamine peak of the *Diluted standard solution*, and disregard any peak that is due to *Solution A*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tropic acid	0.4	2.4	0.1
Scopolamine hydrobromide	0.9	1.0	0.1
Methylatropine bromide[a]	1.2	1.0	0.1
Apomethscopolamine bromide[b]	3.5	1.7	0.1
Any other individual impurity	—	1.0	0.1

USP Monographs

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	0.5

[a] (1*R*,3*r*,5*S*)-3-[(3-Hydroxy-2-phenylpropanoyl)oxy]-8,8-dimethyl-8-azabicyclo[3.2.1]octan-8-ium bromide.
[b] (1*R*,2*R*,4*S*,5*S*,7*s*)-9,9-Dimethyl-7-[(2-phenylacryloyl)oxy]-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-9-ium bromide.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 50 mg/mL in water
  Acceptance criteria: −21° to −25°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methscopolamine Bromide RS
  USP Scopolamine Hydrobromide RS

Methscopolamine Bromide Tablets

DEFINITION

Methscopolamine Bromide Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of methscopolamine bromide ($C_{18}H_{24}BrNO_4$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

pH 7.3 dye–buffer solution: Prepare a solution containing, in each 500 mL, 200 mg of bromothymol blue, 3.2 mL of 0.1 N sodium hydroxide, 577.5 mg of citric acid monohydrate, and 6.3 mg of anhydrous dibasic sodium phosphate.

Standard solution: Prepare 0.025 mg/mL of USP Methscopolamine Bromide RS in water. Transfer 10 mL of this solution to a vessel containing 10 mL of chloroform and 10 mL of *pH 7.3 dye–buffer solution*. Shake vigorously for 3 min, centrifuge, and transfer 8 mL of the chloroform layer to a suitable container. Evaporate to dryness, and dissolve the residue in 1 mL of chloroform.

Sample solution: Finely powder 1 Tablet, and transfer an amount equivalent to 0.5 mg of methscopolamine bromide to a suitable container. Add 20 mL of water, heat for 5 min on a steam bath with frequent agitation, and centrifuge to obtain a clear supernatant. Transfer 10 mL of the supernatant to a vessel containing 10 mL of chloroform and 10 mL of *pH 7.3 dye–buffer solution*. Shake vigorously for 3 min, centrifuge, and transfer 8 mL of the chloroform layer to a suitable container. Evaporate to dryness, and dissolve the residue in 1 mL of chloroform.

Application volume: 50 µL

Developing solvent system: Butyl alcohol, water, and glacial acetic acid (4:5:1). Transfer a measured volume of the upper organic layer to a suitable container, and mix with a volume of alcohol equivalent to 20% of the volume of the organic layer.

Spray reagent: Potassium–bismuth iodide TS

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the solvent front to move three-fourths of the length of the plate, remove the plate from the developing chamber, mark the solvent front, and dry the plate under a current of air for 30 min. Spray the plate evenly with *Spray reagent*.

Acceptance criteria: The chromatogram of the *Sample solution* shows a bright orange spot on a yellow background corresponding in R_F value (0.25) to that of the *Standard solution*. [NOTE—Bromothymol blue produces a dark yellow spot at an R_F value of 0.8.]

Change to read:

- **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

- **PROCEDURE**

▲**Buffer:** A solution containing 5.16 g/L of sodium 1-hexanesulfonate monohydrate and 3.40 g/L of monobasic potassium phosphate in water, adjusted with 1 M phosphoric acid to a pH of 2.8

Solution A: *Buffer*

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	87	13
3	87	13
10	81	19
12	81	19

Diluent: Acetonitrile and *Buffer* (13:87)

Standard solution: 0.125 mg/mL of USP Methscopolamine Bromide RS in *Diluent*

Sample solution: Place 10 Tablets in a 200-mL volumetric flask. Add about 180 mL of *Diluent*, and sonicate for 30 min. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix. Further dilute with *Diluent*, if needed, to obtain the solution with a nominal concentration of 0.125 mg/mL of methscopolamine bromide. Centrifuge for about 10 min and use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10.0-cm; monolithic packing L1
Column temperature: 40°
Flow rate: 3 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%▲ 2S (USP41)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methscopolamine bromide ($C_{18}H_{24}BrNO_4$) in the portion of Tablets taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methscopolamine Bromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methscopolamine bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
pH 3.0 phosphate buffer: 5.44 g/L of monobasic potassium phosphate in water, adjusted with 1 N phosphoric acid to a pH of 3.0
Mobile phase: Methanol and *pH 3.0 phosphate buffer* (1:3)
Standard solution: Solution of USP Methscopolamine Bromide RS in *Medium* having a known concentration similar to the one expected in the *Sample solution*
Sample solution: Use portions of the *Sample solution* that have been passed through a 0.45-µm PTFE filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 204 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methscopolamine bromide ($C_{18}H_{24}BrNO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of the *Medium*, 500 mL
L = label claim (mg/Tablet)

Acceptance criteria: NLT 80% (Q) of the labeled amount of methscopolamine bromide ($C_{18}H_{24}BrNO_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Add the following:

▲• **Organic Impurities**
Buffer, Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
Scopolamine hydrobromide solution: 0.05 mg/mL of USP Scopolamine Hydrobromide RS in *Diluent*
System suitability solution: 1.0 mg/mL of USP Methscopolamine Bromide RS and 1.0 µg/mL of USP Scopolamine Hydrobromide RS in *Diluent*, from the *Scopolamine hydrobromide solution*
Standard stock solution: Prepare as directed for the *Standard solution* in the *Assay*.
Standard solution: 2.0 µg/mL of USP Methscopolamine Bromide RS in *Diluent* from the *Standard stock solution*
Sample solution
For Tablets that contain 2.5 mg of methscopolamine bromide: Place 20 Tablets in a 50-mL volumetric flask. Add 40–45 mL of *Diluent*, and sonicate for 30 min. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix. Centrifuge for about 10 min and use the clear supernatant.
For Tablets that contain 5 mg of methscopolamine bromide: Place 20 Tablets in a 100-mL volumetric flask. Add 80 mL of *Diluent*, and sonicate for 30 min. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix. Centrifuge for about 10 min and use the clear supernatant.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1 between methscopolamine and scopolamine
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of any impurity from the *Sample solution*
r_S = peak area of methscopolamine from the *Standard solution*
C_S = concentration of USP Methscopolamine Bromide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methscopolamine bromide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tropic acid	0.4	2.4	0.2
Scopolamine hydrobromide[a]	0.9	—	—
Methscopolamine bromide	1.0	—	—
Methylatropine bromide[a, b]	1.2	—	—
Apomethscopolamine bromide[a, c]	3.5	—	—
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	0.5

[a] These impurities are controlled in the drug substance and are included in the table for identification only. They are not to be reported for the drug product and should not be included in the total impurities.
[b] (1*R*,3*r*,5*S*)-3-[(3-Hydroxy-2-phenylpropanoyl)oxy]-8,8-dimethyl-8-azabicyclo[3.2.1]octan-8-ium bromide.
[c] (1*R*,2*R*,4*S*,5*S*,7*s*)-9,9-Dimethyl-7-[(2-phenylacryloyl)oxy]-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-9-ium bromide. ▲ 2S (USP41)

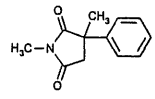
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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methscopolamine Bromide RS
  ▲USP Scopolamine Hydrobromide RS▲ 2S (USP41)

Methsuximide



$C_{12}H_{13}NO_2$ 203.24
2,5-Pyrrolidinedione,1,3-dimethyl-3-phenyl-, (±)-;
(±)-*N*,2-Dimethyl-2-phenylsuccinimide [77-41-8].

DEFINITION
Methsuximide contains NLT 97.0% and NMT 103.0% of methsuximide ($C_{12}H_{13}NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (9:11)
Standard solution: 0.6 mg/mL of USP Methsuximide RS in *Mobile phase*
Sample solution: 0.6 mg/mL of Methsuximide in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5800 theoretical plates
Tailing factor: NMT 1.3
Relative standard deviation: NMT 0.6%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methsuximide ($C_{12}H_{13}NO_2$) in the portion of Methsuximide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methsuximide RS in the *Standard solution* (mg/mL)
C_U = concentration of Methsuximide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.6 mg/mL of Methsuximide in *Mobile phase*
Standard solution: 0.006 mg/mL of USP Methsuximide RS in *Mobile phase*
Sample solution: 6.0 mg/mL of Methsuximide in *Mobile phase*
System suitability
Sample: *System suitability solution*
Suitability requirements
Column efficiency: NTL 5800 theoretical plates
Tailing factor: NMT 1.3
Relative standard deviation: NMT 0.6%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Methsuximide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for methsuximide from the *Standard solution*
C_S = concentration of USP Methsuximide RS in the *Standard solution* (mg/mL)
C_U = concentration of Methsuximide in the *Sample solution* (mg/mL)

Acceptance criteria
Any individual impurity: NMT 0.1%
Total impurities: NMT 2.0%

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample over phosphorus pentoxide for 16 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methsuximide RS

Methsuximide Capsules

DEFINITION
Methsuximide Capsules contain NLT 92.0% and NMT 108.0% of the labeled amount of methsuximide ($C_{12}H_{13}NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Mix a portion of the content of Capsules, equivalent to 200 mg of methsuximide with 25 mL of water in a separator, extract with 50 mL of ether, and discard the aqueous layer. Wash the ether extract with 25 mL of water, and discard the water. Filter the extract, evaporate with the aid of a current of warm air to dryness, and dry the methsuximide over phosphorus pentoxide for 16 h.

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Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (45:55). Filter, and degas.

Standard solution: 0.6 mg/mL of USP Methsuximide RS in *Mobile phase*

Sample stock solution: Place 10 Capsules in a 500-mL volumetric flask, and add 280 mL of water. Sonicate in a water bath at 40°–50°, with occasional shaking, until the Capsules have broken, and cool to room temperature. Dilute with acetonitrile to volume, mix, and filter.

Sample solution: Nominally 0.6 mg/mL of methsuximide prepared from *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2100 theoretical plates

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methsuximide ($C_{12}H_{13}NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methsuximide from the *Sample solution*

r_S = peak response of methsuximide from the *Standard solution*

C_S = concentration of USP Methsuximide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methsuximide in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 120 min

Analysis: Determine the percentage of the labeled amount of methsuximide ($C_{12}H_{13}NO_2$) dissolved by using the procedure set forth in the *Assay*, making any necessary adjustments.

Tolerances: NLT 75% (*Q*) of the labeled amount of methsuximide ($C_{12}H_{13}NO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

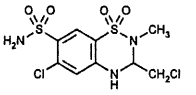
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Methsuximide RS

Methyclothiazide



$C_9H_{11}Cl_2N_3O_4S_2$ 360.24

2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3-(chloromethyl)-3,4-dihydro-2-methyl-, 1,1-dioxide, (±)-.

(±)-6-Chloro-3-(chloromethyl)-3,4-dihydro-2-methyl-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [135-07-9].

» Methyclothiazide contains not less than 97.0 percent and not more than 102.0 percent of $C_9H_{11}Cl_2N_3O_4S_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Methyclothiazide RS

USP Methyclothiazide Related Compound A RS

4-Amino-6-chloro-N^3-methyl-*m*-benzenedisulfonamide.

$C_7H_{10}ClN_3O_4S_2$ 299.76

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution: 20 µg per mL.

Medium: methanol.

C: Fuse about 100 mg of it with a pellet of sodium hydroxide: the ammonia fumes produced turn moistened red litmus paper blue. The fused mixture responds to the test for *Sulfite* ⟨191⟩.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Chloride ⟨221⟩—Shake 750 mg with 25 mL of water for 2 minutes, filter through a suitable membrane filter, and add 4 or 5 drops of 2 N nitric acid: the acidified filtrate shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.02%).

Selenium ⟨291⟩: 0.003%.

Diazotizable substances—

Standard preparation—Transfer about 10 mg of USP Methyclothiazide Related Compound A RS, accurately weighed, to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. Pipet 25 mL of the solution into a 100-mL volumetric flask, dilute with acetonitrile to volume, and mix. Each mL of *Standard preparation* contains about 50 µg of the Reference Standard.

Test preparation—Transfer about 500 mg of Methyclothiazide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Procedure—Pipet 2 mL each of the *Standard preparation* and the *Test preparation* into separate 50-mL volumetric flasks. Pipet 2 mL of acetonitrile into a third 50-mL flask to provide

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the blank. To each flask add 4 mL of 0.1 N hydrochloric acid, and mix. Add 3.0 mL of sodium nitrite solution (1 in 200) to each flask, mix, and place the flasks in an ice bath for 5 minutes, shaking occasionally. Add to each flask 3.0 mL of ammonium sulfamate solution (1 in 50), mix, and allow the flasks to remain in the ice bath for 1 additional minute. Remove the flasks from the ice bath, add 1.0 mL of N-(1-naphthyl) ethylenediamine dihydrochloride solution (1 in 1000), and mix. Allow the flasks to stand at room temperature for 1 minute, then dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions obtained from the *Standard preparation* and the *Test preparation* in 1-cm cells at 525 nm, with a suitable spectrophotometer, using the reagent blank to set the instrument. The absorbance of the solution from the *Test preparation* does not exceed that of the solution from the *Standard preparation*, corresponding to not more than 1.0% of diazotizable substances.

Assay—Transfer about 350 mg of Methyclothiazide, accurately weighed, to a 250-mL conical flask, add 40 mL of a 1 in 20 solution of potassium hydroxide in methanol, and reflux at full boil for 1 hour. Cool, rinse the inner walls of the condenser with 20 mL of water and two 20-mL portions of methanol, add 10 mL of glacial acetic acid and 2 drops of eosin Y TS, and titrate with 0.1 N silver nitrate VS to the first appearance of a definite pink color. Each mL of 0.1 N silver nitrate is equivalent to 36.02 mg of $C_9H_{11}Cl_2N_3O_4S_2$.

Methyclothiazide Tablets

» Methyclothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methyclothiazide ($C_9H_{11}Cl_2N_3O_4S_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Methyclothiazide RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* ***197U***▲ (CN 1-May-2020)—

Solution—Powder a number of Tablets, equivalent to about 50 mg of methyclothiazide, and transfer to a 100-mL volumetric flask with the aid of methanol. Add about 60 mL of methanol, and shake the flask for 1 hour. Dilute with methanol to volume, mix, and centrifuge a portion of the solution. Pipet 2 mL of the clear supernatant into a second 100-mL volumetric flask, dilute with methanol to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima only at the same wavelengths as that of a similar solution of USP Methyclothiazide RS.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.

Procedure—Determine the amount of $C_9H_{11}Cl_2N_3O_4S_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 270 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Methyclothiazide RS in the same *Medium*. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to dissolve USP Methyclothiazide RS prior to dilution with *Dissolution Medium*.

Tolerances—Not less than 70% (*Q*) of the labeled amount of $C_9H_{11}Cl_2N_3O_4S_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Transfer 1 finely powdered Tablet to a 50-mL volumetric flask, add about 30 mL of methanol, and shake by mechanical means for 1 hour. Dilute with methanol to volume, mix, and centrifuge a portion of the mixture. Dilute quantitatively with methanol to obtain a solution containing approximately 10 µg per mL of methyclothiazide. Concomitantly determine the absorbances of this solution and a Standard solution of USP Methyclothiazide RS in the same medium, having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 267 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_9H_{11}Cl_2N_3O_4S_2$ in the Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which T is the labeled quantity, in mg, of methyclothiazide in the Tablet; C is the concentration, in µg per mL, of USP Methyclothiazide RS in the Standard solution; D is the concentration, in µg per mL, of methyclothiazide in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—

Standard preparation—Transfer about 20 mg of USP Methyclothiazide RS, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, add chloroform to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of methyclothiazide, to a 150-mL beaker, add 2.0 mL of methanol, mix, allow the mixture to stand for 30 minutes while taking precautions against loss of solvent, add 2.0 mL of 0.1 M sodium bicarbonate, and mix.

Procedure— [NOTE—Use water-saturated solvents throughout this procedure.] Mix about 3 g of chromatographic siliceous earth with 2.0 mL of 0.1 M sodium bicarbonate in a 150-mL beaker. Pack the mixture into a 25- × 200-mm chromatographic column. Add 4 g of chromatographic siliceous earth to the *Assay preparation*, mix, transfer the mixture to the column, and pack. Dry-wash the beaker with 1 g of the siliceous earth mixed with 3 drops of water, and transfer to the column. Place a small pad of glass wool above the column packing, pass 75 mL of a mixture of isooctane and ether (9:1) through the column, and discard the eluate. Using a 200-mL volumetric flask as a receiver, pass 100 mL of chloroform through the column, wash the tip of column with ether, add 10.0 mL of methanol, dilute with chloroform to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard preparation* at the wavelength of maximum absorbance at about 268 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of methyclothiazide ($C_9H_{11}Cl_2N_3O_4S_2$) in the portion of Tablets taken by the formula:

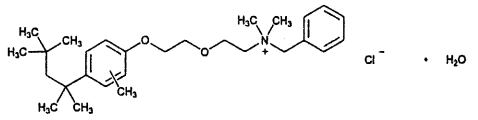
$$0.2C(A_U/A_S)$$

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in which C is the concentration, in µg per mL, of USP Methyclothiazide RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solution from the *Assay preparation* and the *Standard preparation,* respectively.

Methylbenzethonium Chloride



$C_{28}H_{44}ClNO_2 \cdot H_2O$ 480.12

$C_{28}H_{44}ClNO_2$ 462.12

Benzenemethanaminium, *N,N*-dimethyl-*N*-[2-[2-[methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride, monohydrate;

Benzyldimethyl[2-[2-[[4-(1,1,3,3-tetramethylbutyl)tolyl] oxy] ethoxy]ethyl]ammonium chloride monohydrate [1320-44-1].

Anhydrous [25155-18-4].

DEFINITION

Methylbenzethonium Chloride contains NLT 97.0% and NMT 103.0% of $C_{28}H_{44}ClNO_2$, calculated on the dried basis.

IDENTIFICATION

• **A. Procedure**

Analysis: To 1 mL of solution (1 in 100) add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

Acceptance criteria: A white precipitate, which is insoluble in 2 N nitric acid and soluble in 6 N ammonium hydroxide, is formed.

Change to read:

• **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **Procedure**

Buffer: Dilute 20 mL of triethylamine with water to 1000 mL, and adjust the pH to 3.0 with phosphoric acid.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Standard solution: 0.15 mg/mL of USP Methylbenzethonium Chloride RS in *Mobile phase*

System suitability solution: 0.15 mg/mL each of USP Benzethonium Chloride RS and USP Methylbenzethonium Chloride RS in *Mobile phase*

Sample solution: 0.15 mg/mL of methylbenzethonium chloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 10 µL

Run time: 1.5 times the retention time of the methylbenzethonium peak

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for benzethonium and methylbenzethonium are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the benzethonium and methylbenzethonium peaks

Tailing factor: NMT 1.8 for the methylbenzethonium peak

Relative standard deviation: NMT 0.5% for the methylbenzethonium peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$) in the portion of Methylbenzethonium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methylbenzethonium from the *Sample solution*

r_S = peak response of methylbenzethonium from the *Standard solution*

C_S = concentration of USP Methylbenzethonium Chloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylbenzethonium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS

• **Melting Range or Temperature** ⟨741⟩: 159°–163°, the specimen having been previously dried

• **Loss on Drying** ⟨731⟩: Dry a sample at 105° for 4 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards** ⟨11⟩

USP Benzethonium Chloride RS

USP Methylbenzethonium Chloride RS

Methylbenzethonium Chloride Lotion

DEFINITION

Methylbenzethonium Chloride Lotion is an emulsion containing NLT 90.0% and NMT 110.0% of the labeled amount of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$).

IDENTIFICATION

• **A.**

Sample: Suspend 0.5 mL of methylbenzethonium chloride lotion in 20 mL of water.

Analysis: Add 0.1 g of sodium carbonate, 1 mL of bromophenol blue TS, and 10 mL of chloroform to the *Sample*, and shake the mixture.

Acceptance criteria: The chloroform layer is blue.

ASSAY

• **Procedure**

Standard stock solution: 4.446 mg/mL of USP Docusate Sodium RS in isopropyl alcohol (equivalent to 0.01 M of USP Docusate Sodium RS). Store this solution in a tightly-stoppered glass container. Prepare the *Standard solution* on the day of use.

Standard solution 44.46 µg/mL of USP Docusate Sodium RS in water from the *Standard stock solution* (equivalent to 0.1 mM of USP Docusate Sodium RS)

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Sample solution: Transfer an amount of Lotion, equivalent to 0.5 mg of methylbenzethonium chloride, to a glass-stoppered, 50-mL cylinder. Add 5 mL of chloroform (freshly purified by shaking 100 mL with 10 g of silica gel, allowing to settle, and withdrawing the supernatant), 5 mL of phosphoric acid solution (1 in 10), and 1 mL of 0.05 mg/mL of safranin O solution.

Analysis: Titrate the *Sample solution* with the *Standard solution* until 1 mL from the endpoint, then shake the stoppered tube vigorously for about 2 min, and continue the titration in 0.1-mL increments, shaking vigorously after each addition, until a pink color appears in the chloroform layer. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of *Standard solution* is equivalent to 48.01 µg of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$).

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 5.2–6.0

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards** ⟨11⟩

USP Docusate Sodium RS

Methylcellulose

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (• •) to specify this fact.

Cellulose, methyl ether;
Cellulose methyl ether [9004-67-5].

DEFINITION

Methylcellulose is a methyl ether of cellulose. When dried at 105° for 1 h, it contains NLT 26.0% and NMT 33.0% of methoxy ($-OCH_3$) groups.

IDENTIFICATION

• **A.**

Sample: 1 g

Analysis: Evenly distribute the *Sample* onto the surface of 100 mL of water in a beaker, tapping the top of the beaker gently, if necessary, to ensure a uniform layer on the surface, and allow to stand for 1–2 min.

Acceptance criteria: The powdered material aggregates on the surface.

• **B.**

Sample: 1 g

Analysis: Evenly distribute the *Sample* into 100 mL of boiling water and stir the mixture using a magnetic stirrer with a 25-mm long bar. A slurry is formed and the particles do not dissolve. Allow the slurry to cool to 5° and stir using a magnetic stirrer.

Acceptance criteria: A clear or slightly turbid solution occurs with its thickness dependent on the viscosity grade.

• **C.**

Solution A: Sulfuric acid and water (9 in 10).
[Note—Carefully add the sulfuric acid to the water.]

Sample solution: 0.1 mL of the solution prepared for *Identification B*

Analysis: To the *Sample solution*, add 9 mL of *Solution A* and shake. Heat in a water bath for exactly 3 min, immediately cool in an ice bath, and add carefully 0.6 mL of ninhydrin TS. Shake and allow to stand at 25°.

Acceptance criteria: A red color develops immediately and it does not change to purple within 100 min.

• **D.**

Sample solution: 2–3 mL of the solution prepared for *Identification B*

Analysis: Pour the *Sample solution* onto a glass slide as a thin film and allow the water to evaporate.

Acceptance criteria: A coherent, clear film forms on the glass slide.

• **E.**

Sample solution: 50 mL of the solution prepared in *Identification B*

Analysis: Add the *Sample solution* to exactly 50 mL of water in a beaker. Insert a thermometer into the solution. Stir the solution on a magnetic stirrer/hot plate, and begin heating at a rate of 2°–5°/min. Determine the temperature at which a turbidity increase begins to occur and designate this temperature as the flocculation temperature.

Acceptance criteria: The flocculation temperature is higher than 50°.

ASSAY

Change to read:

• **Procedure**

[**Caution**—Perform all steps involving hydriodic acid carefully, in a well-ventilated hood. Use goggles, acid-resistant gloves, and other appropriate safety equipment. Be exceedingly careful when handling the hot vials because they are under pressure. In the event of hydriodic exposure, wash with copious amounts of water, and seek medical attention at once.]

Apparatus

Reaction vial: A 5-mL pressure-tight serum vial, 20 mm in outside diameter, 50 mm in height, and 20 mm in outside diameter and 13 mm in inside diameter at the mouth, equipped with a pressure-tight septum having a polytetrafluoroethylene-faced butyl rubber and an airtight seal using an aluminum crimp or any sealing system that provides sufficient airtightness

Heater: A heating module with a square-shaped aluminum block having holes 20 mm in diameter and 32 mm in depth, so that the reaction vial fits. The heating module is also equipped with a magnetic stirrer capable of mixing the contents of the vial, or a reciprocal shaker that performs a reciprocating motion approximately 100 times/min can be used.

Hydriodic acid: Use a reagent having a specific gravity of at least 1.69, equivalent to 55%–57% hydrogen iodide (HI).

Internal standard solution: 30 mg/mL of *n*-octane in *o*-xylene

Standard solution: Into a suitable serum vial, weigh 60–100 mg of adipic acid, add 2.0 mL of *Hydriodic acid*, and then pipet 2.0 mL of the *Internal standard solution* into the vial. Close the vial securely with a suitable septum stopper. Weigh the vial and contents, add 45 µL of methyl iodide with a syringe through the septum, weigh again, and calculate the weight of methyl iodide added, by difference. Shake, and allow the layers to separate. Use the upper layer as the *Standard solution*.

Sample solution: Transfer 0.065 g of Methylcellulose to a 5-mL thick-walled reaction vial equipped with a pressure-tight septum closure, add 60–100 mg of adipic acid, and pipet 2.0 mL of the *Internal standard solution* into the vial. Cautiously pipet 2.0 mL of *Hydriodic acid* into the mixture, immediately secure the closure, and weigh accurately. Using the magnetic stirrer from the heating module, or using a reciprocal shaker, mix the contents of the vial continuously for 60 min while

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heating the block so that the temperature of the contents is maintained at 130 ± 2°. If a reciprocal shaker or magnetic stirrer cannot be used, shake the vial well by hand at 5-min intervals during the initial 30 min of the heating time. Allow the vial to cool, and weigh again. If the weight loss is less than ▲26 mg▲ (USP 1-May-2019) and there is no evidence of a leak, use the upper layer of the mixture as the *Sample solution*.

Chromatographic system

Mode: GC

Detector: Thermal conductivity or hydrogen flame ionization

Column: ▲0.53-mm × 30-m fused silica capillary, coated with a 3-µm layer of phase G1. Use a guard column if necessary.▲ (USP 1-May-2019)

Temperatures

Injection port: 250°

Detector: 280°

Column: See the temperature program shown in *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	3
50	10	100	—
100	34.9	250	8

Carrier gas: Helium▲▲ (USP 1-May-2019)

Flow rate: Adjust so that the retention time of the internal standard is about 10 min ▲(about 4.3 mL/min).▲ (USP 1-May-2019)

Injection volume: 1 or 2 µL

Injection type: Split ratio, 40:1

Run time: 20.3 min

System suitability

Suitability requirements

Resolution: Methyl iodide and the internal standard are eluted in this order, with resolution NLT 5 between these peaks.

Relative standard deviation: NMT 2.0% for 6 injections of the *Standard solution*, using the peak area ratio between methyl iodide and the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methoxy in the portion of Methylcellulose taken:

$$\text{Result} = X \times (R_U/R_S) \times (W_S/W)$$

X = ratio of the formula weights of methoxy to methyl iodide times 100%, 21.864

R_U = peak area ratio of methyl iodide to the internal standard from the *Sample solution*

R_S = peak area ratio of methyl iodide to the internal standard from the *Standard solution*

W_S = weight of methyl iodide in the *Standard solution* (mg)

W = weight of Methylcellulose, calculated on the dried basis, taken for the *Assay* (mg)

Acceptance criteria: 26.0%–33.0% calculated on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 1.5%

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° for 1 h.

Acceptance criteria: NMT 5.0%

• **Viscosity—Capillary Methods** ⟨911⟩ and **Viscosity—Rotational Methods** ⟨912⟩

[Note—The density is 1.00 g/mL, so there is no necessity for determining the density at every measurement in the case of having the confirmation data.]

Method 1: This method is applied to samples with a viscosity of less than 600 mPa · s. Weigh a quantity of Methylcellulose, equivalent to 4.000 g, calculated on the dried basis, transfer into a wide-mouth bottle, and add hot water (90°–99°) to obtain the total weight of the sample and water of 200.0 g. Cap the bottle and stir by mechanical means at 400 ± 50 rpm for 10–20 min until particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula, if necessary, to ensure that there is no undissolved material on the sides of the bottle. Continue the stirring in a cooling water bath equilibrated at a temperature below 5° for another 20–40 min. Adjust the solution weight, if necessary, to 200.0 g using cold water. Centrifuge the solution, if necessary, to expel any entrapped air bubbles. Using a spatula, remove any foam, if present. Perform the test with this solution at 20 ± 0.1° to obtain the kinematic viscosity (ν). Separately, determine the density (ρ) of the solution, and calculate the viscosity (η) as $\eta = \rho\nu$.

Method 2: This method is applied to samples with a viscosity of 600 mPa · s or higher. Weigh a quantity of Methylcellulose, equivalent to 10.00 g, calculated on the dried basis, transfer into a wide-mouth bottle, and add hot water (90°–99°) to obtain the total weight of the sample and water of 500.0 g. Capping the bottle, stir by mechanical means at 400 ± 50 rpm for 10–20 min until particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula, if necessary, to ensure that there is no undissolved material on the sides of the bottle. Continue the stirring in a cooling water bath equilibrated at a temperature below 5° for another 20–40 min. Adjust the solution weight, if necessary, to 500.0 g using cold water. Centrifuge the solution, if necessary, to expel any entrapped air bubbles. Using a spatula, remove any foam, if present. Determine the viscosity of this solution at 20 ± 0.1° using a single-cylinder type rotational viscometer.

Apparatus: Brookfield type LV model or equivalent. For rotor no., revolution, and calculation multiplier, apply the conditions specified in *Table 2*.

Table 2

Labeled Viscosity[a] (mPa · s)	Rotor No.	Revolution (rpm)	Calculation Multiplier
600 or more and less than 1400	3	60	20
1400 or more and less than 3500	3	12	100
3500 or more and less than 9500	4	60	100
9500 or more and less than 99,500	4	6	1000
99,500 or more	4	3	2000

[a] The *Labeled Viscosity* is based on the manufacturer's specifications.

Operation of apparatus: Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period

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of at least 2 min between subsequent measurements. Repeat the operation to rotate the spindle specified above twice, and average the three readings.

Acceptance criteria: 80.0%–120.0% of that stated on the label for viscosity types less than 600 mPa · s, and 75.0%–140.0% of that stated on the label for viscosity types 600 mPa · s or higher

- **pH** ⟨791⟩

Analysis: Measure the pH of the solution prepared in the test for viscosity. Read the indicated pH value after the probe has been immersed for 5 ± 0.5 min.

Acceptance criteria: 5.0–8.0

ADDITIONAL REQUIREMENTS

- **•PACKAGING AND STORAGE:** Preserve in well-closed containers.•
- **LABELING:** Label it to indicate its nominal viscosity type [viscosity of a solution (1 in 50)] in millipascal seconds (mPa · s).

Methylcellulose Ophthalmic Solution

» Methylcellulose Ophthalmic Solution is a sterile solution of Methylcellulose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of methylcellulose. It may contain suitable antimicrobial, buffering, and stabilizing agents.

Packaging and storage—Preserve in tight containers.

Identification—

A: Heat a few mL of Ophthalmic Solution: the solution becomes cloudy and a flaky precipitate, which redissolves as the solution cools, appears.

B: Pour a few mL of Ophthalmic Solution onto a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

Sterility Tests ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 6.0 and 7.8.

Assay—To boiling flask *A*, as described under *Methoxy Determination* ⟨431⟩, pipet a quantity of Ophthalmic Solution, equivalent to 50 mg of methylcellulose. Evaporate on a steam bath to dryness, cool the flask in an ice bath, add the specified amount of hydriodic acid, and proceed as directed under *Methoxy Determination* ⟨431⟩. Each mL of 0.1 N sodium thiosulfate is equivalent to 1.753 mg of methylcellulose.

Methylcellulose Oral Solution

» Methylcellulose Oral Solution is a flavored solution of Methylcellulose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of methylcellulose.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat. Avoid freezing.

Identification—

A: Heat a few mL of Oral Solution: the solution becomes cloudy and a flaky precipitate, which redissolves as the solution cools, appears.

B: Pour a few mL of Oral Solution onto a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for the absence of *Escherichia coli.*

Alcohol Determination, *Method II* ⟨611⟩: between 3.5% and 6.5% of C_2H_5OH.

Assay—To boiling flask *A*, as described under *Methoxy Determination* ⟨431⟩, transfer an accurately measured volume of Oral Solution, equivalent to 50 mg of methylcellulose. Evaporate on a steam bath to dryness, cool the flask in an ice bath, add the specified amount of hydriodic acid, and proceed as directed under *Methoxy Determination* ⟨431⟩. Each mL of 0.1 N sodium thiosulfate is equivalent to 1.753 mg of methylcellulose.

Methylcellulose Tablets

» Methylcellulose Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methylcellulose.

Packaging and storage—Preserve in well-closed containers.

Identification—

A: Gently add about 250 mg of the residue obtained in the *Assay* to the top of 25 mL of water in a beaker, and allow to disperse over the surface, tapping the top of the container to ensure an even dispersion of the test specimen. Allow the beaker to stand until the specimen becomes transparent and mucilaginous (about 5 hours), swirl the beaker to wet the remaining substance, add a stirring bar, and stir until dissolved: the mixture remains stable when an equal volume of 1 N sodium hydroxide or 1 N hydrochloric acid is added.

B: Heat a few mL of the solution prepared for *Identification* test *A*: the solution becomes cloudy, and a flaky precipitate, which redissolves as the solution cools, appears.

C: Pour a few mL of the solution prepared for *Identification* test *A* onto a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

Disintegration ⟨701⟩: 30 minutes.

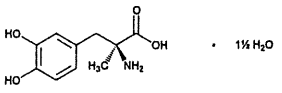
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg of methylcellulose, and transfer to a tared, fine fritted-glass, low-form, 30-mL crucible having a fitted crucible lid. Add 20 mL of alcohol, and macerate the solid for about 5 minutes, mixing intermittently with a glass stirring rod. Repeat the extraction with ten consecutive 10-mL portions of alcohol. Test for completeness of extraction by evaporating the last alcohol extract on a steam bath to dryness, taking up the residue in about 1 mL of water, and adding this to 5 mL of hot alkaline cupric tartrate TS (no red precipitate of cuprous oxide is formed within 5 minutes). If a precipitate is formed, continue with the alcohol extractions until the test is negative. Wash the completely extracted residue with a 10-mL portion of ether, using suction to drain off the liquid. Dry the residue in the crucible in a drying oven at 105° to constant weight. Weigh the crucible with the crucible lid in place. The weight of residue

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is the weight of methylcellulose present in the portion of powdered Tablets taken.

Methyldopa



$C_{10}H_{13}NO_4 \cdot 1½H_2O$ 238.24

$C_{10}H_{13}NO_4$ 211.22

L-Tyrosine, 3-hydroxy-α-methyl-, sesquihydrate;
L-3-(3,4-Dihydroxyphenyl)-2-methylalanine sesquihydrate [41372-08-1].
Anhydrous [555-30-6].

DEFINITION

Methyldopa contains NLT 98.0% and NMT 102.0% of methyldopa ($C_{10}H_{13}NO_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

▲• **A. Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

[Note—Freshly prepare the *Standard solution* and *Sample solution* before use.]

Buffer: 0.1 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: *Buffer* and methanol (850:150)
Diluent: 0.1 N hydrochloric acid
Standard solution: 0.4 mg/mL of USP Methyldopa RS in *Diluent*
Sample solution: 0.4 mg/mL of Methyldopa in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.9–1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methyldopa ($C_{10}H_{13}NO_4$) in the portion of Methyldopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methyldopa from the *Sample solution*
r_S = peak response of methyldopa from the *Standard solution*
C_S = concentration of USP Methyldopa RS in the *Standard solution* (mg/mL)
C_U = concentration of Methyldopa in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Organic Impurities**

[Note—Freshly prepare the *Standard solution* and *Sample solution* before use.]

Buffer, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.
System suitability solution: 4 µg/mL of USP Methyldopa RS and 6 µg/mL each of USP 3-*O*-Methylmethyldopa RS, USP Methyldopa Related Compound B RS, and USP Methyldopa Related Compound C RS in *Diluent*
Standard solution: 4 µg/mL of USP Methyldopa RS in *Diluent*
Sample solution: 4 mg/mL of Methyldopa in *Diluent*
Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.
Run time: NLT 6 times the retention time of methyldopa
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between methyldopa related compound B and methyldopa related compound C, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Methyldopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of methyldopa from the *Standard solution*
C_S = concentration of USP Methyldopa RS in the *Standard solution* (mg/mL)
C_U = concentration of Methyldopa in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peaks below 0.03%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methyldopa	1.0	1.0	—
3-*O*-Methylmethyldopa[a]	1.9	1.0	0.15
Methyldopa related compound B[b]	4.3	0.38	0.15
Methyldopa related compound C[c]	4.9	0.77	0.15
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	–	0.5

[a] (*S*)-2-Amino-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.
[b] (*S*)-2-Amino-3-(4-methoxyphenyl)-2-methylpropanoic acid.
[c] (*S*)-2-Amino-3-(3,4-dimethoxyphenyl)-2-methylpropanoic acid.

USP Monographs

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SPECIFIC TESTS

• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 44 mg/mL, in a solvent that is a solution of aluminum chloride hexahydrate in water (2 in 3) that previously has been treated with activated charcoal, filtered, and adjusted with 0.25 N sodium hydroxide to a pH of 1.5
Acceptance criteria: −25° to −28°

• **Acidity**
Sample solution: Dissolve 1.0 g in carbon dioxide-free water with the aid of heat, and add 1 drop of methyl red TS.
Analysis: Titrate the *Sample solution* with 0.10 N sodium hydroxide to a yellow endpoint.
Acceptance criteria: NMT 0.50 mL is required.

• **Water Determination** ⟨921⟩, *Method I*: 10.0%–13.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

• **USP Reference Standards** ⟨11⟩
USP Methyldopa RS
USP Methyldopa Related Compound B RS
(*S*)-2-Amino-3-(4-methoxyphenyl)-2-methylpropanoic acid hydrochloride.
$C_{11}H_{15}NO_3 \cdot HCl$ 245.70
USP Methyldopa Related Compound C RS
(*S*)-2-Amino-3-(3,4-dimethoxyphenyl)-2-methylpropanoic acid hydrochloride.
$C_{12}H_{17}NO_4 \cdot HCl$ 275.73
USP 3-*O*-Methylmethyldopa RS
(*S*)-2-Amino-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.
$C_{11}H_{15}NO_4$ 225.24

Methyldopa Tablets

DEFINITION

Methyldopa Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$).

IDENTIFICATION

• **A.**
Analysis: To about 10 mg of finely ground Tablets add 3 drops of a solution of ninhydrin in sulfuric acid (1 in 250).
Acceptance criteria: A dark purple color is produced within 5–10 min that changes to pale brownish yellow on addition of 3 drops of water.

• **B.**
Analysis: To about 10 mg of finely ground Tablets add 2 mL of 0.1 N sulfuric acid and 2 mL of *Solution A*, prepared as directed in the *Assay*, followed by 0.25 mL of 6 N ammonium hydroxide.
Acceptance criteria: A dark purple color is produced immediately.

ASSAY

• **Procedure**
Solution A: Freshly dissolve 1 g of ferrous sulfate, 2 g of potassium sodium tartrate, and 100 mg of sodium bisulfite in water to make a 100-mL solution.
Solution B: 50 g/L of ammonium acetate in 20% alcohol. Adjust with 6 N ammonium hydroxide to a pH of 8.5.
Standard solution: 1 mg/mL of anhydrous methyldopa from USP Methyldopa RS in 0.1 N sulfuric acid
Sample solution: Nominally 1 mg/mL of methyldopa in 0.1 N sulfuric acid from NLT 20 powdered Tablets prepared as follows. To a 100-mL volumetric flask add 0.1 N sulfuric acid to fill about 50% of the volume of the flask, agitate by mechanical means for 15 min, and then dilute with 0.1 N sulfuric acid to volume. Filter the solution, and reject the first 20 mL of the filtrate.
Blank: Water
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 520 nm
Cell: 1 cm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Pipet 5 mL each of *Standard solution*, *Sample solution*, and *Blank* into separate 100-mL volumetric flasks. Add to each flask 5 mL of *Solution A*, and dilute with *Solution B* to volume.
Calculate the percentage of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Methyldopa RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methyldopa in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 20 min
Instrumental conditions
Mode: UV
Analytical wavelength: 280 nm
Standard solution: USP Methyldopa RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Analysis: Determine the amount of methyldopa ($C_{10}H_{13}NO_4$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩
USP Methyldopa RS

Methyldopa and Hydrochlorothiazide Tablets

DEFINITION

Methyldopa and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of methyldopa ($C_{10}H_{13}NO_4$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention times of the two major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

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• **B.**

Analysis: To 10 mg of methyldopa from a portion of crushed Tablets add 0.15 mL of a solution of ninhydrin in sulfuric acid (1 in 250).

Acceptance criteria: A dark purple color is produced within 5–10 min. The color changes to pale brownish yellow upon adding 0.15 mL of water.

ASSAY

• **PROCEDURE**

Buffer: 11.04 g/L of monobasic sodium phosphate. Initially add 950 mL of water, adjust with phosphoric acid to a pH of 2.8, and then dilute with water to volume.

Mobile phase: Methanol and *Buffer* (5:95)

Standard solution: Transfer a suitable quantity of USP Methyldopa RS to a suitable volumetric flask to prepare a 1-mg/mL solution of anhydrous methyldopa. Add a quantity of USP Hydrochlorothiazide RS that corresponds to the ratio of hydrochlorothiazide to methyldopa in the Tablets. Dissolve in 25% of the total volume a mixture of water, acetonitrile, and 1 N hydrochloric acid (1:1:0.5). Dilute with water to volume.

Sample solution: Transfer an equivalent to 250 mg of methyldopa (NLT 20 Tablets) to a 250-mL volumetric flask, and add 50 mL of water, 25 mL of acetonitrile, and 13 mL of 1 N hydrochloric acid. Shake the flask for 5 min, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 3.9-mm × 30.0-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—Adjust the flow rate to obtain relative retention times of about 0.38 and 1.0 for methyldopa and hydrochlorothiazide, respectively.]

Suitability requirements

Resolution: NLT 6.0 between methyldopa and hydrochlorothiazide

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methyldopa from the *Sample solution*

r_S = peak response of methyldopa from the *Standard solution*

C_S = concentration of USP Methyldopa RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methyldopa in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*

r_S = peak response of hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Methyldopa

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Solution A: 10 mg/mL of ferrous sulfate, 20 mg/mL of potassium sodium tartrate, and 1 mg/mL of sodium bisulfite in water. [NOTE—Use a freshly prepared solution.]

Solution B: 50 mg/mL of ammonium acetate in dilute alcohol (1 in 5). Adjust with 6 N ammonium hydroxide to a pH of 8.5.

Standard solution: 0.275 mg/mL of anhydrous methyldopa from USP Methyldopa RS in *Medium*

Sample solution: Filter 35 mL of the solution under test through paper.

Instrumental conditions

Mode: Vis

Analytical wavelength: 520 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer an aliquot of the *Sample solution* estimated to contain 2–3 mg of methyldopa to a 100-mL volumetric flask. Adjust the final volume, if necessary, with *Medium* to 10 mL. To a second 100-mL volumetric flask add 10.0 mL of *Standard solution*, and to a third 100-mL volumetric flask add 10.0 mL of *Medium* to provide a blank. Pipet 5.0 mL of *Solution A* into each flask, dilute with *Solution B* to volume, and mix. Determine the absorbances of the *Standard solution* and the *Sample solution* at the wavelength of maximum absorbance, using the blank in the reference cell.

Calculate the percentage of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of anhydrous methyldopa in the *Standard solution* (µg/mL)

L = label claim (mg/Tablet)

V = volume of the medium, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$) is dissolved.

Hydrochlorothiazide

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Instrumental conditions

Mode: Vis

Analytical wavelength: 317 nm

Cell: 1 cm

Standard solution: USP Hydrochlorothiazide RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

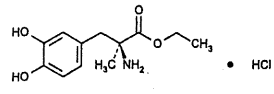
Tolerances: NLT 80% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

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• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity: Proceed as directed in the *Assay*, except use the following *Sample solution*.
Sample solution: Transfer 1 Tablet to a 250-mL volumetric flask, add 50 mL of water, and shake gently, if necessary, to disintegrate the Tablet. Do not sonicate. After the Tablet has completely disintegrated, add 25 mL of acetonitrile, and shake by mechanical means for 30 min. Add 13 mL of 1 N hydrochloric acid, and shake by mechanical means for an additional 5 min. Dilute with water to volume.
Acceptance criteria: Meet the requirements with respect to methyldopa and hydrochlorothiazide

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydrochlorothiazide RS
USP Methyldopa RS

Methyldopate Hydrochloride



$C_{12}H_{17}NO_4 \cdot HCl$ 275.73
L-Tyrosine, 3-hydroxy-α-methyl-, ethyl ester, hydrochloride.
L-3-(3,4-Dihydroxyphenyl)-2-methylalanine ethyl ester hydrochloride [5123-53-5; 2508-79-4].

» Methyldopate Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of $C_{12}H_{17}NO_4 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Methyldopate Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 50 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 280 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: It responds to *Identification* test *C* under *Methyldopa*.
D: It responds to the tests for *Chloride* ⟨191⟩, except that on the addition of the slight excess of 6 N ammonium hydroxide a brown precipitate is formed.
Specific rotation ⟨781S⟩: between −13.5° and −14.9° (λ = 405 nm).
Test solution: 40 mg per mL, in 0.1 N hydrochloric acid.
pH ⟨791⟩: between 3.0 and 5.0, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry it at 100° and at a pressure not exceeding 5 mm of mercury for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Assay—
Mobile solvent—Prepare a suitable solution of 0.02 M monobasic sodium phosphate and 0.015 M phosphoric acid in a water and methanol solution (approximately 15.5:4.5) such that the retention time of methyldopate hydrochloride is approximately 6.5 minutes.
Standard preparation—Dissolve an accurately weighed quantity of USP Methyldopate Hydrochloride RS in the *Mobile solvent* to obtain a solution containing about 1 mg per mL.
Assay preparation—Transfer about 50 mg of Methyldopate Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile solvent* to volume.
Procedure—Introduce separately 20-µL portions of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) operated at 25°, by means of a suitable microsyringe or sampling valve, adjusting the operating parameters such that the peak obtained with the *Standard preparation* is 100% full-scale. Typically, the apparatus is fitted with a 4-mm × 30-cm column that contains packing L1, is equipped with an UV detector capable of monitoring absorption at 280 nm and a suitable recorder, and is capable of operating at a column pressure between 700 and 1700 psi. In a suitable chromatogram, three replicate injections of the *Standard preparation* show a relative standard deviation of not more than 1.5%. Determine the peak areas, at equivalent retention times, obtained with the *Assay preparation* and the *Standard preparation,* and calculate the quantity, in mg, of $C_{12}H_{17}NO_4 \cdot HCl$ in the portion of Methyldopate Hydrochloride taken by the formula:

$$50C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Methyldopate Hydrochloride RS in the *Standard preparation;* and A_U and A_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Methyldopate Hydrochloride Injection

» Methyldopate Hydrochloride Injection is a sterile solution of Methyldopate Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methyldopate hydrochloride ($C_{12}H_{17}NO_4 \cdot HCl$).

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Methyldopate Hydrochloride RS

Identification—
A: Dilute a volume of Injection with a mixture of chloroform and methanol (1:1), if necessary, to obtain a solution containing about 5 mg of methyldopate hydrochloride per mL. Apply separately 10 µL of this solution and 10 µL of a Standard solution of USP Methyldopate Hydrochloride RS in a

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solvent mixture of chloroform and methanol (1:1) containing 5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a saturated chamber with a solvent system consisting of a mixture of butyl alcohol, water, and formic acid (7:2:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with Folin-Ciocalteu phenol TS followed by spraying with sodium carbonate solution (1 in 10): the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

B: It responds to the tests for *Chloride* ⟨191⟩, with the exception that the 6 N ammonium hydroxide is omitted.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 USP Endotoxin Unit per mg of methyldopate hydrochloride.

pH ⟨791⟩: between 3.0 and 4.2.

Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Buffer solution—To 214 g of monobasic potassium phosphate add 700 mL of water, and stir. Cautiously add 75 mL of sodium hydroxide solution (1 in 2), and stir until solution is complete. Adjust with sodium hydroxide solution (1 in 2) to a pH of 8.0, and dilute with water to 1000.0 mL.

Water-saturated tributyl phosphate—Shake 800 mL of tributyl phosphate with 100 mL of water, and discard the lower, aqueous phase. Filter the upper phase.

Standard preparation—Transfer about 25 mg of USP Methyldopate Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask, add water to volume, and mix. Transfer 5 mL of this solution to a 100-mL volumetric flask, add 0.1 N sulfuric acid to volume, and mix. Use a freshly prepared solution. The *Standard preparation* contains about 50 µg per mL.

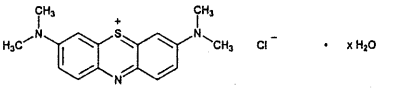
Assay preparation—Transfer to a 50-mL volumetric flask an accurately measured volume of Injection, equivalent to about 50 mg of methyldopate hydrochloride, add water to volume, and mix. Transfer a 5.0-mL aliquot of the solution to a 60-mL separator, add 15 mL of *Buffer solution* and 10 mL of *Water-saturated tributyl phosphate*, and shake for about 1 minute. Allow the phases to separate, and transfer the lower, aqueous phase to a second 60-mL separator. To this separator add a second 10-mL portion of *Water-saturated tributyl phosphate*, shake for about 1 minute, allow the phases to separate, discard the lower, aqueous phase, and add the upper tributyl phosphate phase to the phase retained in the first separator. Rinse the second separator with about 2 mL of *Water-saturated tributyl phosphate*, and add the rinsing to the first separator. Extract the phase contained in the first separator with two 25-mL portions of 0.1 N sulfuric acid. Collect the acid extracts in a 100-mL volumetric flask, add 0.1 N sulfuric acid to volume, and mix. Filter, if necessary, to obtain a clear solution.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 283 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of methyldopate hydrochloride ($C_{12}H_{17}NO_4 \cdot HCl$) in each mL of the Injection taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Methyldopate Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Methylene Blue



$C_{16}H_{18}ClN_3S \cdot xH_2O$

Phenothiazin-5-ium, 3,7-bis(dimethylamino)-, chloride;
3,7-Bis(dimethylamino)phenothiazin-5-ium chloride;

Pentahydrate [32680-41-4].	409.93
Trihydrate [7220-79-3].	373.90
Monohydrate [122965-43-9].	337.90
Anhydrous [61-73-4].	319.85

DEFINITION

Methylene Blue contains NLT 97.0% and NMT 103.0% of methylene blue ($C_{16}H_{18}ClN_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*

Sample solution: Ignite 50 mg of Methylene Blue with 0.5 g of anhydrous sodium carbonate. Cool and dissolve the residue in 10 mL of 2 N nitric acid. Filter and use 2 mL of the filtrate for performing the test.

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

[NOTE—All solutions containing methylene blue are recommended to be prepared fresh before analysis.]

Solution A: 0.1% trifluoroacetic acid in water

Solution B: Acetonitrile

Diluent: *Solution A* and *Solution B* (70:30)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
25	30	70
32	30	70
33	80	20
38	80	20

USP Monographs

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Standard solution: 1 mg/mL of USP Methylene Blue RS in *Diluent*. Stirring and sonication may be necessary for complete dissolution.
Sample solution: 1 mg/mL of Methylene Blue in *Diluent*. Stirring and sonication may be necessary for complete dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L11
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 3.0
Relative standard deviation: NMT 1.10%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methylene blue ($C_{16}H_{18}ClN_3S$) in the portion of Methylene Blue taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methylene blue from the *Sample solution*
r_S = peak response of methylene blue from the *Standard solution*
C_S = concentration of USP Methylene Blue RS in the *Standard solution* (mg/mL)
C_U = concentration of Methylene Blue in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.15%
• **ARSENIC**
Analysis: Proceed as directed in *Elemental Impurities—Procedures* ⟨233⟩, *Procedure 2: ICP-MS*.
Acceptance criteria: 8 ppm
• **COPPER OR ZINC**
Analysis: Proceed as directed in *Elemental Impurities—Procedures* ⟨233⟩, *Procedure 2: ICP-MS*.
Acceptance criteria: NMT 100 ppm of zinc and NMT 200 ppm of copper
• **ORGANIC IMPURITIES**
Diluent, Mobile phase, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 1 mg/mL of USP Methylene Blue RS and 0.025 mg/mL of USP Azure B RS in *Diluent*
Sensitivity solution: 0.5 µg/mL of USP Methylene Blue RS in *Diluent* from the *Standard solution*
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 3.5 between methylene blue and azure B peaks, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of azure B or any unspecified impurity in the portion of Methylene Blue taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of azure B or any unspecified impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azure B[a]	0.8	2.5
Methylene blue	1.0	—
Any unspecified impurity	—	0.10
Total impurities[b]	—	0.5

[a] 3-(Dimethylamino)-7-(methylamino)-phenothiazine-5-ium chloride.
[b] Total impurities does not include azure B.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 5 h.
Acceptance criteria: 8.0%–22.0%
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 2.5 USP Endotoxin Units/mL
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers and protect from light. Store below 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Azure B RS
3-(Dimethylamino)-7-(methylamino)-phenothiazine-5-ium chloride.
$C_{15}H_{16}ClN_3S$ 305.82
USP Endotoxin RS
USP Methylene Blue RS

Methylene Blue Injection

DEFINITION
Methylene Blue Injection is a sterile solution of Methylene Blue in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$).

IDENTIFICATION
• **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
All solutions containing methylene blue are recommended to be freshly prepared prior to analysis.
Solution A: 0.1% trifluoroacetic acid in water
Solution B: Acetonitrile
Diluent: *Solution A* and *Solution B* (70:30)
Mobile phase: *Solution A* and *Solution B* (75:25)

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Standard solution: 100 µg/mL of USP Methylene Blue RS in *Diluent*
Sample solution: Nominally 100 µg/mL of methylene blue in *Diluent* from Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 10-cm; 3.5-µm packing L11
Temperatures
Autosampler: 5°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of methylene blue from the *Sample solution*
r_S = peak response of methylene blue from the *Standard solution*
C_S = concentration of USP Methylene Blue RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methylene blue in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of methylene blue trihydrate, 373.90
M_{r2} = molecular weight of methylene blue anhydrous, 319.85

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• ORGANIC IMPURITIES

Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Mobile phase: For analyzing azure A, azure C, and unspecified impurities, see *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
25	30	70
32	30	70

System suitability solution: 1 mg/mL of USP Methylene Blue RS and 0.03 mg/mL of USP Azure B RS in *Diluent*
Standard solution: 1 µg/mL of USP Methylene Blue RS in *Diluent*
Sensitivity solution: 0.25 µg/mL of USP Methylene Blue RS in *Diluent* from the *Standard solution*
Sample solution: Nominally equivalent to 500 µg/mL of methylene blue (anhydrous) in *Diluent* from Injection
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 3.5 between the methylene blue and azure B peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of methylene blue from the *Standard solution*
C_S = concentration of USP Methylene Blue RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methylene blue in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azure C	0.32	0.20
Azure A	0.52	0.20
Azure B[a]	0.82	3.0
Methylene blue	1.00	—
Any unspecified impurity	—	0.20
Total impurities	—	5.0

[a] Azure B is quantitated using the test for *Limit of Azure B* and is included in the table for identification purposes.

• LIMIT OF AZURE B

Diluent, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 3 µg/mL of USP Methylene Blue RS in *Diluent*
System suitability: Proceed as directed in the *Assay* using the *Standard solution* from the *Assay*.
[NOTE—The relative retention times for azure B and methylene blue are 0.70 and 1.00, respectively.]
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of azure B in the portion of Injection taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of azure B from the *Sample solution*
r_S = peak response of methylene blue from the *Standard solution*
C_S = concentration of USP Methylene Blue RS in the *Standard solution*
C_U = nominal concentration of methylene blue in the *Sample solution*

Acceptance criteria: NMT 3.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.0–4.5
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 2.5 USP Endotoxin Units/mL
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass. Store at room temperature, protected from light. Do not refrigerate or freeze.
- **USP Reference Standards** ⟨11⟩
  USP Azure B RS
  3-(Dimethylamino)-7-(methylamino)phenothiazin-5-ium chloride.
  $C_{15}H_{16}ClN_3S$ 305.82
  USP Methylene Blue RS

Change to read:

Methylene Blue ▲Compounded▲ (USP 1-Aug-2019) Injection, Veterinary

DEFINITION

Change to read:

Methylene Blue ▲Compounded▲ (USP 1-Aug-2019) Injection, Veterinary is a sterile solution of Methylene Blue in Water for Injection. It contains NLT 9.5 mg/mL and NMT 10.5 mg/mL of methylene blue trihydrate ($C_{16}H_{18}ClN_3S \cdot 3H_2O$). Prepare Methylene Blue ▲Compounded▲ (USP 1-Aug-2019) Injection, Veterinary as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Methylene Blue	5 g
Sterile Water for Injection or Sodium Chloride Injection (0.9%), a sufficient quantity to make	500 mL

Dissolve an accurately weighed quantity of *Methylene Blue* in *Sterile Water for Injection* or *Sodium Chloride Injection (0.9%)*, and dilute to volume, with mixing. Sterilize by a suitable means, such as sterile filtration or autoclaving.

IDENTIFICATION
- **A.** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.
- **B. Thin-Layer Chromatographic Identification Test** ⟨201⟩
  Diluent: Methanol and water (1:1)
  Standard solution: 5 mg/mL of USP Methylene Blue RS in *Diluent*
  Sample solution: Dilute a portion of the Injection with an equal volume of methanol.
  Application volume: 1 µL
  Developing solvent system: Alcohol, acetic acid, and water (3:3:4)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Allow the spots to dry, and develop until the solvent front has moved about 10 cm above the line of application. Remove the plate from the chamber, and allow the solvent to evaporate.
  Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY

Change to read:

- **Procedure**
  Standard solution: 2 µg/mL of USP Methylene Blue RS in diluted alcohol
  Sample solution: Dilute a volume of Injection with diluted alcohol to obtain a solution with a nominal concentration of about 2.4 µg of methylene blue trihydrate (2 µg of anhydrous methylene blue)/mL.
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV-Vis
  Detector: 663 nm
  Cell: 1 cm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Concomitantly determine the absorbance of both solutions, using diluted alcohol as the blank.
  Calculate the quantity, in mg, of methylene blue trihydrate ($C_{16}H_{18}ClN_3S \cdot 3H_2O$) in each milliliter of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times L \times (M_{r1}/M_{r2})$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of methylene blue trihydrate in the *Standard solution* (mg/mL)
C_U = nominal concentration of methylene blue trihydrate in the *Sample solution* (mg/mL)
L = labeled amount of methylene blue in the Injection, 10.0 mg/mL
M_{r1} = molecular weight of methylene blue trihydrate, 373.90
M_{r2} = molecular weight of methylene blue anhydrous, ▲319.85▲ (USP 1-Aug-2019)

Acceptance criteria: 9.5–10.5 mg/mL of methylene blue trihydrate ($C_{16}H_{18}ClN_3S \cdot 3H_2O$)

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.0–4.5
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 0.17 USP Endotoxin Units/mg of methylene blue
- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Other Requirements:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

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ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Package in single-dose containers, preferably of Type I ▲▲ (USP 1-Aug-2019) glass. Store at ▲controlled▲ (USP 1-Aug-2019) room temperature, protected from light.

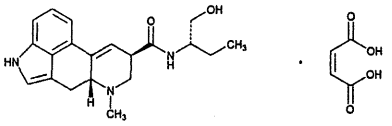
Change to read:

- **BEYOND-USE DATE:** ▲The BUD in ⟨797⟩ applies.▲ (CN 1-May-2020)

Change to read:

- **LABELING:** Label it to indicate that it is to be discarded after its *Beyond-Use Date* ▲▲ (USP 1-Aug-2019) and to indicate the nominal content of methylene blue in the Injection and whether it was prepared in Sterile Water for Injection or in Sodium Chloride Injection (0.9%). ▲▲ (USP 1-Aug-2019) Label it to indicate that it is for veterinary use only and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methylene Blue RS

Methylergonovine Maleate



$C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ 455.50

Ergoline-8-carboxamide, 9,10-didehydro-*N*-[1-(hydroxymethyl)propyl]-6-methyl-, [8β(*S*)]-, (*Z*)-2-butenedioate (1:1) (salt);
9,10-Didehydro-*N*-[(*S*)-1-(hydroxymethyl)propyl]-6-methylergoline-8β-carboxamide maleate (1:1) (salt) [57432-61-8].

DEFINITION

Methylergonovine Maleate contains NLT 97.0% and NMT 103.0% of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The R_F values of the principal fluorescent spot and the principal blue spot of the *Sample solution* correspond to those of the *Standard stock solution*, as obtained in the test for *Related Alkaloids*.

ASSAY

- **PROCEDURE**

Conduct this procedure with a minimum exposure to light.
Mobile phase: Acetonitrile and 2.0 g/L of monobasic potassium phosphate (1:4)
Diluent: 2.5 mg/mL of tartaric acid prepared as follows. Transfer a suitable amount of tartaric acid to a suitable volumetric flask, add 50% of the flask volume of water, and mix with shaking. Dilute with methanol to volume. Allow the mixture to cool before use.
Standard stock solution: 0.1 mg/mL of USP Methylergonovine Maleate RS in *Diluent*. Shake by mechanical means for 15 min.
Standard solution: 4 µg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*.
Sample stock solution: 0.2 mg/mL of Methylergonovine Maleate in *Diluent*. Shake by mechanical means for 15 min or until completely dissolved.
Sample solution: 4 µg/mL of Methylergonovine Maleate from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence with excitation at 315 nm and emission at 423 nm
Column: 4.6-mm × 25-cm; packing L7
Temperature: 30°
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) in the portion of Methylergonovine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methylergonovine Maleate RS in the *Standard solution* (µg/mL)
C_U = concentration of Methylergonovine Maleate in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **RELATED ALKALOIDS**

Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light. Solutions containing methylergonovine maleate should be prepared immediately before use.
Diluent: Alcohol and ammonium hydroxide (9:1)
Standard stock solution: 10 mg/mL of USP Methylergonovine Maleate RS in *Diluent*
Standard solution A: 0.20 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*
Standard solution B: 0.10 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*
Standard solution C: 0.05 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*
Sample solution: 10 mg/mL of Methylergonovine Maleate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Chloroform, methanol, and water (75:25:3), equilibrated for 30 min

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Spray reagent: 10 mg/mL of *p*-dimethylaminobenzaldehyde in a cooled mixture of alcohol and hydrochloric acid (1:1)

Analysis

Samples: *Standard stock solution*, *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Proceed as directed in the chapter. Locate the spots on the plate by spraying thoroughly and evenly with *Spray reagent*. Immediately dry in a stream of nitrogen for 2 min.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard stock solution*. Estimate the concentration of any other spots observed from the *Sample solution* by comparison with *Standard solution A*, *Standard solution B*, and *Standard solution C*. The spots from *Standard solution A*, *Standard solution B*, and *Standard solution C* are equivalent to 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is NMT 2.0%.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Specific Rotation*

Sample solution: 5 mg/mL of Methylergonovine Maleate in water

Acceptance criteria: +44° to +50°

- **pH** ⟨791⟩

Sample solution: 0.2 mg/mL of Methylergonovine Maleate in water

Acceptance criteria: 4.4–5.2

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 80° to constant weight.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cold place.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Methylergonovine Maleate RS

Methylergonovine Maleate Injection

DEFINITION

Methylergonovine Maleate Injection is a sterile solution of Methylergonovine Maleate in Water for Injection. Each mL contains NLT 90.0% and NMT 110.0% of the labeled amount of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$).

IDENTIFICATION

- **A.** The R_F values of the principal fluorescent spot and the principal blue spot of the *Sample solution* correspond to those of the *Standard stock solution*, as obtained in the procedure for *Organic Impurities, Related Alkaloids*.
- **B. PROCEDURE**

Sample solution: 0.67 mg/mL of methylergonovine maleate from Injection in water

Analysis: The *Sample solution* exhibits a bluish fluorescence under UV light. To this solution add 2 mL of a solution of glacial acetic acid in ethyl acetate (1:2), and stratify 2 mL of sulfuric acid, by pipetting, under the solution.

Acceptance criteria: A bluish-purple ring appears at the interface of the two liquids.

ASSAY

- **PROCEDURE**

[NOTE—Conduct this procedure with minimum exposure to light.]

Mobile phase: Acetonitrile and 0.015 M monobasic potassium phosphate solution (1:4)

Diluent: 5 mg/mL of tartaric acid in water and methanol (1:1). Allow the mixture to cool before use. [NOTE—Dissolve tartaric acid with water, then add an equal volume of methanol.]

Standard solution: 100 µg/mL of USP Methylergonovine Maleate RS in *Diluent*. [NOTE—Shake by mechanical means for 15 min or until completely dissolved.]

Sample solution: 100 µg/mL of methylergonovine maleate from Injection in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4-mm × 25-cm; packing L7

Column temperature: 30°

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ in each mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylergonovine Maleate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

- **Procedure: Related Alkaloids**

[NOTE—Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light.]

Diluent: Alcohol and ammonium hydroxide (9:1)

[NOTE—All solutions should be prepared immediately before use.]

Standard stock solution: 10 mg/mL of USP Methylergonovine Maleate RS in *Diluent*

Standard solution A: 0.50 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution B: 0.20 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution C: 0.10 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution D: 0.05 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Sample solution: Transfer the equivalent of 5 mg of methylergonovine maleate from Injection to a separator, and extract with three 5-mL portions of chloroform. Discard the chloroform extracts. Render alkaline to litmus with 6 N ammonium hydroxide, and extract with three 5-mL portions of chloroform. Evaporate the combined extracts with the aid of a current of air, but without heat, to dryness. Dissolve the residue so obtained in 0.5 mL of *Diluent*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

USP Monographs

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Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Chloroform, methanol, and water (75:25:3), equilibrated for 30 min
Spray reagent: 10 mg/mL of *p*-dimethylamino benzaldehyde in a cooled mixture of alcohol and hydrochloric acid (1:1)

Analysis
Samples: *Standard stock solution, Standard solution A, Standard solution B, Standard solution C, Standard solution D,* and *Sample solution*
Proceed as directed in the chapter. Locate the spots on the plate by spraying thoroughly and evenly with *Spray reagent.* Immediately dry in a stream of nitrogen for 2 min.

Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard stock solution.* Estimate the concentration of any other spots observed in the lane for the *Sample solution* by comparison with *Standard solution A, Standard solution B, Standard solution C,* and *Standard solution D*: the spots from the 0.50-, 0.20-, 0.10-, and 0.05-mg/mL dilutions are equivalent to 5.0%, 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is NMT 5.0%.

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 2.7–3.5
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 1.7 USP Endotoxin Units/µg of methylergonovine maleate
- **Other Requirements:** Meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose, light-resistant containers, preferably of Type I glass. Store in a refrigerator.
- **USP Reference Standards** ⟨11⟩
USP Methylergonovine Maleate RS

Methylergonovine Maleate Tablets

» Methylergonovine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Methylergonovine Maleate RS

Identification—
A: The R_F values of the principal fluorescent spot and the principal blue spot in the chromatogram of the *Test preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the test for *Related alkaloids* under *Ergonovine Maleate,* using the Tablets instead of Ergonovine Maleate.

B: Transfer a quantity of powdered Tablets, equivalent to about 4 mg of methylergonovine maleate, to a separator, add 20 mL of water, and render alkaline to litmus with sodium carbonate solution (1 in 10). Extract with three 20-mL portions of chloroform, filter the combined chloroform extracts into a small evaporating dish, and evaporate on a steam bath to dryness. Dissolve the residue in a mixture of 6 mL of water and 0.3 mL of hydrochloric acid, and filter, if necessary: the solution so obtained exhibits a bluish fluorescence under UV light. To this solution, add 2 mL of a solution of glacial acetic acid in ethyl acetate (1 in 2), and stratify 2 mL of sulfuric acid, by pipetting, under the solution: a bluish purple ring appears at the interface of the two liquids.

Dissolution ⟨711⟩—
Medium: tartaric acid solution (1 in 200); 900 mL.
Apparatus 2: 75 rpm.
Time: 30 minutes.
Procedure—Filter a portion of the solution under test into a flask. Concomitantly determine the fluorescence intensity of this solution in comparison with a Standard solution of USP Methylergonovine Maleate RS in the same medium having a known concentration of about 0.22 µg per mL in a fluorometer at an excitation wavelength of about 327 nm and an emission wavelength of about 428 nm, using tartaric acid solution (1 in 200) as the blank.
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩**:** meet the requirements.
Related alkaloids— [Note—Conduct this test without exposure to daylight and with the minimum exposure to artificial light.]
Solvent mixture—Mix 75 volumes of chloroform, 25 volumes of methanol, and 1 volume of ammonium hydroxide.
Detecting reagent—Cautiously dissolve 800 mg of *p*-dimethylaminobenzaldehyde in a mixture of alcohol and sulfuric acid (101:11).
Test preparation—Transfer a quantity of finely powdered Tablets, equivalent to 5.0 mg of methylergonovine maleate, to a suitable container, add 50 mL of *Solvent mixture,* and stir with the aid of a magnetic stirrer for 40 minutes. Filter, rinsing the container with two 10-mL portions of *Solvent mixture.* Evaporate the combined filtrates in vacuum at 25° to 30°, and dissolve the residue in 2.0 mL of *Solvent mixture.*
Standard stock solution—Transfer 25 mg of USP Methylergonovine Maleate RS to a 10-mL volumetric flask, add *Solvent mixture* to volume, and mix to obtain a solution having a known concentration of 2.5 mg per mL.
Standard preparations A, B, C, and *D*—Dilute accurately measured volumes of *Standard stock solution* quantitatively with *Solvent mixture* (designated below as parts by volume of *Standard stock solution* in total parts by volume of the finished *Standard preparation*) to obtain *Standard preparations,* designated below by letter, having the following concentrations and percentage assignments:
A: (1 in 20); 125 µg per mL (5.0%).
B: (1 in 33); 75 µg per mL (3.0%).
C: (1 in 100); 25 µg per mL (1.0%).
D: (1 in 200); 12.5 µg per mL (0.5%).
Procedure—Apply separately 20 µL of the *Test preparation* and 20 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Dry the plate with the aid of a stream of cool air. Position the plate in a chromatographic chamber, and develop the chromatograms in *Solvent mixture* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in a stream of cool air. Examine the plate under long-wavelength UV light. Mark the principal and any secondary fluorescent spots. Spray the plate with *Detecting reagent,* and mark the principal and secondary blue spots. Compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations:* the sum of the intensities of secondary spots obtained from the *Test*

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preparation corresponds to not more than 5.0% of related compounds.

Assay— [NOTE—Conduct this procedure with a minimum exposure to light.]

Mobile phase, Solvent mixture, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Methylergonovine Maleate.*

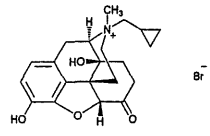
Assay preparation—Place 10 Tablets in 1500-mL volumetric flask, add 400 mL of *Solvent mixture,* and shake by mechanical means for 15 minutes or until completely disintegrated. Dilute with *Solvent mixture* to volume, and mix. Allow the solution to settle for not less than 30 minutes before use, and then filter to obtain the *Assay preparation.*

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) in the portion of Tablets taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of methylergonovine maleate in each Tablet, D is the concentration, in µg per mL, of methylergonovine maleate in the *Assay preparation,* based on the labeled quantity per Tablet and the extent of dilution, C is the concentration, in µg per mL, of USP Methylergonovine Maleate RS in the *Standard preparation,* and r_U and r_S are the responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Methylnaltrexone Bromide



$C_{21}H_{26}BrNO_4$ 436.34

Morphinanium, 17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxy-17-methyl-6-oxo-, bromide, (5α,17*R*)-; (17*R*)-17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-17-methyl-6-oxomorphinanium bromide [916055-92-0].

DEFINITION

Methylnaltrexone Bromide contains NLT 98.0% and NMT 102.0% of methylnaltrexone bromide ($C_{21}H_{26}BrNO_4$), calculated on the anhydrous and solvent-free basis.

Change to read:

IDENTIFICATION

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Bromide*: Meets the requirements of the silver nitrate precipitation test

ASSAY

- **PROCEDURE**

Protect solutions containing methylnaltrexone bromide from light.

Buffer: Dilute 3.0 mL of heptafluorobutyric acid with water to 1000 mL. Adjust with ammonium hydroxide to a pH of 2.4.

Solution A: *Buffer* and methanol (85:15)

Solution B: *Buffer,* methanol, and tetrahydrofuran (85:5:10)

Mobile phase: See *Table 1.* Return to original conditions and re-equilibrate the system for about 15 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	30	70
50	30	70

Diluent: *Solution A*

Standard solution: 3.0 mg/mL of USP Methylnaltrexone Bromide RS in *Diluent*

Sample solution: 3.0 mg/mL of Methylnaltrexone Bromide in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 3-µm packing L1

Column temperature: 50°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylnaltrexone bromide ($C_{21}H_{26}BrNO_4$) in the portion of Methylnaltrexone Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylnaltrexone Bromide RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylnaltrexone Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Protect solutions containing methylnaltrexone bromide from light.

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*

System suitability solution: 3.0 mg/mL of USP Methylnaltrexone Bromide RS and 4.5 µg/mL of USP Naltrexone RS in *Diluent*

Sensitivity solution: 1.5 µg/mL of USP Methylnaltrexone Bromide RS in *Diluent*

Standard solution: 4.5 µg/mL of USP Methylnaltrexone Bromide RS in *Diluent*

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System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between methylnaltrexone bromide and naltrexone, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Methylnaltrexone Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of methylnaltrexone from the *Standard solution*
C_S = concentration of USP Methylnaltrexone Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Methylnaltrexone Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
S-Methylnaltrexone[a]	0.92	0.15
Methylnaltrexone	1.0	—
Naltrexone	1.15	0.15
N-Butenyl oxymorphone[b]	1.21	0.15
Acetylmethylnaltrexone[c]	1.32	0.15
Acetylnaltrexone[d]	1.48	0.15
O-Methyl methylnaltrexone[e]	1.59	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] (17*S*)-17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-17-methyl-6-oxomorphinanium bromide.
[b] (17*RS*)-17-(But-3-en-1-yl)-4,5α-epoxy-3,14-dihydroxy-17-methyl-6-oxomorphinanium bromide.
[c] 3-Acetyloxy-17-cyclopropylmethyl-4,5α-epoxy-14-hydroxy-17-methyl-6-oxomorphinanium bromide.
[d] 17-(Cyclopropylmethyl)-4,5α-epoxy-14-hydroxy-6-oxomorphinan-3-yl acetate.
[e] (17*RS*)-17-(Cyclopropylmethyl)-4,5α-epoxy-14-hydroxy-17-methyl-3-methoxy-6-oxomorphinanium bromide.

• **Limit of Methylnaltrexone Related Compound A**
Buffer: 1.1 g/L solution of 1-octanesulfonate sodium salt in water (5 mM). Adjust with phosphoric acid to a pH of 2.00 ± 0.05.
Solution A: Tetrahydrofuran and *Buffer* (50:950)
Solution B: Acetonitrile, tetrahydrofuran, and *Buffer* (600:50:350)
Mobile phase: See *Table 3*. Return to original conditions and re-equilibrate the system for about 8 min.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
36	86	14
40	50	50
50	50	50

Diluent: Acetonitrile, water, and phosphoric acid (100: 900: 1.2). [Note—This is v:v:v ratio equivalent to (100:900:2, v:v:w).]
Peak identification solution: 5 mg/mL of USP Methylnaltrexone Peak Identification Mixture RS in *Diluent*
Standard solution: 0.5 µg/mL of USP Methylnaltrexone Bromide RS in *Diluent*
Sample solution: 5 mg/mL of Methylnaltrexone Bromide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 3.0-mm × 15-cm; 3.5-µm packing L1
Column temperature: 15°
Flow rate: 0.4 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Peak identification solution*, *Standard solution*, and *Sample solution*
Identify the peak due to methylnaltrexone related compound A from the *Peak identification solution*. [Note—Typical relative retention times for methylnaltrexone and methylnaltrexone related compound A are 1.0 and 1.2, respectively.]
Calculate the amount, in ppm, of methylnaltrexone related compound A in the portion of Methylnaltrexone Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 1000$$

r_U = peak response of methylnaltrexone related compound A from the *Sample solution*
r_S = peak response of methylnaltrexone from the *Standard solution*
C_S = concentration of USP Methylnaltrexone Bromide RS in the *Standard solution* (µg/mL)
C_U = concentration of Methylnaltrexone Bromide in the *Sample solution* (mg/mL)
F = relative response factor for methylnaltrexone related compound A, 1.3

Acceptance criteria: NMT 100 ppm

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 75 mg/mL in water
Acceptance criteria: 4.3–5.3
• **Water Determination** ⟨921⟩, *Method I*, *Method Ia*: NMT 1.0%
• **Optical Rotation** ⟨781S⟩, *Procedures*, *Specific Rotation*
Sample solution: 10 mg/mL in water
Acceptance criteria: −160° to −166°
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic

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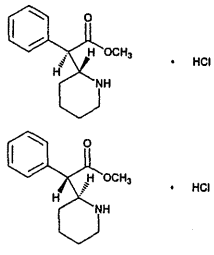
microbial count is NMT 10^3 cfu/g, and the total combined yeasts and molds count is NMT 10^2 cfu/g.

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: The level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Methylnaltrexone Bromide is used can be met. Where the label states that Methylnaltrexone Bromide must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Methylnaltrexone Bromide is used can be met.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers and store at room temperature.
- **LABELING:** Where it must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, Methylnaltrexone Bromide is so labeled. Where it is sterile, it is so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Endotoxin RS
  USP Methylnaltrexone Bromide RS
  USP Methylnaltrexone Peak Identification Mixture RS
  It contains Methylnaltrexone Bromide and a small amount of methylnaltrexone related compound A:
  7,8-Didehydromethylnaltrexone bromide, or ABUK-Methylnaltrexone.
  (17*RS*)-17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-17-methyl-6-oxomorphinan-7-enium bromide.
  $C_{21}H_{24}BrNO_4$ 434.32
  USP Naltrexone RS

Methylphenidate Hydrochloride



$C_{14}H_{19}NO_2 \cdot HCl$ 269.77

2-Piperidineacetic acid, α-phenyl-, methyl ester, hydrochloride, (*R**,*R**)-(±)-;
Methyl α-phenyl-2-piperidineacetate hydrochloride;
(*RS*)-Methyl-2-phenyl-2-[(*RS*)-piperidin-2-yl] acetate, hydrochloride [23655-65-4].

DEFINITION

Methylphenidate Hydrochloride contains NLT 98.0% and NMT 102.0% of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Change to read:

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirement ▲of test *A*▲ (CN 1-May-2018)

Add the following:

▲• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

- **PROCEDURE**

Buffer: 2.7 g/L of monobasic potassium phosphate
Mobile phase: Methanol and *Buffer* (1:2). Adjust with phosphoric acid to a pH of 4.6 ± 0.1.
System suitability solution: 0.005 mg/mL of USP Methylphenidate Related Compound A RS and 0.5 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*
Standard solution: 0.5 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Methylphenidate Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 209 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of methylphenidate
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between methylphenidate related compound A and methylphenidate
Tailing factor: NMT 3.0 for the methylphenidate peak
Relative standard deviation: NMT 2.0% for the methylphenidate peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Methylphenidate Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES, PROCEDURE 1**

Buffer, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Identify each impurity using the relative retention times in *Table 1*.
Calculate the percentage of each impurity in the portion of Methylphenidate Hydrochloride taken:

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$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the responses for all impurity peaks including the methylphenidate peak from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythro isomer[a]	0.58	0.15
Methylphenidate related compound A	0.85	0.5
Methylphenidate	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] (*RS*)-Methyl-2-phenyl-2-[(*SR*)-piperidin-2-yl] acetate.

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 2**

[NOTE—Perform this test only if ethylphenidate or ▲*all-rac*-dimethyl 2,2′-(piperidine-1,2-diyl) bis(2-phenylacetate)▲ 2S (USP41) is a known process impurity.]

Buffer A: 5.7 g of monobasic ammonium phosphate and 1.6 g of octanesulfonic acid sodium salt in 1 L of water

Buffer B: Add 4 mL of triethylamine to 1 L of *Buffer A*. Adjust with phosphoric acid to a pH of 2.9.

Solution A: Acetonitrile and *Buffer B* (7:43)

Solution B: Acetonitrile and *Buffer A* (4:1)

System suitability solution: 0.5 mg/mL of USP Methylphenidate Hydrochloride RS; and 3 µg/mL each of USP Methylphenidate Related Compound A RS, phenylacetic acid, and USP Methylphenidate Hydrochloride Erythro Isomer Solution RS in *Solution A*

Standard solution: 0.5 µg/mL of USP Methylphenidate Hydrochloride RS in *Solution A*

Sample solution: 0.5 mg/mL of Methylphenidate Hydrochloride in *Solution A*. [NOTE—Allow the solution to stand for at least 2 h.]

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
7	65	35
10	50	50
12	50	50
13	90	10
16	90	10

[NOTE—Equilibration of the chromatographic system at the initial conditions for a minimum of 30 min is recommended before the first injection.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 2.8 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.7 between methylphenidate related compound A and phenylacetic acid; NLT 3.6 between phenylacetic acid and erythro isomer

Tailing factor: NMT 2.0 for the methylphenidate peak

Relative standard deviation: NMT 2.0% for the methylphenidate peak; NMT 5.0% for methylphenidate related compound A, phenylacetic acid, and methylphenidate hydrochloride erythro isomer

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Methylphenidate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity peak from the *Sample solution*

r_S = peak response for the methylphenidate peak from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylphenidate Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methylphenidate related compound A	0.55	1.1	0.2
Phenylacetic acid	0.67	1.0	0.1
Erythro isomer[a]	0.80	1.0	0.2
Methylphenidate	1.0	—	—
Ethylphenidate[b]	1.22	0.9	0.1
Bis-methylphenidate[c]	1.80	2.6	0.1
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] (*RS*)-Methyl-2-phenyl-2-[(*SR*)-piperidin-2-yl] acetate.

▲[b] Ethyl(*RS*)-2-phenyl-2-[(*RS*-piperidin-2-yl)]acetate.▲ 2S (USP41)

▲[c] *all-rac*-Dimethyl 2,2′-(piperidine-1,2-diyl)bis(2-phenylacetate).▲ 2S (USP41)

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

USP Monographs

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• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states the procedure with which the article complies.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methylphenidate Hydrochloride RS
USP Methylphenidate Hydrochloride Erythro Isomer Solution RS
This solution contains 0.5 mg/mL of methylphenidate hydrochloride erythro isomer in methanol.
USP Methylphenidate Related Compound A RS
α-Phenyl-2-piperidineacetic acid hydrochloride.
$C_{13}H_{17}NO_2 \cdot HCl$ 255.74

Methylphenidate Hydrochloride Tablets

DEFINITION
Methylphenidate Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: Equivalent to 50 mg of methylphenidate hydrochloride from a portion of powdered Tablets in a 40-mL centrifuge tube. Add 10 mL of chloroform, shake, and centrifuge. Filter the clear extract through a medium-sized sintered-glass funnel into a beaker, and repeat the extraction with an additional 10-mL portion of chloroform. Evaporate the combined chloroform extracts on a steam bath to dryness. Agitate the dried residue with 2 mL of acetonitrile, and filter the mixture through a small sintered-glass funnel. Wash the crystals with an additional 2 mL of acetonitrile, and dry them with the aid of suction.
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Buffer: Dissolve 1.6 g of anhydrous sodium acetate in 900 mL of water. Adjust with acetic acid to a pH of 4.0. Dilute with water to 1 L.
Mobile phase: Methanol, acetonitrile, and *Buffer* (4:3:3)
Internal standard solution: 0.4 mg/mL of phenylephrine hydrochloride in *Mobile phase*
Standard stock solution: 0.2 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*
Standard solution: Mix 10.0 mL of the *Standard stock solution* with 5.0 mL of the *Internal standard solution*
Sample stock solution: 0.2 mg/mL of methylphenidate hydrochloride from finely powdered Tablets (NLT 20 Tablets) prepared as follows. Dissolve in *Mobile phase* using 70% of the final volume. Sonicate for 15 min, and cool to room temperature. Dilute with *Mobile phase* to volume. Pass a portion of this solution through a suitable membrane filter, discarding the first portion of the filtrate. Avoid the use of glass filters. Polypropylene filters are suitable for use.
Sample solution: Mix 10.0 mL of the clear filtrate from the *Sample stock solution* with 5.0 mL of the *Internal standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for phenylephrine hydrochloride and methylphenidate hydrochloride are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the analyte and the internal standard peaks
Relative standard deviation: NMT 2.0% from the peak response ratios of the analyte to the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the analyte to the internal standard from the *Sample solution*
R_S = peak response ratio of the analyte to the internal standard from the *Standard solution*
C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Methylphenidate Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩, ***Procedure for a Pooled Sample***
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Analysis: Determine the amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the procedure in the *Assay*, making any necessary volumetric adjustments.
Tolerances: NLT 75% (*Q*) of the labeled amount of $C_{14}H_{19}NO_2 \cdot HCl$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methylphenidate Hydrochloride RS

Methylphenidate Hydrochloride Extended-Release Tablets

DEFINITION
Methylphenidate Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$).

IDENTIFICATION
• **A. INFRARED ABSORPTION**
Sample: Place a portion of powdered Tablets, equivalent to 100 mg of methylphenidate hydrochloride, in a 100-mL beaker. Add 20 mL of chloroform, stir for 5 min, and filter, collecting the filtrate. Evaporate the filtrate to about 5 mL. Add ethyl ether slowly, with stirring, until crystals form.

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Filter the crystals, wash with ethyl ether, and dry at 80° for 30 min.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the crystals so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Methylphenidate Hydrochloride RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Dissolve 2 g of octanesulfonic acid sodium salt in 730 mL of water. Adjust with phosphoric acid to a pH of 2.7. Mix with 270 mL of acetonitrile.

Solution A: Acidified water; adjusted with phosphoric acid to a pH of 3

Diluent A: Acetonitrile and *Solution A* (25:75)

Diluent B: Acetonitrile and methanol (50:50)

System suitability solution: 80 µg/mL of USP Methylphenidate Hydrochloride RS, 1 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS, and 2 µg/mL of USP Methylphenidate Related Compound A RS in *Diluent A*

Standard solution: 0.1 mg/mL of USP Methylphenidate Hydrochloride RS in *Diluent A*

Sample stock solution: Nominally 1 mg/mL of methylphenidate hydrochloride prepared as follows. Dissolve NLT 10 Tablets in a suitable volumetric flask with 20% of the total flask volume of *Diluent B*. [NOTE—Alternatively, a portion of powder from NLT 10 Tablets may be transferred to a suitable volumetric flask and suspended in 20% of the total flask volume of *Diluent B*.] Stir for 4 h. Dilute with *Solution A* to volume.

Sample solution: Nominally 0.1 mg/mL of methylphenidate hydrochloride in *Solution A* from the *Sample stock solution*. [NOTE—Centrifuge before chromatographic analysis.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 µL

Run time: 2 times the retention time of methylphenidate

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table* ▲8▲ (RB 1-Apr-2017) for relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between methylphenidate related compound A and methylphenidate hydrochloride erythro isomer; NLT 6.0 between the methylphenidate and erythro isomer peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the methylphenidate peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the methylphenidate peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Times: 1, 2, 3.5, 5, and 7 h

Buffer: Dissolve 1.6 g of anhydrous sodium acetate in 900 mL of water. Adjust with acetic acid to a pH of 4.0 and dilute with water to 1000 mL.

Mobile phase: Methanol, acetonitrile, and *Buffer* (40:30:30)

Internal standard solution: 0.4 mg/mL of phenylephrine hydrochloride in *Mobile phase*

Standard stock solution: (1.5 × [*L*/500]) mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase* where *L* is the label claim of methylphenidate hydrochloride in mg/Tablet

Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of the *Internal standard solution*, and mix.

Sample stock solution: Use portions of the solution under test passed through a suitable filter of 0.45-µm pore size. Do not use glass fiber filters.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of the *Internal standard solution*, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for phenylephrine hydrochloride and methylphenidate hydrochloride are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 2.0% for the peak response ratios of the analyte to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the procedure in the *Assay*, making any necessary volumetric adjustments.

Tolerances: See *Table 1*.

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Table 1

Time (h)	Amount Dissolved (%)
1	25–45
2	40–65
3.5	55–80
5	70–90
7	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

For products labeled for dosing every 24 h

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Acidified water; adjusted with phosphoric acid to a pH of 3; 50 mL at 37 ± 0.5°

Apparatus 7: 30 cycles/min; 2–3 cm amplitude. Follow *Drug Release* ⟨724⟩, *General Drug Release Standards, Apparatus 7, Sample preparation A* using a metal spring sample holder (*Drug Release* ⟨724⟩, *Figure 5d*). Place one Tablet in the holder with the Tablet orifice facing down, and cover the top of the holder with Parafilm™. At the end of each specified test interval, the systems are transferred to the next row of new test tubes containing 50 mL of fresh *Medium*.

Times: 1-h intervals for a duration of 10 h

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the following method.

Solution A: Dissolve 2.0 g of sodium 1-octanesulfonate in 700 mL of water, mix well, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Solution A* (30:70)

Diluent: Acetonitrile and *Medium* (25:75)

Standard stock solution: 0.3 mg/mL of USP Methylphenidate Hydrochloride RS in *Diluent*

Standard solutions: Prepare at least six solutions by making serial dilutions of the *Standard stock solution* in *Diluent* to bracket the expected drug concentration range.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.2-mm × 5-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: Middle range concentration of the *Standard solutions*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2% for the peak response of the analyte; NMT 2% for the retention time of the analyte

Analysis

Samples: *Standard solutions* and the solution under test

Construct a calibration curve by plotting the peak response versus the concentration of the *Standard solutions*. Determine the amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in each interval by linear regression analysis of the standard curve.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	12–32
4	40–60
10	NLT 85
3–6 (avg)	9–15 (/h)

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Calculate the average percentage released from 3–6 h:

$$\text{Result} = (Y - X)/3$$

Y = cumulative drug released from 0–6 h

X = cumulative drug released from 0–3 h

For products labeled for dosing every 24 h

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: pH 6.8 phosphate buffer (6.8 g/L of monobasic potassium phosphate in water; adjusted with 2 N sodium hydroxide or 10% phosphoric acid to a pH of 6.80); 900 mL

Apparatus 1: 100 rpm

Times: 0.75, 4, and 10 h

Buffer: pH 4.0 phosphate buffer (2.72 g/L of monobasic potassium phosphate in water; adjusted with 2 N sodium hydroxide or 10% phosphoric acid to a pH of 4.00)

Mobile phase: Acetonitrile and *Buffer* (17.5: 82.5)

Standard solution: 0.06 mg/mL of USP Methylphenidate Hydrochloride RS in 0.1 N hydrochloric acid

Sample solution: Pass a portion of the solution under test through a suitable polytetrafluoroethylene (PTFE) filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.0-mm × 5-cm; 2.5-µm packing L1

Column temperature: 50°

Flow rate: See *Table 3*.

Table 3

Time (min)	Flow Rate (mL/min)
0.0	0.75
2.5	0.75
3.0	2.00
6.0	2.00
6.5	0.75
7.0	0.75

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for methylphenidate related compound A, the erythro isomer, and methylphenidate are 0.47, 0.65, and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0%

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*) shown in *Table 4*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = sum of the peak responses of methylphenidate and methylphenidate related compound A from the *Sample solution*
r_S = peak response of methylphenidate from the *Standard solution*
C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at each time point (*i*) shown in *Table 4*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of methylphenidate hydrochloride in the portion of sample withdrawn at time point (*i*) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	0.75	12–30
2	4	55–80
3	10	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.001 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Times: 1, 2, 6, and 10 h
Mobile phase: Acetonitrile and water (20:80). For every L of *Mobile phase* add 1.0 mL of formic acid and 0.2 mL of trifluoroacetic acid.
Standard solution: 0.02 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*
Sample solution: Pass a portion of the solution under test through a suitable PTFE filter of 0.45-µm pore size. Do not use glass fiber filters.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.0-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 0.75 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*) shown in *Table 5*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of methylphenidate from the *Sample solution*
r_S = peak response of methylphenidate from the *Standard solution*
C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at each time point (*i*) shown in *Table 5*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of methylphenidate hydrochloride in the portion of sample withdrawn at time point (*i*) (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	20–40
2	2	35–55
3	6	65–85
4	10	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: Water; 500 mL
Apparatus 2: 50 rpm
Times: 1, 2, 3.5, and 5 h

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Buffer: 1.6 g/L of anhydrous sodium acetate in water. Adjust with acetic acid to a pH of 4.0.
Mobile phase: Methanol, acetonitrile, and *Buffer* (40:30:30)
Standard stock solution: 0.2 mg/mL of USP Methylphenidate Hydrochloride RS in 0.1 N hydrochloric acid VS
Standard solution: [*L*/500] mg/mL of USP Methylphenidate Hydrochloride RS in 0.1 N hydrochloric acid VS from *Standard stock solution*, where *L* is the label claim of methylphenidate hydrochloride in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, then transfer the filtrate to a suitable container which already contains 10 µL of 2 N hydrochloric acid TS for every 1 mL of solution transferred.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 1.6 times the retention time of methylphenidate
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*) shown in *Table 6*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of methylphenidate from the *Sample solution*
r_S = peak response of methylphenidate from the *Standard solution*
C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at each time point (*i*) shown in *Table 6*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of methylphenidate hydrochloride in the portion of sample withdrawn at time point (*i*) (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	40–60
2	2	55–80
3	3.5	75–95
4	5	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**For products labeled for dosing every 24 h**
Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: Acidified water adjusted with phosphoric acid to a pH of 3; 50 mL
Apparatus 7: 30 cycles/min; 2–3 cm amplitude. Follow *Drug Release* ⟨724⟩, *General Drug Release Standards, Apparatus 7, Sample preparation A* using a metal spring sample holder (*Drug Release* ⟨724⟩, *Figure 5d*). Place 1 Tablet in the holder with the Tablet orifice facing down, and cover the top of the holder with Parafilm™. At the end of each specified test interval, the systems are transferred to the next row of new vessels containing 50 mL of fresh *Medium*.
Times: 1-h intervals for a duration of 10 h
Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the following method.
Buffer: Dissolve 2.0 g of sodium 1-octanesulfonate in 700 mL of water, mix well, and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (30:70)
Diluent A: Acetonitrile and *Medium* (25:75)
Diluent B: Acetonitrile and *Medium* (50:50)
Standard stock solution: 0.3 mg/mL of USP Methylphenidate Hydrochloride RS in *Diluent A*
Standard solution: (*L*/1000) mg/mL of USP Methylphenidate Hydrochloride RS in *Diluent A* from the *Standard stock solution*, where *L* is the label claim of methylphenidate hydrochloride in mg/Tablet
Sample solutions: Following the dissolution, transfer the contents of each vessel to a separate 100-mL volumetric flask. Rinse each vessel three times, using about 15 mL of *Diluent B* each time, and transfer the rinsates to the volumetric flask. Allow to cool and dilute with *Diluent B* to volume. Centrifuge and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.2-mm × 5-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 25 µL
Run time: NLT 2 times the retention time of methylphenidate
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2

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Relative standard deviation: NMT 2.0% for the peak response of methylphenidate; NMT 2% for the retention time of methylphenidate

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the concentration (C_i) of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*) shown in *Table 7*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of methylphenidate from the *Sample solution*

r_S = peak response of methylphenidate from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at each time point (*i*) shown in *Table 7*:

$$\text{Result}_1 = C_1 \times V \times D \times (1/L) \times 100$$

$$\text{Result}_2 = (C_2 + C_1) \times V \times D \times (1/L) \times 100$$

$$\text{Result}_i = (C_i + C_{i-1} + C_{i-2} + C_{i-3} + C_{i-x}) \times V \times D \times (1/L) \times 100$$

C_i = concentration of methylphenidate hydrochloride in the portion of sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 50 mL

D = dilution factor, 2

L = label claim (mg/Tablet)

Calculate the average percentage released from 3–6 h:

$$\text{Result} = (Y - X)/3$$

Y = cumulative drug released from 0–6 h

X = cumulative drug released from 0–3 h

Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved (%)
1	12–32
4	50–75
10	NLT 80
3–6 (avg)	8–13 (%/h)

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Apr-2017)

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Mobile phase: Dissolve 2 g of sodium 1-octanesulfonate in 730 mL of water. Adjust with phosphoric acid to a pH of 2.7. Mix with 270 mL of acetonitrile.

Solution A: Acidified water; adjusted with phosphoric acid to a pH of 3

Diluent A: Acetonitrile and *Solution A* (25:75)

Diluent B: Acetonitrile and methanol (50:50)

System suitability solution: 80 µg/mL of USP Methylphenidate Hydrochloride RS, 1 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS, and 2 µg/mL of USP Methylphenidate Related Compound A RS in *Diluent A*

Standard solution: 0.2 µg/mL of USP Methylphenidate Hydrochloride RS, 0.5 µg/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS, and 1.5 µg/mL of USP Methylphenidate Related Compound A RS in *Diluent A*

Sample stock solution: Nominally 1 mg/mL of methylphenidate hydrochloride prepared as follows. Dissolve NLT 10 Tablets in a suitable volumetric flask with 20% of the total flask volume of *Diluent B*. [NOTE—Alternatively, a portion of powder from NLT 10 Tablets may be transferred to a suitable volumetric flask and suspended in 20% of the total flask volume of *Diluent B*.] Stir for 4 h. Dilute with *Solution A* to volume.

Sample solution: 0.1 mg/mL of methylphenidate hydrochloride in *Solution A* from the *Sample stock solution*. [NOTE—Centrifuge before chromatographic analysis.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 µL

Run time: 2 times the retention time of methylphenidate

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 6.0 between the methylphenidate and erythro isomer peaks

Tailing factor: NMT 2.0 for the methylphenidate peak

Relative standard deviation: NMT 2.0% for the methylphenidate peak; NMT 4.0% each for the methylphenidate related compound A and erythro isomer peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylphenidate related compound A or erythro isomer in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methylphenidate related compound A or erythro isomer from the *Sample solution*

r_S = peak response of methylphenidate related compound A or erythro isomer from the *Standard solution*

C_S = concentration of USP Methylphenidate Related Compound A RS or methylphenidate

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hydrochloride erythro isomer in the *Standard solution* (mg/mL)
C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of USP Methylphenidate Hydrochloride RS from the *Standard solution*
C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table* ▲8.▲ (RB 1-Apr-2017)

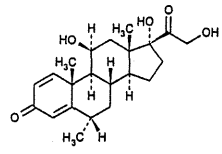
Table ▲8▲ (RB 1-Apr-2017)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylphenidate related compound A	0.47	1.5
Erythro isomer[a]	0.65	0.5
Methylphenidate	1.0	—
Any unspecified degradation product	—	0.2
Total degradation products	—	2.5

[a] Methyl (*RS,SR*)-2-phenyl-2-(piperidin-2-yl)acetate.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** The labeling states the *Dissolution* test with which the product complies if other than *Test 1*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methylphenidate Hydrochloride RS
  USP Methylphenidate Hydrochloride Erythro Isomer Solution RS
  USP Methylphenidate Related Compound A RS
  α-Phenyl-2-piperidineacetic acid hydrochloride.
  $C_{13}H_{17}NO_2 \cdot HCl$ 255.74

Methylprednisolone



$C_{22}H_{30}O_5$ 374.47
Pregna-1,4-diene-3,20-dione, 11,17,21-trihydroxy-6-methyl-, (6α,11β)-.
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione [83-43-2].

» Methylprednisolone contains not less than 97.0 percent and not more than 103.0 percent of $C_{22}H_{30}O_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Methylprednisolone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: alcohol.
Absorptivities at 243 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: Dissolve about 5 mg in 2 mL of sulfuric acid: a red color is produced.
Specific rotation ⟨781S⟩: between +79° and +86°.
Test solution: 5 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

Chromatographic purity—
Mobile phase—Prepare a filtered and degassed mixture of water, tetrahydrofuran, dimethyl sulfoxide, and butanol (149:40:10:1). Make adjustments if necessary (see *System Suitability* in *Chromatography* ⟨621⟩).
Diluting solution—Prepare a filtered mixture of water, tetrahydrofuran, and glacial acetic acid (72:25:3).
Standard solution—Dissolve an accurately weighed quantity of USP Methylprednisolone RS in *Diluting solution.* Dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 0.01 mg per mL.
Test solution—Transfer about 25 mg of Methylprednisolone, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 20-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 800 theoretical plates; and the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Methylprednisolone taken by the formula:

$$100(C_S/C_U)(r_i/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of Methylprednisolone in the *Standard solution* and the *Test solution,* respectively; r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for methylprednisolone in the *Standard solution:* not more than

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1.0% of any individual impurity is found, and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Prepare a solution containing a mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30).

Internal standard solution—Dissolve prednisone in a 3 in 100 solution of glacial acetic acid in chloroform to obtain a solution having a concentration of about 0.2 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Methylprednisolone RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Using about 10 mg of Methylprednisolone, accurately weighed, proceed as directed for *Standard preparation*.

Chromatographic system (see *Chromatography*)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*:the resolution, *R*, between the methylprednisolone and internal standard peaks is not less than 4.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks: the relative retention times are about 0.7 for prednisone and 1.0 for methylprednisolone. Calculate the quantity, in percent, of $C_{22}H_{30}O_5$ in the portion of Methylprednisolone taken by the formula:

$$100(C_S/C_U)(R_U/R_S)$$

in which C_S is the concentration of methylprednisolone, in mg per mL, in the *Standard preparation*; C_U is the nominal concentration, in mg per mL, of Methylprednisolone in the *Assay preparation*; and R_U and R_S are the ratios of the peak responses for the methylprednisolone peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methylprednisolone Tablets

» Methylprednisolone Tablets contain not less than 92.5 percent and not more than 107.5 percent of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Methylprednisolone RS

Identification—Powder a number of Tablets, equivalent to about 40 mg of methylprednisolone, and digest with 25 mL of solvent hexane for 15 minutes. Filter, and discard the filtrate. Digest the residue with 25 mL of chloroform for 15 minutes. Filter, evaporate the filtrate to dryness, and dry at 105° for 2 hours: the residue so obtained responds to *Identification* tests *A* and *C* under *Methylprednisolone*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2 : 50 rpm.
Time: 30 minutes.

Procedure—Measure the UV absorption of filtered aliquots removed from the *Dissolution Medium* and suitably diluted, if necessary, in 1-cm cells at 246 nm, with a suitable spectrophotometer, using water as the blank and utilizing a standard curve, representing the absorbance versus concentration of USP Methylprednisolone RS. [NOTE—Dissolve about 20 mg of USP Methylprednisolone RS, accurately weighed, in 1 mL of alcohol, dilute in a 1000-mL volumetric flask with water to volume, and mix. Prepare quantitative dilutions of this solution for the development of a standard curve.]

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{22}H_{30}O_5$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Methylprednisolone*.

Test preparation—Place 1 Tablet in a suitable container. For tablet labeled strengths of 10 mg or less, add 0.5 mL of water. For tablet labeled strengths greater than 10 mg, add 1.0 mL of water. Allow the tablet to stand for about 2 minutes, then swirl the container to disperse the tablet. Add 5.0 mL of *Internal standard solution* for each mg of labeled tablet strength, shake for 15 minutes, and filter or centrifuge a portion of the test specimen. Analyze the clear solution as directed under *Procedure*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Methylprednisolone*. Calculate the quantity, in mg, of $C_{22}H_{30}O_5$ in the Tablet taken by the formula:

$$(FW_S)(R_U/R_S)$$

in which *F* is the ratio of the volume of *Internal standard preparation*, in mL, in the *Test preparation* to the volume, in mL, of the *Internal standard preparation* in the *Standard preparation*; W_S is the weight, in mg, of USP Methylprednisolone RS taken for the *Standard preparation*; and the other terms are as defined for *Procedure* in the *Assay* under *Methylprednisolone*.

Assay—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Methylprednisolone*.

Assay preparation—Accurately weigh 20 Tablets, and grind to a fine powder in a mortar and pestle. Accurately weigh a portion of the powder, equivalent to about 10 mg of methylprednisolone, and transfer to a suitable container. Add 2.5 mL of water to the ground tablet material and swirl to form a fine slurry. Add 50.0 mL of *Internal standard solution*, and shake for 15 minutes. Filter or centrifuge a portion of the liquid so obtained, if necessary, and analyze the clear solution as directed under *Procedure*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Methylprednisolone*. Calculate the quantity, in mg, of $C_{22}H_{30}O_5$ in the portion of Tablets taken by the formula:

$$50C(R_U/R_S)$$

in which the terms are as defined therein.

Methylprednisolone Acetate

$C_{24}H_{32}O_6$ 416.51

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Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-6-methyl-, (6α,11β)-;
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-acetate [53-36-1].

DEFINITION

Methylprednisolone Acetate contains NLT 97.0% and NMT 103.0% of methylprednisolone acetate ($C_{24}H_{32}O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Analytical wavelength: 243 nm
Standard solution: 10 µg/mL of USP Methylprednisolone Acetate RS in alcohol
Sample solution: 10 µg/mL of methylprednisolone acetate in alcohol
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

- **PROCEDURE**

Mobile phase: *n*-Butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)
Internal standard solution: 6 mg/mL of prednisone prepared as follows. Transfer an appropriate amount of prednisone to a suitable volumetric flask. Add 3% of the flask volume of glacial acetic acid, and sonicate. Dilute with chloroform to volume, slowly adding the chloroform. Sonicate, and shake to dissolve.
Standard solution: 0.2 mg/mL of USP Methylprednisolone Acetate RS prepared as follows. Transfer an appropriate amount of USP Methylprednisolone Acetate RS to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with chloroform to volume, and shake to dissolve.
Sample solution: 0.2 mg/mL of Methylprednisolone Acetate prepared as follows. Transfer an appropriate amount of methylprednisolone acetate to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with chloroform to volume, and shake to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 25-cm; packing L3
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for methylprednisolone acetate and prednisone are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 2.5 between methylprednisolone acetate and prednisone
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Methylprednisolone Acetate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of methylprednisolone acetate to prednisone from the *Sample solution*
R_S = peak height ratio of methylprednisolone acetate to prednisone from the *Standard solution*
C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Methylprednisolone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Mobile phase: Tetrahydrofuran and water (51:149)
Diluent: Tetrahydrofuran, acetonitrile, glacial acetic acid, and water (250:250:1:499)
Standard solution: 20 µg/mL of USP Methylprednisolone Acetate RS in *Diluent*. Sonicate, if necessary, to dissolve.
Sample solution: 1 mg/mL of Methylprednisolone Acetate in *Diluent*. Sonicate, if necessary, to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Methylprednisolone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for methylprednisolone acetate from the *Standard solution*
C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Methylprednisolone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria
Any individual impurity: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL in dioxane
Acceptance criteria: +97° to +105°

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methylprednisolone Acetate RS

Methylprednisolone Acetate Injectable Suspension

DEFINITION
Methylprednisolone Acetate Injectable Suspension is a sterile suspension of Methylprednisolone Acetate in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$).

IDENTIFICATION

Change to read:

• **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Nominally 100 mg of methylprednisolone acetate from Injectable Suspension
Analysis: Filter the *Sample* through paper. Wash the residue with several 5-mL portions of water, and dry at 105° for 3 h.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: *n*-Butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)
Internal standard solution: 6 mg/mL of prednisone prepared as follows. Transfer an appropriate amount of prednisone to a suitable volumetric flask. Add 3% of the flask volume of glacial acetic acid, and sonicate. Dilute with chloroform to volume, slowly adding the chloroform. Sonicate, and shake to dissolve.
Standard solution: 0.2 mg/mL of USP Methylprednisolone Acetate RS prepared as follows. Transfer an appropriate amount of USP Methylprednisolone Acetate RS to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with chloroform to volume, and shake to dissolve.
Sample solution: Swirl Injectable Suspension to ensure uniformity before analysis. Transfer a suitable quantity of Injectable Suspension equivalent to 40 mg of methylprednisolone acetate to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, dilute with chloroform to volume, and shake for 15 min or until the aqueous layer is clear. Transfer 4.0 mL of the chloroform layer to a suitable vial, add 30 mL of chloroform and a small quantity (about 400 mg) of anhydrous sodium sulfate, shake for 5 min. Use the clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 25-cm; packing L3
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for methylprednisolone acetate and prednisone are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 2.5 between methylprednisolone acetate and prednisone
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Injectable Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of methylprednisolone acetate to prednisone from the *Sample solution*
R_S = peak height ratio of methylprednisolone acetate to prednisone from the *Standard solution*
C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of methylprednisolone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS
• **PH** ⟨791⟩: 3.0–7.0
• **PARTICLE SIZE**
Analysis: Transfer 1 drop to a microscope slide, and spread it evenly, diluting with water if necessary, to decrease the density of the field. Examine the slide under a microscope equipped with a calibrated ocular micrometer, using 400× magnification. Scan the entire slide, and note the size of the individual particles.
Acceptance criteria: NLT 99% of the particles are less than 20 µm in length when measured along the longest axis, and NLT 75% of the particles are less than 10 µm.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methylprednisolone Acetate RS

Methylprednisolone Hemisuccinate

$C_{26}H_{34}O_8$ 474.54
Pregna-1,4-diene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-6-methyl-, (6α,11β)-;
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(hydrogen succinate) [2921-57-5].

DEFINITION
Methylprednisolone Hemisuccinate contains NLT 97.0% and NMT 103.0% of methylprednisolone hemisuccinate ($C_{26}H_{34}O_8$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

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Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U** ▲ (CN 1-May-2020)
Standard solution: 20 µg/mL of USP Methylprednisolone Hemisuccinate RS in alcohol
Sample solution: 20 µg/mL of methylprednisolone hemisuccinate in alcohol
Analytical wavelength: 243 nm
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

• **PROCEDURE**
Solution A: Chloroform and glacial acetic acid (97:3)
Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)
Internal standard solution: 6 mg/mL of USP Fluorometholone RS in tetrahydrofuran
Standard solution: 0.4 mg/mL of USP Methylprednisolone Hemisuccinate RS prepared as follows. Transfer a suitable quantity of USP Methylprednisolone Hemisuccinate RS to a suitable volumetric flask, and add 5.0% of the flask volume of *Internal standard solution*. Dilute with *Solution A* to volume.
Sample solution: 0.4 mg/mL of Methylprednisolone Hemisuccinate prepared as follows. Transfer a suitable quantity of Methylprednisolone Hemisuccinate to a suitable volumetric flask, and add 5.0% of the flask volume of *Internal standard solution*. Dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L3
Injection volume: 4–8 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between methylprednisolone hemisuccinate and fluorometholone
Relative standard deviation: NMT 2.0% for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methylprednisolone hemisuccinate ($C_{26}H_{34}O_8$) in the portion of Methylprednisolone Hemisuccinate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of methylprednisolone hemisuccinate to fluorometholone from the *Sample solution*
R_S = peak area ratio of methylprednisolone hemisuccinate to fluorometholone from the *Standard solution*
C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Methylprednisolone Hemisuccinate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**
Mobile phase: Tetrahydrofuran, formic acid, and water (255:1:745)
Diluent: Tetrahydrofuran, acetonitrile, acetic acid, and water (25:25:3:47)
Standard solution: 0.02 mg/mL of USP Methylprednisolone Hemisuccinate RS in *Diluent*
Sample solution: 1 mg/mL of Methylprednisolone Hemisuccinate in *Diluent*. Shake or sonicate to aid in solubilization.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 20-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000 theoretical plates
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Methylprednisolone Hemisuccinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of methylprednisolone hemisuccinate from the *Standard solution*
C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Methylprednisolone Hemisuccinate in the *Sample solution* (mg/mL)

Acceptance criteria
Any individual impurity: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS

• **OPTICAL ROTATION**, *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL of Methylprednisolone Hemisuccinate in dioxane
Acceptance criteria: +87° to +95°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluorometholone RS
USP Methylprednisolone Hemisuccinate RS

Methylprednisolone Sodium Succinate

$C_{26}H_{33}NaO_8$ 496.53
Pregna-1,4-diene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-6-methyl-, monosodium salt, (6α,11β)-; 11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(sodium succinate) [2375-03-3].

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DEFINITION
Methylprednisolone Sodium Succinate contains NLT 97.0% and NMT 103.0% of methylprednisolone sodium succinate ($C_{26}H_{33}NaO_8$), calculated on the dried basis.

IDENTIFICATION
• **A. Infrared Absorption**
Sample: 100 mg of Methylprednisolone Sodium Succinate
Analysis: Transfer the *Sample* to a separator, dissolve in 10 mL of water, add 1 mL of 3 N hydrochloric acid, and extract immediately with 50 mL of chloroform. Filter the chloroform extract through cotton, evaporate on a steam bath to dryness, and dry under vacuum at 60° for 3 h.
Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Methylprednisolone Hemisuccinate RS.

Change to read:

• **B. ▲Spectroscopic Identification Tests ⟨197⟩,** *Ultraviolet-Visible Spectroscopy:* **197U▲** (CN 1-May-2020)
Standard solution: 20 µg/mL of USP Methylprednisolone Hemisuccinate RS in methanol
Sample solution: 20 µg/mL of Methylprednisolone Sodium Succinate in methanol
Analytical wavelength: 243 nm
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
• **C.** The sample imparts an intense yellow color to a nonluminous flame.

ASSAY
• **Procedure**
Solution A: 5 mg/mL of blue tetrazolium in alcohol
Solution B: Alcohol and tetramethylammonium hydroxide TS (9:1)
Standard solution: Proceed as directed for *Assay for Steroids ⟨351⟩, Standard Preparation,* preparing 12.5 µg/mL of USP Methylprednisolone Hemisuccinate RS in alcohol.
Sample solution: 12.5 µg/mL of Methylprednisolone Sodium Succinate in alcohol
Blank: Alcohol
Instrumental conditions
Mode: Vis
Analytical wavelength: 525 nm
Analysis
Samples: *Standard solution, Sample solution,* and *Blank*
Pipet 20.0 mL of the *Blank, Standard solution,* and *Sample solution* into three different glass-stoppered, 50-mL conical flasks. Add 2.0 mL of *Solution A,* and mix. To each flask add 4.0 mL of *Solution B.* Mix, and allow to stand in the dark for 90 min. Add 1.0 mL of glacial acetic acid, and mix. Without delay, determine the absorbances of the *Standard solution* and the *Sample solution* against the *Blank.*
Calculate the percentage of methylprednisolone sodium succinate ($C_{26}H_{33}NaO_8$) in the portion of Methylprednisolone Sodium Succinate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (µg/mL)
C_U = concentration of Methylprednisolone Sodium Succinate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of methylprednisolone sodium succinate, 496.53
M_{r2} = molecular weight of methylprednisolone hemisuccinate, 474.54

Acceptance criteria: 97.0%–103.0% on the dried basis

OTHER COMPONENTS
• **Sodium Content**
Sample solution: Dissolve, with gentle heating, about 1 g of Methylprednisolone Sodium Succinate in 75 mL of glacial acetic acid. Add 20 mL of dioxane, then add 1 drop of crystal violet TS.
Titrimetric system
Mode: Direct
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Visual
Analysis: Titrate with *Titrant* to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 2.299 mg of sodium (Na).
Acceptance criteria: 4.49%–4.77% on the dried basis

SPECIFIC TESTS
• **Optical Rotation ⟨781S⟩,** *Specific Rotation*
Sample solution: 10 mg/mL of Methylprednisolone Sodium Succinate in alcohol
Acceptance criteria: +96° to +104°
• **Loss on Drying ⟨731⟩**
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards ⟨11⟩**
USP Methylprednisolone Hemisuccinate RS

Methylprednisolone Sodium Succinate for Injection

DEFINITION
Methylprednisolone Sodium Succinate for Injection is a sterile mixture of Methylprednisolone Sodium Succinate with suitable buffers. It may be prepared from Methylprednisolone Sodium Succinate or from Methylprednisolone Hemisuccinate with the aid of Sodium Hydroxide or Sodium Carbonate. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$) in the volume of constituted solution designated on the label.

IDENTIFICATION
• **A. Infrared Absorption**
Sample: Nominally 100 mg of methylprednisolone sodium succinate from Methylprednisolone Sodium Succinate for Injection
Analysis: Transfer the *Sample* to a separator, dissolve in 10 mL of water, add 1 mL of 3 N hydrochloric acid, and extract immediately with 50 mL of chloroform. Filter the chloroform extract through cotton, evaporate on a steam bath to dryness, and dry under vacuum at 60° for 3 h.
Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Methylprednisolone Hemisuccinate RS.

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ASSAY

• **PROCEDURE**

Diluent: Chloroform and glacial acetic acid (97:3)

Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

Standard stock solution: 0.30 mg/mL of USP Methylprednisolone RS in *Diluent*

Internal standard solution: 3 mg/mL of USP Fluorometholone RS in tetrahydrofuran

Standard solution: 0.65 mg/mL of USP Methylprednisolone Hemisuccinate RS prepared as follows. Transfer an appropriate amount of USP Methylprednisolone Hemisuccinate RS to a suitable volumetric flask. Pipet 10% of the flask volume of the *Internal standard solution* and 10% of the flask volume of the *Standard stock solution*. Dilute with *Diluent* to volume.

Sample solution: Transfer a suitable quantity of constituted solutions (mix the constituted solutions prepared from the contents of 10 vials of Methylprednisolone Sodium Succinate for Injection) equivalent to 50 mg of methylprednisolone to a suitable flask containing 10.0 mL of the *Internal standard solution*, and dilute with *Diluent* to 100.0 mL. Shake thoroughly for 5 min, then allow the phases to separate, discarding the upper phase.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L3

Flow rate: 1 mL/min

Injection volume: 6 µL

[NOTE—The order of elution of peaks in the *Standard solution* is as follows. Internal standard peak, methylprednisolone hemisuccinate peak, and successive smaller peaks of free methylprednisolone and methylprednisolone 17-hemisuccinate.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$) in the portion of constituted Methylprednisolone Sodium Succinate for Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = ratio of the sum of the peak areas for methylprednisolone hemisuccinate and methylprednisolone 17-hemisuccinate to the peak area of the internal standard from the *Sample solution*

R_S = ratio of the sum of the peak areas for methylprednisolone hemisuccinate and methylprednisolone 17-hemisuccinate to the peak area of the internal standard from the *Standard solution*

C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of methylprednisolone, 374.47

M_{r2} = molecular weight of methylprednisolone hemisuccinate, 474.54

To this calculated amount, add the percentage of free methylprednisolone found in the test for *Free Methylprednisolone*.

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **FREE METHYLPREDNISOLONE**

Analysis

Samples: *Standard solution* and *Sample solution*

Using the chromatograms obtained in the *Assay*, measure the areas of the peaks from the internal standard and free methylprednisolone.

Calculate the percentage of free methylprednisolone in the portion of *Sample solution* taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of the free methylprednisolone to the internal standard from the *Sample solution*

R_S = peak area ratio of the free methylprednisolone to the internal standard from the *Standard solution*

C_S = concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: The amount of free methylprednisolone is NMT 6.6% of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$).

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS

• **PH** ⟨791⟩

Sample solution: 50 mg/mL of methylprednisolone sodium succinate

Acceptance criteria: 7.0–8.0

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.17 USP Endotoxin Units/mg of methylprednisolone

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 2.0%

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** Meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

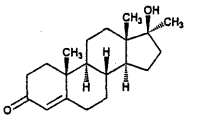
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fluorometholone RS

USP Methylprednisolone RS

USP Methylprednisolone Hemisuccinate RS

Methyltestosterone



$C_{20}H_{30}O_2$ 302.45

Androst-4-en-3-one, 17-hydroxy-17-methyl-, (17β)-; 17β-Hydroxy-17-methylandrost-4-en-3-one [58-18-4].

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DEFINITION

Methyltestosterone contains NLT 97.0% and NMT 103.0% of methyltestosterone ($C_{20}H_{30}O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (55:45)
Standard stock solution: 0.25 mg/mL of USP Methyltestosterone RS in methanol
Standard solution: 20 µg/mL of USP Methyltestosterone RS in *Mobile phase* from the *Standard stock solution*
System suitability stock solution: 250 µg/mL of USP Testosterone RS in methanol
System suitability solution: Dilute 4 mL of the *System suitability stock solution* with the *Standard solution* to 50 mL.
Sample stock solution: 0.50 mg/mL of Methyltestosterone in methanol
Sample solution: 20 µg/mL of Methyltestosterone in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 241 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for testosterone and methyltestosterone are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between testosterone and methyltestosterone, *System suitability solution*
Column efficiency: NLT 2000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.7, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methyltestosterone ($C_{20}H_{30}O_2$) in the portion of Methyltestosterone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methyltestosterone from the *Sample solution*
r_S = peak response of methyltestosterone from the *Standard solution*
C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (mg/mL)
C_U = concentration of Methyltestosterone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: Methanol and water (55:45)
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	60	40
40	0	100
45	0	100
60	100	0

Sample solution: 0.5 mg/mL of Methyltestosterone in methanol
System suitability solution: 0.005 mg/mL of Methyltestosterone in methanol from the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Samples: *Sample solution* and *System suitability solution*
Suitability requirements
Relative standard deviation: NMT 2.0%, *Sample solution*
Signal-to-noise ratio: NLT 100, *System suitability solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Methyltestosterone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

[NOTE—Disregard any impurity peak less than 0.05%.]
Acceptance criteria
Any individual impurity: NMT 0.5%
Total impurities: NMT 1.0%

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL of Methyltestosterone in alcohol
Acceptance criteria: +79° to +85°

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Methyltestosterone RS
USP Testosterone RS

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Methyltestosterone Capsules

DEFINITION

Methyltestosterone Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$).

IDENTIFICATION

• **A. Infrared Absorption**

Sample: Evaporate to dryness 25 mL of the *Sample stock solution* from the *Assay*.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima at the same wavelengths as those of a similar preparation of USP Methyltestosterone RS.

ASSAY

• **Procedure**

Standard solution: 10 µg/mL of USP Methyltestosterone RS in alcohol

Sample stock solution: Nominally 0.2 mg/mL of methyltestosterone prepared as follows. Transfer the equivalent to 10 mg of methyltestosterone from the contents of NLT 20 Capsules to a 125-mL separator with the aid of about 5 mL of water. Extract with four 20-mL portions of chloroform, filtering each through chloroform-washed cotton. Evaporate the combined extracts on a steam bath, with the aid of a current of air, to dryness. Dissolve the residue in alcohol, transfer to a 50-mL volumetric flask, and dilute with alcohol to volume.

Sample solution: Nominally 10 µg/mL of methyltestosterone in alcohol from the *Sample stock solution*

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 241 nm

Cell: 1 cm

Blank: Alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methyltestosterone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: 10 µg/mL of USP Methyltestosterone RS in *Medium*

Sample solution: Filter a portion of the solution under test. Dilute with *Medium* to obtain a solution containing about 10 µg/mL of methyltestosterone.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 248 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 70% (*Q*) of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩

Procedure for content uniformity

Standard solution: 0.010 mg/mL of USP Methyltestosterone RS in methanol

Sample stock solution: Transfer the contents of 1 Capsule to a 100-mL volumetric flask. Add 50 mL of methanol, and shake by mechanical means for 60 min. Dilute with methanol to volume, and filter, discarding the first 20 mL of the filtrate.

Sample solution: Dilute a suitable volume of the *Sample stock solution* with methanol to obtain 0.010 mg/mL of methyltestosterone.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 241 nm

Cell: 1 cm

Blank: Methanol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of methyltestosterone from the *Sample solution*

A_S = absorbance of methyltestosterone from the *Standard solution*

C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of the *Sample solution* (mL)

D = dilution factor of the *Sample solution*

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩

USP Methyltestosterone RS

Methyltestosterone Tablets

DEFINITION

Methyltestosterone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$).

IDENTIFICATION

• **A. Infrared Absorption**

Sample: Evaporate to dryness 25 mL of the *Sample stock solution* from the *Assay*.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima at the same wavelengths as those of a similar preparation of USP Methyltestosterone RS.

ASSAY

• **Procedure**

Standard solution: 10 µg/mL of USP Methyltestosterone RS in alcohol

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Sample stock solution: Nominally 0.2 mg/mL of methyltestosterone prepared as follows. Transfer the equivalent to 10 mg of methyltestosterone from NLT 20 powdered Tablets to a 125-mL separator with the aid of about 5 mL of water. Extract with four 25-mL portions of chloroform, filtering each through chloroform-washed cotton. Evaporate the combined extracts on a steam bath, with the aid of a current of air, to dryness. Dissolve the residue in alcohol, transfer to a 50-mL volumetric flask, and dilute with alcohol to volume.

Sample solution: Nominally 10 µg/mL of methyltestosterone in alcohol from the *Sample stock solution*

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 241 nm

Cell: 1 cm

Blank: Alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methyltestosterone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Time: 30 min

Acceptance criteria: Tablets intended for buccal administration meet the requirements for *Buccal Tablets*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Standard solution: 0.010 mg/mL of USP Methyltestosterone RS in methanol

Sample stock solution: Transfer 1 finely powdered Tablet to a 100-mL volumetric flask. Add 50 mL of methanol, and shake by mechanical means for 60 min. Dilute with methanol to volume, and filter, discarding the first 20 mL of the filtrate.

Sample solution: Dilute a suitable volume of the *Sample stock solution* with methanol to obtain 0.010 mg/mL of methyltestosterone.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 241 nm

Cell: 1 cm

Blank: Methanol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of methyltestosterone from the *Sample solution*

A_S = absorbance of methyltestosterone from the *Standard solution*

C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of the *Sample solution* (mL)

D = dilution factor of the *Sample solution*

Acceptance criteria: Meet the requirements

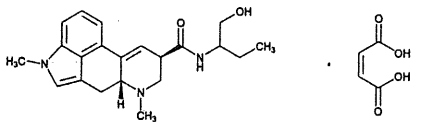
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Methyltestosterone RS

Methysergide Maleate



$C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$ 469.53

Ergoline-8-carboxamide, 9,10-didehydro-*N*-[1-(hydroxy methyl)propyl]-1,6-dimethyl-, (8β)-, (*Z*)-2-butenedioate (1:1) (salt);

9,10-Didehydro-*N*-[1-(hydroxymethyl)propyl]-1,6-dimethylergoline-8β-carboxamide maleate (1:1) (salt) [129-49-7].

DEFINITION

Methysergide Maleate contains NLT 97.0% and NMT 103.0% of methysergide maleate ($C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B. THIN-LAYER CHROMATOGRAPHY**

Conduct this test without exposure to daylight and with minimum exposure to artificial light.

Standard solution: 5 mg/mL of USP Methysergide Maleate RS in methanol

Sample solution: 5 mg/mL of Methysergide Maleate in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 25 µL

Developing solvent: Chloroform and methanol (20:1)

Spray reagent: 8 mg/mL of *p*-dimethylaminobenzaldehyde in a cooled mixture of alcohol and sulfuric acid (8:2)

Analysis

Samples: *Standard solution* and *Sample solution*

In the chromatographic chamber, place a volume of the *Developing solvent* sufficient to develop the chromatogram. Place a beaker containing 25 mL of ammonium hydroxide in the chamber, cover, and allow to equilibrate for 30 min. Apply the *Samples*, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with

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Spray reagent. Allow the plate to dry, then expose it briefly to fumes of a mixture of nitric and hydrochloric acids.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY

• **PROCEDURE**

Sample solution: 200 mg of Methysergide Maleate in 30 mL of glacial acetic acid. Add 1 drop of crystal violet TS.

Analysis: Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 46.95 mg of methysergide maleate ($C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$).

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **ORDINARY IMPURITIES** ⟨466⟩

Standard solution and **Sample solution:** Methanol

Eluant: Use the *Developing solvent* from *Identification* test *B.*

Visualization: 1

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 2.5 mg/mL in water

Acceptance criteria: +35° to +45°

• **pH** ⟨791⟩

Sample solution: 1 in 500 in carbon dioxide-free water

Acceptance criteria: 3.7–4.7

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample under vacuum at 120° for 2 h.

Acceptance criteria: NMT 7.0%

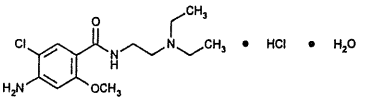
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a cold place.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Methysergide Maleate RS

Metoclopramide Hydrochloride



$C_{14}H_{22}ClN_3O_2 \cdot HCl \cdot H_2O$ 354.27

Benzamide, 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxy-, monohydrochloride, monohydrate;

4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-*o*-anisamide monohydrochloride monohydrate [54143-57-6].

DEFINITION

Metoclopramide Hydrochloride contains NLT 98.0% and NMT 101.0% of metoclopramide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

Sample solution: Dissolve 100 mg in 2 mL of water, and acidify the solution with dilute nitric acid.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample: 250 mg

Analysis: Dissolve the *Sample* in a mixture of 5.0 mL of 0.01 N hydrochloric acid and 50 mL of alcohol. Titrate with 0.1 N sodium hydroxide VS (see *Titrimetry* ⟨541⟩), determining the endpoint potentiometrically. Read the volume of 0.1 N sodium hydroxide added between the two points of inflection. Each mL of 0.1 N sodium hydroxide is equivalent to 33.63 mg of the anhydrous metoclopramide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl$).

Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 700 mL of water. Add 0.2 mL of *N,N*-dimethyloctylamine, and adjust with dilute phosphoric acid to a pH of 4.0. Dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (250:1000)

Standard stock solution: 0.1 mg/mL each of USP Metoclopramide Hydrochloride RS, USP Metoclopramide Related Compound A RS, USP Metoclopramide Related Compound B RS, and USP Metoclopramide Related Compound D RS in *Mobile phase*

Standard solution: 2.0 µg/mL each of USP Metoclopramide Hydrochloride RS, USP Metoclopramide Related Compound A RS, USP Metoclopramide Related Compound B RS, and USP Metoclopramide Related Compound D RS in *Mobile phase* from the *Standard stock solution*

Sample solution: 1.0 mg/mL of Metoclopramide Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: At least 8 times the retention time of the metoclopramide peak

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 for metoclopramide related compound A and metoclopramide

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each of metoclopramide related compounds A, B, and D in the portion of Metoclopramide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the relevant metoclopramide related compound from the *Sample solution*

r_S = peak response of the relevant metoclopramide related compound from the *Standard solution*

C_S = concentration of the relevant USP Metoclopramide Related Compound RS in the *Standard solution* (mg/mL)

C_U = concentration of Metoclopramide Hydrochloride in the *Sample solution* (mg/mL)

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Calculate the percentage of any other individual impurity in the portion of Metoclopramide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of metoclopramide from the *Standard solution*
C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Metoclopramide Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Acetylmetoclopramide (metoclopramide related compound A)[a]	0.8	0.15
Metoclopramide	1.0	—
Methyl 4-acetamido-2-methoxybenzoate (metoclopramide related compound D)	2.4–2.7	0.15
N-Acetyl methyl ester analog (metoclopramide related compound B)[b]	5.2–6.7	0.15
Any other individual impurity	—	0.10
Total impurities	—	0.50

[a] 4-Acetamido-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide.
[b] Methyl 4-acetamido-5-chloro-2-methoxybenzoate.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 4.5%–6.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metoclopramide Hydrochloride RS
  USP Metoclopramide Related Compound A RS
  N-Acetylmetoclopramide.
  4-Acetamido-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide.
  $C_{16}H_{24}ClN_3O_3$ 341.83
  USP Metoclopramide Related Compound B RS
  N-Acetyl methyl ester analog.
  Methyl 4-acetamido-5-chloro-2-methoxybenzoate.
  $C_{11}H_{12}ClNO_4$ 257.67
  USP Metoclopramide Related Compound D RS
  Methyl 4-acetamido-2-methoxybenzoate.
  $C_{11}H_{13}NO_4$ 223.23

Metoclopramide Injection

DEFINITION
Metoclopramide Injection is a sterile solution of Metoclopramide Hydrochloride in Water for Injection. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Dissolve 2.7 g of sodium acetate in 500 mL of water. Add 500 mL of acetonitrile and 2 mL of tetramethylammonium hydroxide solution in methanol (1 in 5), and mix. Adjust with glacial acetic acid to a pH of 6.5, filter, and degas.
System suitability stock solution: Transfer 12.5 mg of benzenesulfonamide to a 25-mL volumetric flask. Add 15 mL of methanol, and shake to dissolve. Dilute with 0.01 M phosphoric acid to volume.
Standard stock solution: 0.9 mg/mL of USP Metoclopramide Hydrochloride RS in 0.01 M phosphoric acid
System suitability solution: Transfer 5 mL of *System suitability stock solution* and 5 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.
Standard solution: 45 µg/mL of USP Metoclopramide Hydrochloride RS (equivalent to 40 µg/mL of metoclopramide) from *Standard stock solution*. Dilute with 0.01 M phosphoric acid.
Sample solution: Nominally 40 µg/mL of metoclopramide, prepared as follows. Transfer a volume of Injection, equivalent to about 40 mg of metoclopramide, to a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm or diode array. [NOTE—Use the diode array detector to perform *Identification* test *B*.]
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
[NOTE—The relative retention times for benzenesulfonamide and metoclopramide are 0.7 and 1.0, respectively.]
Resolution: NLT 1.5 between the benzenesulfonamide and metoclopramide peaks, *System suitability solution*
Tailing factor: NMT 2.0 for the metoclopramide peak, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)

USP Monographs

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C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of metoclopramide, 299.80
M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase: Prepare a 1.88 g/L solution of sodium 1-hexanesulfonate solution (0.01 M solution) in a mixture of acetonitrile and water (60:40), and adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 5.5 µg/mL of USP Metoclopramide Hydrochloride RS in *Mobile phase*

Sample solution: Dilute a volume of Injection with *Mobile phase* to obtain a solution containing about 1.0 mg/mL of metoclopramide.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 5.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of metoclopramide from the *Standard solution*
C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of metoclopramide, 299.80
M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

Acceptance criteria: NMT 0.5% of any individual impurity is found.

SPECIFIC TESTS

• **pH** ⟨791⟩: 2.5–6.5
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 2.5 USP Endotoxin Units/mg of metoclopramide
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. [NOTE—Injection containing an antioxidant agent does not require protection from light.] Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Metoclopramide Hydrochloride RS

Metoclopramide Oral Solution

DEFINITION

Metoclopramide Oral Solution contains an amount of metoclopramide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl \cdot H_2O$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 2.7 g of sodium acetate in 600 mL of water, and add 400 mL of acetonitrile and 4 mL of tetramethylammonium hydroxide solution in methanol (25%). Adjust with glacial acetic acid to a pH of 6.5, filter, and degas.

System suitability stock solution: Transfer 125 mg of benzenesulfonamide to a 25-mL volumetric flask. Add 15 mL of methanol, and shake to dissolve. Dilute with 0.01 M phosphoric acid to volume.

Standard stock solution: 9 mg/mL of USP Metoclopramide Hydrochloride RS in 0.01 M phosphoric acid

System suitability solution: Transfer 15 mL of *System suitability stock solution* and 5 mL of *Standard stock solution* into a 250-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.

Standard solution: 180 µg/mL of USP Metoclopramide Hydrochloride RS (equivalent to 160 µg/mL of metoclopramide) from *Standard stock solution*. Dilute with 0.01 M phosphoric acid.

Sample solution: Nominally 160 µg/mL of metoclopramide, prepared as follows. Transfer a volume of Oral Solution, equivalent to about 4 mg of metoclopramide, to a 25-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm or diode array. [NOTE—Use the diode array detector to perform *Identification* test *B*.]
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
[NOTE—The relative retention times for benzenesulfonamide and metoclopramide are 0.2 and 1.0, respectively.]
Resolution: NLT 1.5 between the benzenesulfonamide and metoclopramide peaks, *System suitability solution*
Tailing factor: NMT 2.0 for the metoclopramide peak, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

USP Monographs

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r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration from USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of metoclopramide, 299.80
M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For oral solution packaged in single-unit containers: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩
For oral solution packaged in multiple-unit containers: Meets the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase: Prepare a 1.88 g/L solution of sodium 1-hexanesulfonate solution (0.01 M solution) in a mixture of acetonitrile and water (60:40), and adjust with glacial acetic acid to a pH of 4.0.
Standard solution: 5.5 µg/mL of USP Metoclopramide Hydrochloride RS in *Mobile phase*
Sample solution: Dilute a volume of Oral Solution with *Mobile phase* to obtain a solution containing about 1.0 mg/mL of metoclopramide.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of metoclopramide from the *Standard solution*
C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of metoclopramide, 299.80
M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

Acceptance criteria: NMT 0.5% of any individual impurity is found. Disregard any peak with a relative retention time of 0.5 or less.

SPECIFIC TESTS
• **PH** ⟨791⟩: 2.0–5.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature. Protect from freezing.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Metoclopramide Hydrochloride RS

Metoclopramide Tablets

DEFINITION
Metoclopramide Tablets contain an amount of metoclopramide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl \cdot H_2O$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Dissolve 2.7 g of sodium acetate in 500 mL of water. Add 500 mL of acetonitrile and 2 mL of tetramethylammonium hydroxide solution in methanol (1 in 5), and mix. Adjust with glacial acetic acid to a pH of 6.5, filter, and degas.
System suitability stock solution: Transfer 12.5 mg of benzenesulfonamide to a 25-mL volumetric flask. Add 15 mL of methanol, and shake to dissolve. Dilute with 0.01 M phosphoric acid to volume.
Standard stock solution: 0.9 mg/mL of USP Metoclopramide Hydrochloride RS in 0.01 M phosphoric acid
System suitability solution: Transfer 5 mL of *System suitability stock solution* and 5 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.
Standard solution: 45 µg/mL of USP Metoclopramide Hydrochloride RS (equivalent to 40 µg/mL of metoclopramide) from *Standard stock solution* diluted with 0.01 M phosphoric acid
Sample solution: Nominally 40 µg/mL of metoclopramide, prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of metoclopramide, to a 100-mL volumetric flask, add about 70 mL of 0.01 M phosphoric acid, and sonicate for 5 min. Cool to room temperature, dilute with 0.01 M phosphoric acid to volume, and mix. Pass the solution through a filter of 0.45-µm pore size, discarding the first portion of the filtrate. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm or diode array. [NOTE—Use the diode array detector to perform *Identification* test *B*.]
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

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Suitability requirements
[NOTE—The relative retention times for benzenesulfonamide and metoclopramide are 0.7 and 1.0, respectively.]
Resolution: NLT 1.5 between the benzenesulfonamide and metoclopramide peaks, *System suitability solution*
Tailing factor: NMT 2.0 for the metoclopramide peak, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of metoclopramide, 299.80
M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 50 rpm
Time: 30 min
Standard solution: USP Metoclopramide Hydrochloride RS at a known concentration in *Medium*
Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium*
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: Wavelength of maximum absorbance at about 309 nm
Tolerances: NLT 75% (*Q*) of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase: Prepare a 1.88 g/L solution of sodium 1-hexanesulfonate solution (0.01 M solution) in a mixture of acetonitrile and water (60:40), and adjust with glacial acetic acid to a pH of 4.0.
Standard solution: 5.5 µg/mL of USP Metoclopramide Hydrochloride RS in *Mobile phase*
Sample solution: Shake a quantity of the powdered Tablets containing the equivalent of 100 mg of metoclopramide with 20 mL of methanol for 5 min, and pass through a suitable filter, discarding the first few mL of the filtrate. Dilute a portion of the filtrate with *Mobile phase* to obtain a solution containing about 1.0 mg/mL of metoclopramide.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

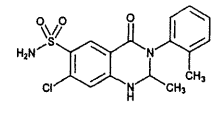
r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of metoclopramide from the *Standard solution*
C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of metoclopramide, 299.80
M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

Acceptance criteria: NMT 0.5% of any individual impurity is found.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Metoclopramide Hydrochloride RS

Metolazone



$C_{16}H_{16}ClN_3O_3S$ 365.83
6-Quinazolinesulfonamide, 7-chloro-1,2,3,4-tetrahydro-2-methyl-3-(2-methylphenyl)-4-oxo-;
7-Chloro-1,2,3,4-tetrahydro-2-methyl-4-oxo-3-*o*-tolyl-6-quinazolinesulfonamide [17560-51-9].

DEFINITION

Metolazone contains NLT 97.0% and NMT 102.0% of metolazone ($C_{16}H_{16}ClN_3O_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 5 µg/mL in methanol
Acceptance criteria: Meets the requirements
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

• **PROCEDURE**

Protect all solutions of Metolazone from light.

Buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile, methanol, and *Buffer* (10:25:65)

Standard stock solution: 0.5 mg/mL of USP Metolazone RS in tetrahydrofuran

Standard solution: 0.05 mg/mL of USP Metolazone RS in alcohol from the *Standard stock solution*

Sample stock solution: 1 mg/mL of Metolazone in tetrahydrofuran

Sample solution: 0.05 mg/mL of Metolazone in alcohol from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.4

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metolazone ($C_{16}H_{16}ClN_3O_3S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metolazone from the *Sample solution*

r_S = peak response of metolazone from the *Standard solution*

C_S = concentration of USP Metolazone RS in the *Standard solution* (µg/mL)

C_U = concentration of Metolazone in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.

Standard stock solution: 0.48 mg/mL of USP Metolazone RS in tetrahydrofuran

Standard solution: 6 µg/mL of USP Metolazone RS in alcohol from the *Standard stock solution*

Sample solution: 0.6 mg/mL of Metolazone, prepared as follows. Dissolve a suitable amount of Metolazone with tetrahydrafuran in 50% of the total volume, and dilute with alcohol to volume.

Chromatographic system: Proceed as directed in the *Assay*, except for the following:

Column: 4.6-mm × 25-cm; 5-µm packing 1

Run time: NLT 3.5 times the retention time of metolazone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.4

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of metolazone from the *Standard solution*

C_S = concentration of USP Metolazone RS in the *Standard solution* (mg/mL)

C_U = concentration of Metolazone in the *Sample solution* (mg/mL)

F = relative response factor of each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desmethyl metolazone[a]	0.7	1.0	0.5
Metolazone benzamide analog[b]	0.8	0.83	0.5
Metolazone	1.0	1.0	—
meta-Metolazone[c]	1.3	0.91	0.5
para-Metolazone[d]	1.4	0.91	0.5
Didehydrometolazone[e]	1.5	0.83	0.5
Any other individual impurity	—	—	0.10
Total impurities[f]	—	—	1.0

[a] 7-Chloro-2-methyl-4-oxo-3-phenyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.
[b] 2-Amino-4-chloro-5-sulfamoyl-*N*-(*o*-tolyl)benzamide.
[c] 7-Chloro-2-methyl-4-oxo-3-(*m*-tolyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.
[d] 7-Chloro-2-methyl-4-oxo-3-(*p*-tolyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.
[e] 7-Chloro-2-methyl-4-oxo-3-(*o*-tolyl)-3,4-dihydroquinazoline-6-sulfonamide.
[f] Sum of all individual impurities. Disregard any peaks less than 0.05%.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Metolazone RS

Metolazone Compounded Oral Suspension

DEFINITION

Metolazone Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of metolazone ($C_{16}H_{16}ClN_3O_3S$).

Prepare Metolazone Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Metolazone	100 mg

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Vehicle: a 1:1 mixture of Vehicle for Oral Solution, (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar and comminute to a fine powder, or use *Metolazone* powder. Add 20 mL of *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions, and transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add *Vehicle* in portions to rinse the mortar, then add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and water (70:30) containing 1.5 g/L of ammonium acetate and 1 mL/L of diisopropylamine. Filter, and degas.

Standard solution: 1.0 µg/mL of USP Metolazone RS

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the sample to a 1000-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 20-cm; 5-µm packing L3

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for metolazone is about 6.0 min.]

Suitability requirements

Relative standard deviation: NMT 2.2% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metolazone ($C_{16}H_{16}ClN_3O_3S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metolazone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metolazone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.6–4.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.

• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator

• **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Metolazone RS

Metolazone Tablets

DEFINITION

Metolazone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metolazone ($C_{16}H_{16}ClN_3O_3S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩**,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Sample solution: Dilute 3 mL of the *Sample solution* in the *Assay* with methanol to 25 mL.

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

[NOTE—Use low-actinic glassware throughout the *Assay*.]

Buffer: 1.38 g of monobasic potassium phosphate monohydrate in 900 mL of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 1000 mL.

Mobile phase: Methanol, acetonitrile, and *Buffer* (28:7:65)

Standard stock solution: 0.25 mg/mL of USP Metolazone RS in methanol

Standard solution: 5 µg/mL of USP Metolazone RS in *Mobile phase* from *Standard stock solution*

Sample stock solution: Transfer 10 Tablets to a 200-mL volumetric flask. Add 3 mL of water and 100 mL of methanol, and sonicate for 30 min. If disintegration is not complete, sonicate for an additional 30 min. Shake by mechanical means for 30 min. Dilute with methanol to volume.

Sample solution: Nominally equivalent to 5 µg/mL of metolazone in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metolazone ($C_{16}H_{16}ClN_3O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metolazone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

[NOTE—Protect all solutions from light.]

Test 1

Medium: 2% w/v sodium lauryl sulfate in 0.05 M monobasic sodium phosphate. Heat the mixture to about

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37° to dissolve the sodium lauryl sulfate, and adjust with 10 N sodium hydroxide to a pH of 7.5; 900 mL, deaerated
Apparatus 2: 75 rpm
Time: 120 min
Buffer: 0.05 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.00.
Mobile phase: Acetonitrile, methanol, and *Buffer* (270:50:680)
Standard stock solution: 0.28 mg/mL of USP Metolazone RS. Initially add methanol to 2% of the volume of the flask. Sonicate to dissolve, and dilute with *Medium* to volume.
Standard solution: (*L*/900) mg/mL in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 2000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metolazone ($C_{16}H_{16}ClN_3O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of metolazone ($C_{16}H_{16}ClN_3O_3S$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Prepare a solution of 0.05 M dibasic sodium phosphate in a suitable flask, and adjust with phosphoric acid to a pH of 7.5. Dissolve a suitable amount of sodium lauryl sulfate to obtain a 20-g/L solution; 900 mL
Apparatus 2: 75 rpm
Time: 120 min
Standard stock solution: 0.275 mg/mL of USP Metolazone RS. Initially add methanol to 10% of the volume of the flask. Sonicate to dissolve, and dilute with *Medium* to volume.
Standard solution: (*L*/900) mg/mL in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Detector: UV 238 nm
Path length: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metalozone ($C_{16}H_{16}ClN_3O_3S$) dissolved:

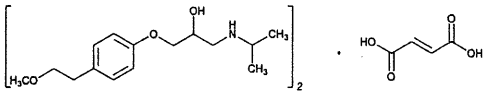
$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of metolazone ($C_{16}H_{16}ClN_3O_3S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store below 30°.
• **LABELING** When more than one test for *Dissolution* is given, the *Labeling* section states the test for *Dissolution* used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Metolazone RS

Metoprolol Fumarate



$(C_{15}H_{25}NO_3)_2 \cdot C_4H_4O_4$ 650.80
2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (*E*)-2-butanedioate (2:1) (salt); (±)-1-(Isopropylamino)-3-[*p*-(2-methoxyethyl)phenoxy]-2-propanol fumarate (2:1) (salt) [119637-66-0].

DEFINITION
Metoprolol Fumarate contains NLT 98.0% and NMT 102.0% of metoprolol fumarate [$(C_{15}H_{25}NO_3)_2 \cdot C_4H_4O_4$], calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid
Solution B: Acetonitrile
Mobile phase: *Solution A* and *Solution B* (60:40)
Standard solution: 1 mg/mL of USP Metoprolol Fumarate RS in *Mobile phase*
Sample solution: 1 mg/mL of Metoprolol Fumarate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL

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Run time: 10 min
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of metoprolol fumarate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_4O_4]$ in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metoprolol from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Metoprolol Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Organic Impurities**

Solution A: 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
8	60	40
13	10	90
13.1	60	40
16	60	40

Diluent: *Solution A* and *Solution B* (60:40)
System suitability solution: 5 µg/mL each of USP Metoprolol Fumarate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, and USP Metoprolol Related Compound C RS in *Diluent*
Standard solution: 2.5 µg/mL each of USP Metoprolol Fumarate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Compound C RS, and USP Metoprolol Related Compound D RS in *Diluent*
Sample solution: 1 mg/mL of Metoprolol Fumarate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between metoprolol related compound A and metoprolol related compound B; NLT 2.5 between metoprolol related compound B and metoprolol related compound C, *System suitability solution*
Relative standard deviation: NMT 2.0% for metoprolol, metoprolol related compound A, metoprolol related compound B, metoprolol related compound C, and metoprolol related compound D, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the corresponding metoprolol related compound in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding metoprolol related compound in the *Sample solution*
r_S = peak response of the corresponding metoprolol related compound in the *Standard solution*
C_S = concentration of the corresponding USP Metoprolol Related Compound RS in the *Standard solution* (µg/mL)
C_U = concentration of Metoprolol Fumarate in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity in the *Sample solution*
r_S = peak response of metoprolol in the *Standard solution*
C_S = concentration of USP Metoprolol Fumarate RS in the *Standard solution* (µg/mL)
C_U = concentration of Metoprolol Fumarate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks below 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fumaric acid	0.2	—
Metoprolol related compound C[a]	0.6	0.10
Metoprolol related compound B[b]	0.7	0.10
Metoprolol related compound A[c]	0.8	0.10
Metoprolol	1.0	—
Metoprolol related compound D[d]	1.5	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

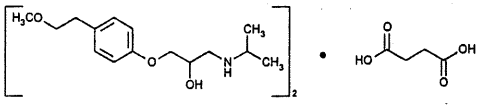
[a] 4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde hydrochloride.
[b] 1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
[c] 1-Ethylamino-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
[d] *N,N*-Bis{2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl}isopropylamine hydrochloride.

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SPECIFIC TESTS
- **pH** ⟨791⟩: 5.5–6.5, in a solution (1 in 10)
- **Loss on Drying** ⟨731⟩
  Analysis: Dry under vacuum at 60° for 4 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Metoprolol Fumarate RS
  USP Metoprolol Related Compound A RS
  1-Ethylamino-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
  $C_{14}H_{23}NO_3$ 253.34
  USP Metoprolol Related Compound B RS
  1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
  $C_{12}H_{17}ClO_3$ 244.71
  USP Metoprolol Related Compound C RS
  4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde hydrochloride.
  $C_{13}H_{19}NO_3 \cdot HCl$ 273.76
  USP Metoprolol Related Compound D RS
  N,N-Bis{2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl}isopropylamine hydrochloride.
  $C_{27}H_{41}NO_6 \cdot HCl$ 512.08

Metoprolol Succinate



$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$ 652.82

2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-, butanedioate (2:1) (salt).
(±)-1-(Isopropylamino)-3-[*p*-(2-methoxyethyl)phenoxy]-2-propanol succinate (2:1) (salt) [98418-47-4].

» Metoprolol Succinate contains not less than 98.0 percent and not more than 102.0 percent of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Metoprolol Related Compound A RS
(±)1-Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.
$C_{14}H_{23}NO_3$ 253.34
USP Metoprolol Related Compound B RS
(±)1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane.
$C_{12}H_{17}ClO_3$ 244.71
USP Metoprolol Related Compound C RS
(±)4-[2-Hydroxy-3-(1-methylethyl)aminopropoxy]benzaldehyde.
$C_{13}H_{19}NO_3$ 237.29
USP Metoprolol Related Compound D RS
(±) *N,N*-Bis[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine.
$C_{27}H_{41}NO_6$ 475.62
USP Metoprolol Succinate RS
Clarity and color of solution—A solution of Metoprolol Succinate having a concentration of 20 mg per mL is not less clear than an equal volume of water in a test tube of similar size. The absorbance of the solution determined at 440 nm in a 5-cm cell, using water as the blank, is not more than 0.1.

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
pH ⟨791⟩: between 7.0 and 7.6, in a solution containing 65 mg per mL.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 0.2% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—
TEST 1—
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.
Test solution—Dissolve an accurately weighed quantity of Metoprolol Succinate in methanol to obtain a solution containing 50 mg per mL.
Standard solution—Dilute the *Test solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of 0.1 mg per mL.
Application volume: 10 µL.
Developing solvent system: a mixture of ethyl acetate and methanol (80:20).
Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Place two 50-mL beakers, each containing 30 mL of ammonium hydroxide, on the bottom of a chromatographic chamber that is lined with filter paper and contains the *Developing solvent system,* and allow to equilibrate for 1 hour. Position the plate in the chromatographic chamber, and develop the chromatogram until the solvent front has moved about two-thirds of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry the plate for 3 hours in a current of warm air. Place the plate in a chamber containing iodine vapor, and allow to react for at least 15 hours. Compare the intensities of the brown spots appearing on the chromatogram: any secondary spot obtained from the *Test solution* is not more intense than the corresponding spot obtained from the *Standard solution.* Not more than 0.2% is found.
TEST 2—
Sodium dodecyl sulfate solution, Mobile phase, and *Resolution solution*—Prepare as directed in the *Assay.*
Standard solution—Dissolve an accurately weighed quantity of USP Metoprolol Succinate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 µg per mL.
Test solution—Transfer about 50 mg of Metoprolol Succinate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay.* Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between metoprolol related compound A and metoprolol related compound B is not less than 2.5; and the resolution, *R,* between metoprolol related compound B and metoprolol related compound C is not less than 1.5. [Note—The relative retention times are about 0.6 for

USP Monographs

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metoprolol related compound C, 0.7 for metoprolol related compound B, 0.8 for metoprolol related compound A, 1.0 for metoprolol, and 5.0 and 5.2 for the two diastereomers of metoprolol related compound D.] Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Metoprolol Succinate taken by the formula:

$$100(C_S/C_T)(r_i/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Metoprolol Succinate RS in the *Standard solution;* C_T is the concentration of metoprolol succinate in the *Test solution;* r_i is the individual peak response of related impurities; and r_S is the peak response obtained from the *Standard solution:* not more than 0.1% of any single impurity is found, and not more than 0.5% of total impurities is found. [NOTE—The sum of the peak responses for the two diastereomers of metoprolol related compound D is used in the above calculation to report the amount of metoprolol related compound D.]

Assay—

Sodium dodecyl sulfate solution—Add 1.3 g of sodium dodecyl sulfate to 1 L of aqueous phosphoric acid, 0.1% (w/v).

Mobile phase—Prepare a filtered and degassed mixture of *Sodium dodecyl sulfate solution* and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Prepare a solution in *Mobile phase* containing about 5 µg each of USP Metoprolol Succinate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Related Compound C RS, and USP Metoprolol Related Compound D RS per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Metoprolol Succinate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg per mL.

Test preparation—Transfer about 80 mg of Metoprolol Succinate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 223-nm detector and a 4-mm × 12.5-cm column that contains 4-µm packing L7. The column temperature is maintained at 30°. The flow rate is about 0.9 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between metoprolol related compound A and metoprolol related compound B is not less than 2.5; and the resolution, *R,* between metoprolol related compound B and metoprolol related compound C is not less than 1.5. [NOTE—The relative retention times are about 0.6 for metoprolol related compound C, 0.7 for metoprolol related compound B, 0.8 for metoprolol related compound A, 1.0 for metoprolol, and 5.0 and 5.2 for the two diastereomers of metoprolol related compound D.] Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject equal volumes (about 10 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms for at least 1.5 times the retention of the metoprolol peak, and measure the peak responses. Calculate the quantity, in mg, of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$ in the portion of Metoprolol Succinate taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Metoprolol Succinate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation,* respectively.

Metoprolol Succinate Extended-Release Tablets

DEFINITION

Metoprolol Succinate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol succinate [$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$].

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample solution: Equivalent to 200 mg of metoprolol succinate from NLT 1 Tablet in a stoppered centrifuge tube. Add 40 mL of pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*) and 40 mL of methylene chloride, and shake for 5 min. Centrifuge, filter, and use the aqueous phase as the *Sample solution.*

Sample: Transfer 3 mL of the *Sample solution* to a separator. Add 2 mL of ammonium hydroxide, and extract with 20 mL of methylene chloride. Filter the methylene chloride phase. Grind 1 mL of the filtrate with 300 mg of potassium bromide, dry in a current of warm air, and prepare a disk.

Acceptance criteria: The IR spectrum of the *Sample* exhibits maxima only at the same wavelengths as those obtained from a similar preparation of USP Metoprolol Succinate RS (presence of metoprolol).

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Transfer 5 mL of the *Sample solution* prepared in *Identification A* to a glass-stoppered test tube. Add 2 mL of 5 N hydrochloric acid, and extract with 5 mL of ether. Filter the ether phase. Grind 2 mL of the filtrate with 300 mg of potassium bromide, dry in a current of warm air, and prepare a disk.

Acceptance criteria: The IR spectrum of the *Sample* exhibits maxima only at the same wavelengths as those obtained from a similar preparation of succinic acid (presence of succinate).

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: Mix 50 mL of 1 M monobasic sodium phosphate and 8.0 mL of 1 M phosphoric acid, and dilute with water to

USP Monographs

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1000 mL. If necessary, adjust with 1 M monobasic potassium phosphate or 1 M phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (250:750)
Standard solution: 0.05 mg/mL of USP Metoprolol Succinate RS in *Mobile phase*
Sample stock solution: Nominally 1 mg/mL of metoprolol succinate prepared as follows. Transfer a suitable number of Tablets to a suitable volumetric flask, add about 5 mL of water, and allow the Tablets to disintegrate. Add a volume of alcohol to fill 30% of the flask volume, and shake for 30 min. Add a portion of 0.1 N hydrochloric acid to fill 50% of the flask volume, and shake for an additional 30 min. Dilute with 0.1 N hydrochloric acid to volume. Filter, and discard the first 10 mL of the filtrate.
Sample solution: Nominally 0.05 mg/mL of metoprolol succinate from the *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4-mm × 12.5-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metoprolol from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metoprolol succinate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 500 mL
Apparatus 2: 50 rpm
Times: 1, 4, 8, and 20 h
Buffer, Mobile phase, and **Standard solution:** Prepare as directed in the *Assay*.
Analysis: Proceed as directed in the *Assay*, except use 5.0 mL of a filtered portion of the solution under test as the *Sample solution*, and use *Medium* as the blank, in comparison with a *Standard solution* with a known concentration of USP Metoprolol Succinate RS in the same *Medium*.
Acceptance criteria: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	NMT 25
4	20–40
8	40–60
20	NLT 80

The percentages of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Simulated gastric fluid without enzyme, pH 1.2; 500 mL
Apparatus 2: 75 rpm
Times: 1, 4, 8, and 20 h
Buffer: 1 M monobasic sodium phosphate, 1 M phosphoric acid, and water (50:8:942). If necessary, adjust with 1 M monobasic sodium phosphate or 1 M phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (250:750)
Standard solution: Prepare a solution of USP Metoprolol Succinate RS in *Medium* as directed in *Table 2*.

Table 2

Tablet Strength (mg, as metoprolol succinate)	Concentration (mg/mL)
200	0.380
100	0.190
50	0.095
25	0.048

Sample solution: Pass the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 12.5-cm; 4-µm packing L7
Flow rate: 1 mL/min
Injection volume: See *Table 3*.

Table 3

Tablet Strength (mg, as metoprolol succinate)	Volume (µL)
25	40
50	20
100	10
200	5

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of metoprolol succinate dissolved in *Medium* at each time point (i):

$$\text{Result} = (r_U/r_S) \times C_S$$

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r_U = peak response of metoprolol from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved (Q_i), at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of metoprolol succinate in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	4	20–40
3	8	55–85
4	20	NLT 80

The percentages of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.
Medium: Phosphate buffer, pH 6.8 (dissolve 6.8 g of monobasic potassium phosphate and 0.93 g of sodium hydroxide in 1 L of water; adjust with sodium hydroxide to a pH of 6.8); 500 mL
Apparatus 2: 50 rpm
Times: 1, 4, 8, and 24 h
Buffer: 5.0 mL/L of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Methanol and *Buffer* (40:60)
Standard solution: Prepare a solution of USP Metoprolol Succinate RS in *Medium* as directed in *Table 5*.

Table 5

Tablet Strength (mg)	Concentration (mg/mL)
200	0.4
100	0.2
50	0.1
25	0.05

Sample solution: Withdraw a 10-mL aliquot at each time point. Pass the solution under test through a suitable filter of 0.45-µm pore size. Replace the portion withdrawn with an equal volume of *Medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 5 µL
Run time: NLT 2 times the retention time of metoprolol
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of metoprolol succinate dissolved in *Medium* at each time point (i):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of metoprolol from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved (Q_i), at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of metoprolol succinate in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (i)	Time (h)	Amount Dissolved (Tablet labeled 25 mg) (%)	Amount Dissolved (Tablets labeled 50, 100, and 200 mg) (%)
1	1	NMT 20	NMT 20
2	4	20–40	15–35
3	8	42–67	38–64
4	24	NLT 80	NLT 80

The percentages of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.

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Medium: Phosphate buffer, pH 6.8 (dissolve 27.22 g of monobasic potassium phosphate and 3.6 g of sodium hydroxide in 4 L of water; adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 6.8); 500 mL
Apparatus 2: 50 rpm, with sinkers
Times: 1, 4, 8, and 20 h
Buffer: Transfer 3.0 mL of triethylamine and 1.0 mL of phosphoric acid to a 1000-mL volumetric flask that contains 600 mL of water. Dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (25:75)
Standard solution: Prepare a solution of USP Metoprolol Succinate RS in *Medium* as directed in *Table 7*.

Table 7

Tablet Strength (mg)	Concentration (mg/mL)
200	0.2
100	0.2
50	0.05
25	0.05

Sample solution: Withdraw a 10-mL aliquot at each time point. Pass the solution under test through a suitable filter of 0.45-µm pore size. Replace the portion withdrawn with an equal volume of *Medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 40 µL for 25 and 50 mg; 10 µL for 100 and 200 mg
Run time: NLT 2 times the retention time of metoprolol
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of metoprolol succinate dissolved in *Medium* at each time point (i):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of metoprolol from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of metoprolol succinate [$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$] dissolved ($Q_i$), at each time point ($i$):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of metoprolol succinate in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 8*.

Table 8

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 10
2	4	5–30
3	8	30–55
4	20	NLT 75

The percentages of the labeled amount of metoprolol succinate [$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$] dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer: 1.15 mL of phosphoric acid in 2 L of water. Add 2.6 g of sodium dodecyl sulfate. Sonicate to dissolve.
Solution A: Methanol and *Buffer* (30:70)
Solution B: Acetonitrile and *Buffer* (75:25)
Mobile phase: See *Table 9*.

Table 9

Time (min)	Solution A (%)	Solution B (%)
0	65	35
20	65	35
25	40	60
30	35	65
35	35	65
37	65	35
50	65	35

Diluent: Acetonitrile and *Buffer* (40:60)
System suitability solution: 3 µg/mL of USP Metoprolol Related Compound A RS and 1 mg/mL of USP Metoprolol Succinate RS in *Diluent*
Standard solution: 3 µg/mL of USP Metoprolol Succinate RS in *Diluent*
Sensitivity solution: 0.5 µg/mL of USP Metoprolol Succinate RS from *Standard solution* in *Diluent*
Sample solution: Nominally 1 mg/mL of metoprolol succinate from Tablets prepared as follows. Transfer a portion of finely powdered Tablets (NLT 20), equivalent to 50 mg of metoprolol succinate, to a 50-mL volumetric flask. Add *Diluent* to fill 60% of the flask volume and sonicate for 30 min with intermittent shaking. Dilute with *Diluent* to volume. Pass the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min

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Injection volume: 10 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between metoprolol related compound A and metoprolol, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Succinate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoprolol succinate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 10*. Reporting threshold: 0.05%.

Table 10

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Succinic acid[a]	0.1	—
Metoprolol related compound A	0.83	—
Metoprolol	1.0	—
Any unspecified degradation product	—	0.20
Total impurities	—	0.75

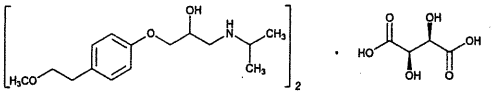
[a] Counter ion included for identification only.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label it to indicate the content of metoprolol succinate and its equivalent, expressed as metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Metoprolol Related Compound A RS
1-Ethylamino-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
$C_{14}H_{23}NO_3$ 253.34
USP Metoprolol Succinate RS

Metoprolol Tartrate

Change to read:



$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ 684.81
2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-, [*R*-(*R**,*R**)]-2,3-dihydroxy butanedioate (2:1) (salt);
▲(±)-1-(Isopropylamino)-3-[*p*-(2-methoxyethyl)phenoxy]-2-propanol (+)-tartrate (2:1) (salt);
1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol L-tartrate▲ (ERR 1-Dec-2018) [56392-17-7].

DEFINITION

Metoprolol Tartrate contains NLT 98.0% and NMT 102.0% of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
[NOTE—Use all solutions within 48 h.]
Buffer: 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid
Mobile phase: Acetonitrile and *Buffer* (400:600)
Standard solution: 1 mg/mL of USP Metoprolol Tartrate RS in *Mobile phase*
Sample solution: 1 mg/mL of Metoprolol Tartrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in the portion of sample taken:

$$\text{Result} = (r_u/r_S) \times (C_S/C_u) \times 100$$

r_u = peak response of metoprolol from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (mg/mL)

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C_U = concentration of Metoprolol Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

[NOTE—Use all solutions within 48 hrs.]

Buffer, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 5 µg/mL each of USP Metoprolol Tartrate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, and USP Metoprolol Related Compound C RS in *Mobile phase*

Standard solution: 1 µg/mL each of USP Metoprolol Tartrate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Related Compound C RS, and USP Metoprolol Related Compound D RS in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between metoprolol related compound A and metoprolol related compound B; NLT 2.5 between metoprolol related compound B and metoprolol related compound C, *System suitability solution*

Relative standard deviation: NMT 5.0% for the metoprolol peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity in the *Sample solution*

r_S = peak response of the corresponding metoprolol related compound or metoprolol (for calculating any individual unspecified impurity) in the *Standard solution*

C_S = concentration of the corresponding USP Metoprolol Related Compound RS or USP Metoprolol Tartrate RS (for calculating any individual unspecified impurity) in the *Standard solution* (mg/mL)

C_U = concentration of Metoprolol Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metoprolol related compound C[a]	0.65	0.10
Metoprolol related compound B[b]	0.72	0.10
Metoprolol related compound A[c]	0.83	0.10
Metoprolol	1.00	—
Metoprolol related compound D[d, e]	4.78/5.00	0.10
Any individual unspecified impurity	—	0.10
Total impurities[f]	—	0.50

[a] (±)-4-[2-Hydroxy-3-(1-isopropyl)aminopropoxy]benzaldehyde).
[b] (±)-1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane.
[c] (±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.
[d] (±)-*N,N*-bis-[2-Hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine hydrochloride.
[e] The sum of the peak responses of the two diastereomers is used to calculate the amount of metoprolol related compound D.
[f] Disregard any peak due to tartaric acid at about RRT 0.17.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 20 mg/mL in water
  Acceptance criteria: +6.5° to +10.5° (t = 20°)
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL of Metoprolol Tartrate in water
  Acceptance criteria: 6.0–7.0
- **LOSS ON DRYING** ⟨731⟩
  Sample solution: Dry a sample in a vacuum at 60° for 4 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metoprolol Tartrate RS
  USP Metoprolol Related Compound A RS
  (±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.
  $C_{14}H_{23}NO_3$ 253.34
  USP Metoprolol Related Compound B RS
  (±)-1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane.
  $C_{12}H_{17}ClO_3$ 244.71
  USP Metoprolol Related Compound C RS
  (±)-4-[2-Hydroxy-3-(1-isopropyl)aminopropoxy] benzaldehyde.
  $C_{13}H_{19}NO_3$ 237.29
  USP Metoprolol Related Compound D RS
  (±)-*N,N*-bis-[2-Hydroxy-3-[4-(2-methoxyethyl)phenoxy] propyl](1-methylethyl)amine hydrochloride.
  $C_{27}H_{41}NO_6$ 512.08

Metoprolol Tartrate Injection

DEFINITION

Metoprolol Tartrate Injection is a sterile solution of Metoprolol Tartrate in Water for Injection. It contains Sodium Chloride as a tonicity-adjusting agent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$.

IDENTIFICATION

Change to read:

- **A.** ▲The retention time of metoprolol of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

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Add the following:

▲• **B.** The UV-Vis spectrum of the major peak of the *Diluted sample stock solution* corresponds to that of the *Diluted standard stock solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**

Solution A: 9.0 mg/mL of sodium chloride in water

Mobile phase: 961 mg of 1-pentanesulfonic acid sodium salt (monohydrate) and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methanol and 470 mL of water. Add 0.57 mL of glacial acetic acid.

Internal standard solution: 0.72 mg/mL of USP Oxprenolol Hydrochloride RS in freshly prepared *Mobile phase*

Standard stock solution: 1 mg/mL of USP Metoprolol Tartrate RS in *Solution A*

Standard solution: *Standard stock solution* and *Internal standard solution* (1:1)

▲**Diluted standard stock solution:** 0.1 mg/mL of USP Metoprolol Tartrate RS from *Standard stock solution* in *Solution A*▲ (USP 1-May-2019)

Sample stock solution: Nominally 1 mg/mL of metoprolol tartrate from Injection prepared as follows. Transfer an accurately measured volume of Injection, if necessary, into *Solution A*.

Sample solution: *Sample stock solution* and *Internal standard solution* (1:1)

▲**Diluted sample stock solution:** Nominally 0.1 mg/mL of metoprolol tartrate from *Sample stock solution* in *Solution A*▲ (USP 1-May-2019)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)

Column: 3.9-mm × 30-cm; ▲10-µm▲ (USP 1-May-2019) packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for metoprolol and oxprenolol are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between metoprolol and oxprenolol

Relative standard deviation: NMT 2.0% from three replicate injections

Analysis

Samples: *Standard solution*, ▲*Diluted standard stock solution*,▲ (USP 1-May-2019) *Sample solution*, and ▲*Diluted sample stock solution*. [NOTE—The *Diluted standard stock solution* and *Diluted sample stock solution* are used for *Identification B*.]▲ (USP 1-May-2019)

Calculate the percentage of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of metoprolol to oxprenolol from the *Sample solution*

R_S = peak response ratio of metoprolol to oxprenolol from the *Standard solution*

C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A: 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid

Solution B: 9.0 mg/mL of sodium chloride in water. [NOTE—This solution is only needed when sample dilution is required.]

Mobile phase: Acetonitrile and *Solution A* (40:60)

System suitability solution: 5 µg/mL each of USP Metoprolol Tartrate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, and USP Metoprolol Related Compound C RS in *Mobile phase*

Standard solution: 2.5 µg/mL each of USP Metoprolol Tartrate RS and USP Metoprolol Related Compound C RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of metoprolol tartrate from a volume of Injection. Transfer an accurately measured volume of Injection, if necessary, into *Solution B*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for metoprolol and metoprolol related compounds are listed in *Table 1*.]

Suitability requirements

Resolution: NLT 1.5 between metoprolol related compound A and metoprolol related compound B; NLT 2.5 between metoprolol related compound B and metoprolol related compound C, *System suitability solution*

Relative standard deviation: NMT 3% for metoprolol and metoprolol related compound C, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metoprolol related compound C in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metoprolol related compound C from the *Sample solution*

r_S = peak response of metoprolol related compound C from the *Standard solution*

C_S = concentration of USP Metoprolol Related Compound C RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Injection taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tartaric acid	0.13	—
Metoprolol related compound C	0.64	0.4
Metoprolol related compound B[a]	0.73	—
Metoprolol related compound A[a]	0.83	—
Metoprolol	1.0	—
Any unspecified degradation product	—	0.2
Total degradation products	—	1.0

[a] Specified impurities controlled in the drug substance. They are not to be included in the calculation of the total degradation products. ▲(USP 1-May-2019)

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.0–8.0
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 25.0 USP Endotoxin Units/mg of metoprolol tartrate
- **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to be Examined, Membrane Filtration*: Meets the requirements
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose, light-resistant containers, preferably of Type I or Type II glass. ▲Store at controlled room temperature.▲(USP 1-May-2019)

Change to read:

- **USP Reference Standards** ⟨11⟩
  USP Metoprolol Tartrate RS
  ▲USP Metoprolol Related Compound A RS
  1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
  $C_{14}H_{23}NO_3$ 253.34
  USP Metoprolol Related Compound B RS
  1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
  $C_{12}H_{17}ClO_3$ 244.71
  USP Metoprolol Related Compound C RS
  4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde hydrochloride.
  $C_{13}H_{19}NO_3 \cdot HCl$ 273.76▲(USP 1-May-2019)
  USP Oxprenolol Hydrochloride RS

Metoprolol Tartrate Compounded Oral Solution

DEFINITION
Metoprolol Tartrate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$.
Prepare Metoprolol Tartrate Compounded Oral Solution 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Metoprolol Tartrate powder	1 g
Vehicle for Oral Solution (regular or sugar-free), *NF*, a sufficient quantity to make	100 mL

Add *Metoprolol Tartrate powder* and 20 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

ASSAY
- **Procedure**

Mobile phase: 961 mg of 1-pentanesulfonic acid sodium salt (monohydrate) and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methanol and 470 mL of water. Add 0.57 mL of glacial acetic acid. Filter, and degas.
Standard solution: 100 µg/mL of USP Metoprolol Tartrate RS
Sample solution: Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the sample to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[Note—The retention time for metoprolol tartrate is about 7.3 min.]
Suitability requirements
Relative standard deviation: NMT 1.3% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

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SPECIFIC TESTS
- **pH** ⟨791⟩: 3.6–4.6

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
- **Labeling:** Label it to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Metoprolol Tartrate RS

Metoprolol Tartrate Compounded Oral Suspension

DEFINITION
Metoprolol Tartrate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$.
Prepare Metoprolol Tartrate Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Metoprolol Tartrate	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar, and comminute to a fine powder, or use *Metoprolol Tartrate* powder. Add the *Vehicle* in small portions, and mix well. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add the *Vehicle* in portions to rinse the mortar. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY
- **Procedure**

Mobile phase: 961 mg of 1-pentanesulfonic acid sodium salt (monohydrate) and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methanol and 470 mL of water. Add 0.57 mL of glacial acetic acid. Filter, and degas.
Standard solution: 100 µg/mL of USP Metoprolol Tartrate RS
Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[Note—The retention time for metoprolol tartrate is about 7.3 min.]
Suitability requirements
Relative standard deviation: NMT 1.3% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.6–4.6

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator
- **Labeling:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Metoprolol Tartrate RS

Metoprolol Tartrate Tablets

DEFINITION
Metoprolol Tartrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$.

IDENTIFICATION
- **A.**

Standard solution: 0.1 mg/mL of USP Metoprolol Tartrate RS in water
Sample solution: Transfer an amount equivalent to 50 mg of metoprolol tartrate from a quantity of finely powdered Tablets to a 500-mL volumetric flask, dilute with water to volume, and mix. Pass a portion of the solution through a filter of 1-µm or finer pore size.
Acceptance criteria: The UV spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution.*
- **B.** The retention time of the metoprolol peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **Procedure**

Mobile phase: 961 mg of sodium 1-pentanesulfonate and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methanol and 470 mL of water. Add 0.57 mL of glacial acetic acid.
Diluent: Methanol and 0.1 N hydrochloric acid (1:1)
System suitability stock solution: 0.72 mg/mL of USP Oxprenolol Hydrochloride RS in *Diluent*

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Standard stock solution: 1 mg/mL of USP Metoprolol Tartrate RS in *Diluent*
System suitability solution: *System suitability stock solution* and *Standard stock solution* (1:1)
Standard solution: 0.5 mg/mL of USP Metoprolol Tartrate RS from the *Standard stock solution* in *Mobile phase*
Sample stock solution: Nominally 1 mg/mL of metoprolol tartrate from Tablets prepared as follows. Transfer a portion of finely powdered Tablets (NLT 20), equivalent to about 50 mg of metoprolol tartrate, to a 50-mL volumetric flask, add 30 mL of *Diluent*, shake by mechanical means for 30 min, sonicate for 15 min, and heat on a steam bath for 10 min. Allow the solution to cool to room temperature, dilute with *Diluent* to volume, and centrifuge a portion of the solution. Use the supernatant.
Sample solution: Nominally 0.5 mg/mL of metoprolol tartrate prepared from the *Sample stock solution* in *Mobile phase*. Pass a portion of the solution through a filter of 0.5-µm or finer pore size. Discard the first few milliliters of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 30 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for metoprolol and oxprenolol are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between metoprolol and oxprenolol, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metoprolol from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*
C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Simulated gastric fluid TS (without enzyme); 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: USP Metoprolol Tartrate RS with a known concentration in *Medium*
Sample solution: Sample per the chapter. Dilute with *Medium* as needed.
Instrumental conditions
Mode: UV
Analytical wavelength: 275 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 75% (*Q*) of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: 3.9 g of ammonium acetate in 810 mL of water. Add 2.0 mL of triethylamine, 10.0 mL of glacial acetic acid, and 3.0 mL of phosphoric acid. [NOTE—Adjust the pH of the solution to 3.7 if needed.]
Solution B: Acetonitrile and *Solution A* (70:30)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15.0	80	20
30.0	40	60
40.0	40	60
40.1	90	10
45.0	90	10

Diluent: Acetonitrile and *Solution A* (17:83)
System suitability solution: 0.01 mg/mL each of tyrosol and USP Metoprolol Related Compound C RS in *Diluent*
Standard solution: 0.01 mg/mL of USP Metoprolol Tartrate RS in *Diluent*
Sensitivity solution: 0.7 µg/mL of USP Metoprolol Tartrate RS from the *Standard solution* in *Diluent*
Sample solution: Nominally 1 mg/mL of metoprolol tartrate from Tablets prepared as follows. Transfer 100 mg of metoprolol tartrate from a quantity of finely powdered Tablets (NLT 20) to a 100-mL volumetric flask, and add 50 mL of *Diluent*. Sonication and stirring may be necessary for complete dissolution. Dilute with *Diluent* to volume. Centrifuge a portion of the solution. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between tyrosol and metoprolol related compound C, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of metoprolol from the *Standard solution*

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C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks below 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tyrosol[a]	0.41	—
Metoprolol related compound C[b]	0.43	—
Metoprolol related compound A[b, c]	0.83	—
Metoprolol	1.00	—
Metoprolol related compound D[b, d]	1.57	—
Metoprolol related compound D[b, d]	1.58	—
Metoprolol related compound B[b, e]	1.68	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.0

[a] 2-(4-Hydroxyphenyl)ethanol. For resolution measurement only.
[b] Specified impurities controlled in the drug substance.
[c] (±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.
[d] (±)-*N*,*N*-Bis-[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine hydrochloride. It has two diastereomers.
[e] (±)-1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metoprolol Related Compound C RS
  4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde hydrochloride.
  $C_{13}H_{19}NO_3 \cdot HCl$ 273.76
  USP Metoprolol Tartrate RS
  USP Oxprenolol Hydrochloride RS

Metoprolol Tartrate and Hydrochlorothiazide Tablets

DEFINITION

Metoprolol Tartrate and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A.** The retention times of the metoprolol and hydrochlorothiazide peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectra of the metoprolol and hydrochlorothiazide peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 4.1 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	85	15
4.0	85	15
10.0	10	90
10.1	85	15
13	85	15

Diluent: Acetonitrile and *Solution A* (15:85)
Standard solution: 0.1 mg/mL of USP Metoprolol Tartrate RS and 0.05 mg/mL of USP Hydrochlorothiazide RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of metoprolol tartrate and 0.05 mg/mL of hydrochlorothiazide in *Diluent* prepared as follows. Transfer a portion of the powder from NLT 20 finely powdered Tablets to a suitable volumetric flask and dissolve in a suitable amount of *Diluent*. Sonication may be necessary for complete dissolution. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 10-cm; 3.5-µm packing L1
Autosampler temperature: 4°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for hydrochlorothiazide and metoprolol are 0.56 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for metoprolol and hydrochlorothiazide
Relative standard deviation: NMT 1.0% for metoprolol and hydrochlorothiazide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metoprolol or hydrochlorothiazide from the *Sample solution*
r_S = peak response of metoprolol or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Metoprolol Tartrate RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

USP Monographs

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Acceptance criteria: 90.0%–110.0% of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Simulated gastric fluid TS (without enzyme); 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Determination of dissolved metoprolol tartrate

Standard solution: 0.05 mg/mL of USP Metoprolol Tartrate RS in *Medium*

Sample solution: Remove 125 mL of the solution under test, allow to cool to room temperature, and filter, discarding the first 25 mL of the filtrate. [NOTE—Retain 30 mL of the remaining filtrate of the solution under test for the *Determination of dissolved hydrochlorothiazide*.] If necessary, prepare 0.05 mg/mL of metoprolol tartrate from the filtrate in fresh *Medium*.

Instrumental conditions

Mode: UV

Analytical wavelength: 276 nm

Cell: 2 cm

Blank: *Medium*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer to separate separators 50.0 mL each of the *Standard solution*, *Sample solution*, and *Blank*. Add 10 mL of 2.5 N sodium hydroxide to each separator, and extract each with three 15-mL portions of chloroform, filtering the chloroform extracts through pledgets of chloroform-prerinsed glass wool into individual 50-mL volumetric flasks. Dilute the contents of each flask with chloroform to volume, and mix. Determine the absorbances of the solutions obtained.

Calculate the percentage of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Determination of dissolved hydrochlorothiazide

Standard solution: 0.03 mg/mL of USP Hydrochlorothiazide RS in *Medium*

Sample solution: Pass a portion of the filtrate retained from the *Determination of dissolved metoprolol tartrate* through a filter of 0.8-µm or finer pore size, and discard the first 5 mL of the filtrate. If necessary, prepare 0.03 mg/mL of hydrochlorothiazide in fresh *Medium*.

Instrumental conditions

Mode: UV

Analytical wavelength: 316 nm

Cell: 2 cm

Blank: *Medium*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Determine the absorbances of the *Standard solution* and the *Sample solution* at the wavelength of maximum absorbance.

Calculate the percentage of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ and NLT 80% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements with respect to metoprolol tartrate and hydrochlorothiazide

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Dissolve 3.9 g of ammonium acetate in 810 mL of water. Add 2.0 mL of triethylamine, 10.0 mL of glacial acetic acid, and 3.0 mL of phosphoric acid. [NOTE—Adjust the solution to a pH of 3.7 if needed.]

Solution B: Acetonitrile and *Solution A* (70:30)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15.0	80	20
30.0	40	60
40.0	40	60
40.1	90	10
45	90	10

Diluent: Acetonitrile and *Solution A* (17:83)

System suitability solution: 0.7 µg/mL of USP Maltol RS, 5 µg/mL of USP Benzothiadiazine Related Compound A RS, and 0.5 mg/mL of USP Hydrochlorothiazide RS in *Diluent*

Standard solution: 10.0 µg/mL of USP Metoprolol Tartrate RS and 2.5 µg/mL of USP Hydrochlorothiazide RS in *Diluent*

Sensitivity solution: 1.0 µg/mL of USP Metoprolol Tartrate RS and 0.25 µg/mL of USP Hydrochlorothiazide RS in *Diluent* from the *Standard solution*

Sample solution: Nominally 1 mg/mL of metoprolol tartrate in *Diluent* prepared as follows. Transfer a suitable portion of NLT 20 finely powdered Tablets, equivalent to 200 mg of metoprolol tartrate, into a suitable volumetric flask and add *Diluent* to about 50% of the flask volume. Sonicate for 20 min with occasional swirling. Stir for 15 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution. Use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detectors

Metoprolol and related impurities: UV 275 nm

Hydrochlorothiazide and related impurities: UV 320 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

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System suitability

Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*

[NOTE—See *Table 3* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between maltol and benzothiadiazine related compound A; NLT 3.0 between benzothiadiazine related compound A and hydrochlorothiazide, *System suitability solution*

Relative standard deviation: NMT 5.0% for metoprolol and hydrochlorothiazide, *Standard solution*

Signal-to-noise ratio: NLT 10 for metoprolol and hydrochlorothiazide, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

For impurities detected at UV 275 nm

Calculate the percentage of maltol and any unspecified degradation product (excluding the peaks that appear at 320 nm) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of maltol or any unspecified degradation product from the *Sample solution*

r_S = peak response of metoprolol from the *Standard solution*

C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (µg/mL)

F = relative response factor (relative to metoprolol)

For impurities detected at UV 320 nm

Calculate the percentage of benzothiadiazine related compound A and any unspecified degradation product (excluding the peaks that appear at 275 nm) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of benzothiadiazine related compound A or any unspecified degradation product from the *Sample solution*

r_S = peak response of hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (µg/mL)

F = relative response factor (relative to hydrochlorothiazide)

Acceptance criteria: See *Table 3*. Disregard peaks below 0.1%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maltol	0.29	17	0.2
Benzothiadiazine related compound A[a]	0.31	1.0	1.0
Hydrochlorothiazide	0.46	—	—
Metoprolol	1.00	—	—
Any unspecified degradation product[b]	—	1.0	0.2
Total degradation products[c]	—	—	1.0

[a] Not to be included in the total degradation products.

[b] Based on the sum of unspecified degradation products determined at 275 nm and at 320 nm.

[c] Excluding benzothiadiazine related compound A.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

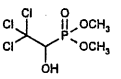
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73

USP Hydrochlorothiazide RS

USP Maltol RS
3-Hydroxy-2-methyl-4-pyrone.
$C_6H_6O_3$ 126.11

USP Metoprolol Tartrate RS

Metrifonate



$C_4H_8Cl_3O_4P$ 257.44

Phosphonic acid, (2,2,2-trichloro-1-hydroxyethyl)-, dimethyl ester.

Dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate [52-68-6].

» Metrifonate contains not less than 98.0 percent and not more than 100.5 percent of $C_4H_8Cl_3O_4P$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers at a temperature not exceeding 25°.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Metrifonate RS
Trichlorfon.

Completeness of solution ⟨641⟩: meets the requirements, 0.5 g of it being dissolved in methanol.

Color of solution ⟨631⟩—The solution obtained in the test for *Completeness of solution* has no more color than *Matching Fluid F.*

Change to read:

Identification—

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Thin-Layer Chromatographic Identification Test ⟨201⟩—

Test solution: Dissolve 10 mg of Metrifonate in methanol, and dilute with methanol to 10.0 mL.

Developing solvent sytstem: a mixture of toluene, dioxane, and glacial acetic acid (70:25:5)

Procedure—Proceed as directed in the chapter. After allowing the plate to air-dry, spray the plate with a 5% solution of

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4-(*p*-nitrobenzyl)pyridine in acetone, and heat at 120° for 15 minutes. Before the plate cools, spray it with a 10% solution of tetraethylenepentamine in acetone, and immediately examine the plate: the principal spot in the chromatogram obtained from the *Test solution* corresponds in R_F value, size, and blue color to that in the chromatogram obtained from the Standard solution.

C: Dissolve 20 mg of Metrifonate in 1 mL of 2 N sodium hydroxide, add 1 mL of pyridine, shake, and heat on a water bath for 2 minutes: a red color develops in the pyridine layer.

D: To 100 mg of Metrifonate add 0.5 mL of nitric acid, 0.5 mL of a 50% solution of ammonium nitrate, and 0.1 mL of 30 percent hydrogen peroxide, and heat on a water bath for 10 minutes. Heat to boiling, and add 1 mL of ammonium molybdate TS: a yellow color precipitate is formed.

Acidity—Dissolve 2.5 g of it in carbon dioxide-free water, dilute with carbon dioxide-free water to 50 mL, and add 0.1 mL of methyl red TS. Not more than 1.0 mL of 0.1 N sodium hydroxide is required to change the color of the indicator.

Water Determination, *Method I* ⟨921⟩**:** not more than 0.3%.

Limit of free chloride—Dissolve 5.0 g of Metrifonate in 30 mL of alcohol, and add a mixture of 100 mL of water and 15 mL of nitric acid. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically using a silver electrode. Not more than 0.7 mL of 0.1 N silver nitrate is consumed (0.05%).

Chromatographic purity—

Solution A—Dissolve 1.36 g of monobasic potassium phosphate in water, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 3.0.

Solution B—Use acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of acetonitrile and water (1:1).

Standard preparation—Prepare a solution of USP Metrifonate RS in *Diluent* containing 20 mg per mL.

Test solution—Transfer 500 mg of Metrifonate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4-mm × 25-cm column that contains 5-µm packing L7. The column is maintained at a constant temperature of about 40°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	90	10	equilibration (10 minutes)
0–5	90	10	isocratic
5	90→85	10→15	step gradient
5–25	85	15	isocratic
25	85→45	15→55	step gradient
25–end*	45	55	isocratic

* The elution concludes at 3 times the retention time of metrifonate.

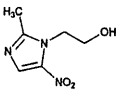
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of each impurity taken by the formula:

$$100F(r_i/r_S)$$

in which F is a response factor, being 0.38 for the desmethylmetrifonate peak, if present at a retention time of 0.5 relative to that of Metrifonate, 0.03 for the dichlorvos peak, if present, at a retention time of 1.9 relative to that of Metrifonate, and 1.0 for any other impurity; r_i is the peak area for the individual impurity obtained from the *Test solution;* and r_S is the peak area for Metrifonate obtained from the *Standard solution:* not more than 1.0% of desmethylmetrifonate, 0.2% of dichlorvos, and 0.5% of any other impurity are found; and a total of not more than 1.0% of impurities other than desmethylmetrifonate and dichlorvos is found.

Assay—Dissolve about 300 mg of Metrifonate, accurately weighed, in 30 mL of alcohol. Add 10 mL of monoethanolamine, and allow to stand for 1 hour at 21 ± 1°. Cool while adding a mixture of 100 mL of water and 15 mL of nitric acid. While maintaining the temperature at 21 ± 1°, titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically using a silver electrode. Each mL of 0.1 N silver nitrate is equivalent to 25.74 mg of $C_4H_8Cl_3O_4P$.

Metronidazole



$C_6H_9N_3O_3$ 171.15

1*H*-Imidazole-1-ethanol, 2-methyl-5-nitro-;
2-Methyl-5-nitroimidazole-1-ethanol [443-48-1].

DEFINITION

Metronidazole contains NLT 99.0% and NMT 101.0% of metronidazole ($C_6H_9N_3O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K.**▲ (CN 1-May-2020) Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and water (1:4)
Standard solution: 0.03 mg/mL of USP Metronidazole RS in *Mobile phase*
Sample solution: 0.03 mg/mL of Metronidazole in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 319 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 30 µL
Run time: Twice the retention time of metronidazole
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

USP Monographs

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of metronidazole ($C_6H_9N_3O_3$) in the portion of Metronidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*. The run time is 30 min.
Standard solution: 1 µg/mL of metronidazole from USP Metronidazole RS and 2 µg/mL of tinidazole related compound A from USP Tinidazole Related Compound A RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Metronidazole in *Mobile phase*
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between tinidazole related compound A and metronidazole
Tailing factor: NMT 2.0 for the metronidazole peak
Relative standard deviation: NMT 6.0% for both tinidazole related compound A and metronidazole; six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tinidazole related compound A in the portion of Metronidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*
r_S = peak response of tinidazole related compound A from the *Standard solution*
C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Metronidazole in the *Sample solution* (mg/mL)

Calculate the percentage of any single unspecified impurity in the portion of Metronidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any single unspecified impurity from the *Sample solution*
r_S = peak response of metronidazole from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

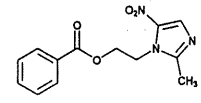
Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.75	0.1
Metronidazole	1.00	—
Any single unspecified impurity	—	0.1
Total impurities	—	0.2

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Metronidazole RS
USP Tinidazole Related Compound A RS
2-Methyl-5-nitroimidazole.
$C_4H_5N_3O_2$ 127.10

Metronidazole Benzoate



$C_{13}H_{13}N_3O_4$ 275.26
2-(2-Methyl-5-nitroimidazol-1-yl)ethyl benzoate [13182-89-3].

DEFINITION
Metronidazole Benzoate contains NLT 98.5% and NMT 101.0% of metronidazole benzoate ($C_{13}H_{13}N_3O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The principal spot of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**

Sample solution: Dissolve with stirring 250 mg of Metronidazole Benzoate in 50.0 mL of glacial acetic acid.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid
Endpoint detection: Potentiometric
Analysis: Titrate the *Sample solution* with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.53 mg of metronidazole benzoate ($C_{13}H_{13}N_3O_4$).
Acceptance criteria: 98.5%–101.0% on the dried basis

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IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Standard solution A: 0.1 mg/mL of USP Metronidazole Benzoate RS in acetone

Standard solution B: 0.04 mg/mL of USP Metronidazole Benzoate RS in acetone from *Standard solution A*

Standard solution C: 0.2 mg/mL each of USP Metronidazole RS and USP Tinidazole Related Compound A RS in acetone

Sample solution: 20 mg/mL of Metronidazole Benzoate in acetone

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.2-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Ethyl acetate

System suitability

Sample: *Standard solution C*

Suitability requirements: The test is valid only if the metronidazole and tinidazole related compound A spots are clearly separated.

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Proceed as directed in the chapter. Examine the plate under short-wavelength UV light.

Acceptance criteria: No secondary spot of the *Sample solution* is larger or more intense than the principal spot of *Standard solution A* (0.5%); and NMT three spots, excluding the principal spot, of the *Sample solution* are larger or more intense than the principal spot of *Standard solution B* (0.2%).

SPECIFIC TESTS

• **ACIDITY**

Sample: 2.0 g

Analysis: Neutralize 40 mL of a mixture of dimethylformamide and water (1:1) with hydrochloric acid or 0.02 M sodium hydroxide. Add 0.2 mL of methyl red TS and the *Sample*, mix to dissolve, and titrate with 0.02 M sodium hydroxide.

Acceptance criteria: NMT 0.25 mL is required to produce a color change.

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 80° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Metronidazole RS

USP Metronidazole Benzoate RS

USP Tinidazole Related Compound A RS

2-Methyl-5-nitroimidazole.

$C_4H_5N_3O_2$ 127.10

Metronidazole Benzoate Compounded Oral Suspension

DEFINITION

Metronidazole Benzoate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

Prepare Metronidazole Benzoate Compounded Oral Suspension containing 50 mg/mL of metronidazole as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Metronidazole (as the Benzoate) powder	5 g (8 g)
Ora-Blend[a], a sufficient quantity to make	100 mL

[a] Perrigo, Minneapolis, MN.

Place the *Metronidazole Benzoate powder* into a suitable mortar. Wet the powder with a small amount of *Ora-Blend*, and triturate to make a smooth paste. Add the *Ora-Blend* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container. Add sufficient *Ora-Blend* to bring the preparation to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: 0.1% (v/v) glacial acetic acid in water

Mobile phase: Acetonitrile and *Solution A* (40:60). Filter, and degas.

Standard solution: 0.4 mg/mL of metronidazole prepared from USP Metronidazole Benzoate RS in *Mobile phase*. Mix well until dissolved.

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 0.8 mL of the Oral Suspension into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 316 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for metronidazole is about 7.7 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of metronidazole in the *Standard solution* (mg/mL)

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C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.6–4.6

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metronidazole Benzoate RS

Metronidazole Capsules

DEFINITION
Metronidazole Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Wavelength range: Between 1600 and 1000 cm^{-1}
  Acceptance criteria: Capsule contents show maxima only at the same wavelengths as those of similarly prepared USP Metronidazole RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Methanol and water (1:4)
  Standard solution: 0.03 mg/mL of USP Metronidazole RS in *Mobile phase*
  Sample stock solution: Nominally 1 mg/mL of metronidazole prepared as follows. Mix the contents of Capsules (NLT 20). Transfer an amount equivalent to 100 mg of metronidazole to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, and sonicate with intermittent shaking for 10 min. Shake for 30 min, and dilute with *Mobile phase* to volume. Centrifuge a portion of the solution.
  Sample solution: 0.03 mg/mL of metronidazole in *Mobile phase*, from the *Sample stock solution*. Pass a portion of the solution through a nylon membrane filter of 0.45-µm or finer pore size. Discard the first 10 mL of the filtrate, and use the remainder.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 319 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L7
  Column temperature: 30°
  Flow rate: 1 mL/min
  Injection volume: 30 µL
  Run time: 2 times the retention time of the metronidazole peak
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0% for five replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 1: 100 rpm
  Time: 30 min
  Standard solution: 0.4 mg/mL of USP Metronidazole RS in *Medium*. Sonicate to dissolve if necessary.
  Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first few mL.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum at about 278 nm
  Cell: 0.05 cm
  Blank: *Medium*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (1/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of metronidazole ($C_6H_9N_3O_3$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**
  Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Standard solution: 1 µg/mL of metronidazole from USP Metronidazole RS and 2 µg/mL of tinidazole related compound A from USP Tinidazole Related Compound A RS in *Mobile phase*
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for tinidazole related compound A and metronidazole are 0.75 and 1.0, respectively.]

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Suitability requirements
Resolution: NLT 2.0 between tinidazole related compound A and metronidazole
Tailing factor: NMT 2.0 for metronidazole
Relative standard deviation: NMT 6.0% for both tinidazole related compound A and metronidazole

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tinidazole related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*
r_S = peak response of tinidazole related compound A from the *Standard solution*
C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of metronidazole from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.75	0.1
Metronidazole	1.0	—
Each unspecified degradation product	—	0.1
Total impurities	—	0.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metronidazole RS
  USP Tinidazole Related Compound A RS
  2-Methyl-5-nitroimidazole.
  $C_4H_5N_3O_2$ 127.10

Metronidazole Gel

DEFINITION

Metronidazole Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

- **A.** The UV spectrum of the metronidazole peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Methanol and water (20:80)
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10.0	100	0
15.0	10	90
15.1	100	0
20.0	100	0

System suitability solution: 0.6 µg/mL of USP Metronidazole RS and 0.6 µg/mL of USP Tinidazole Related Compound A RS in *Solution A*
Standard solution: 30 µg/mL of USP Metronidazole RS in *Solution A*
Sample stock solution: Nominally 300 µg/mL of metronidazole in *Solution A* prepared as follows. Transfer a portion of Gel to a suitable volumetric flask. Add *Solution A* equivalent to 50% of the flask volume and sonicate or vortex until dissolved. Dilute with *Solution A* to volume. [NOTE—On the basis of formulation, if necessary, centrifuge a portion of the solution at 3000 rpm for 10 min and pass a portion of the supernatant through a filter of 0.45-µm pore size. Use the filtrate.]
Sample solution: Nominally 30 µg/mL of metronidazole in *Solution A* prepared from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 319 nm. For *Identification* test *A*, use a diode-array detector in the range of 210–500 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 30 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between metronidazole and tinidazole related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metronidazole from the *Sample solution*

USP Monographs

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r_S = peak response of metronidazole from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: Use the *System suitability solution* from the *Assay*.

Sample solution: Use the *Sample stock solution* from the *Assay*.

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between metronidazole and tinidazole related compound A

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole related compound A in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*
r_S = peak response of tinidazole related compound A from the *Standard solution*
C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of metronidazole from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.76	0.2
Metronidazole	1.0	—
Any individual unspecified impurity	—	0.3
Total impurities	—	1.0

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS

• **PH** ⟨791⟩: The apparent pH determined potentiometrically is between 4.0 and 6.5.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in laminated collapsible tubes at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Metronidazole RS
USP Tinidazole Related Compound A RS
2-Methyl-5-nitroimidazole.
$C_4H_5N_3O_2$ 127.10

Metronidazole Injection

DEFINITION

Metronidazole Injection is a sterile, isotonic, buffered solution of Metronidazole in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

• **A.** The UV (UV-Vis) spectrum of the metronidazole peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and water (20:80)

System suitability solution: 1 µg/mL of USP Metronidazole RS and 2 µg/mL of USP Tinidazole Related Compound A RS in *Mobile phase*

Standard solution: 0.03 mg/mL of USP Metronidazole RS in *Mobile phase*

Sample solution: Nominally 0.03 mg/mL of metronidazole in *Mobile phase* prepared as follows. Transfer a portion of Injection to a suitable volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 319 nm. For *Identification* test *A*, use a diode array detector in the range of 210–800 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between metronidazole and tinidazole related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Injection taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Chromatographic system, and System suitability solution: Proceed as directed in the *Assay*.

Standard solution: 0.75 µg/mL each of USP Metronidazole RS and USP Tinidazole Related Compound A RS in *Mobile phase*

Sample solution: Nominally 500 µg/mL of metronidazole prepared as follows. Transfer a portion of Injection to a suitable volumetric flask. Add *Mobile phase* equivalent to 50% of the flask size. Sonicate for 2 min. Dilute with *Mobile phase* to volume, and pass a portion of the solution through a filter of 0.45-µm pore size. Use the filtrate.

System suitability

Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between metronidazole and tinidazole related compound A, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole related compound A in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*
r_S = peak response of tinidazole related compound A from the *Standard solution*
C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each unspecified impurity from the *Sample solution*
r_S = peak response of metronidazole from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.7	0.15
Metronidazole	1.0	—
Any individual unspecified degradation product	—	0.15
Total impurities	—	2.0

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.5–7.0

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.35 USP Endotoxin Units/mg of metronidazole

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers of Type I or Type II glass, or in suitable plastic containers, protected from light. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Metronidazole RS
USP Tinidazole Related Compound A RS
2-Methyl-5-nitroimidazole.
$C_4H_5N_3O_2$ 127.10

Metronidazole Tablets

DEFINITION

Metronidazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Sample stock solution: Equivalent to 15 mg/mL of metronidazole from powdered Tablets in dilute hydrochloric acid (1 in 100). Shake for several min, and filter.

Medium: Sulfuric acid in methanol (1 in 350)

Sample solution: 20 µg/mL of metronidazole in *Medium* from the *Sample stock solution*

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and water (20:80)

Standard solution: 0.5 mg/mL of USP Metronidazole RS in *Mobile phase*

Sample stock solution: Nominally 10 mg/mL of metronidazole in methanol from Tablets prepared as follows. Transfer 10 Tablets, whole or ground, to a suitable size volumetric flask. Add methanol, and shake by mechanical means for 30 min or until the Tablets are

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disintegrated. Dilute with methanol to volume, and allow the solution to stand until the insoluble material has settled.
Sample solution: Nominally 0.5 mg/mL of metronidazole in *Mobile phase* prepared from the clear supernatant of the *Sample stock solution*. Filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metronidazole from the *Sample solution*
r_S = peak response of metronidazole from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Standard solution: USP Metronidazole RS in *Medium*
Sample solution: Filter a portion of the solution under test, and dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 278 nm
Blank: *Medium*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) dissolved.

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
D = dilution factor to prepare the *Sample solution*

Tolerances: NLT 85% (Q) of the labeled amount of metronidazole ($C_6H_9N_3O_3$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Diluent: Diluted hydrochloric acid (1 in 100)
Standard solution: 20 µg/mL of USP Metronidazole RS in *Diluent*
Sample stock solution: Transfer 1 Tablet to a 250-mL volumetric flask. Add about 100 mL of *Diluent*, and shake for 30 min. Dilute with *Diluent* to volume. Filter, discarding the first 15 mL of the filtrate. Nominally 200 µg/mL of metronidazole is prepared by transferring the filtrate quantitatively with the *Diluent*.
Sample solution: 20 µg/mL of metronidazole in *Diluent* from *Sample stock solution*
Instrumental conditions
Mode: UV
Analytical wavelength: 278 nm
Cell: 1 cm
Blank: *Diluent*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the quantity, in mg, of metronidazole ($C_6H_9N_3O_3$) in each Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times L$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)
L = label claim (mg/Tablet)

Acceptance criteria: Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase: Methanol and water (20:80)
Standard solution: 0.5 µg/mL of USP Metronidazole RS and 2.5 µg/mL of USP Tinidazole Related Compound A RS in *Mobile phase*
Sample solution: Nominally 500 µg/mL of metronidazole prepared as follows. Transfer a suitable amount of powdered Tablets (NLT 20) to a suitable volumetric flask. Add *Mobile phase* equivalent to 80% of the flask size. Sonicate for 10 min. Dilute with *Mobile phase* to volume, and pass a portion of the solution through a suitable filter. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 319 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 30 µL
System suitability
Sample: *Standard solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between metronidazole and tinidazole related compound A
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tinidazole related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*

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r_S = peak response of tinidazole related compound A from the *Standard solution*
C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of metronidazole from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.7	0.5
Metronidazole	1.0	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	2.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metronidazole RS
  USP Tinidazole Related Compound A RS
  2-Methyl-5-nitroimidazole.
  $C_4H_5N_3O_2$ 127.10

Metronidazole Extended-Release Tablets

DEFINITION

Metronidazole Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Diluent: Methanol and sulfuric acid (350:1)
  Standard stock solution: 15 mg/mL of USP Metronidazole RS in dilute hydrochloric acid (1 in 100). Sonicate to dissolve and pass through a suitable filter.
  Standard solution: 18.8 µg/mL of USP Metronidazole RS in *Diluent* from *Standard stock solution*
  Sample stock solution: Nominally 15 mg/mL of metronidazole prepared as follows. Finely powder NLT 5 Tablets and transfer an amount equivalent to 300 mg of metronidazole into a 20-mL volumetric flask. Add about 15 mL of dilute hydrochloric acid (1 in 100) and shake mechanically for 30 min. Dilute with dilute hydrochloric acid (1 in 100) to volume and shake well. Pass through a suitable filter.
  Sample solution: Nominally equivalent to 18.8 µg/mL of metronidazole in *Diluent* from *Sample stock solution*
  Wavelength range: 200–400 nm
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 1.4 g/L of monobasic potassium phosphate in water
  Mobile phase: Methanol and *Buffer* (30:70)
  Standard solution: 0.1 mg/mL of USP Metronidazole RS in *Mobile phase*
  Sample stock solution: Nominally 2.0 mg/mL of metronidazole from NLT 20 finely powdered Tablets in *Mobile phase*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume with *Mobile phase*, and shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume. Allow the solution to stand until the insoluble material settles.
  Sample solution: Nominally 0.1 mg/mL of metronidazole in *Mobile phase* from the *Sample stock solution* supernatant. Pass the solution through a suitable filter of 0.45-µm pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 315 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Temperatures
  Column: 30°
  Autosampler: 15°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  Run time: 15 min
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metronidazole from the *Sample solution*
r_S = peak response of metronidazole from the *Standard solution*
C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

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PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 2, 6, 10, and 16 h

Standard solution: 16.65 µg/mL of USP Metronidazole RS in *Medium*

Sample solution: At the times specified, withdraw 10 mL of the solution under test and pass through a suitable filter of 0.45-µm pore size. Replace the aliquots withdrawn for analysis with equal volumes of fresh portions of *Medium* maintained at 37°. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.

Blank: *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: 320 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of metronidazole ($C_6H_9N_3O_3$) in the sample withdrawn from the vessel at each time point (*i*).

$$\text{Result} = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

D = dilution factor, if needed

Calculate the percentage of the labeled amount (Q_i) of metronidazole ($C_6H_9N_3O_3$) dissolved at each time point (*i*).

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of metronidazole in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	20–35
2	6	45–60
3	10	60–75
4	16	NLT 75

The percentages (Q) of the labeled amount of metronidazole ($C_6H_9N_3O_3$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Buffer: Dissolve 1.5 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.2, and dilute with water to 1000 mL.

Diluent: Acetonitrile and *Buffer* (45:55)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Buffer (%)	Acetonitrile (%)
0	95	5
5	95	5
25	50	50
30	95	5
35	95	5

System suitability solution: 0.5 mg/mL of USP Metronidazole RS and 2.5 µg/mL of USP Tinidazole Related Compound A RS in *Diluent*. Sonicate, if necessary, to dissolve.

Standard solution: 0.75 µg/mL of USP Metronidazole RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of metronidazole from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add *Diluent* equivalent to 70% of the flask volume, sonicate for 15 min with intermittent shaking, and dilute with *Diluent* to volume. Allow the solution to stand until the insoluble material settles, and pass the supernatant through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 315 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Autosampler temperature: 20°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between tinidazole related compound A and metronidazole, *System suitability solution*

Relative standard deviation: NMT 5.0% for metronidazole, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*

r_S = peak response of metronidazole from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.05%.

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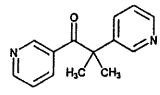
Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.79	0.15
Metronidazole	1.0	—
Any individual unspecified degradation product	—	0.10
Total degradation products	—	0.50

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metronidazole RS
  USP Tinidazole Related Compound A RS
  2-Methyl-5-nitroimidazole.
  $C_4H_5N_3O_2$ 127.10

Metyrapone



$C_{14}H_{14}N_2O$ 226.27
1-Propanone, 2-methyl-1,2-di-3-pyridinyl-;
2-Methyl-1,2-di-3-pyridyl-1-propanone [54-36-4].

DEFINITION

Metyrapone contains NLT 98.0% and NMT 102.0% of metyrapone ($C_{14}H_{14}N_2O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 10 µg/mL in 1 N sulfuric acid
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Diluent: 1 N sulfuric acid
Standard solution: 10 µg/mL of USP Metyrapone RS in *Diluent*
Sample solution: 10 µg/mL of Metyrapone in *Diluent*
Instrumental conditions
Mode: UV
Analytical wavelength: 260 nm
Cell: 1 cm
Blank: *Diluent*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of metyrapone ($C_{14}H_{14}N_2O$) in the portion of Metyrapone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Metyrapone RS in the *Standard solution* (µg/mL)
C_U = concentration of Metyrapone in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Standard stock solution: 0.2 mg/mL of USP Metyrapone RS in methanol
Standard solution A: 40 µg/mL of USP Metyrapone RS in methanol
Standard solution B: 20 µg/mL of USP Metyrapone RS in methanol
Sample solution: 20 mg/mL of Metyrapone in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Chloroform and methanol (48:3)
Analysis
Samples: *Standard stock solution*, *Standard solution A*, *Standard solution B*, and *Sample solution*
Apply each of the *Samples* separately to the TLC plate. Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry under a stream of nitrogen for about 10 min. Position the dried plate once again in the same chromatographic chamber, and again develop the chromatograms until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry under a current of warm air for about 15 min. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spots in the chromatograms of the *Standard solutions*.
Acceptance criteria: The intensity of any secondary spot from the *Sample solution* is NMT the principal spot from *Standard solution A* (0.2%), and the sum of the intensities of the secondary spots from the *Sample solution* corresponds to NMT 1.0%.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample under vacuum at room temperature for 6 h.
Acceptance criteria: NMT 0.5%

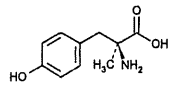
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from heat and light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metyrapone RS

USP Monographs

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Metyrosine



$C_{10}H_{13}NO_3$ 195.22
L-Tyrosine, α-methyl-, (−)-.
(−)-α-Methyl-L-tyrosine [672-87-7].

» Metyrosine contains not less than 98.6 percent and not more than 101.0 percent of $C_{10}H_{13}NO_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Metyrosine RS

Change to read:

Identification—
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).
B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 15 µg per mL.
Medium:0.1 N hydrochloric acid.
Absorptivities at 224 nm, calculated on the dried basis, do not differ by more than 3.0%.
Specific rotation ⟨781S⟩: between +185° and +195° (t = 30°; λ = 546 nm; l = 0.5 dm).
Test solution: 5 mg per mL, in *Diluent*, with the aid of sonication if necessary. Prepare the *Diluent* as follows.
Solution A—Dissolve 20.0 g of anhydrous sodium acetate in about 150 mL of water in a 250-mL volumetric flask. Add 50.0 mL of glacial acetic acid, dilute with water to volume, and mix.
Solution B—Dissolve 62.5 g of cupric sulfate in water in a 200-mL volumetric flask, dilute with water to volume, and mix.
Diluent—Mix *Solution A* and *Solution B* in a 1000-mL volumetric flask, dilute with water to volume, and mix.
Loss on drying ⟨731⟩—Dry it at a pressure not exceeding 5 mm of mercury at 100° for two hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Standard solutions—Dissolve USP Metyrosine RS in a solvent mixture of methanol and ammonium hydroxide (7:3) to obtain a solution having a concentration of 10 mg per mL (*Standard solution A*). Pipet 1 mL of *Standard solution A* into a 100-mL volumetric flask, dilute with the same solvent mixture to volume, and mix (*Standard solution B*). Pipet 5 mL of *Standard solution B* into a 10-mL volumetric flask, dilute with the same solvent mixture to volume, and mix (*Standard solution C*). Pipet 5 mL of *Standard solution C* into a 10-mL volumetric flask, dilute with the same solvent mixture to volume, and mix (*Standard solution D*).
Test solution—Dissolve Metyrosine in the solvent mixture of methanol and ammonium hydroxide (7:3) to obtain a solution having a concentration of 10 mg per mL.
Procedure—Apply 10-µL portions of *Standard solutions A, B, C,* and *D* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously washed with methanol. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol and ammonium hydroxide (7:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate. Expose the plate to iodine vapors, and examine under short-wavelength UV light: the chromatogram shows principal spots at about the same R_F value. Estimate the levels of any additional spots observed in the chromatogram of the *Test solution* by comparison with the spots in the chromatograms of *Standard solutions B, C,* and *D:* the sum of the intensities of any spots observed is not greater than that of the principal spot obtained from *Standard solution B*, corresponding to not more than 1%.
Assay—Dissolve about 300 mg of Metyrosine, accurately weighed, in about 100 mL of glacial acetic acid, sonicate for about 5 minutes, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a platinum ring electrode and a sleeve-type calomel electrode containing 0.1 N lithium perchlorate in glacial acetic acid (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 19.52 mg of $C_{10}H_{13}NO_3$.

Metyrosine Capsules

» Metyrosine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of metyrosine ($C_{10}H_{13}NO_3$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Metyrosine RS
Identification—The UV absorption spectrum of a 1 in 10,000 solution of the Capsule contents in dilute hydrochloric acid (1 in 100) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Metyrosine RS, concomitantly measured.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 750 mL.
Apparatus 1: 100 rpm.
Time: 60 minutes.
Procedure—Determine the amount of $C_{10}H_{13}NO_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 274 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Metyrosine RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{10}H_{13}NO_3$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Standard preparation—Dissolve a suitable quantity of USP Metyrosine RS, accurately weighed, in dilute hydrochloric acid (1 in 100) to obtain a solution having a known concentration of about 100 µg per mL.
Assay preparation—Combine the contents of not less than 20 Capsules, and transfer an accurately weighed portion of the combined contents, equivalent to about 100 mg of metyrosine, to a 100-mL volumetric flask. Add 50 mL of dilute hydrochloric acid (1 in 100), shake by mechanical means for 45 minutes, dilute with dilute hydrochloric acid (1 in 100) to volume, mix, and filter. Transfer 10.0 mL of the filtrate to a

USP Monographs

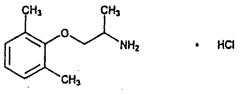
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100-mL volumetric flask, dilute with dilute hydrochloric acid solution (1 in 100) to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard preparation* at the wavelength of maximum absorbance at about 274 nm, with a suitable spectrophotometer, using dilute hydrochloric acid solution (1 in 100) as the blank. Calculate the quantity, in mg, of metyrosine ($C_{10}H_{13}NO_3$) in the portion of Capsules taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Metyrosine RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mexiletine Hydrochloride



$C_{11}H_{17}NO \cdot HCl$ 215.72

2-Propanamine, 1-(2,6-dimethylphenoxy)-, hydrochloride, (±)-;
(±)-1-Methyl-2-(2,6-xylyloxy)ethylamine hydrochloride [5370-01-04].

DEFINITION

Mexiletine Hydrochloride contains NLT 98.0% and NMT 102.0% of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**

Sample solution: 3 mL of a solution (1 in 60)
Analysis: Add 1 mL of 6 N ammonium hydroxide to the *Sample solution*, filter, and acidify the filtrate with 2 mL of nitric acid. Then add 1 mL of silver nitrate TS.
Acceptance criteria: A curdy, white precipitate is formed, and it is soluble in an excess of 6 N ammonium hydroxide (presence of chloride).

ASSAY

- **PROCEDURE**

Buffer: Dissolve 11.5 g of anhydrous sodium acetate in 500 mL of water. Add 3.2 mL of glacial acetic acid, mix, and allow to cool. Adjust with hydrochloric acid to a pH of 4.8 ± 0.1, and dilute with water to 1000 mL.
Mobile phase: Methanol and *Buffer* (600:400)
Standard solution: 2 mg/mL of USP Mexiletine Hydrochloride RS in *Mobile phase*
System suitability solution: 1 mg/mL of 2-phenylethylamine hydrochloride in *Standard solution*
Sample solution: 2 mg/mL of Mexiletine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Columns
Guard: Packing L1
Analytical: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for 2-phenylethylamine and mexiletine are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the 2-phenylethylamine and mexiletine peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$) in the portion of Mexiletine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of mexiletine from the *Sample solution*
r_S = peak area of mexiletine from the *Standard solution*
C_S = concentration of USP Mexiletine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Mexiletine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.2 mg/mL of USP Mexiletine Hydrochloride RS in *Mobile phase*, from the *Standard solution* in the *Assay*
Sample solution: 20 mg/mL of Mexiletine Hydrochloride in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for 2-phenylethylamine and mexiletine are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the 2-phenylethylamine and mexiletine peaks, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Mexiletine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of mexiletine from the *Standard solution*
C_S = concentration of USP Mexiletine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Mexiletine Hydrochloride in the *Sample solution* (mg/mL)

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Acceptance criteria
Any individual impurity: NMT 1%
Total impurities: NMT 1.5%

SPECIFIC TESTS
- **pH** ⟨791⟩
Sample solution: 100 mg/mL
Acceptance criteria: 3.5–5.5
- **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩
USP Mexiletine Hydrochloride RS

Mexiletine Hydrochloride Capsules

» Mexiletine Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Mexiletine Hydrochloride RS

Identification—
A: Transfer a quantity of Capsule contents, equivalent to about 250 mg of mexiletine hydrochloride, to a suitable test tube, add 10 mL of methanol, and mix on a vortex mixer for 1 minute. Filter the mixture, evaporate the filtrate under a stream of nitrogen to dryness, and dry the residue in vacuum at 60° for 1 hour: the IR absorption spectrum of a mineral oil dispersion of the dried residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mexiletine Hydrochloride RS.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{11}H_{17}NO \cdot HCl$ dissolved from the difference between first derivative values at the wavelengths of maximum and minimum first derivative absorbance in the wavelength range from 230 to 290 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Mexiletine Hydrochloride RS in the same *Medium.*
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{11}H_{17}NO \cdot HCl$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity—
Mobile phase, Standard preparation, and *Resolution solution*—Prepare as directed in the *Assay* under *Mexiletine Hydrochloride.*
Standard solution—Transfer 10.0 mL of the *Standard preparation* prepared as directed in the *Assay* under *Mexiletine Hydrochloride* to a 1000-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 20 µg of USP Mexiletine Hydrochloride RS per mL.
Test solution—Use the *Assay preparation* prepared as directed in the *Assay.*
Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay* under *Mexiletine Hydrochloride,* except that the relative standard deviation of replicate injections of the *Standard solution* is not more than 3.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph; record the chromatograms using a high sensitivity setting for the recorder; and measure the areas for the peaks. Calculate the percentage of each impurity observed by the formula:

$$100(C/L)(r_U/r_S)$$

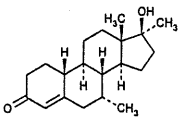
in which C is the concentration, in mg per mL, of USP Mexiletine Hydrochloride RS in the *Standard solution; L* is the quantity, in mg, of mexiletine hydrochloride in each mL of the *Test solution,* based on the labeled amount in the portion of Capsule contents used to prepare the *Assay preparation* and the extent of dilution; r_U is the peak area obtained from an individual impurity observed in the chromatogram of the *Test solution;* and r_S is the mexiletine peak area obtained from the *Standard solution:* not more than 1% of any individual impurity is found; and the total of all observed impurities is not more than 1.5%.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Mexiletine Hydrochloride.*
Assay preparation—Weigh the contents of not fewer than 20 Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an accurately weighed portion, equivalent to about 50 mg of mexiletine hydrochloride, to a stoppered, 50-mL centrifuge tube. Add 25.0 mL of *Mobile phase,* insert the stopper, and shake by mechanical means for 15 minutes. Centrifuge, and use the clear supernatant as the *Assay preparation.*
[NOTE—Reserve a portion of this solution for use as the *Test solution* in the test for *Chromatographic purity.*]
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Mexiletine Hydrochloride.* Calculate the quantity, in mg, of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$) in the portion of Capsule contents taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Mexiletine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the mexiletine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Mibolerone



$C_{20}H_{30}O_2$ 302.45
Estr-4-en-3-one, 17-hydroxy-7,17-dimethyl-, (7α,17β)-.
17β-Hydroxy-7α,17-dimethylestr-4-en-3-one [3704-09-4].

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» Mibolerone contains not less than 96.0 percent and not more than 106.0 percent of $C_{20}H_{30}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Mibolerone RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests,*** ⟨197⟩, *Infrared Spectroscopy*: 197M▲ (CN 1-May-2020)
Specific rotation ⟨781S⟩: between +34° and +40°.
Test solution: 10 mg per mL, in chloroform.
Loss on drying ⟨731⟩—Dry about 1 g, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.5%.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of water, tetrahydrofuran, and methanol (60:25:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Prepare a solution of progesterone in methanol containing 0.6 mg per mL.
Standard preparation—Prepare a solution of USP Mibolerone RS in *Internal standard solution* having a known concentration of about 0.4 mg per mL. Mix, and sonicate if necessary to achieve complete solution.
Assay preparation—Transfer about 10 mg of Mibolerone, accurately weighed, to a 25-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix. Sonicate if necessary to achieve complete solution.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for mibolerone and 1.0 for progesterone; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{20}H_{30}O_2$ in the portion of Mibolerone taken by the formula:

$$25C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Mibolerone RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak responses of the mibolerone peak and the progesterone peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mibolerone Oral Solution

» Mibolerone Oral Solution contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of mibolerone ($C_{20}H_{30}O_2$).

Packaging and storage—Preserve in tight containers, protected from light.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Mibolerone RS
Identification—The chromatogram of the *Assay preparation* exhibits a major peak for mibolerone, the retention time of which corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
Specific gravity ⟨841⟩: between 1.030 and 1.045.

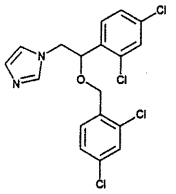
Assay—
Internal standard solution—Prepare a solution of 1,3,5-triphenylbenzene in chloroform containing about 0.25 mg per mL.
Standard preparation—Prepare a solution of USP Mibolerone RS in *Internal standard solution* having a known concentration of about 0.5 mg per mL.
Assay preparation—Transfer an accurately weighed portion of Oral Solution, equivalent to about 1000 µg of mibolerone, to a 125-mL separator containing 60 mL of water, and swirl to disperse. Add 30 mL of methylene chloride, shake gently for about 5 minutes, and allow the phases to separate. Drain the lower methylene chloride layer through a pledget of methylene chloride-washed cotton into a 50-mL conical flask. Evaporate to dryness under a current of air. Re-extract the aqueous layer remaining in the separator with an additional 30-mL portion of methylene chloride, draining the filtered methylene chloride extract into the 50-mL conical flask, and evaporating it to dryness. Add 2.0 mL of *Internal standard solution*, and swirl to dissolve.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 3-mm × 61-cm column packed with 1% liquid phase G6 on support S1AB. The column is maintained at about 175° and the detector at 195° to 225°. Helium is used as the carrier gas at a flow rate of about 60 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for the internal standard and 1.0 for mibolerone; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of mibolerone ($C_{20}H_{30}O_2$) in each mL of the Oral Solution taken by the formula:

$$2000(C/W_U)(D)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Mibolerone RS in the *Standard preparation;* W_U is the weight, in g, of Oral Solution taken to prepare the *Assay preparation;* D is the specific gravity of the Oral Solution; and R_U and R_S are the ratios of the peak height response of the mibolerone peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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Miconazole



$C_{18}H_{14}Cl_4N_2O$ 416.13

1*H*-Imidazole, 1-2-[(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)]methoxy]ethyl]-, (±)-;
(±)-1-[2,4-Dichloro-β-[(2,4-dichlorobenzyl)oxy] phenethyl] imidazole [22916-47-8].

DEFINITION

Miconazole contains NLT 98.0% and NMT 102.0% of miconazole ($C_{18}H_{14}Cl_4N_2O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**

Sample solution: Dissolve 40 mg in 50 mL of isopropyl alcohol in a 100-mL volumetric flask, and add 10 mL of 0.1 N hydrochloric acid. Dilute with isopropyl alcohol to volume.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Miconazole RS, concomitantly measured.

ASSAY

- **PROCEDURE**

Sample solution: 300 mg of Miconazole in 40 mL of glacial acetic acid. Add 4 drops of *p*-naphtholbenzein TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant* to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.61 mg of miconazole ($C_{18}H_{14}Cl_4N_2O$).

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Standard solution A: 10 mg/mL of USP Miconazole RS in chloroform

Standard solution B: 100 µg/mL of USP Miconazole RS from *Standard solution A* in chloroform

Sample solution: 10 mg/mL in chloroform

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: *n*-Hexane, chloroform, methanol, and ammonium hydroxide (60:30:10:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* separately to the starting line of the plate. Develop the chromatogram in a suitable chamber with freshly prepared *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for 30 min, and locate the spots.

Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from *Standard solution A*, and any other spot from the *Sample solution* does not exceed, in size or intensity, the principal spot from *Standard solution B* (1.0%).

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Miconazole RS

Miconazole Compounded Ophthalmic Solution

DEFINITION

Miconazole Compounded Ophthalmic Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of miconazole ($C_{18}H_{14}Cl_4N_2O$).

Prepare Miconazole Compounded Ophthalmic Solution 1% (10 mg/mL) as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Miconazole	1 g
Polyoxyl 40 Hydrogenated Castor Oil	11.5 mL
Lactic Acid Solution (88%)	0.4 mL
Sterile Water for Injection, a sufficient amount to make	100 mL

Add the *Miconazole* to a sterile container and gradually add the *Polyoxyl 40 Hydrogenated Castor Oil*. Mix into a smooth viscous mixture. Add the *Lactic Acid Solution (88%)* and mix thoroughly. Add 80 mL of the *Sterile Water for Injection* and stir vigorously until the *Miconazole* is completely dissolved. Transfer the contents stepwise and quantitatively to a sterile calibrated container and bring to final volume with *Sterile Water for Injection*. Pass through a sterile filter of 0.22-µm pore size into an empty sterile dropper bottle. [NOTE—Room temperature *Sterile Water for Injection* should be used to assist in solubilization.]

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 5.7 g of ammonium acetate in 380 mL of water, and add 320 mL of methanol and 300 mL of acetonitrile. Mix well.

Standard solution: 0.05 mg/mL of miconazole prepared from USP Miconazole RS in methanol

Sample solution: Transfer 0.5 mL of Ophthalmic Solution to a 100-mL volumetric flask, dilute with methanol to volume, and vortex to mix.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 2.0-mm × 10-cm; 2.5-µm packing L1
Column temperature: 55°
Flow rate: 0.35 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for miconazole is about 18.0 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of miconazole ($C_{18}H_{14}Cl_4N_2O$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of miconazole from the *Sample solution*
r_S = peak response of miconazole from the *Standard solution*
C_S = concentration of USP Miconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of miconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

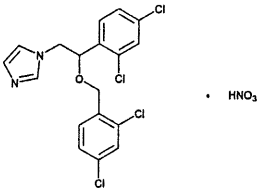
SPECIFIC TESTS
- **pH** ⟨791⟩: 2.9–3.9
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **PARTICULATE MATTER IN OPHTHALMIC SOLUTIONS** ⟨789⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in sterile plastic ophthalmic dropper bottles for single-use in one patient only. Store in a refrigerator (2°–8°) or at controlled room temperature.

Change to read:

- **BEYOND-USE DATE:** ▲In the absence of passing a sterility test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test is passed, NMT 30 days after the date on which it was compounded when stored in a refrigerator (2°–8°) or NMT 21 days after the date on which it was compounded when stored at controlled room temperature.▲ (CN 1-May-2020)
- **LABELING:** Label it to indicate that it is for ophthalmic use only and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Miconazole RS

Miconazole Nitrate



$C_{18}H_{14}Cl_4N_2O \cdot HNO_3$ 479.14
1*H*-Imidazole, 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-, mononitrate;
1-[2,4-Dichloro-β-[(2,4-dichlorobenzyl)oxy]phenethyl] imidazole mononitrate [22832-87-7].

DEFINITION
Miconazole Nitrate contains NLT 98.0% and NMT 102.0% of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: Methanol, water, and 1 M triethylammonium acetate (30:70:1)
Solution B: Acetonitrile, methanol, and 1 M triethylammonium acetate (25: 75: 0.2)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
10	44	56
27	44	56
30	25	75
35	25	75
36	70	30
40	70	30

Diluent: Methanol and water (70:30)
System suitability solution: 0.1 mg/mL of USP Miconazole Nitrate RS and 6 µg/mL of USP Miconazole Related Compound F RS in *Diluent*. Sonication may be needed to aid dissolution.
Standard solution: 0.1 mg/mL of USP Miconazole Nitrate RS in *Diluent*. Sonication may be needed to aid dissolution.
Sample solution: 0.1 mg/mL of Miconazole Nitrate in *Diluent*. Sonication may be needed to aid dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm

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Column: 4.6-mm × 10-cm; 2.6-µm packing L11
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for miconazole related compound F and miconazole are 0.96 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between miconazole related compound F and miconazole, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Miconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of miconazole from the *Sample solution*
r_S = peak response of miconazole from the *Standard solution*
C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Miconazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1.2 µg/mL each of USP Miconazole Nitrate RS, USP Econazole Nitrate RS, USP Miconazole Related Compound C RS, USP Miconazole Related Compound F RS, and USP Miconazole Related Compound I RS in *Diluent*
Sample solution: 600 µg/mL of Miconazole Nitrate in *Diluent*. Sonication may be needed to aid dissolution.
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between miconazole related compound C and miconazole related compound I; NLT 1.5 between miconazole related compound I and econazole; and NLT 1.5 between miconazole related compound F and miconazole
Relative standard deviation: NMT 3.0% for miconazole
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of miconazole related compound C, miconazole related compound F, miconazole related compound I, or econazole nitrate in the portion of Miconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of miconazole related compound C, miconazole related compound F, miconazole related compound I, or econazole nitrate from the *Sample solution*
r_S = peak response of miconazole related compound C, miconazole related compound F, miconazole related compound I, or econazole nitrate from the *Standard solution*
C_S = concentration of USP Miconazole Related Compound C RS, USP Miconazole Related Compound F RS, USP Miconazole Related Compound I RS, or USP Econazole Nitrate RS in the *Standard solution* (µg/mL)
C_U = concentration of Miconazole Nitrate in the *Sample solution* (µg/mL)

Calculate the percentage of each specified and any individual unspecified impurity in the portion of Miconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified and any individual unspecified impurity from the *Sample solution*
r_S = peak response of miconazole from the *Standard solution*
C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (µg/mL)
C_U = concentration of Miconazole Nitrate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deschlorobenzyl econazole[a]	0.22	0.25
Miconazole quarternary salt[b]	0.57	0.25
Miconazole benzyl analog[c]	0.65	0.25
Miconazole related compound C	0.74	0.25
Miconazole related compound I	0.76	0.25
Econazole nitrate	0.78	0.25
Miconazole 2,6-isomer[d]	0.87	0.25
Miconazole 2,5-isomer[e]	0.94	0.25
Miconazole related compound F	0.96	0.25
Miconazole	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] 1-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol.
[b] 2-(3-{2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazol-3-ium-1-yl)-2-methylpropanoate.
[c] 1-[2-(Benzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.
[d] 1-{2-[(2,6-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.
[e] 1-{2-[(2,5-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

SPECIFIC TESTS

Change to read:

- **▲OPTICAL ROTATION** ⟨781A⟩, *Procedures, Angular Rotation*▲ (RB 1-May-2019)

Sample solution: 10 mg/mL of Miconazole Nitrate in methanol

USP Monographs

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Acceptance criteria: −0.10° to +0.10° ▲at 20°▲ (RB 1-May-2019)

• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩
USP Econazole Nitrate RS
USP Miconazole Nitrate RS
USP Miconazole Related Compound C RS
2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethan-1-amine hydrochloride.
$C_{15}H_{13}Cl_4NO \cdot HCl$ 401.53
USP Miconazole Related Compound F RS
1-{2-[(3,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethyl}-1*H*-imidazole.
$C_{18}H_{14}Cl_4N_2O$ 416.13
USP Miconazole Related Compound I RS
1-{2-[(2-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole mononitrate.
$C_{18}H_{15}Cl_3N_2O \cdot HNO_3$ 444.69

Miconazole Nitrate Cream

DEFINITION

Miconazole Nitrate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Buffer: Triethylamine and water (10:1000). Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Methanol, acetonitrile, tetrahydrofuran, and *Buffer* (5:4:3:8)
Standard solution: 0.28 mg/mL of USP Miconazole Nitrate RS and 0.02 mg/mL of benzoic acid in *Mobile phase*
Sample solution: Nominally 0.28 mg/mL of miconazole nitrate in *Mobile phase* prepared as follows. Dissolve a weighed quantity of Cream in *Mobile phase*, and sonicate in a water bath at 40°–45° until the sample is completely dispersed. Cool the solution to below room temperature, and pass through a 0.45-µm Teflon filter into an HPLC vial.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L11
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 13 between miconazole nitrate and benzoic acid
Column efficiency: NLT 7500 theoretical plates for the miconazole nitrate peak
Tailing factor: NMT 2.0 for the miconazole nitrate peak
Relative standard deviation: NMT 2.0% from the miconazole nitrate peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of miconazole nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Minimum Fill** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in collapsible tubes or tight containers, and store at controlled room temperature.

• **Labeling:** Cream that is packaged and labeled for use as a vaginal preparation shall be labeled Miconazole Nitrate Vaginal Cream.

• **USP Reference Standards** ⟨11⟩
USP Miconazole Nitrate RS

Miconazole Nitrate Topical Powder

DEFINITION

Miconazole Nitrate Topical Powder contains NLT 90.0% and NMT 110.0% of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

IDENTIFICATION

• **A.**
Sample: Transfer nominally 100 mg of miconazole nitrate from Topical Powder to a 50-mL beaker, disperse in 40 mL of *methanol*, and mix for a minimum of 5 min. Allow to settle for 5–10 min and filter into a 100-mL beaker. Evaporate on a steam bath or by a rotary evaporator to dryness. Dry the residue at 105° for 10 min.
Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue obtained from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Miconazole Nitrate RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Buffer: 4.6 g/L of dibasic potassium phosphate trihydrate in *water*. Adjust with phosphoric acid to a pH of 7.5.
Solution A: *Acetonitrile* and *Buffer* (65:35)
Solution B: *Acetonitrile*
Solution C: *Acetonitrile*, *water*, and phosphoric acid (50: 50: 0.05)
Mobile phase: See *Table 1*.

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Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)	Flow rate (mL/min)
0	100	0	0	1.0
17.0	86	14	0	1.0
17.1	0	0	100	3.0
28.0	0	0	100	3.0
28.1	100	0	0	2.0
38.0	100	0	0	2.0
38.1	100	0	0	1.0
40	100	0	0	1.0

Diluent: *Acetonitrile* and *water* (50:50)
Standard stock solution: 2 mg/mL of USP Miconazole Nitrate RS in *Diluent*. Sonication may be needed to aid dissolution.
Standard solution: 0.2 mg/mL of USP Miconazole Nitrate RS in *Diluent* from the *Standard stock solution*
Sample solution: Nominally 0.2 mg/mL of miconazole nitrate in *Diluent* prepared as follows. Transfer an appropriate amount of miconazole nitrate from a portion of the Topical Powder to a suitable volumetric flask. Add *Diluent* equivalent to 50% of the flask volume, shake for 30 min, and sonicate for 10 min. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 32°
Flow rate: See *Table 1*.
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Topical Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of miconazole from the *Sample solution*
r_S = peak response of miconazole from the *Standard solution*
C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of miconazole nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Solution B, Solution C, Diluent, Standard stock solution, and **Sample solution:** Prepare as directed in the *Assay*.
Solution A: *Acetonitrile* and *Buffer* (35:65)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)	Flow rate (mL/min)
0	100	0	0	1.0
55.0	30.8	69.2	0	1.0
55.1	0	0	100	3.0
68.0	0	0	100	3.0
68.1	100	0	0	2.0
83.0	100	0	0	2.0
83.1	100	0	0	1.0
85.0	100	0	0	1.0

System suitability solution: 0.2 mg/mL of USP Miconazole Nitrate RS from the *Standard stock solution* and 0.2 µg/mL of USP Miconazole Related Compound C RS in *Diluent*
Standard solution: 2 µg/mL of USP Miconazole Nitrate RS in *Diluent* from the *Standard stock solution*
Sensitivity solution: 0.1 µg/mL of USP Miconazole Nitrate RS in *Diluent* from the *Standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 32°
Flow rate: See *Table 2*.
Injection volume: 15 µL
System suitability
Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between miconazole related compound C and miconazole, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of miconazole related compound C and any individual unspecified degradation product in the portion of Topical Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of miconazole related compound C or any individual unspecified degradation product from the *Sample solution*
r_S = peak response of miconazole from the *Standard solution*
C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of miconazole nitrate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Miconazole	1.0	—
Miconazole related compound C	1.06	0.25

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Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified degradation product	—	0.10
Total impurities	—	1.0

PERFORMANCE TESTS
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total count does not exceed 10^2 cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Miconazole Nitrate RS
  USP Miconazole Related Compound C RS
  2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethan-1-amine ▲hydrochloride.
  $C_{15}H_{13}Cl_4NO \cdot HCl$ 401.53▲ (ERR 1-Jun-2018)

Miconazole Nitrate Vaginal Suppositories

DEFINITION
Miconazole Nitrate Vaginal Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

IDENTIFICATION
- **A.**

Sample: Place a portion of the *Sample stock solution* prepared as directed in the *Assay*, containing about 25 mg of miconazole nitrate, in a 50-mL beaker. Evaporate on a steam bath to dryness with the aid of a current of filtered air. Dry the residue at 105° for 10 min.
Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue obtained from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Miconazole Nitrate RS.

ASSAY
- **PROCEDURE**

Internal standard solution: 1 mg/mL of cholestane in a mixture of chloroform and methanol (1:1)
Standard solution: 2.5 mg/mL of USP Miconazole Nitrate RS prepared as follows. Transfer a 10.0-mL aliquot of a solution containing 500 µg/mL of USP Miconazole Nitrate RS in methanol to a test tube, and evaporate on a steam bath to dryness with the aid of a current of filtered air. Dissolve the residue in 2.0 mL of *Internal standard solution*.
Sample stock solution: Nominally 2.5 mg/mL of miconazole nitrate prepared as follows. Transfer 1 Suppository to a stoppered, 50-mL centrifuge tube. Add 30 mL of pentane, and shake by mechanical means for 20 min to dissolve the suppository base and to disperse the miconazole nitrate. Centrifuge to obtain a clear supernatant. Aspirate, and discard the clear liquid. Wash the residue with three 20-mL portions of pentane, shaking, centrifuging, and aspirating in the same manner. Discard the pentane washings. Evaporate the residual pentane from the residue with the aid of a current of filtered air. Using small portions of methanol, transfer the residue to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume.
Sample solution: Transfer an aliquot containing nominally the equivalent to 5 mg of miconazole nitrate from the *Sample stock solution* to a suitable container, and evaporate on a steam bath to dryness with the aid of a current of filtered air. Dissolve the residue in 2.0 mL of *Internal standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.2-m glass; packed with 3% phase G32 on support S1A
Temperatures
Column: 250°
Injection port: 250°
Detector: 300°
Carrier gas: Helium
Flow rate: 50 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for cholestane and miconazole nitrate are 0.44 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the cholestane and miconazole nitrate peaks
Relative standard deviation: NMT 3.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Suppository taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of miconazole nitrate to cholestane from the *Sample solution*
R_S = peak response ratio of miconazole nitrate to cholestane from the *Standard solution*
C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of miconazole nitrate in the *Sample solution* (mg/mL)

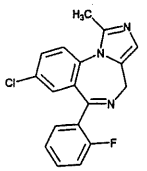
Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Miconazole Nitrate RS

USP Monographs

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Midazolam



$C_{18}H_{13}ClFN_3$ 325.77

4-*H*-Imidazo[1,5-*a*][1,4]benzodiazepine, 8-chloro-6-(2-fluorophenyl)-1-methyl;
8-Chloro-6-(*o*-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine [59467-70-8].

DEFINITION

Midazolam contains NLT 98.5% and NMT 101.5% of $C_{18}H_{13}ClFN_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 7.7 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.5 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (1:2)
Standard solution: 0.04 mg/mL of USP Midazolam RS in *Mobile phase*
Sample solution: 0.04 mg/mL of Midazolam in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L60
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 10,000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{18}H_{13}ClFN_3$ in the portion of Midazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)
C_U = concentration of Midazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

- **Procedure**

Buffer, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity check solution: Dilute the *Standard solution* with *Mobile phase* to obtain a 0.2-µg/mL solution.
Sample solution: 0.2 mg/mL of Midazolam in *Mobile phase*
System suitability
Samples: *Standard solution* and *Sensitivity check solution*
Suitability requirements
Column efficiency: NLT 10,000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Peak ratio: The ratio of the area of the midazolam peak of the *Standard solution* to the area of the midazolam peak of the *Sensitivity check solution* should be within 160–240.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Midazolam taken:

$$\text{Result} = (r_U/F)/[\Sigma(r_U/F) + r_T] \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = peak response of Midazolam from the *Sample solution*
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Reduced midazolam[a]	0.20	1.0	0.1
Reduced reduced midazolam[b]	0.24	1.0	0.1
Amino compound[c]	0.25	0.5	0.1
Oxide midazolam[d]	0.46	1.3	0.1
Nitromethylene compound[e]	0.76	1.0	0.1
Dihydromidazolam[f]	0.83	0.5	0.1
Midazolam	1.0	—	—
Desfluoromidazolam[g]	1.14	1.0	0.2
6*H*-isomer[h]	2.48	0.7	0.1
Unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] 8-Chloro-3a,4-dihydro-6-(2-fluorophenyl)-1-methyl-3*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.
[b] 8-Chloro-6-(2-fluorophenyl)-3a,4,5,6-tetrahydro-1-methyl-3*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.
[c] 2-Aminomethyl-7-chloro-2,3-dihydro-5-(2-fluorophenyl)-1*H*-1,4-benzodiazepine.
[d] 8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine-5-oxide.
[e] 7-Chloro-1,3-dihydro-2-nitromethylene-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepine-4-oxide.
[f] 8-Chloro-6-(2-fluorophenyl)-5,6-dihydro-1-methyl-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.
[g] 8-Chloro-6-phenyl-1-methyl-4*H*-imidazo-[1,5-*a*][1,4]-benzodiazepine.
[h] 8-Chloro-6-(2-fluorophenyl)-1-methyl-6*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

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SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩: Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Midazolam RS

Midazolam Injection

DEFINITION

Midazolam Injection is a sterile solution of Midazolam Hydrochloride in Water for Injection or of Midazolam in Water for Injection prepared with the aid of Hydrochloric Acid. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of midazolam ($C_{18}H_{13}ClFN_3$). It may contain Sodium Chloride, Benzyl Alcohol, and/or a chelating agent.

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

[Note—Protect all prepared Standard and sample solutions from light.]

- **Procedure**

Buffer: 6.7 g/L of dibasic sodium phosphate heptahydrate in water. Adjust with phosphoric acid to a pH of 5.0 ± 0.1.
Solution A: Prepare a filtered and degassed mixture of acetonitrile, methanol and *Buffer* (8:3:9).
Solution B: Acetonitrile and *Buffer* (3:1)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	0	100
35	0	100
37	100	0
45	100	0

Standard solution: Dissolve USP Midazolam RS in about 2 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a 0.2-mg/mL solution.
Sample solution: [Note—The midazolam present in the Injection converts from the open-ring form to the closed-ring form when diluted with *Solution A*. The midazolam potency is determined based on the peak area of the closed-ring form. It takes approximately 60 min at 40° or 2–3 h at room temperature to complete the conversion. The *Standard solution* is not subject to this conversion process.] Transfer a volume of Injection to a suitable volumetric flask, and dilute with *Solution A* to obtain a solution containing about 0.2 mg/mL of midazolam. Transfer the resulting solution into suitable crimp top vials, seal tightly, and heat at about 40° for 60 min. Allow this solution to cool to room temperature before injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5500 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of labeled amount of $C_{18}H_{13}ClFN_3$ in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Midazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

[Note—Protect all prepared Standard and sample solutions from light.]

- **Procedure**

Buffer, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: Use *Standard solution* in the *Assay*.
Standard solution: 0.5 µg/mL USP Midazolam RS in *Solution A* from *Standard stock solution*
Control solution: 0.1 µg/mL USP Midazolam RS in *Solution A* from *Standard solution*
System suitability
Samples: *Standard solution* and *Control solution*
Suitability requirements
Tailing factor: NMT 2.5 for midazolam peak, *Standard solution*
Column efficiency: NLT 5500 theoretical plates, *Standard solution*
Signal-to-noise ratio: NLT 10, *Control solution*
Relative standard deviation: NMT 8.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*
r_S = peak response of midazolam from the *Standard solution*
C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Midazolam in the *Sample solution* (mg/mL)
F = relative response factor; 0.51 for the peak eluting at a relative retention between 0.79 and

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0.97 with respect to midazolam; 1.0 for all other peaks

Acceptance criteria
Individual known impurity: NMT 0.5%
Individual unknown impurity: NMT 0.1%
Total impurities: NMT 1.0%
[NOTE—Disregard all solvent- and excipient-related peaks.]

SPECIFIC TESTS
• **BENZYL ALCOHOL CONTENT** (if present)
Buffer: 3.4 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer* (7:13)
System suitability solution: 0.05 mg/mL of USP Midazolam RS and 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*
Standard solution: 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*
Sample solution: Transfer a measured volume of Injection to a suitable volumetric flask. Dilute with *Mobile phase* to obtain a concentration of about 0.5 mg/mL of benzyl alcohol, based on the labeled content of benzyl alcohol in the Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; L1 packing
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 6.0 between benzyl alcohol and midazolam
Tailing factor: NMT 2.0 for benzyl alcohol
Relative standard deviation: NMT 2.0% for benzyl alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzyl alcohol in the volume of Injection taken:

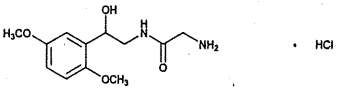
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzyl alcohol from the *Sample solution*
r_S = peak response of benzyl alcohol from the *Standard solution*
C_S = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzyl alcohol in the *Sample solution* (mg/mL)

Acceptance criteria: The content of benzyl alcohol meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Vehicles and added substances*.
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 8.33 USP Endotoxin Units/mg of midazolam.
• **pH** ⟨791⟩**:** 2.5–3.7
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
• **OTHER REQUIREMENTS:** It meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type 1 glass. Store between 15° and 30°.
• **LABELING:** Label to indicate the vehicle used and the names and concentrations of any added preservatives. Indicate if the product is preservative free.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzyl Alcohol RS
USP Midazolam RS

Midodrine Hydrochloride



$C_{12}H_{18}N_2O_4 \cdot HCl$ 290.74
Acetamide, 2-amino-*N*-[2-(2,5-dimethoxyphenyl)-2-hydroxyethyl]-, monohydrochloride, (±)-;
(±)-2-Amino-*N*-(β-hydroxy-2,5-dimethoxyphenethyl)acetamide monohydrochloride [3092-17-9].

DEFINITION
Midodrine Hydrochloride contains NLT 98.0% and NMT 102.0% of midodrine hydrochloride ($C_{12}H_{18}N_2O_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** A 10 mg/mL solution of Midodrine Hydrochloride in water meets the requirements.

ASSAY
• **PROCEDURE**
Buffer: 13.6 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 4.00 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (3:22)
Standard solution: 0.05 mg/mL of USP Midodrine Hydrochloride RS in *Mobile phase*
Sample solution: 0.05 mg/mL of Midodrine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{12}H_{18}N_2O_4 \cdot HCl$ in the portion of Midodrine Hydrochloride taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of midodrine from the *Sample solution*
r_S = peak response of midodrine from the *Standard solution*
C_S = concentration of USP Midodrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Midodrine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.2%. A 1-g sample is used.

ORGANIC IMPURITIES

- **Procedure**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: 1.0 µg/mL of USP Midodrine Hydrochloride RS and 2.0 µg/mL of USP Midodrine Related Compound A RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Midodrine Hydrochloride in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay* except for the following:
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between midodrine hydrochloride and midodrine hydrochloride related compound A
Tailing factor: NMT 2.0 for midodrine hydrochloride
Relative standard deviation: NMT 2.0% for both midodrine hydrochloride and midodrine related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of midodrine related compound A in the portion of Midodrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of midodrine related compound A from the *Sample solution*
r_S = peak response of midodrine related compound A from the *Standard solution*
C_S = concentration of USP Midodrine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Midodrine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual impurity in the portion of Midodrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of midodrine from the *Standard solution*
C_S = concentration of USP Midodrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Midodrine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Midodrine related compound A[a]	0.8	0.2%
Midodrine hydrochloride	1	—
Individual unspecified impurity	—	0.1%
Total impurities	—	0.5%

[a] 1-(2,5 Dimethoxyphenyl)-2-aminoethanol.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%
- **pH** ⟨791⟩: 4.0–5.0. Use 50 mg/mL of the midodrine hydrochloride sample.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Midodrine Hydrochloride RS
  USP Midodrine Related Compound A RS
  1-(2,5 Dimethoxyphenyl)-2-aminoethanol.
  $C_{10}H_{15}NO_3$ 197.23

Midodrine Hydrochloride Tablets

DEFINITION

Midodrine Hydrochloride Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of Midodrine Hydrochloride ($C_{12}H_{18}N_2O_4 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample specimen: Weigh a quantity, from finely powdered Tablets (NLT 20), equivalent to 15 mg of midodrine hydrochloride, into a 50-mL disposable centrifuge tube. Add 20 mL of water, and stir for 2 min using a vortex mixer. Pass the mixture through filter paper into a 50-mL beaker, and boil it until about 2 mL of the solution is left. Evaporate the final solution in an oven at 105° for 1 h.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 13.6 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 4.00 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (3:22)
Standard solution: 0.05 mg/mL of USP Midodrine Hydrochloride RS in *Mobile phase*
Sample solution: 0.05 mg/mL of midodrine hydrochloride in *Mobile phase* from NLT 5 Tablets (for 10-mg Tablet strength) or NLT 10 Tablets (for 5-mg and 2.5-mg Tablet strength). Initially add *Mobile phase* up to 80% of the volume of the flask. Sonicate for 10 min, stir for 15 min, and then dilute to volume, mix, and let stand for 10 min. Pass through a suitable PVDF filter of 0.45-µm pore size, and discard the first 5 mL.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of midodrine hydrochloride ($C_{12}H_{18}N_2O_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N HCl; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 15 min
Buffer: Proceed as directed in the *Assay*.
Mobile phase: Acetonitrile and *Buffer* (3:17)
Standard solution: L/900 mg/mL of USP Midodrine Hydrochloride RS in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of midodrine hydrochloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of midodrine hydrochloride is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Standard stock solution 1: 25 µg/mL of USP Midodrine Hydrochloride RS in *Mobile phase*
Standard stock solution 2: 25 µg/mL of USP Midodrine Related Compound A RS in *Mobile phase*
Standard solution: 1.25 µg/mL each of USP Midodrine Hydrochloride RS and USP Midodrine Related Compound A RS in *Mobile phase* from *Standard stock solution 1* and *Standard stock solution 2*
Sample solution: 0.25 mg/mL in *Mobile phase* from NLT 5 Tablets (for 10-mg Tablet strength) and NLT 10 Tablets (for 5-mg and 2.5-mg Tablet strength). Initially add *Mobile phase* to about 80% of the volume of the flask. Sonicate for 10 min, stir for 15 min, and then dilute to volume. Pass through a suitable PVDF filter of 0.45-µm pore size, and discard the first 5 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Proceed as directed in the *Assay* except for the following:
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
[NOTE—The relative retention times for midodrine related compound A and midrodrine hydrochloride are 0.83 and 1, respectively.]
Resolution: NLT 2.0 between midodrine hydrochloride and midodrine related compound A
Column efficiency: NLT 2000 theoretical plates for the midodrine peak
Tailing factor: NMT 2.0 for the midodrine peak
Relative standard deviation: NMT 2.0% for the midodrine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of midodrine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of midodrine related compound A from the *Sample solution*
r_S = peak response of midodrine related compound A from the *Standard solution*
C_S = concentration of USP Midodrine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of midodrine hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any other unknown impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

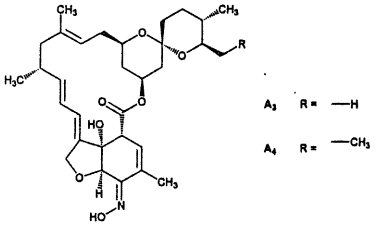
r_U = peak response of any other unknown impurity from the *Sample solution*
r_S = peak response of midodrine from the *Standard solution*
C_S = concentration of USP Midodrine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of midodrine hydrochloride in the *Sample solution* (µg/mL)

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Acceptance criteria
Individual impurities: NMT 0.5% of midodrine related compound A; NMT 0.2% of any other individual impurity
Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Midodrine Hydrochloride RS
  USP Midodrine Related Compound A RS
  1-(2,5 Dimethoxyphenyl)-2-aminoethanol.
  $C_{10}H_{15}NO_3$ 197.23

Milbemycin Oxime



Mixture of milbemycin A_3 oxime and milbemycin A_4 oxime [129496-10-2].
Milbemycin A_3 Oxime

$C_{31}H_{43}NO_7$ 541.68
Milbemycin B, 5-*O*-demethyl-28-deoxy-25-methyl-6,28-epoxy-23-hydroxyimino-, [6*R*,23*S*,25*S*(*E*)]-; (2α*E*,4*E*,5′*S*,6*R*,6′*S*,8*E*,11*R*,13*R*,15*S*,17α*R*,20α*R*,20β*S*)-3′,4′,5′,6,6′,7,10,11,14,15,17α,20,20α,20β-tetradecahydro-20β-hydroxy-5′,6′,6,8,19-pentamethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17-one 20-oxime.
Milbemycin A_4 Oxime

$C_{32}H_{45}NO_7$ 555.70
Milbemycin B, 5-*O*-demethyl-28-deoxy-25-ethyl-6,28-epoxy-23-hydroxyimino-, [6*R*,23*S*,25*S*(*E*)]-; (2α*E*,4*E*,5′*S*,6*R*,6′*S*,8*E*,11*R*,13*R*,15*S*,17α*R*,20α*R*,20β*S*)-6′-ethyl-3′,4′,5′,6,6′,7,10,11,14,15,17α,20,20α,20β-tetradecahydro-20β-hydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17-one 20-oxime.

DEFINITION
Milbemycin Oxime is a mixture of milbemycin A_4 oxime and milbemycin A_3 oxime. It contains NLT 95.0% and NMT 102.0% of the sum of milbemycin A_4 oxime and milbemycin A_3 oxime, calculated on the anhydrous basis. The ratio of milbemycin A_4 oxime is NLT 0.75.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: Water
  Solution B: Methanol
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	26	74
2	26	74
27	24.5	75.5
47	24.5	75.5
47.1	26	74
60	26	74

Diluted phosphoric acid solution: 0.3–0.5 mL/L of phosphoric acid in water
Diluent: Acetonitrile and *Diluted phosphoric acid solution* (75:25)
Standard solution: 0.2 mg/mL of USP Milbemycin Oxime RS in *Diluent*. Sonicate if necessary to facilitate dissolution.
Sample solution: 0.2 mg/mL of Milbemycin Oxime in *Diluent*. Sonicate if necessary to facilitate dissolution. This solution must be stored at 5° protected from light.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 3.0-mm × 10-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The elution order is milbemycin A_3 oxime followed by milbemycin A_4 oxime. (See *Table 2* for the relative retention times.)]
Suitability requirements
Resolution: NLT 9 between milbemycin A_3 oxime and milbemycin A_4 oxime
Tailing factor: NMT 2.0 each for milbemycin A_3 oxime and milbemycin A_4 oxime
Relative standard deviation: NMT 0.73% each for milbemycin A_3 oxime and milbemycin A_4 oxime
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of milbemycin A_3 oxime ($C_{31}H_{43}NO_7$) in the portion of Milbemycin Oxime taken:

$$\text{Result} = (r_U/r_S) \times [(C_S \times F_{A3})/C_U] \times 100$$

r_U = peak response of milbemycin A_3 from the *Sample solution*
r_S = peak response of milbemycin A_3 from the *Standard solution*
C_S = concentration of USP Milbemycin Oxime RS in the *Standard solution* (mg/mL)
F_{A3} = potency of milbemycin A_3 oxime in USP Milbemycin Oxime RS (mg/mg)
C_U = concentration of Milbemycin Oxime in the *Sample solution* (mg/mL)

Calculate the percentage of milbemycin A_4 oxime ($C_{32}H_{45}NO_7$) in the portion of Milbemycin Oxime taken:

$$\text{Result} = (r_U/r_S) \times [(C_S \times F_{A4})/C_U] \times 100$$

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r_U = peak response of milbemycin A_4 from the *Sample solution*
r_S = peak response of milbemycin A_4 from the *Standard solution*
C_S = concentration of USP Milbemycin Oxime RS in the *Standard solution* (mg/mL)
F_{A4} = potency of milbemycin A_4 oxime in USP Milbemycin Oxime RS (mg/mg)
C_U = concentration of Milbemycin Oxime in the *Sample solution* (mg/mL)

Calculate the percentage of the sum of milbemycin A_3 oxime and milbemycin A_4 oxime in the portion of Milbemycin Oxime taken:

$$\text{Result} = P_{A3} + P_{A4}$$

P_{A3} = percentage of milbemycin A_3 oxime
P_{A4} = percentage of milbemycin A_4 oxime

Calculate the ratio of milbemycin A_3 oxime in the portion of Milbemycin Oxime taken:

$$\text{Result} = P_{A3}/(P_{A3} + P_{A4})$$

P_{A3} = percentage of milbemycin A_3 oxime
P_{A4} = percentage of milbemycin A_4 oxime

Calculate the ratio of milbemycin A_4 oxime in the portion of Milbemycin Oxime taken:

$$\text{Result} = P_{A4}/(P_{A3} + P_{A4})$$

P_{A4} = percentage of milbemycin A_4 oxime
P_{A3} = percentage of milbemycin A_3 oxime

Acceptance criteria
Sum of milbemycin A_4 oxime and milbemycin A_3 oxime: 95.0%–102.0% on the anhydrous basis
Ratio of milbemycin A_4 oxime: NLT 0.75

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
- **ORGANIC IMPURITIES**

Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Diluted standard solution: 0.002 mg/mL of USP Milbemycin Oxime RS in *Diluent* from *Standard solution*
Analysis
Samples: *Sample solution* and *Diluted standard solution*
Calculate the percentage of each impurity in the portion of Milbemycin Oxime taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the peak responses of milbemycin A_3 oxime and milbemycin A_4 oxime from the *Diluted standard solution*
C_S = concentration of USP Milbemycin Oxime RS in the *Diluted standard solution* (mg/mL)
C_U = concentration of Milbemycin Oxime in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.10%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Milbemycin A_3 oxime	1.00	—
11′-Desmethylmilbemycin A_4 oxime[a]	1.17	0.7
(20′*R*)-Hydroxymilbemycin A_4 keto form[b]	1.33	0.5
Milbemycin A_4 oxime	1.43	—
Milbemycin A_4 keto form[c]	1.51	0.7
Milbemycin D oxime[d]	2.18	3.0
Any other individual impurity	—	0.5
Total impurities (excluding milbemycin D oxime)	—	3.5

[a] (1′*R*,2*R*,4′*S*,5*S*,6*R*,8′*R*,10′*E*,13′*R*,14′*E*,16′*E*,20′*R*,21′*Z*,24′*S*)-6-Ethyl-24′-hydroxy-21′-(hydroxyimino)-5,13′,22′-trimethyl-3,4,5,6-tetrahydrospiro[pyran-2,6′-[3,7,19]trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa[10,14,16,22]tetraene]-2′-one.
[b] (1′*R*,2*R*,4′*S*,5*S*,6*R*,8′*R*,10′*E*,13′*R*,14′*E*,16′*E*,20′*R*,21′*Z*,24′*S*)-6-Ethyl-20′,24′-dihydroxy-21′-(hydroxyimino)-5,11′,13′,22′-tetramethyl-3,4,5,6-tetrahydrospiro[pyran-2,6′-[3,7,19]trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa[10,14,16,22]tetraene]-2′-one.
[c] (1′*R*,2*R*,4′*S*,5*S*,6*R*,8′*R*,10′*E*,13′*R*,14′*E*,16′*E*,20′*S*,24′*S*)-6-Ethyl-24′-hydroxy-5,11′,13′,22′-tetramethyl-3,4,5,6-tetrahydrospiro[pyran-2,6′-[3,7,19]trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa[10,14,16,22]tetraene]-2′,21′-dione.
[d] (1′*R*,2*R*,4′*S*,5*S*,6*R*,8′*R*,10′*E*,13′*R*,14′*E*,16′*E*,20′*R*,21′*Z*,24′*S*)-24′-Hydroxy-21′-(hydroxyimino)-6-(propan-2-yl)-5,11′,13′,22′-tetramethyl-3,4,5,6-tetrahydrospiro[pyran-2,6′-[3,7,19]trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa[10,14,16,22]tetraene]-2′-one.

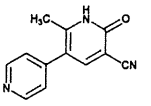
SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature, protected from light.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Milbemycin Oxime RS

Milrinone



$C_{12}H_9N_3O$ 211.22
[3,4′-Bipyridine]-5-carbonitrile, 1,6-dihydro-2-methyl-6-oxo-; 1,6-Dihydro-2-methyl-6-oxo[3,4′-bipyridine]-5-carbonitrile [78415-72-2].

DEFINITION

Milrinone contains NLT 98.0% and NMT 102.0% of milrinone ($C_{12}H_9N_3O$), calculated on the anhydrous basis.
[**CAUTION**—Milrinone is a cardiotonic agent.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

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• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

Buffer: To 72.44 g of sodium tetraborate, ▲anhydrous▲ (ERR 1-Jun-2019) add 900 mL of water. Adjust with hydrochloric acid to a pH of 6.5. The solution should become nearly transparent after adjustment. Dilute with water to 1 L.

Mobile phase: Methanol, *Buffer*, and water (320:40:640)

Diluent: Methanol, water, and lactic acid (320: 679: 1.2)

Standard solution: 0.1 mg/mL of USP Milrinone RS in *Diluent*. Sonicate until dissolved.

Sample solution: 0.1 mg/mL of Milrinone in *Diluent*. Sonicate until dissolved.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 268 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of milrinone ($C_{12}H_9N_3O$) in the portion of Milrinone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of milrinone from the *Sample solution*

r_S = peak response of milrinone from the *Standard solution*

C_S = concentration of USP Milrinone RS in the *Standard solution* (mg/mL)

C_U = concentration of Milrinone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%

• **ORGANIC IMPURITIES**

Buffer: To 2.7 g of dibasic potassium phosphate in 800 mL of water add 2.4 mL of triethylamine, and adjust with phosphoric acid to a pH of 7.5.

Mobile phase: Acetonitrile and *Buffer* (200:800)

System suitability stock solution: 0.2 mg/mL of USP Milrinone Related Compound A RS in *Mobile phase*. Heat in a water bath at approximately 80°, and/or sonicate if necessary to dissolve.

Standard stock solution: 2 mg/mL of USP Milrinone RS in *Mobile phase*. Heat in a water bath at approximately 80°, and/or sonicate if necessary to dissolve.

System suitability solution: 10.0 mL of *System suitability stock solution* and 1.0 mL of *Standard stock solution* in a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

Standard solution: 0.006 mg/mL of USP Milrinone RS, from the *Standard stock solution*, in *Mobile phase*

Sample solution: 2 mg/mL of Milrinone in *Mobile phase*. Heat in a water bath at approximately 80°, if necessary to dissolve.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for milrinone related compound A and milrinone are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between milrinone related compound A and milrinone

Relative standard deviation: NMT 5.0% from the milrinone peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Milrinone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of milrinone from the *Standard solution*

C_S = concentration of USP Milrinone RS in the *Standard solution* (mg/mL)

C_U = concentration of Milrinone in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual impurity: NMT 0.3%

Total impurities: NMT 1.0%

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Milrinone RS

USP Milrinone Related Compound A RS

1,6-Dihydro-2-methyl-6-oxo-(3,4′-bipyridine)-5-carboxamide.

$C_{12}H_{11}N_3O_2$ 229.23

Add the following:

▲Milrinone Lactate Injection

DEFINITION

Milrinone Lactate Injection is a sterile aqueous solution of Milrinone and a suitable osmolality-adjusting substance in Water for Injection, prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of milrinone ($C_{12}H_9N_3O$).

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IDENTIFICATION

- **A.** The retention time of the milrinone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectrum of the major peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 3.3 g of dibasic potassium phosphate in 1 L of water and add 3 mL of triethylamine. Adjust with phosphoric acid to a pH of 7.5.

Mobile phase: Acetonitrile and *Buffer* (20:80)

Standard solution: 0.05 mg/mL of USP Milrinone RS in *Mobile phase*. Sonication may be necessary for complete dissolution.

Sample solution: Nominally equivalent to 0.05 mg/mL of milrinone prepared from a volume of Injection suitably diluted with *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 1.7 times the retention time of milrinone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of milrinone ($C_{12}H_9N_3O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of milrinone from the *Sample solution*

r_S = peak response of milrinone from the *Standard solution*

C_S = concentration of USP Milrinone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of milrinone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

System suitability solution: 0.5 µg/mL each of USP Milrinone RS and USP Milrinone Related Compound A RS in *Mobile phase*

Standard solution: 0.5 µg/mL of USP Milrinone RS in *Mobile phase*

Sensitivity solution: 0.1 µg/mL of USP Milrinone RS in *Mobile phase* from *Standard solution*

Sample solution: Nominally 500 µg/mL of milrinone from a volume of Injection in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, except for the *Run times*.

Run times

Standard solution: NLT 1.7 times the retention time of milrinone

Sample solution: NLT 4 times the retention time of milrinone

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 5.0 between milrinone and milrinone related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 20, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \text{ ▲▲ (ERR 1-Sep-2018)} \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of milrinone from the *Standard solution*

C_S = concentration of USP Milrinone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of milrinone in the *Sample solution* (µg/mL)

▲▲ (ERR 1-Sep-2018)

Acceptance criteria: See *Table 1*. Disregard peaks below 0.01%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Milrinone related compound A[a]	0.6	—
Milrinone	1.0	—
Any unspecified degradation product	—	0.20
Total impurities[b]	—	0.5

[a] Process-related impurity; 1,6-Dihydro-2-methyl-6-oxo(3,4′-bipyridine)-5-carboxamide.

[b] Total impurities include both process-related and degradation products.

SPECIFIC TESTS

- **CONTENT OF LACTIC ACID**

Mobile phase: Water adjusted with phosphoric acid to a pH of 2.1

Standard solution: 0.2 mg/mL of USP Sodium Lactate RS in *Mobile phase*

Sample solution: Nominally equivalent to 0.2 mg/mL of milrinone prepared as follows. Transfer a suitable volume of Injection into a suitable volumetric flask and add about 8% of the flask volume of 1.0 N sodium hydroxide solution. Shake well and keep for 10 min. Neutralize with an equal amount of 1.0 N sulfuric acid and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

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Detector UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run times
Standard solution: NLT 2.4 times the retention time of lactic acid
Sample solution: NLT 4 times the retention time of lactic acid
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lactic acid in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of lactic acid from the *Sample solution*
r_S = peak response of lactic acid from the *Standard solution*
C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of milrinone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lactic acid, 90.08
M_{r2} = molecular weight of sodium lactate, 112.06

Acceptance criteria: 95.0%–129.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 25 USP Endotoxin Units/mg of milrinone
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 3.2–4.0
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be suitably diluted prior to administration.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Milrinone RS
USP Milrinone Related Compound A RS
1,6-Dihydro-2-methyl-6-oxo(3,4′-bipyridine)-5-carboxamide.
$C_{12}H_{11}N_3O_2$ 229.24
USP Sodium Lactate RS
Sodium 2-hydroxypropanoate.
$C_3H_5NaO_3$ 112.06 ▲(USP41)

Mineral Oil

DEFINITION
Mineral Oil is a purified mixture of liquid hydrocarbons obtained from petroleum. It may contain a suitable stabilizer.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F** ▲(CN 1-May-2020)
- **B.** It meets the requirements in *Specific Tests* for *Viscosity—Capillary Methods* ⟨911⟩.

IMPURITIES
- **LIMIT OF POLYCYCLIC AROMATIC HYDROCARBONS**
Dimethyl sulfoxide: Use spectrophotometric grade dimethyl sulfoxide.
***n*-Hexane:** Use *n*-hexane that has been washed by being shaken previously twice with one-fifth its volume of *Dimethyl sulfoxide*.
Standard solution: 7.0 µg/mL of USP Naphthalene RS in isooctane (2,2,4-trimethylpentane)
Standard blank: 2,2,4-Trimethylpentane
Sample solution: Transfer 25.0 mL of Mineral Oil and 25 mL of *n-Hexane* to a 125-mL separator, and mix. [NOTE—Use no lubricants other than water on the stopcock, or use a separator equipped with a suitable polymeric stopcock.]
Add 5.0 mL of *Dimethyl sulfoxide*, and shake the mixture vigorously for 1 min. Allow to stand until the lower layer is clear, transfer the lower layer to another 125-mL separator, add 2 mL of *n-Hexane*, and shake vigorously. Use the lower layer.
Sample blank: *Dimethyl sulfoxide* that has been shaken previously vigorously for 1 min with *n-Hexane* in the ratio of 5 mL of *Dimethyl sulfoxide* to 25 mL of *n-Hexane*
Instrumental conditions
Mode: UV
Analytical wavelengths
Standard solution: 275 nm
Sample solution: 260–350 nm
Cell: 1 cm
Analysis
Samples: *Standard solution, Standard blank, Sample solution,* and *Sample blank*
Acceptance criteria: The absorbance at any wavelength in the specified range of the *Sample solution* is NMT one-third of the absorbance of the *Standard solution*.

SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨841⟩: 0.845–0.905
- **VISCOSITY—CAPILLARY METHODS** ⟨911⟩: 34.5–150.0 $mm^2 \cdot s^{-1}$ for kinematic viscosity, measured with a capillary viscometer at 40 ± 0.1°
- **ACIDITY**
Sample: 10 mL
Analysis: Add 20 mL of boiling water to the *Sample*, and shake vigorously for about 1 min. Allow to cool, and draw off the separated water. To 10 mL of the filtered aqueous layer add 0.1 mL of phenolphthalein TS.
Acceptance criteria: The solution does not produce a pink color. NMT 1.0 mL of 0.01 N sodium hydroxide is required to to produce a pink color.
- **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩
Sample: 5 mL
Standard solution: In a glass-stoppered test tube that previously has been rinsed with hot nitric acid (see *Cleaning Glass Apparatus* ⟨1051⟩), mix 3 mL of ferric chloride CS, 1.5 mL of cobaltous chloride CS, and 0.5 mL of cupric sulfate CS then overlaid with 5 mL of Mineral Oil.
Analysis: Place the *Sample* in a glass-stoppered test tube that previously has been rinsed with hot nitric acid (see *Cleaning Glass Apparatus* ⟨1051⟩), then rinsed with water, and dried. Add 5 mL of sulfuric acid containing 94.5%–

USP Monographs

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94.9% of H_2SO_4, and heat in a boiling water bath for 10 min. After the test tube has been in the bath for 30 s, remove it quickly, and while holding the stopper in place, give three vigorous, vertical shakes over an amplitude of about 5 in. Repeat every 30 s. Do not keep the test tube out of the bath longer than 3 s for each shaking period. At the end of 10 min from the time when first placed in the water bath, remove the test tube.

Acceptance criteria: The oil portion of the *Sample* may turn hazy, but it remains colorless or shows a slight pink or yellow color, and the acid portion of the *Sample* does not become darker than the *Standard solution*.

- **SOLID PARAFFIN**

Sample: Mineral Oil that has been dried previously in a beaker at 105° for 2 h and cooled to room temperature in a desiccator over silica gel

Analysis:

Fill a tall, cylindrical, standard oil-sample bottle of colorless glass of 120-mL capacity with the *Sample*, insert the stopper, and immerse in an ice bath for 4 h.

Acceptance criteria: The *Sample* is sufficiently clear that a black line 0.5 mm in width, on a white background, held vertically behind the bottle, is clearly visible.

- **LIMIT OF SULFUR COMPOUNDS**

Solution A: Saturated solution of lead(II) oxide in sodium hydroxide (200 mg/mL)

Sample: 4.0 mL

Analysis: Combine the *Sample*, 2 mL of dehydrated alcohol, and 2 drops of *Solution A*, heat at 70° for 10 min with frequent shaking, and cool.

Acceptance criteria: No dark brown color develops.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. No storage requirements specified.
- **LABELING:** Label it to indicate the name and quantity of any substance added as a stabilizer.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Mineral Oil RS
USP Naphthalene RS

Mineral Oil Emulsion

DEFINITION

Prepare Mineral Oil Emulsion as follows.

Mineral Oil	500 mL
Acacia, in very fine powder	125 g
Syrup	100 mL
Vanillin	40 mg
Alcohol	60 mL
Purified Water, a sufficient quantity to make	1000 mL

Mix the *Mineral Oil* with the *Acacia* in a dry mortar. Add 250 mL of *Purified Water* all at once, and emulsify the mixture. Add, in divided portions, triturating after each addition, a mixture of the *Syrup*, 50 mL of *Purified Water*, and *Vanillin* dissolved in *Alcohol*. Add *Purified Water* to bring the preparation to final volume, and mix.

The *Vanillin* may be replaced by NMT 1% of any other official flavoring substance or any mixture of official flavoring substances. *Alcohol* may be replaced with 60 mL of sweet orange peel tincture or 2 g of benzoic acid as a preservative.

SPECIFIC TESTS

- **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩: 4.0%–6.0% of C_2H_5OH

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers.

Mineral Oil, Rectal

DEFINITION

Mineral Oil, Rectal, is Mineral Oil that has been suitably packaged.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F** ▲ (CN 1-May-2020)
- **B.** It meets the requirements in *Specific Tests* for *Viscosity—Capillary Methods* ⟨911⟩.

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 0.845–0.905
- **VISCOSITY—CAPILLARY METHODS** ⟨911⟩: 34.5–150.0 $mm^2 \cdot s^{-1}$ for kinematic viscosity, measured with a capillary viscometer at 40 ± 0.1°
- **ACIDITY**

Sample: 10 mL

Analysis: Add 20 mL of boiling water to the *Sample*, and shake vigorously for about 1 min. Allow to cool, and draw off the separated water. To 10 mL of the filtered aqueous layer add 0.1 mL of phenolphthalein TS.

Acceptance criteria: The solution does not produce a pink color. NMT 1.0 mL of 0.01 N sodium hydroxide is required to produce a pink color.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, single-unit containers. No storage requirements specified.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Mineral Oil RS

Topical Light Mineral Oil

DEFINITION

Topical Light Mineral Oil is Light Mineral Oil that has been suitably packaged.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F** ▲ (CN 1-May-2020)
- **B.** It meets the requirements in *Specific Tests* for *Viscosity—Capillary Methods* ⟨911⟩.

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 0.818–0.880
- **VISCOSITY—CAPILLARY METHODS** ⟨911⟩: 3.0–34.4 $mm^2 \cdot s^{-1}$ for kinematic viscosity, measured with a capillary viscometer at 40 ± 0.1°
- **ACIDITY**

Sample: 10 mL

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Analysis: Add 20 mL of boiling water to the *Sample*, and shake vigorously for about 1 min. Allow to cool, and draw off the separated water. To 10 mL of the filtered aqueous layer add 0.1 mL of phenolphthalein TS.
Acceptance criteria: The solution does not produce a pink color. NMT 1.0 mL of 0.01 N sodium hydroxide is required to produce a pink color.

• **SOLID PARAFFIN**
Sample: Topical Light Mineral Oil that has been dried previously in a beaker at 105° for 2 h and cooled to room temperature in a desiccator over silica gel
Analysis: Fill a tall, cylindrical, standard oil-sample bottle of colorless glass of 120-mL capacity with the *Sample*. Insert the stopper, and immerse the bottle in a mixture of ice and water for 4 h.
Acceptance criteria: The *Sample solution* is sufficiently clear that a black line 0.5 mm in width, on a white background, held vertically behind the bottle, is clearly visible.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. No storage requirements specified.
• **LABELING:** Label it to indicate the name and quantity of any substance added as a stabilizer, and label packages intended for direct use by the public to indicate that it is not intended for internal use.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mineral Oil RS

Minocycline for Injection

DEFINITION

Minocycline for Injection is sterile, freeze-dried Minocycline Hydrochloride suitable for parenteral use. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.
System suitability solution: Dissolve 10 mg of USP Minocycline Hydrochloride RS in 20 mL of 0.2 M ammonium oxalate. Heat on a water bath at 60° for 3 h, allow to cool, and dilute with water to 25.0 mL.
Standard solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in water. Use this solution within 3 h.
Sample solution 1 (where it is represented as being in a single-dose container): Nominally 0.5 mg/mL of minocycline, prepared as follows. Constitute Minocycline for Injection in a volume of water, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and dilute with water.
Sample solution 2 (where the label states the quantity of minocycline in a given volume of constituted solution): Nominally 0.5 mg/mL of minocycline, prepared as follows. Constitute Minocycline for Injection in a volume of water, corresponding to the volume of solvent specified in the labeling. Dilute a portion of constituted solution with water.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]
Suitability requirements
Capacity factor: 5.0–11.5, *Standard solution*
Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*
Tailing factor: 0.9–2.0 for minocycline, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution*, and *Sample solution 1* or *Sample solution 2*
Calculate the percentage of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) in the container, or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from *Sample solution 1* or *Sample solution 2*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of *Sample solution 1* or *Sample solution 2* (mg/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **LIMIT OF EPIMINOCYCLINE**
Mobile phase, System suitability solution, Standard solution, Sample solution 1 or **Sample solution 2, Chromatographic system,** and **System suitability:** Proceed as directed in the *Assay*.
[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]
Analysis: Calculate the percentage of epiminocycline in the portion of Minocycline for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of epiminocycline from *Sample solution 1* or *Sample solution 2*
r_T = total area of all the peaks from *Sample solution 1* or *Sample solution 2*

Acceptance criteria: NMT 6.0%

SPECIFIC TESTS

• **PH** ⟨791⟩
Sample solution: Nominally 10 mg/mL of minocycline
Acceptance criteria: 2.0–3.5

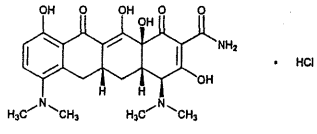
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- **WATER DETERMINATION,** *Method I* ⟨921⟩
  Test preparation: Prepare as directed for a hygroscopic specimen.
  Acceptance criteria: NMT 3.0%
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 1.25 USP Endotoxin Units/mg of minocycline.
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **OTHER REQUIREMENTS:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Minocycline Hydrochloride RS

Minocycline Hydrochloride



$C_{23}H_{27}N_3O_7 \cdot HCl$ 493.94

2-Naphthacenecarboxamide, 4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11dioxo-, monohydrochloride, [4*S*-(4α,4aα,5aα,12aα)]-;
4,7-Bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [13614-98-7].

DEFINITION

Minocycline Hydrochloride contains the equivalent of NLT 890 µg and NMT 950 µg of minocycline ($C_{23}H_{27}N_3O_7$) per mg, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K.▲ (CN 1-May-2020) Dry the Standard and Sample at 100° for 2 h before use.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**
  [NOTE—Protect the *Standard solution* and *Sample solution* from light, store in a refrigerator, and use within 3 h.]
  Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium TS (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.
  System suitability solution: Dissolve 10 mg of USP Minocycline Hydrochloride RS in 20 mL of 0.2 M ammonium oxalate. Heat on a water bath at 60° for 3 h, allow to cool, and dilute with water to 25.0 mL.
  Standard solution: Equivalent to 500 µg/mL of minocycline ($C_{23}H_{27}N_3O_7$) from USP Minocycline Hydrochloride RS in water
  Sample solution: Equivalent to 500 µg/mL of minocycline ($C_{23}H_{27}N_3O_7$) from Minocycline Hydrochloride in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 280 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*
  Tailing factor: 0.9–2.0, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the quantity, in µg/mg, of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of Minocycline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of minocycline in the *Standard solution* (µg/mL)
C_U = concentration of minocycline in the *Sample solution* (µg/mL)
P = potency of USP Minocycline Hydrochloride RS (µg/mg)

Acceptance criteria: 890–950 µg/mg on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.15%
- **ORGANIC IMPURITIES**
  Mobile phase, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*
  [NOTE—Protect the *Standard solution* and the *Sample solutions* from light, store in a refrigerator, and use within 3 h.]
  Sample solution 1: 0.25 mg/mL of Minocycline Hydrochloride in water
  Sample solution 2: 5 µg/mL of Minocycline Hydrochloride in water
  Sample solution 3: 3 µg/mL of Minocycline Hydrochloride in water
  Run time: 2.6 times the retention time of minocycline, *Sample solution 1*
  Analysis
  Samples: *Sample solution 1, Sample solution 2,* and *Sample solution 3*
  Calculate the percentage of epiminocycline in the portion of Minocycline Hydrochloride taken:

$$\text{Result} = (r_{E1}/r_{M3}) \times D_1 \times 100$$

r_{E1} = peak response of epiminocycline from *Sample solution 1*

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r_{M3} = peak response of minocycline from *Sample solution 3*
D_1 = dilution factor for *Sample solution 3*

Calculate the total percentage of impurities other than epiminocycline in the portion of Minocycline Hydrochloride taken:

$$\text{Result} = (r_T/r_{M2}) \times D_2 \times 100$$

r_T = sum of the peak responses of all impurities other than epiminocycline from *Sample solution 1*
r_{M2} = peak response of minocycline from *Sample solution 2*
D_2 = dilution factor for *Sample solution 2*

Acceptance criteria
Individual impurities: NMT 1.2% epiminocycline
Total impurities (excluding epiminocycline): NMT 2.0%

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩
  Sample: 10 mg/mL of minocycline in solution
  Acceptance criteria: 3.5–4.5
- **WATER DETERMINATION** ⟨921⟩, *Method I*: 4.3%–8.0%
- **STERILITY TESTS** ⟨71⟩: Where the label states that Minocycline Hydrochloride is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Minocycline Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 1.25 USP Endotoxin Units/mg of minocycline.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Minocycline Hydrochloride RS

Minocycline Hydrochloride Capsules

DEFINITION
Minocycline Hydrochloride Capsules contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.
  System suitability solution: Dissolve 10 mg of USP Minocycline Hydrochloride RS in 20 mL of 0.2 M ammonium oxalate. Heat on a water bath at 60° for 3 h, allow to cool, and dilute with water to 25.0 mL.
  Standard solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in water. Use this solution within 3 h.
  Sample solution: Nominally 0.5 mg/mL of minocycline in water from combined contents of NLT 20 Capsules. Pass through a suitable filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]
  Suitability requirements
  Capacity factor: 5.0–11.5, *Standard solution*
  Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*
  Tailing factor: 0.9–2.0 for minocycline, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm
  Time: 45 min
  Detector: UV maximum at about 348 nm
  Standard solution: USP Minocycline Hydrochloride RS in *Medium*
  Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
  Tolerances: NLT 75% (*Q*) of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 12.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Minocycline Hydrochloride RS

Delete the following:

▲Minocycline Hydrochloride Oral Suspension

DEFINITION
Minocycline Hydrochloride Oral Suspension contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) and one or more suitable diluents, flavors, preservatives, and wetting agents in an aqueous vehicle.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.
System suitability solution: 2 mg/mL of USP Minocycline Hydrochloride RS in water. Transfer 5 mL of this solution to a small beaker, and heat on a steam bath for 60 min. Evaporate to dryness, and dissolve the residue in 25 mL of *Mobile phase*. Pass through a filter.
Standard solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Mobile phase*. Use the solution within 1 h.
Sample solution: Nominally 0.5 mg/mL of minocycline from Oral Suspension, freshly mixed and free from air bubbles, in *Mobile phase*. Use the solution within 1 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]
Suitability requirements
Capacity factor: 5.0–11.5, *Standard solution*
Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*
Tailing factor: 0.9–2.0 for minocycline, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of minocycline in the *Sample solution* (mg/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–130.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For single-unit containers
Acceptance criteria: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 7.0–9.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Minocycline Hydrochloride RS▲ (USP 1-May-2020)

Minocycline Hydrochloride Tablets

DEFINITION
Minocycline Hydrochloride Tablets contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.
System suitability solution: Dissolve 10 mg of USP Minocycline Hydrochloride RS in 20 mL of 0.2 M ammonium oxalate. Heat on a water bath at 60° for 3 h, allow to cool, and dilute with water to 25.0 mL.
Standard solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in water. Use this solution within 3 h.
Sample solution: Nominally 0.5 mg/mL of minocycline from NLT 20 finely powdered Tablets in water. Shake for 1 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]

USP Monographs

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Suitability requirements
Capacity factor: 5.0–11.5, *Standard solution*
Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*
Tailing factor: 0.9–2.0 for minocycline, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Detector: UV maximum at about 348 nm
Standard solution: USP Minocycline Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: NLT 75% (Q) of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 12.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Minocycline Hydrochloride RS

Minocycline Hydrochloride Extended-Release Tablets

DEFINITION
Minocycline Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV absorption spectrum of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Protect solutions containing minocycline from light.
Buffer: 3.5 g/L of tetrabutylammonium hydrogen sulfate, 2 g/L of anhydrous citric acid, and 6.8 g/L of monobasic potassium phosphate. Adjust with 10 N sodium hydroxide to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (24:76)
Diluent: Acetonitrile and water (20:80)
Standard solution: 0.045 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Diluent*. Store at 4° and use within 24 h.
Sample stock solution: Nominally about 0.9 mg/mL of minocycline from Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets (NLT 10) to a suitable volumetric flask. Add acetonitrile, using 20% of the final volume, and mix vigorously for 15 min. Add water, using 65% of the final volume, and mix vigorously for 30 min. Dilute with water to volume and mix.
Sample solution: Nominally 0.045 mg/mL of minocycline from *Sample stock solution* in *Diluent*. Centrifuge and use the clear supernatant. Store at 4° and use within 24 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 277 nm. When this procedure is used for *Identification* test *B*, use a diode-array detector set at 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 35°
Autosampler: 4°
Flow rate: 1.3 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of minocycline in the *Sample solution* (mg/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Protect solutions containing minocycline from light.
Medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 2: 50 rpm
Times: 1, 2, and 5 h

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Standard stock solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Medium*
Standard solution: (*L*/900) mg/mL of minocycline from *Standard stock solution* in *Medium*, where *L* is the label claim of minocycline in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Analytical wavelength: 348 nm
Cell: See *Table 1*.

Table 1

Tablet Strength (mg)	Cell Path Length (cm)
45	0.5
90	0.2
135	0.2

Blank: *Medium*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Autozero the instrument using the *Blank*.
Calculate the concentration (C_i) of minocycline ($C_{23}H_{27}N_3O_7$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (A_U/A_S) \times C_S \times P \times F$$

A_U = absorbance of the *Sample solution* at time point *i*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount (Q_i) of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of minocycline in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	20–45
2	2	40–70
3	5	NLT 85

The percentages of the labeled amounts of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2
If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Protect solutions containing minocycline from light.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Times: 1, 2, and 4 h
Standard solution: 0.0225 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Medium*
Sample solution: At the times specified, withdraw 10 mL of the solution under test and replace with 10 mL of *Medium*. Pass through a suitable filter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 348 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Autozero the instrument using the *Blank*.
Calculate the concentration (C_i) of minocycline ($C_{23}H_{27}N_3O_7$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (A_U/A_S) \times C_S \times D \times P \times F$$

A_U = absorbance of the *Sample solution* at time point *i*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor (mL/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount (Q_i) of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of minocycline in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)	
		45 mg/Tablet	90 mg/Tablet and 135 mg/Tablet
1	1	40–60	40–60
2	2	70–95	70–90
3	4	NLT 85	NLT 85

USP Monographs

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The percentages of the labeled amounts of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Protect solutions containing minocycline from light.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Times: 0.5, 1.5, and 4 h

Standard solution: 0.021 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Medium*

Sample solution: At the times specified, withdraw 10 mL of the solution under test and replace with 10 mL of *Medium*. Pass through a suitable filter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 265 nm

Cell: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Autozero the instrument using the *Blank*.

Calculate the concentration (C_i) of minocycline ($C_{23}H_{27}N_3O_7$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times D \times P \times F$$

A_U = absorbance of the *Sample solution* at time point i
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor (mL/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount (Q_i) of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of minocycline in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (h)	Amount Dissolved (%)
1	0.5	NMT 40
2	1.5	50–95
3	4	NLT 85

The percentages of the labeled amounts of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Protect solutions containing minocycline from light.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, and 4 h

Standard solution: (L/900) mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Medium*, where L is the label claim of minocycline in mg/Tablet

Sample solution: At the times specified, withdraw 5 mL of the solution under test and replace with 5 mL of *Medium*. Pass through a suitable filter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 353 nm

Cell: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Autozero the instrument using the *Blank*.

Calculate the concentration (C_i) of minocycline ($C_{23}H_{27}N_3O_7$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times D \times P \times F$$

A_U = absorbance of the *Sample solution* at time point i
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor (mL/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount (Q_i) of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of minocycline in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (i)	Time (h)	Amount Dissolved (%)	
		45/Tablet and 90 mg/Tablet	135 mg/Tablet
1	1	35–50	35–50
2	2	63–78	67–82

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Table 5 *(continued)*

Time Point (*i*)	Time (h)	Amount Dissolved (%)	
		45/Tablet and 90 mg/Tablet	135 mg/Tablet
3	4	NLT 90	NLT 90

The percentages of the labeled amounts of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Protect solutions containing minocycline from light.

Buffer, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.

Standard stock solution: Use the *Standard solution* as directed in the *Assay*.

Standard solution: 0.009 mg/mL of minocycline from *Standard stock solution* in *Diluent*. Store at 4° and use within 24 h.

Sample solution: Use the *Sample stock solution* as directed in the *Assay*.

Sensitivity solution: 0.9 µg/mL of minocycline from *Standard solution* in *Diluent*. Store at 4° and use within 24 h.

System suitability solution: Heat a portion of the *Standard stock solution* at 60° for about 2 h and cool. This solution contains a mixture of 4-epiminocycline and minocycline. Store at 4° and use within 24 h.

Chromatographic system: Proceed as directed in the *Assay*, except use a flow rate of 1 mL/min.

System suitability

Samples: *Standard solution, Sensitivity solution,* and *System suitability solution*

Suitability requirements

Resolution: NLT 4.6 between minocycline and 4-epiminocycline, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of minocycline from the *Standard solution*

C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of minocycline in the *Sample solution* (mg/mL)

P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 6*. The reporting threshold is 0.1%.

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Epiminocycline[a]	0.38	4.0
Desmethyl minocycline[b, c]	0.46	—
Sancycline[b, d]	0.68	—
5a,6-Anhydrominocycline[b, e]	0.81	—
Hydroxymethylminocycline[b, f]	0.92	—
Minocycline	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products[g]	—	2.0

[a] (4*R*,4a*S*,5a*R*,12a*S*)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

[b] Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total degradation products.

[c] (4*S*,4a*S*,5a*R*,12a*S*)-4-Dimethylamino-3,10,12,12a-tetrahydroxy-7-methylamino-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

[d] 6-Demethyl-6-deoxytetracycline; (4*S*,4a*S*,5a*R*,12a*S*)-4-Dimethylamino-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

[e] (4*S*,4a*S*,12a*S*)-4,7-Bis(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide.

[f] (4*S*,4a*S*,5a*R*,12a*S*)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-*N*-(hydroxymethyl)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

[g] Total degradation products does not include 4-epiminocycline.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in tightly closed containers at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Minocycline Hydrochloride RS

Minocycline Periodontal System

DEFINITION

Minocycline Periodontal System is an extended-release formulation of Minocycline Hydrochloride containing the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Wavelength range: 250–450 nm

Standard stock solution: Transfer USP Minocycline Hydrochloride RS to a suitable volumetric flask. Dissolve first in dimethylformamide, using about 20% of the final volume, then dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.48 mg/mL of minocycline.

Standard solution: 0.024 mg/mL minocycline hydrochloride from *Standard stock solution* in water

Sample stock solution: Transfer Minocycline Periodontal System equivalent to 12 mg of minocycline hydrochloride to a 25-mL volumetric flask. Add 5.0 mL of

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dimethylformamide, and mix to dissolve. Dilute with water to volume and filter.
Sample solution: 0.024 mg/mL minocycline hydrochloride from *Sample stock solution* in water
Acceptance criteria: The *Sample solution* exhibits maxima at the same wavelengths as the *Standard solution*, concomitantly measured.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Dimethylformamide, 0.2 M ammonium oxalate, and 0.1 M edetate disodium (25:55:20), adjusted with 0.4 M aqueous tetrabutylammonium hydroxide solution to a pH of 6.3 ± 0.2
Diluent: Dimethylformamide and methanol (1:1)
System suitability solution: Prepare a solution in water containing 2 mg/mL USP Minocycline Hydrochloride RS. Heat over a steam bath for 60 min. To one part of this solution add four parts of *Mobile phase* and mix. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler.
Standard solution: 0.4 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Diluent*. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler. [NOTE—Use low-actinic glassware.]
Sample solution: Mix the contents of NLT 10 dispensing units of Minocycline Periodontal System. Transfer a portion of the mixture, equivalent to 10 mg of minocycline, into a 25-mL volumetric flask. Add *Diluent* and sonicate for 2–5 min, or until the sample is dissolved. Dilute with *Diluent* to volume, and mix to obtain a solution having a nominal concentration of 0.4 mg/mL of minocycline, based on the label claim. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler. [NOTE—Use low-actinic glassware.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Guard column: 4.6-mm × 3-cm; 10-µm packing L7
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 2 mL/min
Autosampler temperature: 5°
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of epiminocycline and minocycline are 0.81 and 1, respectively.]
Suitability requirements
Resolution: NLT 2.0 between epiminocycline and minocycline, *System suitability solution*
Tailing factor: NMT 2.0 for the minocycline peak, *System suitability solution*
Relative standard deviation: NMT 2.0% for the minocycline peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of the Minocycline Periodontal System taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of minocycline from the *Sample solution*
r_S = peak response of minocycline from the *Standard solution*
C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of minocycline in the *Sample solution* (mg/mL)
P = potency of minocycline in USP Minocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION**
Medium: 6.9 g/L of monobasic sodium phosphate monohydrate in water, adjusted with phosphoric acid to a pH of 4.2. This solution is stable for 10 days.
Apparatus: Tube rotator. [NOTE—Suitable equipment is available as Labquake® tube rotator, catalog number 400110.]
0.1 M Edetate disodium: 37.2 g/L of edetate disodium in water
0.2 M Ammonium oxalate: 28.4 g/L of ammonium oxalate in water
Mobile phase: Mix 310 mL of *0.1 M Edetate disodium* and 500 mL of *0.2 M Ammonium oxalate*, adjust with 0.4 M aqueous tetrabutylammonium hydroxide to a pH of 6.2, and add 175 mL of dimethylformamide. The injector wash solution is a mixture of dimethylformamide and water (25:75).
Standard stock solution: 0.11 mg/mL of USP Minocycline Hydrochloride RS in *Medium*
Standard solutions: Dilute the *Standard stock solution* with *Medium* to obtain solutions with final concentrations of 0.088 mg/mL, 0.0528 mg/mL, 0.0352 mg/mL, 0.022 mg/mL, and 0.0176 mg/mL.
System suitability solution: Transfer 10 mg of USP Minocycline Hydrochloride RS to a 50-mL beaker. Add 5 mL of water and heat on a steam bath for 60 min. Add 20 mL of *Medium* or *Mobile phase*, and mix well. Store at 5°.
Sample solution: Use borosilicate glass tubes, 25 mm outside diameter and 15 cm long. Close the tubes with a snap type cell with a Teflon prong consisting of a Teflon closure and holder that snap together, two 25-µm stainless steel screens, two silicone gaskets, and a Teflon spacer (see *Figure 1*). Prepare six tubes as follows: partially assemble a release tube and tare its weight; dispense one dose of Minocycline Periodontal System into a partially assembled release cell (see *Figure 1*); record the sample weight in mg; assemble the cell so that the sample is enclosed between the two 25-µm screens; close the cells and place each one of them into separate glass tubes containing 10 mL of *Medium* previously equilibrated at 37°; add the Teflon prong, and cap the tube with Teflon faced rubber-lined caps; seal with Teflon tape. Place the tubes in the tube rotator. Place the tube rotator in a convection incubator that is maintained at 37°. Allow the tubes to rotate for 4 h. Remove the solution under test, and add 10 mL of *Medium* previously equilibrated at 37°. Replace the tubes in the apparatus and rotate for 20 h (24 h total). Repeat the sampling procedure after 24 h (48 h total), and after another 24 h (72 h total).

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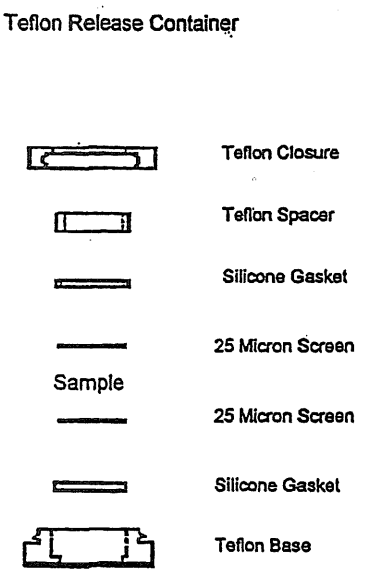


Figure 1. Sample Extraction Configuration

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Guard column: 4.6-mm × 3-cm; 10-µm packing L7
Column: 4.6-mm × 3.3-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Autosampler temperature: 5°
Injection size: 20 µL for the 4 and 24 h time points; 50 µL for the 48 and 72 h time points

Suitability requirements
Samples: *System suitability solution* and *Standard solutions*
Resolution: NLT 2.0 between epiminocycline and minocycline. Inject 20 µL of the *System suitability solution*.
Tailing factor: NMT 2.0. Inject 20 µL of the *System suitability solution*.
Relative standard deviation: NMT 2.0% for the minocycline peak, any of the *Standard solutions*

Analysis: Construct a calibration curve for each sampling interval by plotting the concentration of the *Standard solutions* versus peak area. Calculate the slopes and y-intercepts using linear regression analysis. Calculate the release rate of minocycline:

$$\text{Result}_i = [(r_{Ui} - y_i)/S_i] \times 10/(i \times W \times A)$$

i = sampling time, 4, 24, 48, 72 h
r_{Ui} = peak response from each of the *Standard solutions* at time i
y_i = y-intercept of the calibration curve at sampling time i
S_i = slope of the calibration curve at sampling time i
W = weight of the sample (mg)
A = amount of minocycline in the sample (mg/mg of sample) as determined in the *Assay*

Tolerances

Time (h)	Release Rate (µg/h) Average of 6 Measurements
0–4	NLT 25
4–24	NLT 1.0
24–48	NLT 0.2
48–72	NLT 0.05

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES
Organic Impurities
• **Procedure**
Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each related compound in the portion of Minocycline Periodontal System taken:

$$\text{Result} = (r_U/r_T) \times 100$$

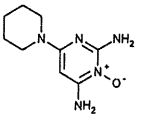
r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the peak responses from the *Sample solution*. [Note—Exclude peaks eluting in the solvent front.]

Acceptance criteria
Individual impurities: NMT 6.0% of epiminocycline
Total impurities (excluding epiminocycline): NMT 3.5%

SPECIFIC TESTS
• **Water Determination,** *Method I* ⟨921⟩: NMT 5.0%
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Organisms** ⟨62⟩: The total aerobic microbial count does not exceed 1000 cfu/g; the total combined molds and yeasts count does not exceed 100 cfu/g; and the product meets the requirements of the test for the absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in a tight, light-resistant container. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Minocycline Hydrochloride RS

Minoxidil



$C_9H_{15}N_5O$ 209.25
2,4-Pyrimidinediamine, 6-(1-piperidinyl)-, 3-oxide;
2,4-Diamino-6-piperidinopyrimidine 3-oxide [38304-91-5].

DEFINITION
Minoxidil contains NLT 97.0% and NMT 103.0% of minoxidil ($C_9H_{15}N_5O$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)**:** ▲[NOTE—Methods described in 197A or 197M may be used.]▲ (USP 1-May-2020)Do not dry specimens.

Add the following:

▲• **B.** The retention time of the minoxidil peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (70:1:30). Add 3.0 g/L of docusate sodium. Adjust with perchloric acid to a pH of 3.0. ▲▲ (USP 1-May-2020)

Standard solution: 0.25 mg/mL of USP Minoxidil RS in ▲*Mobile phase*▲ (USP 1-May-2020)

Sample solution: 0.25 mg/mL of Minoxidil in ▲*Mobile phase*▲ (USP 1-May-2020)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 25-cm; ▲5-µm▲ (USP 1-May-2020) packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
▲Run time: NLT 2 times the retention time of the minoxidil peak▲ (USP 1-May-2020)

System suitability
Sample: *Standard solution*▲▲ (USP 1-May-2020)
Suitability requirements
▲Tailing factor: NMT 2.0▲ (USP 1-May-2020)
Relative standard deviation: NMT ▲1.10%▲ (USP 1-May-2020)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of minoxidil ($C_9H_{15}N_5O$) in the portion of Minoxidil taken:

$$\text{Result} = {}^{\blacktriangle}(r_U/r_S)_{\blacktriangle\,(\text{USP 1-May-2020})} \times (C_S/C_U) \times 100$$

$^{\blacktriangle}r_{U\blacktriangle\,(\text{USP 1-May-2020})}$ = peak response ▲of minoxidil▲ (USP 1-May-2020) from the *Sample solution*

$^{\blacktriangle}r_{S\blacktriangle\,(\text{USP 1-May-2020})}$ = peak response ▲of minoxidil▲ (USP 1-May-2020) from the *Standard solution*

C_S = concentration of ▲USP Minoxidil RS in▲ (USP 1-May-2020) the *Standard solution* (mg/mL)

C_U = concentration of ▲Minoxidil in▲ (USP 1-May-2020) the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.5%

Change to read:

• **ORGANIC IMPURITIES**

▲Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in a mixture of 600 mL of methanol, 10 mL of glacial acetic acid, and 400 mL of water. Adjust with perchloric acid to a pH of 3.0 ± 0.1.

Diluent: Methanol and water (50:50)

Standard solution: 0.005 mg/mL of USP Minoxidil RS in *Diluent*

Sensitivity solution: 0.125 µg/mL of USP Minoxidil RS in *Diluent* from the *Standard solution*

Sample solution: 0.25 mg/mL of Minoxidil in *Diluent* prepared as follows. Transfer a suitable amount of Minoxidil in an appropriate volumetric flask. Initially add *Diluent* to about 60% of the flask volume, shake on a mechanical shaker for 20 min, and then dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 40 µL
Run time: NLT 3 times the retention time of the minoxidil peak

System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of the Minoxidil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of minoxidil from the *Standard solution*

C_S = concentration of USP Minoxidil RS in the *Standard solution* (mg/mL)

C_U = concentration of Minoxidil in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pyrimidine oxide analog[a]	0.19	0.35	0.2
Pyrimidine analog[b]	0.37	0.49	0.2
Minoxidil	1.00	1.0	—
Deoxyminoxidil[c]	1.45	1.32	0.2
Any individual unspecified impurity	—	1.0	0.1

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	1.0

[a] 4-Chloropyrimidine-2,6-diamine-1-oxide.
[b] 6-Chloropyrimidine-2,4-diamine.
[c] 6-(Piperidin-1-yl)pyrimidine-2,4-diamine. ▲(USP 1-May-2020)

SPECIFIC TESTS
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 50° and at a pressure not exceeding 5 mm of mercury for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩
  USP Minoxidil RS

Minoxidil Tablets

DEFINITION
Minoxidil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of minoxidil ($C_9H_{15}N_5O$).

IDENTIFICATION

Change to read:

- **▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Transfer a portion of finely powdered Tablets, equivalent to 10 mg of minoxidil, to a separator. Add 25 mL of water, and extract with three 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a stream of nitrogen. Wash the inside of the container with 5 mL of alcohol, add 300 mg of potassium bromide, and evaporate under vacuum at 50° until dry.
  Acceptance criteria: The IR absorption spectrum of the potassium bromide dispersion prepared from the *Sample* exhibits maxima at the same wavelengths as that of a similar preparation of USP Minoxidil RS.

ASSAY
- **Procedure**
  Mobile phase: Methanol, glacial acetic acid, and water (70:1:30). Add 3.0 g/L of docusate sodium, and adjust with perchloric acid to a pH of 3.0.
  Internal standard solution: 0.2 mg/mL of medroxyprogesterone acetate in *Mobile phase*
  Standard solution: 0.25 mg/mL of USP Minoxidil RS in *Internal standard solution*
  Sample solution: Nominally 0.25 mg/mL of minoxidil in *Internal standard solution*, prepared as follows. Dissolve the equivalent to 5 mg of minoxidil, from powdered Tablets (NLT 10), in 20.0 mL of *Internal standard solution*, and shake for 5 min.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 25-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for the internal standard and minoxidil are 0.8 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between the internal standard and minoxidil peaks
  Relative standard deviation: NMT 2.0% from NLT four replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of minoxidil ($C_9H_{15}N_5O$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of minoxidil to the internal standard from the *Sample solution*
R_S = peak response ratio of minoxidil to the internal standard from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩
  Medium: *pH 7.2 Phosphate Buffer* (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
  Apparatus 1: 75 rpm
  Time: 15 min
  Spectrometric conditions
  Mode: UV
  Detector: UV 231 nm for Tablets containing up to 10 mg of minoxidil, and UV 287 nm for Tablets containing more than 10 mg of minoxidil
  Standard solution: USP Minoxidil RS in *Medium*
  Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
  Tolerances: NLT 75% (*Q*) of the labeled amount of minoxidil ($C_9H_{15}N_5O$) is dissolved.
- **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES
- **Organic Impurities**
  Diluent: Methanol and water (50:50)
  Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in a mixture of methanol, glacial acetic acid, and water (60:1:40). Adjust with perchloric acid to a pH of 3.0 ± 0.1, and pass through a suitable filter of 0.45-µm pore size.
  Standard solution: 5 µg/mL of USP Minoxidil RS in *Diluent*
  Sample solution: Nominally equivalent to 0.25 mg/mL of minoxidil in *Diluent* from powdered tablets (NLT 20). Initially add *Diluent* to about 60% of the volume of the flask, shake on a mechanical shaker for 20 min, and then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 3.9-mm × 15-cm; 3–10-µm packing L1
  Flow rate: 0.5 mL/min
  Run time: NLT 3 times the retention time of the main peak
  Injection volume: 40 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each individual impurity in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_U/C_S) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of minoxidil from the *Standard solution*
C_S = concentration of USP Minoxidil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of minoxidil in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyrimidine oxide analog[a]	0.19	—**
Minoxidil	1.00	—
Pyrimidine analog[b]	0.37	—**
Deoxominoxidil[c]	1.45	—**
Any other unknown impurity	—	0.2
Total impurities*	—	2.0

* Total impurities is the sum of all the impurities, including process-related impurities. Disregard peaks less than 0.05%.
[a] 2,4,-Diamin-6-chloro-pyrimidine 3 oxide.
[b] 2,4,-Diamin-6-chloro-pyrimidine.
[c] 2,4,-Diamin-6-pipridino-pyrimidine.
** A process-related impurity that is controlled in the drug substance.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Minoxidil RS

Minoxidil Topical Solution

DEFINITION
Minoxidil Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of minoxidil ($C_9H_{15}N_5O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: Evaporate 1 mL of the Topical Solution under a stream of nitrogen while heating at 50°.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Add 0.65 mL of heptafluorobutyric acid to a 1000-mL volumetric flask. Dilute with water to volume.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
10.0	60	40
10.1	100	0
15.0	100	0

Diluent: Methanol and water (50:50)
System suitability solution: 0.4 mg/mL of USP Minoxidil RS and 0.001 mg/mL of USP Minoxidil Related Compound E RS in *Diluent*
Standard solution: 0.05 mg/mL of USP Minoxidil RS in *Diluent*
Sample solution: Nominally 0.05 mg/mL of minoxidil in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 35°
Flow rate: 0.4 mL/min
Injection volume: 1 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between minoxidil and minoxidil related compound E, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of minoxidil ($C_9H_{15}N_5O$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Minoxidil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of minoxidil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.4 µg/mL of USP Minoxidil RS in *Diluent*
Sample solution: Nominally 0.4 mg/mL of minoxidil in *Diluent*. Pass through a suitable filter of 0.2-µm pore size.
System suitability
Samples: *System suitability solution* and *Standard solution*

USP Monographs

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Suitability requirements
Resolution: NLT 1.5 between minoxidil and minoxidil related compound E, *System suitability solution*
Relative standard deviation: NMT 2.8%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unspecified impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of minoxidil from the *Standard solution*
C_S = concentration of USP Minoxidil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of minoxidil in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

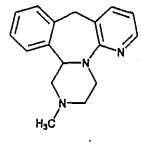
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Minoxidil related compound A (pyrimidine oxide analog)[a, b]	0.36	—
Pyrimidine analog[b, c]	0.51	—
Minoxidil	1.00	—
Minoxidil related compound E (deoxyminoxidil)[b, d]	1.03	—
Individual unspecified impurity	—	0.2
Total impurities	—	2.0

[a] 2,6-Diamino-4-chloropyrimidine 1-oxide.
[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.
[c] 6-Chloropyrimidine-2,4-diamine.
[d] 6-(Piperidin-1-yl)pyrimidine-2,4-diamine.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Minoxidil RS
  USP Minoxidil Related Compound E RS
  6-(Piperidin-1-yl)pyrimidine-2,4-diamine.
  $C_9H_{15}N_5$ 193.25

Mirtazapine



$C_{17}H_{19}N_3$ 265.35

Pyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine,1,2,3,4,10,14b-hexahydro-2-methyl-;
1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]-benzazepine [85650-52-8].

DEFINITION
Mirtazapine contains NLT 98.0% and NMT 102.0% of mirtazapine ($C_{17}H_{19}N_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Diluent: Acetonitrile and water (1:1)
Buffer: Dissolve 18 g of tetramethylammonium hydroxide pentahydrate in 950 mL of water. Adjust with phosphoric acid to a pH of 7.4. Dilute with water to 1 L.
Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Buffer* (15: 12.5: 7.5: 65)
Standard solution: 0.3 mg/mL of USP Mirtazapine RS in *Diluent*
Sample solution: 0.3 mg/mL of Mirtazapine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 7000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mirtazapine ($C_{17}H_{19}N_3$) in the portion of Mirtazapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)
C_U = concentration of Mirtazapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Diluent, Buffer, and **Mobile phase:** Prepare as directed in the *Assay*.
System suitability solution: 1.5 mg/mL of USP Mirtazapine Resolution Mixture RS in *Diluent*
Standard solution: 0.0015 mg/mL of USP Mirtazapine RS in *Diluent*
Sample solution: 1.5 mg/mL of Mirtazapine in *Diluent*

USP Monographs

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: Twice the retention time of mirtazapine
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times are listed in *Table 1*.]
Suitability requirements
Resolution: NLT 1.5 between acyclomirtazapine methyl derivative (impurity E) and 10-ketomirtazapine (impurity F), *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Mirtazapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of mirtazapine from the *Standard solution*
C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)
C_U = concentration of Mirtazapine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

[NOTE—Disregard any peak with a result of 0.05% or less, as calculated using the formula given above.]
Acceptance criteria: See *Table 1*.

Table 1

Impurity Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mirtazapine *N*-oxide[a]	0.2	0.8	0.1
Acyclomirtazapine alcohol[b]	0.3	0.8	0.1
1-Ketomirtazapine[c]	0.35	1.0	0.1
Desmethylmirtazapine[d]	0.4	1.0	0.1
Mirtazapine	1.0	—	—
Acyclomirtazapine methyl derivative[e]	1.3	1.0	0.1
10-Ketomirtazapine[f]	1.35	5.0	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[NOTE—Disregard any peak representing less than 0.05% of the main peak and any peak that is due to the *Diluent*.]
[a] 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide (Impurity A).
[b] (2-(4-Methyl-2-phenylpiperazin-1-yl)pyridin-3-yl)methanol (Impurity B).
[c] (2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one (Impurity C).
[d] 1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine (Impurity D).
[e] 4-Methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine (Impurity E).
[f] 2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14b*H*)-one (Impurity F).

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 3.5%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL, in denatured alcohol
Acceptance criteria: +2° to −2°

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label it to indicate whether it is anhydrous or hemihydrate.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Mirtazapine RS
USP Mirtazapine Resolution Mixture RS
This resolution mixture contains approximately 0.1% w/w each of the following:
Impurity A: 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide.
Impurity B: (2-(4-Methyl-2-phenylpiperazin-1-yl)pyridin-3-yl)methanol.
Impurity C: (2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one.
Impurity D: 1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine.
Impurity E: 4-Methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine.
Impurity F: 2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14b*H*)-one.

Add the following:

▲Mirtazapine Compounded Oral Suspension, Veterinary

DEFINITION
Mirtazapine Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$).
Prepare Mirtazapine Compounded Oral Suspension, Veterinary 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Mirtazapine tablet(s),[a] equivalent to	1.2 g of mirtazapine

USP Monographs

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Vehicle: 1:1 mixture of Ora-Plus[b] and Ora-Sweet,[b] a sufficient quantity to make	120 mL

[a] Mirtazapine 30-mg tablets, Mylan Pharmaceuticals, Inc., Morgantown, WV.
[b] Perrigo, Allegan, MI.

Place the *Mirtazapine tablets* in a suitable container and triturate to a fine powder. Add a small amount of *Vehicle* and mix well to form a smooth paste. Add a sufficient amount of *Vehicle* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Add 23.5 mL of 25% tetramethylammonium hydroxide solution to 626.5 mL of water. Adjust with phosphoric acid to a pH of 7.4. Add 150 mL of acetonitrile, 125 mL of methanol, and 75 mL of tetrahydrofuran.

Diluent: Acetonitrile and water (50:50)

Standard solution: 0.5 mg/mL of USP Mirtazapine RS in *Diluent*

Sample solution: Transfer 0.5 mL of Oral Suspension, Veterinary to a 10-mL volumetric flask, and add *Diluent* to volume. Pass through a filter of 0.22-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 60°
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for mirtazapine is about 25.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mirtazapine from the *Sample solution*
r_S = peak response of mirtazapine from the *Standard solution*
C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mirtazapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 5.6–6.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator.

• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mirtazapine RS▲ 2S (USP41)

Mirtazapine Tablets

DEFINITION
Mirtazapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Extraction mixture: *n*-Hexane and water (1:1)
Sample: Transfer an amount equivalent to 30 mg of mirtazapine from finely powdered Tablets to a suitable centrifuge tube. Add *Extraction mixture* to obtain a solution of 1 mg/mL of mirtazapine in *n*-hexane. Shake for 5 min, and centrifuge. Decant, and evaporate the supernatant.
Standard: Dissolve USP Mirtazapine RS in *Extraction mixture* to obtain a solution having a concentration of about 1 mg/mL of mirtazapine in *n*-hexane. Shake for 5 min, and centrifuge. Decant, and evaporate the supernatant.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Acetonitrile and water (1:1)

Buffer: Dissolve 18.0 g of tetramethylammonium hydroxide pentahydrate in 950 mL of water. Adjust with phosphoric acid to a pH of 7.4, and dilute with water to 1 L.

Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Buffer* (15: 12.5: 7.5: 65)

Standard solution: 0.3 mg/mL of USP Mirtazapine RS in *Diluent*

Sample solution: Nominally 0.3 mg/mL of mirtazapine (from an amount equivalent to the weight of 1 Tablet from NLT 20 finely powdered Tablets) in *Diluent*. Shake vigorously for 10 min, centrifuge an aliquot, and use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 7000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Standard solution: USP Mirtazapine RS in *Medium* in a concentration similar to the one expected in the *Sample solution*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 315 nm
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Diluent, Buffer, and **Mobile phase:** Proceed as directed in the *Assay*.
System suitability solution: 1.5 mg/mL of USP Mirtazapine Resolution Mixture RS in *Diluent*
Standard solution: 0.015 mg/mL of USP Mirtazapine RS in *Diluent*
Sample solution: 1.5 mg/mL of mirtazapine (from an amount equivalent to the weight of 1 Tablet from NLT 20 finely powdered Tablets) in *Diluent*. Shake vigorously for 10 min, centrifuge an aliquot, and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of mirtazapine
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times are listed in *Table 1*.]
Suitability requirements
Resolution: NLT 1.5 between acyclomirtazapine methyl derivative (impurity E) and 10-ketomirtazpine (impurity F), *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = mirtazapine peak response from the *Standard solution*
C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mirtazapine *N*-oxide[a]	0.2	0.8	0.2
Acyclomirtazapine alcohol[b, g]	0.3	—	—
1-Ketomirtazpine[c]	0.35	1.0	0.2
Desmethylmirtazapine[d, g]	0.4	—	—
Mirtazapine	1.0	—	—
Acyclomirtazapine methyl derivative[e, g]	1.3	—	—
10-Ketomirtazapine[f]	1.35	5.0	0.2
Any individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

[Note—Disregard any peak representing less than 0.05% of the main peak and any peak that is due to the *Diluent*.]
[a] 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide. (Impurity A)
[b] (2-(4-Methyl-2-phenylpiperazin-1-yl)pyridin-3-yl)methanol. (Impurity B)
[c] (2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one. (Impurity C)
[d] 1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine. (Impurity D)
[e] 4-Methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine. (Impurity E)
[f] 2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14b*H*)-one. (Impurity F)
[g] Process impurity. Included for identification purposes only. Not to be included in *Total impurities*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Mirtazapine RS
USP Mirtazapine Resolution Mixture RS

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This resolution mixture contains approximately 0.1% w/w of each of the following:
Impurity A: 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide.
Impurity B: (2-(4-Methyl-2-phenylpiperazin-1-yl)pyridin-3-yl)methanol.
Impurity C: (2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one.
Impurity D: 1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine.
Impurity E: 4-Methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine.
Impurity F: 2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14b*H*)-one.

Mirtazapine Orally Disintegrating Tablets

DEFINITION

Mirtazapine Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Standard solution: Dissolve 30 mg of USP Mirtazapine RS in a separatory funnel containing 30 mL of water, and add 30 mL of *n*-hexane. Shake vigorously for 5 min. Allow the solution to separate into two layers. Filter the *n*-hexane layer through glass wool, and evaporate to dryness.
Sample solution: Transfer a quantity of finely powdered Tablets, equivalent to 30 mg of mirtazapine, to a separatory funnel. Add 30 mL of water and 30 mL of *n*-hexane. Shake vigorously for 5 min. Allow the solution to separate into two layers. Filter the *n*-hexane layer through glass wool, and evaporate to dryness.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Acetonitrile and water (50:50)
Diluted phosphoric acid: Water and phosphoric acid (1000:3)
Buffer: Dissolve 1 g of monobasic potassium phosphate and 1.7 g of pentanesulfonic acid sodium salt in 1 L of water. Adjust with *Diluted phosphoric acid* to a pH of 4.7 ± 0.1, and filter.
Mobile phase: Acetonitrile and *Buffer* (25:75)
Standard stock solution: 0.3 mg/mL of USP Mirtazapine RS in *Diluent*
Standard solution: 0.036 mg/mL of USP Mirtazapine RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 0.3 mg/mL of mirtazapine in *Diluent* (from NLT 20 Tablets, finely powdered). Sonicate for 15 min with occasional swirling, and shake for 30 min. [NOTE—Alternatively, dissolve 10 Tablets in a volume of a mixture of acetonitrile and water (90:10) to obtain a 0.3 mg/mL solution of mirtazapine. Shake or stir until the mixture is free from lumps.]
Sample solution: Nominally, 0.036 mg/mL of mirtazapine in *Mobile phase* obtained as follows: transfer 40 mL of the *Sample stock solution* into a centrifuge tube, and centrifuge at 3000 rpm for 10 min. Transfer 6.0 mL of the supernatant into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass the portion through a polypropylene membrane filter of 0.45-µm pore size. Discard at least the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mirtazapine RS (mg/mL)
C_U = nominal concentration of mirtazapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩: NMT 60 s
- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Sample solution: Sample per *Dissolution* ⟨711⟩. Pass through a filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.
Standard solution: 33 µg/mL of USP Mirtazapine RS in *Medium*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 316 nm
Blank: *Medium*
Cell: 0.5 cm
Analysis: Determine the percentage of mirtazapine ($C_{17}H_{19}N_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)
V = volume, 900 mL
L = label claim of mirtazapine (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

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IMPURITIES

• ORGANIC IMPURITIES

Solution A: Dissolve 7.2 g of tetramethylammonium hydroxide pentahydrate in 4 L of water. Add 1 mL of triethylamine. Adjust with phosphoric acid to a pH of 7.4.

Solution B: Acetonitrile, methanol, and tetrahydrofuran (170:145:85)

Diluent: Acetonitrile and water (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	61	39
6.0	61	39
10.0	46	54
18.4	46	54
18.5	61	39
22.0	61	39

System suitability solution: 0.3 mg/mL of USP Mirtazapine RS in *Diluent*

Standard solution: 0.015 mg/mL each of USP Mirtazapine RS, USP Mirtazapine Related Compound A RS, USP Mirtazapine Related Compound B RS, USP Mirtazapine Related Compound C RS, and USP Mirtazapine Related Compound D RS in *Diluent*

Sample solution: Nominally, 1.5 mg/mL of mirtazapine in *Diluent* from NLT 5 Tablets

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0, *System suitability solution*

Relative standard deviation: NMT 2.0%, *System suitability solution*

Resolution: NLT 4.0 between the mirtazapine and mirtazapine related compound D peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual specified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual specified impurity from the *Sample solution*

r_S = peak response of the corresponding related compound from the *Standard solution*

C_S = concentration of each individual impurity in the *Standard solution* (mg/mL)

C_U = nominal concentration of mirtazapine in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of mirtazapine from the *Standard solution*

C_S = concentration of mirtazapine in the *Standard solution* (mg/mL)

C_U = nominal concentration of mirtazapine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. [NOTE—Disregard any peak less than 0.05%.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mirtazapine related compound B	0.23	0.5
Mirtazapine related compound C	0.51	0.5
Mirtazapine related compound A	0.62	0.5
Mirtazapine	1.0	—
Mirtazapine related compound D	1.3	0.5
Any individual unspecified degradation product	—	0.5
Total impurities	—	3.0

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Store at controlled room temperature. Protect from light and moisture.

• USP REFERENCE STANDARDS ⟨11⟩

USP Mirtazapine RS

USP Mirtazapine Related Compound A RS

1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine.

$C_{16}H_{17}N_3$ 251.33

USP Mirtazapine Related Compound B RS

1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide monohydrate.

$C_{17}H_{19}N_3O \cdot H_2O$ 299.36

USP Mirtazapine Related Compound C RS

2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one.

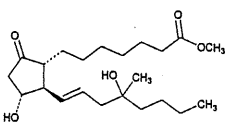
$C_{17}H_{17}N_3O$ 279.34

USP Mirtazapine Related Compound D RS

2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14b*H*)-one.

$C_{17}H_{17}N_3O$ 279.34

Misoprostol



$C_{22}H_{38}O_5$ 382.53

Prost-13-en-1-oic acid, 11,16-dihydroxy-16-methyl-9-oxo-, methyl ester, (1*R**,2*R**,3*R**,*E*)-;

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(±)-Methyl (1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(4*RS*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate [59122-46-2].

DEFINITION

Misoprostol contains NLT 97.0% and NMT 102.0% of $C_{22}H_{38}O_5$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197S▲** (CN 1-May-2020)

Sample solution: 30 mg/mL
Medium: Chloroform

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 2,2,4-Trimethylpentane, dioxane, and acetonitrile (78: 21.5: 0.5)
Standard solution: 5.0 mg/mL of USP Misoprostol RS in *Mobile phase*
Sample solution: 5.0 mg/mL of Misoprostol in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—Identify the impurities based on the retention times shown in *Impurity Table 1*.]
Suitability requirements
Resolution: NLT 1.2, between the second diastereomer peak for 12-epimisoprostol and the Misoprostol peak
Relative standard deviation: NMT 1.0%, for three replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{38}O_5$ in the portion of Misoprostol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

ORGANIC IMPURITIES

- **Procedure 1**

Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatogram for at least 3 times the retention time of the Misoprostol peak, and measure the peak responses. Identify the impurities based on the retention times shown in *Impurity Table 1*.

Calculate the percentage of each impurity in the portion of Misoprostol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL)
C_U = concentration of Misoprostol in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
A-Type misoprostol[a]	0.22	7.8	0.1
B-Type misoprostol[b]	0.33	0.80	0.1
Norprostol[c]	0.51	8.4	0.1
8-Epimisoprostol[d]	0.71	1.05	0.3
12-Epimisoprostol[e]	0.86 and 0.92[f]	1.08	1.0[f]
Misoprostol	1.0	—	—
Any other individual impurity	—	1.0	0.1

[a] Methyl 7-{(1*R**,2*S**)-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl}heptanoate.
[b] (*E*)-Methyl 7-[2-(4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopent-1-enyl] heptanoate.
[c] Methyl 7-(3-hydroxy-5-oxocyclopent-1-enyl)heptanoate.
[d] Methyl (1*S**,2*R**,3*R**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.
[e] Methyl (1*S**,2*R**,3*S**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.
[f] 12-Epimisoprostol consists of two diastereomers that are separated under these conditions; integrate both peaks together for the impurity calculations.

- **Procedure 2: Content of Diastereomers**

Mobile phase: Hexane, ethanol, and isopropyl alcohol (94:4:2)
Sample solution: 1.0 mg/mL of Misoprostol in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Sample solution*
[NOTE—Identify the components based on their relative retention times which are about 0.92 for the first diastereomer peak and 1.0 for the second diastereomer peak.]
Suitability requirements
Resolution: NLT 2.0, between the two diastereomer peaks
Relative standard deviation: NMT 2.0% from the area of the first diastereomer peak
Analysis
Sample: *Sample solution*

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Calculate the fraction of the first diastereomer in the portion of Misoprostol taken:

$$\text{Result} = r_1/(r_1 + r_2)$$

r_1 = peak response for the first diastereomer
r_2 = peak response for the second diastereomer

Acceptance criteria
Fraction of the first diastereomer: 0.51–0.56

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a freezer.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Misoprostol RS

Misoprostol Dispersion

DEFINITION
Misoprostol Dispersion is a mixture of Misoprostol and Hypromellose. It contains NLT 95.0% and NMT 104.0% of the labeled amount of misoprostol ($C_{22}H_{38}O_5$).

IDENTIFICATION
• **A. ULTRAVIOLET ABSORPTION**
Perform both *Procedure 1* and *Procedure 2*.
Procedure 1
Medium: Methanol and water (4:1)
Sample solution: Nominally 16 µg/mL of misoprostol in *Medium* prepared as follows. Dissolve the amount of Misoprostol Dispersion, equivalent to 400 µg of misoprostol, in 25 mL of *Medium*.
Blank: Prepare a solution of hypromellose in *Medium* having the same concentration as in the *Sample solution*.
Analysis: Determine UV absorption spectrum of *Sample solution* against the *Blank* from 330–230 nm.
Acceptance criteria 1: It exhibits no maximum near 280 nm.
Procedure 2
Medium: Methanol and 1 N potassium hydroxide (4:1)
Sample solution: Add 10 mL of *Medium* to 10 mL of the *Sample solution* prepared from *Procedure 1*. Allow to stand for 30 min at room temperature.
Blank: Add 10 mL of *Medium* to 10 mL of the *Blank* prepared from *Procedure 1*. Allow to stand for 30 min at room temperature.
Analysis: Determine UV absorption spectrum of *Sample solution* against the *Blank* from 330–230 nm.
Acceptance criteria 2: It exhibits a maximum near 280 nm.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
[NOTE—During addition of water to a solution of misoprostol in isopropyl alcohol, an exothermic reaction takes place. After each addition of water, invert the flask to mix isopropyl alcohol and water. Allow the solution to cool to room temperature before the final dilution.]
Buffer: 1.36 g/L of monobasic potassium phosphate in water, adjusted with phosphoric acid to a pH of 3.0 ± 0.1
Mobile phase: Isopropyl alcohol and *Buffer* (27:73)
Standard stock solution: 0.5 mg/mL of USP Misoprostol RS in isopropyl alcohol. [NOTE—This solution is stable up to 28 days when stored at 5 ± 3°.]
Standard solution: 0.1 mg/mL of USP Misoprostol RS in water from *Standard stock solution*. [NOTE—This solution is stable up to 7 days when stored at 5 ± 3°.]
Sample solution: Nominally 0.1 mg/mL of misoprostol prepared as follows. Place an amount of Misoprostol Dispersion, equivalent to about 10 mg of misoprostol, into a 100-mL volumetric flask, and add 25 mL of isopropyl alcohol. Shake to disperse the solid, place the solution in an ice bath, swirl, and allow to cool for 10 min. Carefully add about 70 mL of water, previously chilled in a refrigerator, remove from the ice bath, and shake. Sonicate as necessary at a temperature not exceeding 20°. Equilibrate to room temperature, dilute with water to volume, and immediately cool the solution to 5°. [NOTE—This solution is stable up to 7 days when stored at 5 ± 3°.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperatures
Column: 50 ± 2°; the *Mobile phase* must be preheated prior to introduction on the column.
Autosampler: 5 ± 3°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—USP Misoprostol RS contains 12-epimisoprostol as a minor component. The relative retention times for 12-epimisoprostol and misoprostol are 0.84 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.7 between 12-epimisoprostol and misoprostol
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of misoprostol ($C_{22}H_{38}O_5$) in the portion of Misoprostol Dispersion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of misoprostol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–104.0%

IMPURITIES
• **ORGANIC IMPURITIES**
[NOTE—During the addition of water to a solution of misoprostol in isopropyl alcohol, an exothermic reaction takes place. After each addition of water, invert the flask to mix isopropyl alcohol and water. Allow the solution to cool to room temperature before the final dilution.]
Buffer, Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Diluent: Isopropyl alcohol and water (27:73)

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Blank: Prepare a solution of hypromellose in the *Diluent* having the same concentration as in the *Sample solution*.
Diluted standard solution: 0.5 µg/mL of USP Misoprostol RS in *Diluent* from *Standard solution*
Sensitivity solution: 0.1 µg/mL of USP Misoprostol RS in *Diluent* from *Diluted standard solution*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.7 between 12-epimisoprostol and misoprostol, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution*, *Blank*, and *Diluted standard solution*
Calculate the percentage of any individual impurity in the portion of Misoprostol Dispersion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of misoprostol from the *Diluted standard solution*
C_S = concentration of USP Misoprostol RS in the *Diluted standard solution* (µg/mL)
C_U = nominal concentration of misoprostol in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard a peak from the *Diluent*, eluting at about 4 min, and any other peak observed in the *Blank*.

Table 1

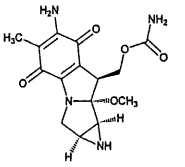
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
12-Epimisoprostol	0.84	—	—[a]
8-Epimisoprostol[b]	0.90	0.90	0.50
Misoprostol	1.0	—	—
B-Type misoprostol[c]	1.6	0.78	0.30
A-Type misoprostol[d]	1.9	2.6	0.50
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	1.8

[a] This is a process impurity in the manufacturing of misoprostol. It is controlled in the misoprostol, which is the starting material for the Misoprostol Dispersion. This impurity is included in the table for identification only, and it is not to be reported or included in the total impurities for the Misoprostol Dispersion.
[b] Methyl (1*S**,2*R**,3*R**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.
[c] (*E*)-Methyl 7-[2-(4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopent-1-enyl] heptanoate.
[d] Methyl 7-[(1*R**,2*S**)-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl]heptanoate.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩
Sample: 300 mg
Analysis: Perform the test immediately after opening the sample container. Use the evaporation technique in which water is released and evaporated from the *Sample* by heating it in an external oven at 105° and transferring it to the reaction cell with the aid of an inert gas.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and store in a refrigerator.
• **LABELING:** The label states that this article is not intended for direct administration to humans or animals. Label it to indicate the nominal concentration or percentage of misoprostol in the Misoprostol Dispersion.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Misoprostol RS

Mitomycin



$C_{15}H_{18}N_4O_5$ 334.33
Azirino[2′,3′:3,4]pyrrolo[1,2-*a*]indole-4,7-dione, 6-amino-8-[[(aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methyl-, [1a*S*-(1aα,8β,8aα,8bα)]-; (1a*S*,8*S*,8a*R*,8b*S*)-(6-Amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4] pyrrolo[1,2-*a*]indol-8-yl)methyl carbamate; Mitomycin C [50-07-7].

DEFINITION
Mitomycin has a potency of NLT 970 µg/mg of mitomycin ($C_{15}H_{18}N_4O_5$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Analysis: Do not dry the sample and standard.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Dissolve 1.54 g of ammonium acetate in 250 mL of methanol. Add 5.0 mL of 0.83 N acetic acid and water to make 1000 mL.
System suitability solution: 0.5 mg/mL of USP Mitomycin RS and 7.5 mg/mL of 3-ethoxy-4-hydroxybenzaldehyde in *N,N*-dimethylacetamide
Standard solution: 0.5 mg/mL of USP Mitomycin RS in *N,N*-dimethylacetamide
Sample solution: 0.5 mg/mL of Mitomycin in *N,N*-dimethylacetamide
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 3.9-mm × 30-cm; 10-µm packing L11
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

USP Monographs

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[NOTE—The relative retention times for mitomycin and 3-ethoxy-4-hydroxybenzaldehyde are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 1.8 between mitomycin and 3-ethoxy-4-hydroxybenzaldehyde, *System suitability solution*

Tailing factor: NMT 1.3, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of mitomycin ($C_{15}H_{18}N_4O_5$) in the portion of Mitomycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Mitomycin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of mitomycin in USP Mitomycin RS (µg/mg)

Acceptance criteria: NLT 970 µg/mg

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 0.77 g/L of ammonium acetate

Solution B: Methanol and *Solution A* (20:80)

Solution C: Methanol and *Solution A* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
30	0	100
45	0	100
50	100	0

Standard solution: 0.025 mg/mL of USP Mitomycin RS in methanol

Sensitivity solution: 2.5 µg/mL of USP Mitomycin RS in methanol

Sample solution: 5 mg/mL of Mitomycin in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Mitomycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of mitomycin from the *Standard solution*
C_S = concentration of USP Mitomycin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of mitomycin in USP Mitomycin RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Albomitomycin C[a]	0.6	1.0	0.5
Mitomycin	1.0	—	—
Mitomycin B[b]	1.2	1.0	0.5
Cinnamamide	1.3	2.9	0.5
Mitomycin A[c]	1.6	1.0	0.5
Any individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	2.0

[a] {(1*S*,2*S*,4a*S*,8a*R*,9*S*,9a*R*)-7-Amino-9a-methoxy-6-methyl-5,8-dioxo-1,2,3,5,8,8a,9,9a-octahydro-1,2,4a-metheno-(epinitrilo)pyrrolo[1,2-*a*]indol-9-yl}methyl carbamate.

[b] {(1a*S*,8*S*,8a*R*,8b*S*)-8a-Hydroxy-6-methoxy-1,5-dimethyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-yl}methyl carbamate.

[c] {(1a*S*,8*S*,8a*R*,8b*S*)-6,8a-Dimethoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-yl}methyl carbamate.

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩: Meets the requirements

• **pH** ⟨791⟩

Sample: 5-mg/mL suspension in water

Acceptance criteria: 6.0–7.5

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.5%

• **STERILITY TESTS** ⟨71⟩: Where the label states that Mitomycin is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Mitomycin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 10.0 USP Endotoxin Units/mg of mitomycin.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Mitomycin RS

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Mitomycin for Injection

DEFINITION

Mitomycin for Injection contains NLT 90.0% and NMT 120.0% of the labeled amount of mitomycin ($C_{15}H_{18}N_4O_5$).

IDENTIFICATION

- **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 1.54 g of ammonium acetate in 250 mL of methanol. Add 5.0 mL of 0.83 N acetic acid and water to make 1000 mL.

System suitability solution: 0.5 mg/mL of USP Mitomycin RS and 7.5 mg/mL of 3-ethoxy-4-hydroxy benzaldehyde in *N,N*-dimethylacetamide

Standard solution: 0.5 mg/mL of USP Mitomycin RS in *N,N*-dimethylacetamide

Sample solution: Add an accurately measured volume of *N,N*-dimethylacetamide to 1 container of Mitomycin for Injection to obtain a solution that is nominally 0.5 mg/mL of mitomycin.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; 10-µm packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mitomycin and 3-ethoxy-4-hydroxybenzaldehyde are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 1.8 between mitomycin and 3-ethoxy-4-hydroxybenzaldehyde, *System suitability solution*

Tailing factor: NMT 1.3, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mitomycin ($C_{15}H_{18}N_4O_5$) in the container of Mitomycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Mitomycin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mitomycin in the *Sample solution* (mg/mL)

P = potency of mitomycin in USP Mitomycin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 6.0–8.0 where it contains mannitol, and 5.5–8.5 where it contains hydroxypropyl betadex

- **WATER DETERMINATION,** *Method Ia* ⟨921⟩

Sample solution: Prepare as directed for a hygroscopic specimen, using the pooled contents of five containers.

Acceptance criteria: NMT 5.0%

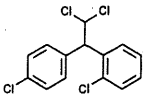
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Contains NMT 10.0 USP Endotoxin Units/mg of mitomycin
- **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Mitomycin RS

Mitotane



$C_{14}H_{10}Cl_4$ 320.04

Benzene, 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]-, (*RS*)-;

(±)-1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane [53-19-0].

DEFINITION

Mitotane contains NLT 97.0% and NMT 103.0% of mitotane ($C_{14}H_{10}Cl_4$), calculated on the anhydrous basis.

[**CAUTION**—Handle Mitotane with exceptional care, because it is a highly potent agent.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 1.38 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Buffer* (75:25)

System suitability solution: 0.2 mg/mL of USP Mitotane RS and 0.2 mg/mL of the *p,p'*-isomer of mitotane in *Mobile phase*. [NOTE—The *p,p'*-isomer of mitotane is 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane known as *p,p'*-DDD.]

Standard solution: 0.2 mg/mL of USP Mitotane RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Mitotane in *Mobile phase*. [NOTE—Inject within 48 h of preparation.]

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for the *p,p'*-isomer of mitotane and mitotane are about 0.92 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the *p,p'*-isomer of mitotane and mitotane, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mitotane ($C_{14}H_{10}Cl_4$) in the portion of Mitotane taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mitotane RS in the *Standard solution* (mg/mL)
C_U = concentration of Mitotane in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mitotane RS

Mitotane Tablets

» Mitotane Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mitotane ($C_{14}H_{10}Cl_4$).

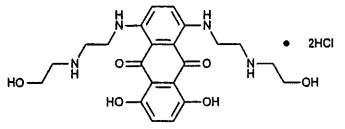
Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Mitotane RS
Identification—Triturate a quantity of finely powdered Tablets, equivalent to about 500 mg of mitotane, with 10 mL of water, filter on a sintered-glass filter funnel, and wash the residue with two 5-mL portions of water. Transfer the residue to a small beaker, add 4 mL of alcohol, heat to boiling, and filter immediately. Allow the filtrate to cool, filter the crystals of mitotane, wash once with 2 mL of alcohol, and dry in vacuum at 60° for 2 hours: the IR absorption spectrum of a mineral oil dispersion of the mitotane so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mitotane RS.
Disintegration ⟨701⟩: 15 minutes, the use of disks being omitted.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Standard preparation—Dissolve about 50 mg of USP Mitotane RS, accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 200 µg per mL.
Assay preparation—Weigh and finely powder not less than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of mitotane, to a 250-mL volumetric flask, add 100 mL of methanol, and shake occasionally for 5 minutes, then dilute with methanol to volume, and mix. Filter, rejecting the first portion of the filtrate, transfer 25.0 mL of the filtrate to a 50-mL volumetric flask, dilute with methanol to volume, and mix.
Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 268 nm, with a suitable spectrophotometer, using methanol as the blank.
Calculate the quantity, in mg, of $C_{14}H_{10}Cl_4$ in the portion of Tablets taken by the formula:

$$0.5C(A_U/A_S)$$

in which C is the concentration, in mg/mL, of USP Mitotane RS in the *Standard preparation*, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Mitoxantrone Hydrochloride



$C_{22}H_{28}N_4O_6 \cdot 2HCl$ 517.40
9,10-Anthracenedione, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-, dihydrochloride;
1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]anthraquinone dihydrochloride [70476-82-3].

DEFINITION
Mitoxantrone Hydrochloride contains NLT 97.0% and NMT 102.0% of mitoxantrone hydrochloride ($C_{22}H_{28}N_4O_6 \cdot 2HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*, 197A or 197K▲ (CN 1-May-2020)**:** Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 0.09 g/mL of sodium 1-heptanesulfonate prepared as follows. Dissolve 22.0 g of sodium

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1-heptanesulfonate in 150 mL of water. Pass through a filter of NMT 0.5-µm pore size and transfer the filtrate to a 250-mL volumetric flask. Wash the filter with 50 mL of water and add the washings to the flask. Add 32.0 mL of glacial acetic acid to the flask, and dilute with water to volume.

Mobile phase: Acetonitrile, *Solution A*, and water (10:1:30)

System suitability solution: 0.2 mg/mL of monoalkyl mitoxantrone hydrochloride and 0.1 mg/mL of mitoxantrone hydrochloride from USP Mitoxantrone System Suitability Mixture RS in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Mitoxantrone Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Mitoxantrone Hydrochloride RS to an appropriate volumetric flask. Add 80% of the flask volume of *Mobile phase*. Sonicate for about 5 min to dissolve. Cool to room temperature and dilute with *Mobile phase* to volume.

Sample solution: 0.4 mg/mL of Mitoxantrone Hydrochloride prepared as follows. Transfer a suitable amount of Mitoxantrone Hydrochloride to an appropriate volumetric flask. Add 80% of the flask volume of *Mobile phase*. Sonicate for about 5 min to dissolve. Cool to room temperature and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L11
[NOTE—After use, wash the column with a mixture of acetonitrile and water (50:50), and store in this mixture.]
Flow rate: 3 mL/min
Injection volume: 50 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for mitoxantrone and monoalkyl mitoxantrone hydrochloride are about 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 3.0 between mitoxantrone and monoalkyl mitoxantrone hydrochloride, *System suitability solution*
Tailing factor: NMT 2.0 for mitoxantrone, *System suitability solution*
Capacity factor, *k'*: NLT 3.5 for mitoxantrone, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mitoxantrone dihydrochloride ($C_{22}H_{28}N_4O_6 \cdot 2HCl$) in the portion of Mitoxantrone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of mitoxantrone from the *Sample solution*
r_S = peak area of mitoxantrone from the *Standard solution*
C_S = concentration of USP Mitoxantrone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Mitoxantrone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**
Analysis: Using the chromatogram of the *Sample solution* obtained as directed in the *Assay*, calculate the percentage of each impurity in the portion of Mitoxantrone Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of all peak responses

Acceptance criteria
Individual impurities: NMT 1.0%
Total impurities: NMT 2.0%

• **LIMIT OF ALCOHOL**
Proceed as directed in *Residual Solvents* ⟨467⟩.
Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 6.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mitoxantrone Hydrochloride RS
USP Mitoxantrone System Suitability Mixture RS
0.2 mg of 1-Amino-5,8-dihydroxy-4-({2-[(2-hydroxyethyl)amino]ethyl}amino)anthracene-9,10-dione hydrochloride [monoalkyl mitoxantrone hydrochloride ($C_{18}H_{19}N_3O_5 \cdot HCl$, 393.82)] and 0.1 mg of USP Mitoxantrone Hydrochloride RS

Mitoxantrone Injection

DEFINITION
Mitoxantrone Injection is a sterile solution of Mitoxantrone Hydrochloride in Water for Injection. It contains the equivalent of NLT 90.0% and NMT 105.0% of the labeled amount of mitoxantrone ($C_{22}H_{28}N_4O_6$).

IDENTIFICATION

Change to read:

• **▲A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*:
197U▲ (CN 1-May-2020)▲ (USP 1-May-2019)
Standard solution: 0.01 mg/mL of USP Mitoxantrone Hydrochloride RS (equivalent to 0.01 mg/mL of mitoxantrone) prepared as follows. Transfer a suitable amount of USP Mitoxantrone Hydrochloride RS to an appropriate volumetric flask. Add 50% of the flask volume of water and 10% of the flask volume of 1 N hydrochloric acid VS. Dilute with water to volume.
Sample solution: Nominally 0.01 mg/mL of mitoxantrone from Injection prepared as follows. Transfer a volume of Injection equivalent to 2 mg of mitoxantrone to a 200-mL volumetric flask containing about 50% of the flask volume of water and 10% of the flask volume of 1 N hydrochloric acid VS. Dilute with water to volume.
Acceptance criteria: ▲Meets the requirements▲ (USP 1-May-2019)

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Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**

Solution A: 0.09 mg/mL of sodium 1-heptanesulfonate prepared as follows. Dissolve 22.0 g of sodium 1-heptanesulfonate in 150 mL of water. Pass through a filter of NMT 0.5-µm pore size and transfer the filtrate to a 250-mL volumetric flask. Wash the filter with 50 mL of water and add the ▲washings▲ (USP 1-May-2019) to the flask. Add 32.0 mL of glacial acetic acid to the flask, and dilute with water to volume.

Mobile phase: Acetonitrile, *Solution A*, and water ▲(10:1:30)▲ (USP 1-May-2019)

System suitability solution: 0.2 mg/mL of ▲monoalkyl mitoxantrone hydrochloride▲ (USP 1-May-2019) and 0.1 mg/mL of mitoxantrone hydrochloride from USP Mitoxantrone System Suitability Mixture RS in *Mobile phase*

Standard solution: 0.46 mg/mL of USP Mitoxantrone Hydrochloride RS (equivalent to 0.4 mg/mL of mitoxantrone) prepared as follows. Transfer a suitable amount of USP Mitoxantrone Hydrochloride RS to an appropriate volumetric flask. Add 80% of the flask volume of *Mobile phase*. Sonicate for about 5 min to dissolve. Cool to room temperature and dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.4 mg/mL of mitoxantrone from Injection, in *Mobile phase*

Chromatographic system

Chromatography ⟨621⟩, *System Suitability*

Mode: LC

Detector: UV 254 nm

Column: 3.9 mm × 30 cm; ▲10-µm▲ (USP 1-May-2019) packing L11

[NOTE—After use, wash the column with a mixture of acetonitrile and water (50:50), and store in this mixture.]

Flow rate: 3 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mitoxantrone and ▲monoalkyl mitoxantrone hydrochloride▲ (USP 1-May-2019) are about ▲1.0 and 1.4,▲ (USP 1-May-2019) respectively.]

Suitability requirements

Resolution: NLT 3.0 between mitoxantrone and ▲monoalkyl mitoxantrone hydrochloride,▲ (USP 1-May-2019) *System suitability solution*

Tailing factor: NMT 2.0 for mitoxantrone, *System suitability solution*

Capacity factor, *k'*: NLT 3.5 for mitoxantrone, *Standard solution*

Relative standard deviation: NMT ▲1.0%,▲ (USP 1-May-2019) *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mitoxantrone ($C_{22}H_{28}N_4O_6$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of mitoxantrone from the *Sample solution*

r_S = peak area of mitoxantrone from the *Standard solution*

C_S = concentration of USP Mitoxantrone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mitoxantrone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of mitoxantrone, 444.49

M_{r2} = molecular weight of mitoxantrone hydrochloride, 517.40

Acceptance criteria: 90.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Analysis: Using the chromatogram of the *Sample solution* obtained as directed in the *Assay*, calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity

r_T = sum of all peak responses

Acceptance criteria

Individual impurities: NMT 1.5%

Total impurities: NMT 3.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.0–4.5

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements▲ (USP 1-May-2019)

Change to read:

• **STERILITY TESTS** ⟨71⟩: Meets the requirements▲▲ (USP 1-May-2019)

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. ▲Do not freeze. Store at controlled room temperature.▲ (USP 1-May-2019)

Change to read:

• **LABELING:** Label Injection to state both the content of the active moiety and the name of the salt used in formulating the article. Label Injection to indicate that it is to be diluted to appropriate ▲volume with▲ (USP 1-May-2019) suitable fluid before administration.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

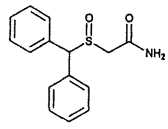
USP Mitoxantrone Hydrochloride RS

USP Monographs

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USP Mitoxantrone System Suitability Mixture RS
▲0.2 mg of 1-Amino-5,8-dihydroxy-4-({2-[(2-hydroxyethyl)amino]ethyl}amino)anthracene-9,10-dione hydrochloride [monoalkyl mitoxantrone hydrochloride ($C_{18}H_{19}N_3O_5 \cdot HCl$, 393.82)] and 0.1 mg of USP Mitoxantrone Hydrochloride RS▲ (USP 1-May-2019)

Modafinil



$C_{15}H_{15}NO_2S$ 273.35
Acetamide, 2-[(diphenylmethyl)sulfinyl]-;
2-[(Diphenylmethyl)sulfinyl]-acetamide [68693-11-8].

DEFINITION
Modafinil contains NLT 98.0% and NMT 101.5% of $C_{15}H_{15}NO_2S$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Buffer: 6.8 g/L of potassium dihydrogen phosphate in water. Adjust with phosphoric acid to a pH of 2.3.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Diluent: Acetonitrile and water (35:65)
System suitability solution: 5 µg/mL of USP Modafinil RS and 10 µg/mL of USP Salicylic Acid RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Modafinil RS in *Diluent*
Sample solution: 0.1 mg/mL of Modafinil in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for modafinil and salicylic acid are about 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.3 between modafinil and salicylic acid
Tailing factor: NMT 1.5 for the modafinil peak
Relative standard deviation: NMT 2.0% for the modafinil peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of modafinil ($C_{15}H_{15}NO_2S$) in the portion of Modafinil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Modafinil RS in the *Standard solution* (mg/mL)
C_U = concentration of Modafinil in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Sample solution, System suitability solution, and **Chromatographic system:** Prepare as directed in the *Assay*.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.3 between modafinil and salicylic acid
Tailing factor: NMT 1.5 for the modafinil peak
Relative standard deviation: NMT 2.0% for the modafinil peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Modafinil taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity
r_T = sum of the responses of all the peaks
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Modafinil	1.0	—	—
Salicylic acid[a]	1.1	—	—
Modafinil acid[b]	1.4	1.0	0.5
Modafinil sulfone[c]	1.7	0.9	0.5
Modafinil ester[d]	3.0	1.0	0.5
Any other individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	1.0

[a] Salicylic acid is used for calculating resolution and is not a potential impurity.
[b] 2-[(Diphenylmethyl)sulfinyl]acetic acid.
[c] 2-[(Diphenylmethyl)sulfonyl]acetamide.
[d] 2-[(Diphenylmethyl)sulfinyl]acetic acid methyl ester.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.2%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Modafinil RS
USP Salicylic Acid RS

USP Monographs

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Modafinil Tablets

DEFINITION

Modafinil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Standard specimen: Transfer a quantity, in mg, of USP Modafinil RS, equivalent to the labeled amount of modafinil, to a suitable container. Add 50 mL each of dichloromethane and water. Shake the mixture, and allow the layers to separate. Filter a portion of the lower (dichloromethane) layer, and evaporate to dryness, using a stream of nitrogen if necessary. Prepare a potassium bromide pellet of the residue.

Sample specimen: Grind 1 Tablet, and add 50 mL each of dichloromethane and water. Shake the mixture, and allow the layers to separate. Filter a portion of the lower (dichloromethane) layer, and evaporate to dryness, using a stream of nitrogen if necessary. Prepare a potassium bromide pellet of the residue.

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Buffer: 6.8 g/L of potassium dihydrogen phosphate in water. Adjust with phosphoric acid to a pH of 2.3.

Mobile phase: Acetonitrile and *Buffer* (35:65)

Diluent A: Acetonitrile and water (35:65)

Diluent B: Acetonitrile, water, and acetic acid (35:65:1)

System suitability solution: 5 µg/mL of USP Modafinil RS and 10 µg/mL of USP Salicylic Acid RS in *Diluent A*

Standard solution: 0.4 mg/mL of USP Modafinil RS in *Diluent B*

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 100 mg of modafinil, to a 250-mL volumetric flask, add 200 mL of *Diluent B*, and sonicate for about 5 min with intermittent manual shaking. Dilute with *Diluent B* to volume, and mix. Pass through a suitable filter of 0.45-µm or finer pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for modafinil and salicylic acid are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.3 between modafinil and salicylic acid

Tailing factor: NMT 1.5 for the modafinil peak

Relative standard deviation: NMT 2.0% for the modafinil peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Modafinil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of modafinil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Prepare a solution having a known concentration of USP Modafinil RS in *Medium*.

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium* if necessary

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: Absorption maximum at about 222 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the amount of modafinil ($C_{15}H_{15}NO_2S$) dissolved.

Tolerances: NLT 75% (*Q*) of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: (*L*/900) mg/mL of USP Modafinil RS, where *L* is the label claim (mg/Tablet). Prepare by dissolving the standard in a volume of methanol equivalent to 5%–10% of the final volume and then diluting with *Medium* to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: Absorption maximum at about 225 nm

Cell: 0.1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of modafinil ($C_{15}H_{15}NO_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated

Apparatus 2: 75 rpm

Time: 30 min

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Standard solution: (*L*/900) mg/mL of USP Modafinil RS, where *L* is the label claim (mg/Tablet). Prepare by dissolving the standard in a volume of methanol equivalent to 5%–10% of the final volume and then diluting with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Absorption maximum at about 220 nm
Cell: 0.1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of modafinil ($C_{15}H_{15}NO_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.3 between modafinil and salicylic acid
Relative standard deviation: NMT 2.0% for the modafinil peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity
r_T = sum of the responses of all the peaks
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

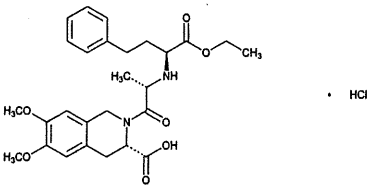
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Modafinil	1.0	—	—
Salicylic acid[a]	1.1	—	—
Modafinil acid[b]	1.4	1.0	0.5
Modafinil sulfone[c]	1.7	0.90	0.5
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.5

[a] Salicylic acid is used for calculating resolution and is not a potential impurity.
[b] 2-[(Diphenylmethyl)sulfinyl]acetic acid.
[c] 2-[(Diphenylmethyl)sulfonyl]acetamide.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Modafinil RS
USP Salicylic Acid RS

Moexipril Hydrochloride



$C_{27}H_{34}N_2O_7 \cdot HCl$ 535.03

3-Isoquinolinecarboxylic acid, 2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-, monohydrochloride, [3*S*-[2[*R**(*R**)],3*R**]]-; (3*S*)-2-[(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alanyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid, 2-ethyl ester, monohydrochloride [82586-52-5].

DEFINITION

Moexipril Hydrochloride contains NLT 98.0% and NMT 102.0% of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements

ASSAY

• **PROCEDURE**
Buffer: 1.32 g/L of dibasic ammonium phosphate. Adjust with diluted phosphoric acid to a pH of 7.5.
Solution A: Acetonitrile and tetrahydrofuran (95:5)
Mobile phase: *Solution A* and *Buffer* (30:70)
Standard solution: 0.1 mg/mL of USP Moexipril Hydrochloride RS in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]
Sample solution: 0.1 mg/mL of Moexipril Hydrochloride in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 3.2 times the retention time of moexipril
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) in the portion of Moexipril Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Moexipril Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.20%
- **Organic Impurities**

[Note—Use freshly prepared samples for analysis.]
Solution A and **Chromatographic system:** Proceed as directed in the *Assay*.
Solution B: Proceed as directed for the *Buffer* in the *Assay*.
Diluent: *Solution A* and *Solution B* (20:80)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	20	80
5	20	80
35	55	45
65	55	45
70	20	80
80	20	80

Standard solution 1: 4 µg/mL of USP Moexipril Hydrochloride RS in *Diluent*. [Note—Sonication may be necessary for complete dissolution.]
Standard solution 2: 2 mg/mL of USP Moexipril Hydrochloride RS and 3 µg/mL each of USP Moexipril Related Compound A RS, USP Moexipril Related Compound B RS, USP Moexipril Related Compound C RS, USP Moexipril Related Compound D RS, USP Moexipril Related Compound E RS, USP Moexipril Related Compound F RS, and USP Moexipril Related Compound G RS in *Diluent*. [Note—Sonication may be necessary for complete dissolution.]
Sample solution: 2 mg/mL of Moexipril Hydrochloride in *Diluent*. [Note—Sonication may be necessary for complete dissolution.]

System suitability
Samples: *Standard solution 1* and *Standard solution 2*
Suitability requirements
Resolution: NLT 3.5 between moexipril related compound A and moexipril related compound E; NLT 2.5 between moexipril and moexipril related compound G, *Standard solution 2*
Relative standard deviation: NMT 5.0% for moexipril, *Standard solution 1*
Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Calculate the percentage of each specified impurity in the portion of Moexipril Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from *Standard solution 2*
C_S = concentration of each specified impurity in *Standard solution 2* (mg/mL)
C_U = concentration of Moexipril Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Moexipril Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of moexipril from *Standard solution 1*
C_S = concentration of USP Moexipril Hydrochloride RS in *Standard solution 1* (mg/mL)
C_U = concentration of Moexipril Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moexipril related compound E[a]	0.14	0.15
Moexipril related compound A[b]	0.28	0.2
Moexipril related compound F[c]	0.62	0.15
Moexipril related compound G[d]	0.90	0.15
Moexipril	1.00	—
Moexipril related compound D[e]	1.28	0.15
Moexipril related compound B[f]	1.62	0.2
Moexipril related compound C[g]	2.26	0.15
Any other individual unspecified impurity	—	0.10

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Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities[h]	—	1.0

[a] (*S*)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
[b] (3*S*)-2-{(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alanyl}-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.
[c] (*S*)-2-[(*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoic acid.
[d] (*S*)-6,7-Dimethoxy-2-{(*S*)-2-[(*S*)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl}-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
[e] (*S*)-2-{(*S*)-2-[(*S*)-4-Cyclohexyl-1-ethoxy-1-oxobutan-2-ylamino]propanoyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
[f] (*S*)-Ethyl 2-{(3*S*,11a*S*)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1*H*-pyrazino[1,2-*b*]isoquinolin-2(6*H*,11*H*,11a*H*)-yl}-4-phenylbutanoate.
[g] (*S*)-*tert*-Butyl 2-{(*S*)-2-[(*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.
[h] Sum of all specified and unspecified impurities.

• **CONTENT OF IMIDAZOLE**
Mobile phase: Hexane, isopropyl alcohol, and diethyl amine (52: 48: 0.025)
Standard solution: 0.01 mg/mL of USP Imidazole RS in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]
Sample solution: 2 mg/mL of Moexipril Hydrochloride in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution. Use freshly prepared *Sample solution* for analysis.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 3.3 times the retention time of imidazole
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of imidazole in the portion of Moexipril Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of imidazole from the *Sample solution*
r_S = peak response of imidazole from the *Standard solution*
C_S = concentration of USP Imidazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Moexipril Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.03%

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: NMT 1.5%
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 0.011 g/mL of Moexipril Hydrochloride in alcohol. Sonicate to dissolve the sample.
Acceptance criteria: +30.0° to +38.0°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from moisture. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Imidazole RS
USP Moexipril Hydrochloride RS
USP Moexipril Related Compound A RS
(3*S*)-2-{(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alanyl}-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.
$C_{25}H_{30}N_2O_7$ 470.51
USP Moexipril Related Compound B RS
(*S*)-Ethyl 2-{(3*S*,11a*S*)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1*H*-pyrazino[1,2-*b*]isoquinolin-2(6*H*,11*H*,11a*H*)-yl}-4-phenylbutanoate.
$C_{27}H_{32}N_2O_6$ 480.55
USP Moexipril Related Compound C RS
(*S*)-*tert*-Butyl 2-{(*S*)-2-[(*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.
$C_{31}H_{42}N_2O_7$ 554.67
USP Moexipril Related Compound D RS
(*S*)-2-{(*S*)-2-[(*S*)-4-Cyclohexyl-1-ethoxy-1-oxobutan-2-ylamino]propanoyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
$C_{27}H_{40}N_2O_7$ 504.62
USP Moexipril Related Compound E RS
(*S*)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
$C_{12}H_{15}NO_4$ 237.25
USP Moexipril Related Compound F RS
(*S*)-2-[(*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoic acid.
$C_{15}H_{21}NO_4$ 279.33
USP Moexipril Related Compound G RS
(*S*)-6,7-Dimethoxy-2-{(*S*)-2-[(*S*)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl}-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
$C_{26}H_{32}N_2O_7$ 484.54

Moexipril Hydrochloride Tablets

DEFINITION

Moexipril Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 0.01 M potassium dihydrogen phosphate
Diluent: Acetonitrile and water (30:70)
Mobile phase: Acetonitrile and *Buffer* (350:650)
Standard solution: 0.075 mg/mL of USP Moexipril Hydrochloride RS in *Diluent*. Initially fill with *Diluent* to about 70% of the total volume, and sonicate. Further dilute with *Diluent* to volume.
Sample solution: Nominally 0.075 mg/mL of moexipril hydrochloride in *Diluent*, prepared from a sufficient number of crushed Tablets as follows. Add *Diluent* to about 75% of the total volume, and sonicate for 30 min with intermittent

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shaking. Dilute with *Diluent* to volume, and pass through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 4 times the retention time of the moexipril peak

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Test 1
Buffer, Diluent, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Standard stock solution: 0.16 mg/mL of USP Moexipril Hydrochloride RS in *Diluent*. [Note—Sonication may be necessary for complete dissolution.]
Standard solution: 0.016 mg/mL of USP Moexipril Hydrochloride RS in *Medium* from the *Standard stock solution* for 15-mg Tablet strength and 0.008 mg/mL of USP Moexipril Hydrochloride RS in *Medium* from the *Standard stock solution* for 7.5-mg Tablet strength
Sample solution: Pass 10 mL of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of moexipril from the *Sample solution*
r_S = peak response of moexipril from the *Standard solution*
C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution*
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Buffer, Diluent, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Medium, Apparatus 2, Standard stock solution, Standard solution, Sample solution, and Analysis: Proceed as directed in *Test 1*.
Time: 30 min
Tolerances: NLT 80% (*Q*) of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements for *Content Uniformity*

IMPURITIES

• **Organic Impurities**
Solution A: 0.025% trifluoroacetic acid in water
Solution B: Acetonitrile and tetrahydrofuran (90:10)
Diluent: Acetonitrile and water (30:70)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
50	30	70
60	95	5
70	95	5

Impurity stock solution: 0.12 mg/mL of USP Moexipril Related Compound G RS in *Diluent*. [Note—Sonication may be necessary for complete dissolution.]
System suitability solution: 1.2 mg/mL of USP Moexipril Hydrochloride RS and 2.4 µg/mL of USP Moexipril Related Compound G RS from the *Impurity stock solution* in *Diluent*. [Note—Sonication may be necessary for complete dissolution.]
Standard stock solution: 1.2 mg/mL of USP Moexipril Hydrochloride RS in *Diluent*. Initially add *Diluent* to about 60% of the volume of the flask, and sonicate with intermittent shaking for complete dissolution.
Standard solution: 6 µg/mL each of USP Moexipril Related Compound A RS and USP Moexipril Related Compound B RS, and 1.2 µg/mL of USP Moexipril Hydrochloride RS in *Diluent* from the *Standard stock solution*. [Note—Sonication may be necessary for complete dissolution.]
Sample solution: Nominally 1.2 mg/mL of moexipril hydrochloride in *Diluent*, prepared from a sufficient number of crushed Tablets. Initially add *Diluent* to about 60% of the volume of the flask, and sonicate for 20 min with intermittent shaking in ice cold water. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*

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Suitability requirements

Resolution: NLT 2.5 between moexipril and moexipril related compound G, *System suitability solution*

Tailing factor: NMT 2.0 for the moexipril peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of moexipril related compound A and moexipril related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of moexipril related compound A and moexipril related compound B from the *Sample solution*

r_S = peak response of moexipril related compound A and moexipril related compound B from the *Standard solution*

C_S = concentration of USP Moexipril Related Compound A RS and USP Moexipril Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*

r_S = peak response of moexipril from the *Standard solution*

C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moexipril related compound E[a]	0.31	—
Moexipril related compound F[a]	0.77	—
Moexipril related compound A[b]	0.85	2.0
Moexipril related compound G[a]	0.94	—
Moexipril	1.00	—
Moexipril related compound D[a]	1.17	—
Moexipril related compound C[a]	1.27	—
Moexipril related compound B[c]	1.43	1.5
Any unspecified degradation product	—	0.2
Total impurities[d]	—	2.0

[a] Process-related impurities controlled in the drug substance.
[b] (3*S*)-2-{(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alanyl}-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.
[c] (*S*)-Ethyl 2-{(3*S*,11a*S*)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1*H*-pyrazino[1,2-*b*]isoquinolin-2(6*H*,11*H*,11a*H*)-yl}-4-phenylbutanoate.
[d] Total impurities do not include moexipril related compound A.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store at controlled room temperature in tight, well-closed containers, and protect from moisture.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Moexipril Hydrochloride RS

USP Moexipril Related Compound A RS
(3*S*)-2-{(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alanyl}-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.
$C_{25}H_{30}N_2O_7$ 470.51

USP Moexipril Related Compound B RS
(*S*)-Ethyl 2-{(3*S*,11a*S*)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1*H*-pyrazino[1,2-*b*]isoquinolin-2(6*H*,11*H*,11a*H*)-yl}-4-phenylbutanoate.
$C_{27}H_{32}N_2O_6$ 480.55

USP Moexipril Related Compound G RS
(*S*)-6,7-Dimethoxy-2-{(*S*)-2-[(*S*)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl}-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
$C_{26}H_{32}N_2O_7$ 484.54

Moexipril Hydrochloride and Hydrochlorothiazide Tablets

DEFINITION

Moexipril Hydrochloride and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% each of the labeled amounts of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.01 M potassium dihydrogen phosphate

Mobile phase: Acetonitrile and *Buffer* (350:650)

Diluent: Acetonitrile and water (30:70)

Standard solution: Prepare solutions of USP Moexipril Hydrochloride RS and USP Hydrochlorothiazide RS in *Diluent*, of concentrations stated in *Table 1*. Initially add *Diluent* to 70% of the total volume, sonicate to dissolve, and then dilute with *Diluent* to volume.

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Table 1

Tablet Strength Moexipril Hydrochloride/ Hydrochlorothiazide (mg/mg)	Concentration of Moexipril Hydrochloride (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
7.5/12.5	0.06	0.1
15/12.5	0.06	0.05
15/25	0.06	0.1

Sample solution: Prepare the *Sample solutions* of nominal concentrations given in *Table 1* from NLT 20 powdered Tablets as follows. Initially add *Diluent* to about 60% of the total volume, sonicate for 45 min with intermittent shaking, and then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 2.2 times the retention time of the moexipril peak

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2500 theoretical plates for moexipril and NLT 4000 theoretical plates for the hydrochlorothiazide peaks
Tailing factor: NMT 2.0 for both moexipril and hydrochlorothiazide peaks
Relative standard deviation: NMT 2.0% for both moexipril and hydrochlorothiazide peaks

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of moexipril or hydrochlorothiazide from the *Sample solution*
r_S = peak response of moexipril or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Moexipril Hydrochloride RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of moexipril hydrochloride or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of moexipril hydrochloride and hydrochlorothiazide

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Buffer, Mobile phase, Diluent, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min

Standard solution: Prepare solutions of USP Moexipril Hydrochloride RS and USP Hydrochlorothiazide RS in *Medium* of concentrations stated in *Table 2*.

Table 2

Tablet Strength Moexipril Hydrochloride/ Hydrochlorothiazide (mg/mg)	Concentration of USP Moexipril Hydrochloride RS (µg/mL)	Concentration of USP Hydrochlorothiazide RS (µg/mL)
7.5/12.5	8	14
15/12.5	16	14
15/25	16	28

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of moexipril or hydrochlorothiazide from the *Sample solution*
r_S = peak response of moexipril or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Moexipril Hydrochloride RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
L = label claim for moexipril hydrochloride or hydrochlorothiazide (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (Q) of the labeled amounts each of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: Add 1 mL of trifluoroacetic acid to 4 L of water.
Solution B: Acetonitrile and tetrahydrofuran (90:10)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
50	30	70
60	95	5
70	95	5

Diluent: Proceed as directed in the *Assay*.
System suitability solution: 1.2 mg/mL of USP Moexipril Hydrochloride RS, 2 mg/mL of USP Hydrochlorothiazide RS, and 2.4 µg/mL of USP Moexipril Related Compound G RS in *Diluent*. Initially add *Diluent* to 70% of the total volume, sonicate to dissolve, and then dilute with *Diluent* to volume.
Standard solution: 1.2 µg/mL of USP Moexipril Hydrochloride RS, 12 µg/mL each of USP Moexipril Related Compound A RS and USP Moexipril Related

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Compound B RS, 2 µg/mL of USP Hydrochlorothiazide RS, and 40 µg/mL each of USP Benzothiadiazine Related Compound A RS and USP Chlorothiazide RS in *Diluent*. Initially add *Diluent* to 70% of the total volume, sonicate to dissolve, and then dilute with *Diluent* to volume.

Sample solution: Prepare solutions of nominal concentration given in *Table 4*. Initially add *Diluent* to 70% of the total volume, and sonicate for 15 min with intermittent shaking in ice cold water. Dilute with *Diluent* to volume, and pass through a suitable filter of 0.45-µm pore size.

Table 4

Tablet Strength Moexipril Hydrochloride/ Hydrochloro-thiazide (mg/mg)	Number of Tablets (NLT)	Nominal Concentration of Moexipril Hydrochloride (mg/mL)	Nominal Concentration of Hydrochloro-thiazide (mg/mL)
7.5/12.5	20	1.2	2
15/12.5	10	1.8	1.5
15/25	10	1.2	2

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between the moexipril and moexipril related compound G peaks, *System suitability solution*
Tailing factor: NMT 2.0 for both moexipril and hydrochlorothiazide peaks, *Standard solution*
Relative standard deviation: NMT 5.0% for both moexipril and hydrochlorothiazide peaks, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of moexipril related compound A and moexipril related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of moexipril related compound A or moexipril related compound B from the *Sample solution*
r_S = peak response of USP Moexipril Related Compound A RS or USP Moexipril Related Compound B RS from the *Standard solution*
C_S = concentration of USP Moexipril Related Compound A RS and USP Moexipril Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of benzothiadiazine related compound A or chlorothiazide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A or chlorothiazide from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A or chlorothiazide from the *Standard solution*
C_S = concentration of USP Benzothiadiazine Related Compound A RS or USP Chlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of moexipril from the *Standard solution*
C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moexipril related compound E[a, b]	0.31	—
Benzothiadiazine related compound A[c]	0.47	1.0
Chlorothiazide[d]	0.53	0.5
Hydrochlorothiazide	0.57	—
5-Chlorohydrochlorothiazide[e, b]	0.82	—
Moexipril related compound F[f, b]	0.77	—
Moexipril related compound A[g]	0.85	1.0
Moexipril related compound G[h, b]	0.94	—
Moexipril	1.00	—
Moexipril related compound D[i, b]	1.17	—
Moexipril related compound C[j, b]	1.27	—
Moexipril related compound B[k]	1.43	1.5
Any other individual unspecified impurity	—	0.2

USP Monographs

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Table 5 *(continued)*

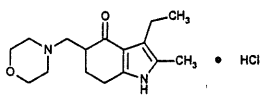
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	4.0[l]

[a] (*S*)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
[b] Process-related impurity controlled in the drug substance.
[c] 4-Amino-6-chloro-1,3-benzenedisulfonamide.
[d] 2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide.
[e] 5-Chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-, 1,1-dioxide.
[f] (*S*)-2-[(*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoic acid.
[g] (3*S*)-2-{(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alanyl}-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.
[h] (*S*)-6,7-Dimethoxy-2-{(*S*)-2-[(*S*)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl}-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
[i] (*S*)-2-{(*S*)-2-[(*S*)-4-Cyclohexyl-1-ethoxy-1-oxobutan-2-ylamino]propanoyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
[j] (*S*)-*tert*-Butyl 2-{(*S*)-2-[(*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.
[k] (*S*)-Ethyl 2-{(3*S*,11a*S*)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1*H*-pyrazino[1,2-b]isoquinolin-2(6*H*,11*H*,11a*H*)-yl}-4-phenylbutanoate.
[l] Total impurities is a sum total of all specified and unspecified impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and protect from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzothiadiazine Related Compound A RS
  4-Amino-6-chloro-1,3-benzenedisulfonamide.
  $C_6H_8ClN_3O_4S_2$ 285.73
  USP Chlorothiazide RS
  2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-,1,1-dioxide.
  $C_7H_6ClN_3O_4S_2$ 295.73
  USP Hydrochlorothiazide RS
  USP Moexipril Hydrochloride RS
  USP Moexipril Related Compound A RS
  (3*S*)-2-{(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alanyl}-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.
  $C_{25}H_{30}N_2O_7$ 470.51
  USP Moexipril Related Compound B RS
  (*S*)-Ethyl 2-{(3*S*,11a*S*)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1*H*-pyrazino[1,2-b]isoquinolin-2(6*H*,11*H*,11a*H*)-yl}-4-phenylbutanoate.
  $C_{27}H_{32}N_2O_6$ 480.55
  USP Moexipril Related Compound G RS
  (*S*)-6,7-Dimethoxy-2-{(*S*)-2-[(*S*)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl}-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
  $C_{26}H_{32}N_2O_7$ 484.54

Molindone Hydrochloride



$C_{16}H_{24}N_2O_2 \cdot HCl$ 312.83
4*H*-Indol-4-one, 3-ethyl-1,5,6,7-tetrahydro-2-methyl-5-(4-morpholinylmethyl)-, monohydrochloride.
3-Ethyl-6,7-dihydro-2-methyl-5-(morpholinomethyl)indol-4(5*H*)-one monohydrochloride [15622-65-8].

» Molindone Hydrochloride contains not less than 98.0 percent and not more than 101.5 percent of $C_{16}H_{24}N_2O_2 \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Molindone Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020). Do not dry specimens.
B: Prepare a solution in methanol containing 10 mg of molindone hydrochloride per mL. Separately apply 1 µL of this solution and 1 µL of a Standard solution containing 10 mg per mL of USP Molindone Hydrochloride RS in methanol to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Protect the chromatogram from light, and develop in a solvent system consisting of a mixture of alcohol, methanol, and 1 N hydrochloric acid (90:5:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a freshly prepared solution containing 100 mg of potassium ferricyanide dissolved in 20 mL of 10% ferric chloride solution: the principal spot obtained from the test solution corresponds in R_F value and intensity to that obtained from the Standard solution.
C: It responds to the tests for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 4.0 and 5.0, in a solution (1 in 100).
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Residue on ignition ⟨281⟩: not more than 0.25%.

Chromatographic purity—
Mobile phase—Dissolve 1.1 g of sodium octanesulfonate in 600 mL of water, add 400 mL of methanol, 1 mL of glacial acetic acid, and 0.5 mL of triethylamine. Mix, filter through a filter having a porosity of 0.45 µm or less, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Solvent mixture—Proceed as directed in the *Assay*.
Standard solution—Prepare a solution of USP Molindone Hydrochloride RS in *Solvent mixture* having a known concentration of about 0.01 mg per mL.
Test solution—Transfer about 100 mg of Molindone Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Solvent mixture* to volume.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L11. The column temperature is maintained at 35°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses of all peaks: no peak from the *Test solution*, other than the molindone peak, is greater than the molindone peak from the *Standard preparation* (0.5%), and the sum of all the impurity peaks is not greater than 2.0%.

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Assay—

Mobile phase—Dissolve 1.1 g of sodium octanesulfonate in 480 mL of water, add 520 mL of methanol, 2 mL of glacial acetic acid, and 0.4 mL of triethylamine. Mix, filter through a 0.45-µm filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Solvent mixture—Prepare a mixture of 0.01 N hydrochloric acid and methanol (60:40).

Internal standard solution—Dissolve 200 mg of butylparaben in 40 mL of methanol in a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation—Transfer about 25 mg of USP Molindone Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

Assay preparation—Transfer about 50 mg of Molindone Hydrochloride, accurately weighed, to a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L11. The column temperature is maintained at 35°. The flow rate is 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the resolution, *R*, between the molindone and butylparaben peaks is not less than 2, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for molindone and 1.0 for butylparaben. Calculate the quantity, in mg, of $C_{16}H_{24}N_2O_2 \cdot HCl$ in the portion of Molindone Hydrochloride taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Molindone Hydrochloride RS in the *Standard preparation*, and R_U and R_S are the ratios of the peak response of molindone to that of butylparaben obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Molindone Hydrochloride Tablets

» Molindone Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of molindone hydrochloride ($C_{16}H_{24}N_2O_2 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Molindone Hydrochloride RS

Identification—Dissolve a portion of finely powdered Tablets in methanol to obtain a test solution containing about 2.5 mg of molindone hydrochloride per mL. Separately apply 5 µL of the test solution and 5 µL of a Standard solution of USP Molindone Hydrochloride RS in methanol containing 2.5 mg per mL to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, protect the chromatogram from light, and develop in a solvent system consisting of a mixture of alcohol and 1 N hydrochloric acid (95:5). Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with Dragendorff's reagent, prepared as directed for *Visualization Technique 3* under *Ordinary Impurities* ⟨466⟩: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Solvent A—Mix 300 mL of methanol and 700 mL of 0.1 N hydrochloric acid.

Solvent B—Mix 75 mL of methanol and 25 mL of 0.1 N hydrochloric acid.

Standard solution—Transfer about 100 mg of USP Molindone Hydrochloride RS, accurately weighed, to a 250-mL volumetric flask, and dissolve in and dilute with *Solvent A* to volume. Pipet 5.0 mL of this stock solution into a 250-mL volumetric flask, and dilute with *Solvent A* to volume. Pipet 15.0 mL of the diluted stock solution into a 50-mL volumetric flask, and dilute with *Solvent A* to volume.

Test solution—Withdraw a portion of the solution under test, and filter, discarding the first 3 mL of filtrate. Pipet 15.0 mL of this solution into a 25-mL volumetric flask, and dilute with *Solvent B* to volume.

Mobile phase—Dissolve 1.08 g of sodium 1-octanesulfonate in 480 mL of water. Add 520 mL of methanol, 2.0 mL of acetic acid, and 0.4 mL of triethylamine, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm UV detector and a 4.6-mm × 25-cm column that contains packing L11. The flow rate is about 1.5 mL per minute.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak heights. Determine the amount of molindone hydrochloride ($C_{16}H_{24}N_2O_2 \cdot HCl$) dissolved.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{16}H_{24}N_2O_2 \cdot HCl$ is dissolved in 30 minutes.

Assay—

Mobile phase , Solvent mixture, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Molindone Hydrochloride.*

Assay preparation—Accurately weigh not less than 20 Tablets, grind the Tablets to a homogeneous mixture, and transfer an accurately weighed portion, equivalent to about 50 mg of molindone hydrochloride, to a 250-mL conical flask. Add 10.0 mL of *Internal standard solution* and 90.0 mL of *Solvent mixture*, shake for 30 minutes, and filter.

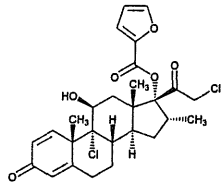
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Molindone Hydrochloride.* Calculate the quantity, in mg, of molindone hydrochloride ($C_{16}H_{24}N_2O_2 \cdot HCl$) in the portion of Tablets taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Molindone Hydrochloride RS in the *Standard preparation,* and R_U and R_S are the ratios of the peak response of molindone to that of butylparaben obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Mometasone Furoate



$C_{27}H_{30}Cl_2O_6$ 521.43

Pregna-1,4-diene-3,20-dione, 9,21-dichloro-17-[(2-furanylcarbonyl)oxy]-11-hydroxy-16-methyl-, (11β,16α)-;
9,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate) [83919-23-7].

DEFINITION

Mometasone Furoate contains NLT 97.0% and NMT 102.0% of mometasone furoate ($C_{27}H_{30}Cl_2O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and water (65:35)
Diluent: Methanol, acetic acid, and water (65: 0.2: 35)
Internal standard solution: 0.4 mg/mL of beclomethasone dipropionate in *Diluent*
Standard stock solution: 0.1 mg/mL of USP Mometasone Furoate RS, prepared by dissolving USP Mometasone Furoate RS in methanol and diluting quantitatively and stepwise, if necessary, with *Diluent*
Standard solution: 0.02 mg/mL of USP Mometasone Furoate RS and 0.08 mg/mL of beclomethasone dipropionate, prepared by pipetting equal volumes of *Standard stock solution* and *Internal standard solution* into a suitable volumetric flask and diluting with *Diluent* to volume, if necessary
Sample stock solution: 0.1 mg/mL of mometasone furoate, prepared by dissolving Mometasone Furoate in methanol and diluting quantitatively and stepwise, if necessary, with *Diluent*
Sample solution: 0.02 mg/mL of mometasone furoate and 0.08 mg/mL of beclomethasone dipropionate, prepared by pipetting 10 mL each of *Sample stock solution* and *Internal standard solution* into a 50-mL volumetric flask and diluting with *Diluent* to volume
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1.7 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for mometasone furoate and beclomethasone dipropionate are about 1.0 and 1.6, respectively.]
Suitability requirements
Resolution: NLT 4.0 between the mometasone furoate and beclomethasone dipropionate peaks
Tailing factor: NMT 1.8 for the mometasone furoate peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Mometasone Furoate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of mometasone furoate to the internal standard from the *Sample solution*
R_S = peak response ratio of mometasone furoate to the internal standard from the *Standard solution*
C_S = concentration of USP Mometasone Furoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Mometasone Furoate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Standard stock solution: 10 mg/mL of USP Mometasone Furoate RS in dichloromethane
Standard solution A (5%): 0.5 mg/mL of USP Mometasone Furoate RS in dichloromethane from the *Standard stock solution*
Standard solution B (2%): 0.2 mg/mL of USP Mometasone Furoate RS in dichloromethane, from the *Standard stock solution*
Standard solution C (1%): 0.1 mg/mL of USP Mometasone Furoate RS in dichloromethane, from the *Standard stock solution*
Standard solution D (0.2%): 0.02 mg/mL of USP Mometasone Furoate RS in dichloromethane, from the *Standard stock solution*
Standard solution E (0.1%) 0.01 mg/mL of USP Mometasone Furoate RS in dichloromethane, from the *Standard stock solution*
Sample solution: 10 mg/mL of Mometasone Furoate in dichloromethane
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 40 µL
Developing solvent system: Chloroform and ethyl acetate (3:1)
Analysis
Samples: *Standard solutions* and *Sample solution*
Proceed as directed in the chapter. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots from the *Sample solution* with those of the principal spots from the *Standard solutions*.
Acceptance criteria: No secondary spot from the *Sample solution* is larger or more intense than the principal spot from *Standard solution C*; and the sum of the intensities of the secondary spots from the *Sample solution* is NMT 2.0%.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 5 mg/mL in dioxane
Acceptance criteria: +56° to +62°

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• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Mometasone Furoate RS

Mometasone Furoate Cream

DEFINITION
Mometasone Furoate Cream is Mometasone Furoate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mometasone furoate ($C_{27}H_{30}Cl_2O_6$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
• **B. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Standard solution: 0.2 mg/mL of USP Mometasone Furoate RS in acetonitrile
Sample solution: 0.2 mg/mL of mometasone furoate from Cream in acetonitrile
Developing solvent system: Chloroform and ethyl acetate (3:1)
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
• **Procedure**
[Note—Protect from light.]
Diluent A: Tetrahydrofuran and glacial acetic acid (100:1)
Diluent B: Acetonitrile, water, and glacial acetic acid (50:50:1)
Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
45	45	55
46	70	30
50	70	30

Internal standard solution: 1.4 mg/mL of diethyl phthalate in acetonitrile
Standard stock solution: 0.2 mg/mL of USP Mometasone Furoate RS in *Diluent A*
Standard solution: 0.05 mg/mL of mometasone furoate and 0.35 mg/mL of diethyl phthalate from equal quantities of the *Standard stock solution* and the *Internal standard solution*, in *Diluent B*
Sample solution: Transfer a portion of Cream, equivalent to 1.0 mg of mometasone furoate, to a 50-mL, screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 5.0 mL of *Internal standard solution*, and mix. Add 10.0 mL of *Diluent B*, mix on a vortex mixer for 1 min, and centrifuge for 10 min. Pass the aqueous phase through a polypropylene filter of 0.2-µm pore size, discarding the first 1–2 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L60
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for diethyl phthalate and mometasone furoate are 0.4 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 1.5 for the mometasone furoate peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the mometasone furoate peak response to the diethyl phthalate peak response from the *Sample solution*
R_S = ratio of the mometasone furoate peak response to the diethyl phthalate peak response from the *Standard solution*
C_S = concentration of USP Mometasone Furoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mometasone furoate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

IMPURITIES
• **Organic Impurities**
[Note—Protect from light.]
Diluent A, Solution A, Solution B, Mobile phase, and **Standard stock solution:** Prepare as directed in the *Assay*.
Diluent C: Acetonitrile, water, and glacial acetic acid (30:70:1)
System suitability solution: 0.1 µg/mL of USP Mometasone Furoate RS from *Standard stock solution* in *Diluent C*
Blank solution: *Diluent C* and *Diluent A* (3:1)
Sample solution: Transfer a portion of Cream, equivalent to 2.0 mg of mometasone furoate, to a 50-mL, screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 15.0 mL of *Diluent C*, and mix. Centrifuge for 10 min. Pass the aqueous phase through a 0.2-µm polypropylene filter, discarding the first 1–2 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L60
Column temperature: 25 ± 5°
Flow rate: 2 mL/min
Injection size: 50 µL
System suitability
Sample: *System suitability solution*

USP Monographs

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Suitability requirements
Relative standard deviation: NMT 10%
Analysis
Samples: *System suitability solution*, *Blank solution*, and *Sample solution*
[NOTE—Exclude any peak areas less than those from the *System suitability solution*. Also exclude any peaks with the same retention time as that observed in the *Blank solution*. Any peaks having a relative retention time of about 1.04 or 1.13 are controlled in the *Mometasone Furoate* monograph and, therefore, are not included in the total specified and unspecified impurities limit.]
Calculate the percentage of each impurity in the portion of Cream taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
9α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	0.1
9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	0.73	0.1
21-Chloro-17-hydroxy-16α- methyl-pregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	0.1
21-Chloro-9β,11β-epoxy-17-hydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.94	1.0
Mometasone furoate	1.0	—
Unspecified individual impurity	—	0.2
Total specified and unspecified impurities	—	1.0

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Mometasone Furoate RS

Mometasone Furoate Ointment

DEFINITION
Mometasone Furoate Ointment is Mometasone Furoate in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mometasone furoate ($C_{27}H_{30}Cl_2O_6$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHY**
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Standard solution: 0.6 mg/mL of USP Mometasone Furoate RS in methanol
Sample solution: Transfer the equivalent to 3 mg of mometasone furoate from Ointment to a 50-mL screw-capped centrifuge tube. Pipet 5.0 mL of methanol into the tube, and attach the cap. Heat in a steam bath until the Ointment completely melts, and shake vigorously until the Ointment resolidifies. Place in an ice-water bath for 10 min. Centrifuge, and filter a portion of the supernatant. Extract 1 mL of the filtrate with 1 mL of hexane, and use the lower phase.
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system A: Methanol
Developing solvent system B: Chloroform and ethyl acetate (3:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in *Developing solvent system A* until the solvent front has moved 2 cm from the origin. Remove the plate from the developing chamber, and air-dry. Develop the chromatogram in *Developing solvent system B* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the spots to air-dry. Examine the plate under short-wavelength UV light.
Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
- **PROCEDURE**
[NOTE—Protect from light.]
Diluent A: Tetrahydrofuran and glacial acetic acid (100:1)
Diluent B: Acetonitrile, water, and glacial acetic acid (50:50:1)
Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
45	45	55
46	70	30
50	70	30

Internal standard solution: 1.4 mg/mL of diethyl phthalate in acetonitrile
Standard stock solution: 0.2 mg/mL of USP Mometasone Furoate RS in *Diluent A*
Standard solution: 0.05 mg/mL of mometasone furoate and 0.35 mg/mL of diethyl phthalate from equal quantities of the *Standard stock solution* and the *Internal standard solution*, in *Diluent B*

USP Monographs

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Sample solution: Transfer a portion of Ointment, equivalent to 1.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 5.0 mL of *Internal standard solution*, and mix. Add 10.0 mL of *Diluent B*, mix on a vortex mixer for 1 min, and centrifuge for 10 min. Pass the aqueous phase through a polypropylene filter of 0.2-µm pore size, discarding the first 1–2 mL of filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L60
Flow rate: 2 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for diethyl phthalate and mometasone furoate are 0.4 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 1.5 for the mometasone furoate peak
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mometasone fuorate ($C_{27}H_{30}Cl_2O_6$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the mometasone furoate peak response to the diethyl phthalate peak response from the *Sample solution*
R_S = ratio of the mometasone furoate peak response to the diethyl phthalate peak response from the *Standard solution*
C_S = concentration of USP Mometasone Furoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mometasone furoate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**
[NOTE—Protect from light.]
Diluent A, Solution A, Solution B, Mobile phase, and **Standard stock solution:** Prepare as directed in the *Assay*.
Diluent C: Acetonitrile, water, and glacial acetic acid (30:70:1)
System suitability solution: 0.1 µg/mL of USP Mometasone Furoate RS from *Standard stock solution* in *Diluent C*
Sample solution: Transfer a portion of Ointment, equivalent to 2.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 15.0 mL of *Diluent C*, and mix. Centrifuge for 10 min. Pass the aqueous phase through a polypropylene filter of 0.2-µm pore size, discarding the first 1–2 mL of filtrate.
Blank solution: *Diluent C* and *Diluent A* (3:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L60
Column temperature: 25 ± 5°
Flow rate: 2 mL/min
Injection size: 50 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Relative standard deviation: NMT 10%

Analysis
Samples: *System suitability solution*, *Sample solution*, and *Blank solution*
[NOTE—Exclude any peak areas less than those from the chromatogram of the *System suitability solution*. Also exclude any peaks with the same retention time as that observed in the chromatogram of the *Blank solution*. Any peaks having a relative retention time of about 1.04 or 1.13 are controlled in the monograph for *Mometasone Furoate*, and therefore are not included in the total specified and unspecified impurities limit.]
Calculate the percentage of each impurity in the portion of Ointment taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
9α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	0.2
9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	0.73	0.2
21-Chloro-17-hydroxy-16α-methylpregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	0.2
21-Chloro-9β,11β-epoxy-17-hydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.94	1.0
Mometasone furoate	1.0	—
Unspecified individual impurity	—	0.2
Total specified and unspecified impurities	—	1.0

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mometasone Furoate RS

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Mometasone Furoate Topical Solution

DEFINITION

Mometasone Furoate Topical Solution is Mometasone Furoate in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mometasone furoate ($C_{27}H_{30}Cl_2O_6$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 1 mg/mL of USP Mometasone Furoate RS in a mixture of chloroform and methanol (4:1)

Sample solution: Transfer the equivalent of 2 mg of mometasone furoate from Topical Solution to a 50-mL centrifuge tube. Add 10 mL of water. Extract the aqueous solution with 20 mL of chloroform. Remove the chloroform layer, dry over anhydrous sodium sulfate, and filter through a cotton pledget. Repeat the chloroform extraction, and combine the dried extracts. Evaporate the chloroform solution to dryness on a steam bath under a stream of nitrogen. Allow the sample specimen to cool to room temperature. Dissolve the residue in a mixture of chloroform and methanol (4:1) to obtain 1 mg/mL of *Sample solution*.

Application volume: 20 µL

Developing solvent system: Chloroform and ethyl acetate (3:1)

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to the that of the *Standard solution*.

ASSAY

- **PROCEDURE**

[NOTE—Protect from light.]

Diluent: Acetonitrile, water, and glacial acetic acid (50:50:1)

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
45	45	55
46	70	30
50	70	30

Standard solution: 0.1 mg/mL of USP Mometasone Furoate RS in *Solution B*

Sample solution: Transfer a portion of Topical Solution, equivalent to about 2.5 mg of mometasone furoate, to a 25-mL flask. Dilute with *Diluent* to volume, and mix. Pass a portion of the solution through a polypropylene filter of 0.2-µm pore size, discarding the first 1–2 mL of filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L60
Flow rate: 2 mL/min
Injection size: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements:
Tailing factor: NMT 1.5 for the mometasone furoate peak
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mometasone Furoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mometasone furoate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

[NOTE—Protect from light.]

Diluent, Solution A, Solution B, Mobile phase, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.

System suitability solution: 0.1 µg/mL of USP Mometasone Furoate RS from *Standard solution* in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L60
Column temperature: 25 ± 5°
Flow rate: 2 mL/min
Injection size: 50 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Relative standard deviation: NMT 10%

Analysis
Samples: *Diluent*, *System suitability solution*, and *Sample solution*

[NOTE—Exclude any peak areas less than that of the *System suitability solution*. Also, exclude any peaks with the same retention times as those observed in the *Diluent*. Any peaks having a relative retention time of 1.04 or 1.13 are controlled in the *Mometasone Furoate* monograph, and therefore are not included in the total specified and unspecified impurities limit.]

Calculate the percentage of each impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

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Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
9α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	0.3
9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	0.73	0.1
21-Chloro-17-hydroxy-16α- methylpregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	0.1
21-Chloro-9β,11β-epoxy-17-hydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.94	1.0
Mometasone furoate	1.0	—
Unspecified individual impurity	—	0.5
Total specified and unspecified impurities	—	2.0

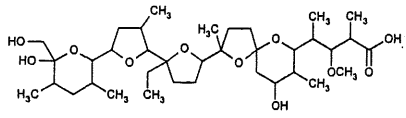
SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mometasone Furoate RS

Monensin



$C_{36}H_{62}O_{11}$ (monensin A) 670.87

$C_{35}H_{60}O_{11}$ (monensin B) 656.84

$C_{37}H_{64}O_{11}$ (monensin C) 684.90

Monensin.

Stereoisomer of 2-[2-ethyloctahydro-3′-methyl-5′-[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2*H*-pyran-2-yl][2,2′-bifuran-5-yl]]-9-hydroxy-β-methoxy-α,γ,2,8-tetramethyl-1,6-dioxaspiro[4.5]decan-7-butanoic acid [17090-79-8].

» Monensin is a mixture of antibiotic substances produced by the growth of *Streptomyces cinnamonensis*. It has a potency of not less than 110 µg of monensin per mg.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for veterinary use only. Label it also to state that it is for manufacturing, processing, or repackaging.

USP Reference standards ⟨11⟩—
USP Monensin Sodium RS
USP Narasin RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for monensin A and a minor peak for monensin B, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 2 hours: it loses not more than 10% of its weight.

Content of monensin A and B activity—Using the results of the calculations in the *Assay*, calculate the percentage of monensin A activity in the Monensin under test by the formula:

$$100A/P$$

in which A is the potency, in µg per mg, of monensin A in the Monensin under test, as determined in the *Assay*, and P is the potency, in µg of monensin, in each mg of the Monensin under test, as determined in the *Assay*:not less than 90% is found. Calculate the percentage of monensin A activity plus monensin B activity in the Monensin under test by the formula:

$$100(A + B)/P$$

in which B is the potency, in µg per mg, of monensin B in the Monensin under test, as determined in the *Assay*, and the other terms are as defined above: not less than 95% is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (94: 6: 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Neutralized methanol—Add 1 g of sodium bicarbonate to 4 liters of methanol, mix, and filter.

Diluent—Prepare a mixture of methanol and water (9:1).

Derivatizing reagent—Dissolve 3 g of vanillin in a mixture of 95 mL of methanol and 2 mL of sulfuric acid. [*Caution—To avoid splattering, add the sulfuric acid carefully and slowly with a pipet; do not pour. Allow the mixture of methanol and sulfuric acid to cool before adding vanillin.*]

Standard preparation—Dissolve an accurately weighed quantity of USP Monensin Sodium RS quantitatively in methanol to obtain a solution containing the equivalent of 1000 µg of monensin per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Diluent* to obtain a solution containing 20.0 µg of monensin per mL.

Assay preparation—Transfer about 500 mg of Monensin, accurately weighed, to a 250-mL flask, add 200.0 mL of *Diluent*, and shake by mechanical means for 1 hour. Allow the solids to settle, and dilute an accurately measured volume of the supernatant quantitatively with *Diluent* to obtain a solution containing about 20 µg of monensin per mL.

Resolution solution—Prepare a solution in *Neutralized methanol* containing about 1 mg of USP Monensin Sodium RS and 3 mg of USP Narasin RS per mL. Transfer 2 mL of this solution to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 4.6-mm × 25-cm column that contains packing L1 and the outlet of which is attached to a tee, the opposing arm of which is attached to a tube from which is pumped the *Derivatizing reagent*, and the outlet of which is connected to a 2-mL postcolumn reaction coil maintained at 98°. The outlet of the reaction coil is connected to a detector set at 520 nm. The *Mobile phase* and

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the *Derivatizing reagent* flow at the rate of about 0.7 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure:* the relative retention times are about 0.9 for monensin B, 1.0 for monensin A, 1.3 for narasin A, and 1.5 for narasin I, the resolution, *R*, between the monensin B peak and the monensin A peak is not less than 1.25, and between the monensin A peak and the narasin A peak is not less than 3.5. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the tailing factor is not more than 1.4, and the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—After use, flush the system with methanol.]

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 200 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks, including a peak for monensin C/D, if present, at a retention time of about 1.1 relative to that of the main monensin A peak in the chromatogram obtained from the *Assay preparation*. Calculate the quantity, in µg, of monensin A in each mg of the Monensin taken by the formula:

$$(CFD/100{,}000W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of monensin activity in the *Standard preparation*, based on the quantity of USP Monensin Sodium RS taken, its designated potency, in µg per mg, and the extent of dilution, *F* is the designated percentage of monensin A in USP Monensin Sodium RS, *D* is the dilution factor used in preparing the *Assay preparation*, *W* is the quantity, in g, of Monensin taken to prepare the *Assay preparation*, and r_U and r_S are the monensin A peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in µg, of monensin B in each mg of the Monensin taken by the same formula, except that r_U is the monensin B peak response obtained from the *Assay preparation* and r_S is the monensin A peak response obtained from the *Standard preparation*. Calculate the quantity, in µg, of monensin C/D in each mg of the Monensin taken by the same formula, except that r_U is the monensin C/D peak response obtained from the *Assay preparation*. Calculate the potency, in µg of monensin, in each mg of the Monensin taken by the formula:

$$A + 0.28B + 1.5C/D$$

in which *A* is the quantity, in µg, of monensin A in each mg of the Monensin taken, as calculated above, and *B* is the quantity, in µg, of monensin B in each mg of the Monensin taken, and *C/D* is the quantity, in µg, of monensin C/D in each mg of Monensin taken, as calculated above.

Monensin Granulated

» Monensin Granulated contains Monensin mixed with suitable diluents, carriers, and inactive ingredients prepared in a granulated form that is free-flowing and free from aggregates. It may contain added Monensin Sodium. It contains not less than 140 mg of monensin per g.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for veterinary use only. Label it also to state that it is for manufacturing, processing, or repackaging.

USP Reference standards ⟨11⟩—
USP Monensin Sodium RS
USP Narasin RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for monensin A and a minor peak for monensin B, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 2 hours: it loses not more than 10% of its weight.

Content of monensin A and B activity—Using the results of the calculations in the *Assay*, calculate the percentage of monensin A activity in the Monensin Granulated under test by the formula:

$$100A/P$$

in which *A* is the potency, in µg per mg, of monensin A in the Monensin Granulated under test, as determined in the *Assay*, and *P* is the potency, in µg of monensin, in each mg of the Monensin Granulated under test, as determined in the *Assay*: not less than 90% is found. Calculate the percentage of monensin A activity plus monensin B activity in the Monensin Granulated under test by the formula:

$$100(A + B)/P$$

in which *B* is the potency, in µg per mg, of monensin B in the Monensin Granulated under test, as determined in the *Assay*, and the other terms are as defined above: not less than 95% is found.

Assay—

Mobile phase, Neutralized methanol, Diluent, Derivatizing reagent, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Monensin*.

Assay preparation—Transfer about 5 g of Monensin Granulated, accurately weighed, to a 250-mL flask, add 200.0 mL of *Diluent*, and shake by mechanical means for 1 hour. Allow the solids to settle, and dilute an accurately measured volume of the supernatant quantitatively with *Diluent* to obtain a solution containing about 20 µg of monensin per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Monensin*. Calculate the quantity, in mg, of monensin A in each g of the Monensin Granulated taken by the formula:

$$(CFD/100{,}000W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of monensin activity in the *Standard preparation*, based on the quantity of USP Monensin Sodium RS taken, its designated potency, in µg per mg, and the extent of dilution, *F* is the designated percentage of monensin A in USP Monensin Sodium RS, *D* is the dilution factor used in preparing the *Assay preparation*, *W* is the quantity, in g, of Monensin Granulated taken to prepare the *Assay preparation*, and r_U and r_S are the monensin A peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of monensin B in each g of the Monensin Granulated taken by the same formula, except that r_U is the monensin B peak response obtained from the *Assay preparation* and r_S is the monensin A peak response obtained from the *Standard preparation*. Calculate the quantity, in mg, of monensin C/D in each g of the Monensin Granulated taken

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by the same formula, except that r_U is the monensin C/D peak response obtained from the *Assay preparation*. Calculate the potency, in µg of monensin, in each mg of the Monensin Granulated taken by the formula:

$$A + 0.28B + 1.5C/D$$

in which A is the quantity, in mg, of monensin A in each g of the Monensin Granulated taken, as calculated above, and B is the quantity, in mg, of monensin B in each g of the Monensin Granulated taken, and C/D is the quantity, in mg, of monensin C/D in each g of Monensin Granulated taken, as calculated above.

Monensin Type A Medicated Article

DEFINITION

Monensin Type A Medicated Article contains Monensin Granulated mixed with suitable diluents and inactive ingredients. It contains the equivalent of NLT 85.0% and NMT 115.0% of the labeled amount of monensin.

IDENTIFICATION

• **A.** The retention times of the major peak for monensin A and minor peak for monensin B of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (940:1:60)

Neutralized methanol: Add 1 g of sodium bicarbonate to 4 L of methanol, mix, and filter.

Diluent: Methanol and water (9:1)

Derivatizing reagent: Dissolve 3 g of vanillin in a mixture of 95 mL of methanol and 2 mL of sulfuric acid. [CAUTION—To avoid splattering, add the sulfuric acid carefully and slowly with a pipet; do not pour. Allow the mixture of methanol and sulfuric acid to cool before adding vanillin.]

System suitability solution: 1 mg/mL of USP Monensin Sodium RS and 3 mg/mL of USP Narasin RS in *Neutralized methanol*. Dilute 2 mL of this solution with *Diluent* to 200 mL.

Standard stock solution: 1000 µg/mL of monensin from USP Monensin Sodium RS in methanol

Standard solution: 20 µg/mL of monensin from *Standard stock solution* in *Diluent*

Sample stock solution: Dilute 5 g of Monensin Type A Medicated Article in 200.0 mL of *Diluent*, and shake by mechanical means for 1 h. Allow the solids to settle.

Sample solution: Nominally 20 µg/mL of monensin, from the clear supernatant of the *Sample stock solution*, in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 520 nm

Column: 4.6-mm × 25-cm; packing L1. The column outlet is attached to a tee, the opposing arm is attached to a tube from which is pumped the *Derivatizing reagent*, and the outlet is connected to a 2-mL postcolumn reaction coil maintained at 98°. The outlet of the reaction coil is connected to the *Detector*.

Flow rate: 0.7 mL/min for *Mobile phase* and *Derivatizing reagent*

Injection volume: 200 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for monensin B, monensin A, narasin A, and narasin I are about 0.9, 1.0, 1.3, and 1.5, respectively.]

Suitability requirements

Resolution: NLT 1.25 between the monensin B and the monensin A peaks; NLT 3.5 between the monensin A and the narasin A peaks, *System suitability solution*

Tailing factor: NMT 1.4, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

[NOTE—After use, flush the system with methanol.]

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Use peak areas where peak responses are indicated.]

Measure the responses for the major peaks, including a peak for monensin C/D, if present, at a retention time of 1.1 relative to that of the main monensin A peak in the chromatogram from the *Sample solution*.

Calculate the quantity, in mg, of monensin A, monensin B, and monensin C/D in each g of Monensin Type A Medicated Article taken:

$$\text{Result} = (r_U/r_S) \times (C_S \times F \times D)/(100{,}000 \times W)$$

r_U = peak response of monensin A, monensin B, or monensin C/D from the *Sample solution*

r_S = peak response of monensin A from the *Standard solution*

C_S = concentration of monensin activity in the *Standard solution*, based on the quantity of USP Monensin Sodium RS taken, its designated potency (µg/mg) and extent of dilution (µg/mL)

F = designated percentage of monensin A in USP Monensin Sodium RS

D = dilution factor used in preparing the *Sample solution*

W = quantity of Monensin Type A Medicated Article taken to prepare the *Sample solution* (g)

Calculate the potency, in mg, of monensin in each g of Monensin Type A Medicated Article taken:

$$\text{Result} = (A \times F_A) + (B \times F_B) + (C/D \times F_{C/D})$$

A = quantity of monensin A in each g of Monensin Type A Medicated Article taken, as calculated previously (mg)

F_A = biopotency conversion factor for monensin A, 1.00

B = quantity of monensin B in each g of Monensin Type A Medicated Article taken, as calculated previously (mg)

F_B = biopotency conversion factor for monensin B, 0.28

C/D = quantity of monensin C/D in each g of Monensin Type A Medicated Article taken, as calculated previously (mg)

$F_{C/D}$ = biopotency conversion factor for monensin C/D, 1.50

Acceptance criteria: 85.0%–115.0%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 60° for 2 h.

Acceptance criteria: NMT 10%

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Avoid moisture and excessive heat.
- **LABELING:** Label it to indicate that it is for veterinary use only. The label bears the statement "Do not feed undiluted".
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Monensin Sodium RS
  USP Narasin RS

Monensin Sodium

$C_{36}H_{61}NaO_{11}$ (monensin A sodium) 692.85

$C_{35}H_{59}NaO_{11}$ (monensin B sodium) 678.83

$C_{37}H_{63}NaO_{11}$ (monensin C sodium) 706.88

Monensin, sodium salt.

Stereoisomer of 2-[2-ethyloctahydro-3′-methyl-5′-[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2*H*-pyran-2-yl][2,2′-bifuran-5-yl]]-9-hydroxy-β-methoxy-α,γ,2,8-tetramethyl-1,6-dioxaspiro[4.5]decan-7-butanoic acid sodium salt [22373-78-0].

» Monensin Sodium has a potency of not less than 800 µg per mg.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for veterinary use only. Label it also to state that it is for manufacturing, processing, or repackaging.

USP Reference standards ⟨11⟩—
USP Monensin Sodium RS
USP Narasin RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for monensin A and a minor peak for monensin B, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 4% of its weight.

Content of monensin A and B activity—Using the results of the calculations in the *Assay,* calculate the percentage of monensin A activity in the Monensin Sodium under test by the formula:

$$100A/P$$

in which A is the potency, in mg per g, of monensin A in the Monensin Sodium under test, as determined in the *Assay,* and P is the potency, in mg of monensin, in each g of the Monensin Sodium under test, as determined in the *Assay:* not less than 90% is found. Calculate the percentage of monensin A activity plus monensin B activity in the Monensin Sodium under test by the formula:

$$100(A + B)/P$$

in which B is the potency, in mg per g, of monensin B in the Monensin Sodium under test, as determined in the *Assay,* and the other terms are as defined above: not less than 95% is found.

Assay—

Mobile phase , Neutralized methanol, Diluent, Derivatizing reagent, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Monensin.*

Assay preparation—Transfer about 100 mg of Monensin Sodium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume. If necessary, to achieve complete dissolution, sonicate for about 1 minute, and mix. Dilute an accurately measured volume of this solution quantitatively with *Diluent* to obtain a solution containing about 20 µg of monensin per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Monensin.* Calculate the quantity, in mg, of monensin A in each g of the Monensin Sodium taken by the formula:

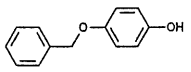
$$(CFD/100{,}000W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of monensin activity in the *Standard preparation,* based on the quantity of USP Monensin Sodium RS taken, its designated potency, in µg per mg, and the extent of dilution; F is the designated percentage of monensin A in USP Monensin Sodium RS; D is the dilution factor used in preparing the *Assay preparation; W* is the quantity, in g, of Monensin Sodium taken to prepare the *Assay preparation;* and r_U and r_S are the monensin A peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the quantity, in mg, of monensin B in each g of the Monensin Sodium taken by the same formula, except that r_U is the monensin B peak response obtained from the *Assay preparation,* and r_S is the monensin A peak response obtained from the *Standard preparation.* Calculate the quantity, in mg, of monensin C/D in each g of the Monensin Sodium taken by the same formula, except that r_U is the monensin C/D peak response obtained from the *Assay preparation.* Calculate the potency, in mg of monensin, in each g of the Monensin Sodium taken by the formula:

$$A + 0.28B + 1.5C/D$$

in which A is the quantity, in mg, of monensin A in each g of the Monensin Sodium taken, as calculated above; B is the quantity, in mg, of monensin B in each g of the Monensin Sodium taken; and C/D is the quantity, in mg, of monensin C/D in each g of Monensin Sodium taken, as calculated above.

Monobenzone



$C_{13}H_{12}O_2$ 200.23

Phenol, 4-(phenylmethoxy)-.

p-(Benzyloxy)phenol [103-16-2].

» Monobenzone, dried at 105° for 3 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{12}O_2$.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to temperatures above 30°.

USP Reference standards ⟨11⟩—
USP Monobenzone RS

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 10 µg per mL.

Medium: methanol.

Absorptivities at 292 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Transfer about 500 mg of Monobenzone, previously dried, to a 150-mL flask fitted with a reflux condenser, employing a suitable glass joint. Add 5 mL of pyridine and 3 mL of acetic anhydride, reflux for 10 minutes, and cool. Add 100 mL of water and 6 mL of acetone to the flask, and insert a stopper. Cool the contents of the flask in a refrigerator for 1 hour, collect the precipitate in a sintered-glass crucible, and wash the precipitate with water until no odor of pyridine remains. Dry the precipitate for 16 hours in a vacuum desiccator over phosphorus pentoxide. The monobenzone acetate so obtained melts between 110° and 113° when determined as directed for *Class I* (see *Melting Range or Temperature* ⟨741⟩).

Melting range, *Class I* ⟨741⟩: between 117° and 120°.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Monobenzone RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 40 µg per mL.

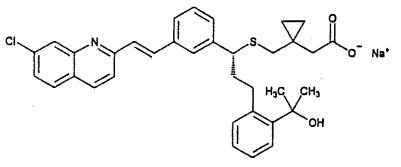
Assay preparation—Transfer about 100 mg of Monobenzone, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 4 mL of this solution into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—With a suitable spectrophotometer, using methanol as a blank, concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 292 nm. Calculate the quantity, in mg, of $C_{13}H_{12}O_2$ in the portion of Monobenzone taken by the formula:

$$2500C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Monobenzone RS in the *Standard preparation*; and A_U and A_S are the absorbances obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Montelukast Sodium



$C_{35}H_{35}ClNNaO_3S$ 608.17

Cyclopropaneacetic acid, 1-[[[1-[3-[2-(7-chloro-2-quinolinyl) ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propyl]thio]methyl]-, sodium salt, [*R*-,(*E*)]-;

Sodium 1-[[[(*R*)-*m*-[(*E*)-2-(7-chloro-2-quinolyl)vinyl]-α-[*o*-(1-hydroxy-1-methylethyl)phenethyl]benzyl]thio]-methyl] cyclopropaneacetate [151767-02-1].

$C_{35}H_{36}ClNO_3S$ 586.18

Montelukast [158966-92-8].

DEFINITION

Montelukast Sodium contains NLT 98.0% and NMT 102.0% of $C_{35}H_{35}ClNNaO_3S$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

Sample: 100 mg

Analysis: Ignite the *Sample* in a crucible until an almost white residue is obtained. Take up the residue in 2 mL of water, and filter.

Acceptance criteria: The filtrate meets the requirements of test *A*.

- **C.** Meets the requirements of the test for *Enantiomeric Purity*.

ASSAY

[NOTE—Avoid exposure of the samples to light. Use low-actinic glassware.]

- **PROCEDURE**

Solution A: Add 1.5 mL of trifluoroacetic acid to 1 L of water.

Solution B: Add 1.5 mL of trifluoroacetic acid to 1 L of acetonitrile.

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the column.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
3.0	60	40
16.0	49	51

Diluent: Methanol and water (9:1)

Standard solution: 0.13 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*

Sample solution: 0.1 mg/mL of Montelukast Sodium in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 238 nm

Column: 4.6-mm × 5-cm; 1.8-µm packing L11

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of montelukast sodium ($C_{35}H_{35}ClNNaO_3S$) in the portion of Montelukast Sodium taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast sodium, 608.17
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **ORGANIC IMPURITIES**

[NOTE—Avoid exposure of the samples to light. Use low-actinic glassware.]

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Impurity solution: 1 mg/mL of USP Montelukast for Peak Identification RS in *Diluent*

System suitability solution: Transfer 1 mL of the *Impurity solution* to a colorless glass vial, and expose to ambient light for approximately 20 min to generate the *cis*-isomer of montelukast.

Sample solution: 1 mg/mL of Montelukast Sodium in *Diluent*

Sensitivity solution: 0.5 µg/mL of Montelukast Sodium in *Diluent* from the *Sample solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.5 between the *cis*-isomer and montelukast; NLT 1.5 between montelukast and the methylketone impurity, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Montelukast Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Reporting level for impurities: 0.05%

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulfoxide impurity[a]	0.4	0.2
Cis-isomer[b]	0.8	0.15
Michael Adducts 1[c] and 2[d]	0.9	0.15*
Montelukast	1.0	—
Methylketone impurity[e]	1.2	0.15
Methylstyrene impurity[f]	1.9	0.3
Any other individual impurity	—	0.10
Total impurities	—	0.6

* These two impurities are not resolved by the method and need to be integrated together to determine conformance.

[a] [1-[[[1-[3-[(*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfinyl]methyl]cyclopropyl]acetic acid.

[b] [1-[[[(1*R*)-1-[3-[(*Z*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

[c] 1-[[[(1*R*)-1-[3-[(1*R*)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

[d] 1-[[[(1*R*)-1-[3-[(1*S*)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

[e] [1-[[[(1*R*)-3-(2-Acetylphenyl)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

[f] [1-[[[(1*R*)-1-[3-[(*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-methylethenyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

• **ENANTIOMERIC PURITY**

[NOTE—Avoid exposure of the samples to light. Use low-actinic glassware.]

Solution A: 2.3 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.7.

Solution B: Methanol and acetonitrile (60:40)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	70	30
30	60	40
35	60	40

Diluent: Acetonitrile and water (1:1)

System suitability solution: 0.1 mg/mL of USP Montelukast Racemate RS in *Diluent*

Sample solution: 1 mg/mL of Montelukast Sodium in *Diluent*

Sensitivity solution: 1 µg/mL of Montelukast Sodium in *Diluent* from the *Sample solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 15-cm; 5-µm packing L41

Column temperature: 30°

Flow rate: 0.9 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times are 1.0 for montelukast, which is the *R*-enantiomer, and 0.7 for the *S*-enantiomer.]

Suitability requirements

Resolution: NLT 2.9 between the *S*-enantiomer and montelukast, *System suitability solution*

Signal-to-noise ratio: NLT 10 for the montelukast peak, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of *S*-enantiomer in the portion of Montelukast Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

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r_U = peak response of the *S*-enantiomer from the *Sample solution*
r_T = sum of the peak responses of the *S*-enantiomer and montelukast from the *Sample solution*

Acceptance criteria: NMT 0.2% of the *S*-enantiomer

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 4.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Montelukast Sodium RS
  USP Montelukast Dicyclohexylamine RS
  $C_{35}H_{36}ClNO_3S \cdot C_{12}H_{23}N$ 767.50
  USP Montelukast Racemate RS
  USP Montelukast for Peak Identification RS
  (montelukast containing sulfoxide impurity, michael adducts 1 and 2, methylketone impurity, and methylstyrene impurity)

USP Monographs

Montelukast Sodium Oral Granules

DEFINITION
Montelukast Sodium Oral Granules contain Montelukast Sodium equivalent to NLT 90.0% and NMT 108.0% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$).
[NOTE—Avoid exposure of samples containing montelukast to light.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Diluent: Methanol and water (3:1)
  Standard solution: 3.3 µg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
  Sample stock solution: Nominally 0.02 mg/mL of montelukast prepared as follows. Transfer the contents of one packet to a suitable volumetric flask, add 66% of the flask volume of *Diluent*, shake well, and sonicate for 15 min with occasional shaking. Cool to room temperature, dilute with *Diluent* to volume, and mix well.
  Sample solution: Nominally 2 µg/mL of montelukast in *Diluent* from the *Sample stock solution*. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size or centrifuge to obtain a clear solution.
  Wavelength range: 210–400 nm
  Acceptance criteria: The *Sample solution* exhibits maxima only at the same wavelengths as the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Diluent: Methanol and water (3:1)
  Solution A: 0.2% (v/v) trifluoroacetic acid in water
  Solution B: Methanol and acetonitrile (3:2)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	48	52
5	45	55
12	45	55
22	25	75
23	25	75
25	48	52
30	48	52

Standard solution: 0.33 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
System suitability solution: Transfer 10 mL of the *Standard solution* to a clear 10-mL volumetric flask, add 4 µL of hydrogen peroxide, and mix well. Expose the flask for at least 4 h to ambient light or 10 min to a 4 klx cool white light. [NOTE—Montelukast is partially converted to the *cis*-isomer under these conditions.]
Sensitivity solution: 0.33 µg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent* from the *Standard solution*
Sample solution: Nominally 0.24 mg/mL of montelukast prepared as follows. Transfer the equivalent of 60 mg of montelukast from the contents of the packets (NLT 15) to a 500-mL volumetric flask, and add 250 mL of *Diluent*. Shake well and sonicate for 30 min, with occasional shaking. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size or centrifuge to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 255 nm
Columns
Guard: 3.0-mm × 4-mm; packing L11
Analytical: 4.6-mm × 10-cm; 3-µm packing L11
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of montelukast
System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
[NOTE—The relative retention times for the *cis*-isomer and montelukast are about 0.92 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the *cis*-isomer and montelukast, *System suitability solution*
Relative standard deviation: NMT 2.0% for five injections, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the portion of Oral Granules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: 90.0%–108.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Test 1

Medium: 0.5% (w/v) sodium dodecyl sulfate in water; 900 mL. Do not deaerate.
Apparatus 1: 100 mesh; 50 rpm
Time: 15 min
Solution A: 0.2% (v/v) trifluoroacetic acid in water
Solution B: 0.2% (v/v) trifluoroacetic acid in acetonitrile
Mobile phase: *Solution A* and *Solution B* (1:1)
Standard stock solution: 0.33 mg/mL of USP Montelukast Dicyclohexylamine RS in methanol (equivalent to 0.25 mg/mL of montelukast)
Standard solution: (*L*/900) mg/mL of montelukast in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/packet of montelukast
Sample solution: Place the entire contents of one packet in the basket. At the appropriate time point, pass a portion of the solution under test through a suitable filter to obtain a clear solution. Discard the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 389 nm
Column: 3.0-mm × 10-cm; 5-µm packing L11
Column temperature: 50°
Flow rate: 0.9 mL/min
Injection volume: 25 µL
Run time: 1.5 times the retention time of montelukast
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of montelukast from the *Sample solution*
r_S = peak response of montelukast from the *Standard solution*
C_S = concentration of montelukast in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/packet)

Tolerances: NLT 85% (*Q*) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.5% (w/v) sodium dodecyl sulfate in water; 900 mL
Apparatus 1: 100 mesh; 50 rpm
Time: 15 min
Solution A: 0.07 g/L of monobasic sodium phosphate
Solution B: Acetonitrile
Mobile phase: *Solution A* and *Solution B* (45:55). Add 1.33 mL/L of triethylamine and adjust with phosphoric acid to a pH of 6.7.
Standard stock solution: 0.1 mg/mL of montelukast from montelukast sodium hydrate prepared as follows. Transfer a suitable amount of montelukast sodium hydrate to an appropriate volumetric flask. Dissolve in 4% of the flask volume of methanol and dilute with *Medium* to volume. Determine the water content of montelukast sodium hydrate at the time of use.
Standard solution: 0.004 mg/mL of montelukast in *Medium* from the *Standard stock solution*
Sample solution: Place the entire contents of one packet in the basket. At the appropriate time point, centrifuge a portion of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 5-cm; 1.8-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: 1.5 times the retention time of montelukast
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of montelukast in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/packet)

Tolerances: NLT 80% (*Q*) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.5% (w/v) sodium dodecyl sulfate in water; 900 mL
Apparatus 2: 50 rpm
Time: 10 min
Solution A: 2.72 g/L of monobasic potassium phosphate in water
Mobile phase: Acetonitrile and *Solution A* (70:30)
Diluent: Acetonitrile and water (50:50)
System suitability solution: Expose a portion of *Standard solution* in a clear glass vial to direct room light for about 30 min.
Standard stock solution: 0.524 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent* (equivalent to 0.4 mg/mL of montelukast)
Standard solution: 0.0065 mg/mL of USP Montelukast Dicyclohexylamine RS in *Medium* from the *Standard stock solution* (equivalent to 0.005 mg/mL of montelukast)
Sample solution: Transfer the entire contents of one packet to the dissolution vessel. At the specified time point, withdraw 10 mL of sample from the dissolution vessel. Pass a portion of the solution under test through a suitable filter. Discard the first 5 mL of the filtrate.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 281 nm
Column: 4.6-mm × 3-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 25 µL
Run time: About 1.5 times the retention time of montelukast
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for *Z*-isomer and montelukast are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the *Z*-isomer and montelukast, *System suitability solution*
Tailing factor: NMT 2.0 for montelukast, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/packet)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Tolerances: NLT 80% (*Q*) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.5% (w/v) sodium dodecyl sulfate in water; 900 mL
Apparatus 1: 100 mesh; 50 rpm
Time: 20 min
Solution A: 3.9 g/L of sodium phosphate monobasic dihydrate in water. Adjust with dilute phosphoric acid to a pH of 3.7.
Mobile phase: Acetonitrile and *Solution A* (80:20)
Diluent: *Medium*
Standard solution: 0.005 mg/mL of montelukast in *Diluent* from montelukast sodium hydrate. Determine the water content of montelukast sodium hydrate at the time of use.
Sample solution: Place the entire contents of one packet in the basket. At the specified time point, withdraw 10 mL of sample from the dissolution vessel. Pass a portion of the solution under test through a suitable filter paper. Discard the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 20°
Column: 28°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 1.5 times the retention time of montelukast
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for montelukast
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of montelukast in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/packet)

Tolerances: NLT 80% (*Q*) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Solution A, Solution B, Mobile phase, and **System suitability:** Proceed as directed in *Dissolution Test 1*.
Standard solution: 26.4 µg/mL of USP Montelukast Dicyclohexylamine RS in methanol
Sample solution: Nominally 0.02 mg/mL of montelukast prepared as follows. Transfer the contents of one packet to a suitable volumetric flask, add 66% of the flask volume of methanol, shake well, and sonicate for 15 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size or centrifuge to obtain a clear solution.
Chromatographic system: Proceed as directed in *Dissolution Test 1*, except use an *Injection volume* of 5 µL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the packet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Oral Granules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*
r_S = peak response of montelukast from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak with an area less than that of the *Sensitivity solution*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfoxide impurity[a, b]	0.45	1.0	1.0
Montelukast ketone impurity[c]	0.71	1.7	0.2
cis-Isomer[d]	0.92	1.0	0.2
Montelukast	1.0	—	—
Methylketone impurity[e, f]	1.04	—	—
Michael adduct 1[g, e]	1.16	—	—
Michael adduct 2[h, e]	1.18	—	—
Methylstyrene impurity[i, e]	1.55	—	—
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	1.5

[a] These two impurities are not resolved by the method and need to be integrated together to determine conformance.
[b] [1-[[[1-[3-[(*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfinyl]methyl]cyclopropyl]acetic acid.
[c] (*E*)-1-{3-[2-(7-Chloroquinolin-2-yl)vinyl]phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propan-1-one.
[d] [1-[[[(1*R*)-1-[3-[(*Z*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[e] This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.
[f] [1-[[[(1*R*)-3-(2-Acetylphenyl)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[g] (1-{[(*R*)-1-(3-[(*R*)-1-{[1-(Carboxymethyl)cyclopropyl]methylthio}-2-(7-chloroquinolin-2-yl)ethyl]phenyl)-3-[2-(2-hydroxypropan-2-yl)phenyl]propylthio]methyl}cyclopropyl)acetic acid.
[h] (1-{[(*R*)-1-(3-[(*S*)-1-{[1-(Carboxymethyl)cyclopropyl]methylthio}-2-(7-chloroquinolin-2-yl)ethyl]phenyl)-3-[2-(2-hydroxypropan-2-yl)phenyl]propylthio]methyl}cyclopropyl)acetic acid.
[i] [1-[[[(1*R*)-1-[3-[(*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-methylethenyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Montelukast Dicyclohexylamine RS
  $C_{35}H_{36}ClNO_3S \cdot C_{12}H_{23}N$ 767.50

Montelukast Sodium Tablets

DEFINITION
Montelukast Sodium Tablets contain Montelukast Sodium equivalent to NLT 92.5% and NMT 107.5% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$).
[NOTE—Avoid exposure of samples containing montelukast to light.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Diluent: Methanol and water (3:1)
  Standard solution: 0.026 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
  Sample solution: Nominally 0.02 mg/mL of montelukast prepared as follows. Transfer one Tablet equivalent to 10 mg of montelukast to a suitable volumetric flask, add 25% of the flask volume of water, and let stand for 5–10 min until the Tablet has disintegrated. Add 60% of the flask volume of methanol, shake well, and sonicate for 70 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Centrifuge a portion of the resulting solution to obtain a clear solution.
  Wavelength range: 210–400 nm
  Acceptance criteria: The *Sample solution* exhibits maxima only at the same wavelengths as the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Diluent: Methanol and water (3:1)
  Solution A: 0.2% (v/v) Trifluoroacetic acid in water
  Solution B: Methanol and acetonitrile (3:2)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	48	52
5	45	55
12	45	55
22	25	75
23	25	75
25	48	52
30	48	52

Standard solution: 0.52 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*

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System suitability solution: Transfer 10 mL of the *Standard solution* to a clear 10-mL volumetric flask, add 4 µL of hydrogen peroxide, and mix well. Expose the flask for at least 4 h to ambient light or 10 min to a 4 klx cool white light. [NOTE—Montelukast is partially converted to the *cis*-isomer under these conditions.]
Sensitivity solution: 0.52 µg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent* from the *Standard solution*
Sample solution: Nominally 0.4 mg/mL of montelukast prepared as follows. Transfer a number of Tablets equivalent to 100 mg of montelukast to a suitable volumetric flask, add 70% of the flask volume of *Diluent*, and sonicate for 30 min. Shake for 30 min on a platform shaker. Dilute with *Diluent* to volume and stir for 30 min. Pass a portion through a suitable filter of 0.45-µm pore size, discarding the first mL of filtrate. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 255 nm
Columns
Guard: 3.0-mm × 4-mm; packing L11
Analytical: 4.6-mm × 10-cm; 3-µm packing L11
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection volume: 15 µL
Run time: 2 times the retention time of montelukast
System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
[NOTE—The relative retention times for the *cis*-isomer and montelukast are about 0.92 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the *cis*-isomer and montelukast, *System suitability solution*
Relative standard deviation: NMT 2% for five injections, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL. Do not deaerate.
Apparatus 2: 50 rpm
Time: 20 min
Solution A: 0.2% (v/v) Trifluoroacetic acid in water
Solution B: 0.2% (v/v) Trifluoroacetic acid in acetonitrile
Mobile phase: *Solution A* and *Solution B* (1:1)
Standard stock solution: 0.35 mg/mL of USP Montelukast Dicyclohexylamine RS in methanol (equivalent to 0.27 mg/mL of montelukast)
Standard solution: (*L*/900) mg/mL of montelukast in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet of montelukast
Sample solution: Pass a portion of the solution under test through a suitable filter or centrifuge to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 389 nm
Column: 3.0-mm × 10-cm; 5-µm packing L11
Column temperature: 50°
Flow rate: 0.9 mL/min
Injection volume: 20 µL
Run time: 1.5 times the retention time of montelukast
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of montelukast in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Solution A: 0.07 g/L of monobasic sodium phosphate
Solution B: Acetonitrile
Mobile phase: *Solution A* and *Solution B* (45:55). Add 1.33 mL/L of triethylamine and adjust with phosphoric acid to a pH of 6.7.
Standard stock solution: 0.1 mg/mL of montelukast from montelukast sodium hydrate prepared as follows. Transfer a suitable amount of montelukast sodium hydrate to a suitable volumetric flask. Dissolve in 5% of the flask volume of methanol and dilute with *Medium* to volume. Determine the water content of montelukast sodium hydrate at the time of use.
Standard solution: 0.01 mg/mL of montelukast in *Medium* from the *Standard stock solution*
Sample solution: Centrifuge a portion of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 5-cm; 1.8-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min

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Injection volume: 100 µL
Run time: 1.5 times the retention time of montelukast
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of montelukast in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Solution A: Acetonitrile and water (80:20)
Solution B: 3% Trifluoroacetic acid in *Solution A* prepared as follows. Transfer 3 mL of trifluoroacetic acid to a 100-mL volumetric flask and dilute with *Solution A* to volume.
Mobile phase: Acetonitrile, water, and *Solution B* (75: 25: 0.05).
Standard solution: (L/900) mg/mL of montelukast in *Medium* from montelukast sodium hydrate, where L is the label claim in mg/Tablet of montelukast. Determine the water content of montelukast sodium hydrate at the time of use.
Sample solution: Pass a portion of the solution under test through a suitable filter to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 346 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: NLT 1.5 times the retention time of montelukast
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of montelukast in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Solution A, Solution B, Mobile phase, and **System suitability:** Proceed as directed in *Dissolution Test 1*.
Diluent: Methanol and water (3:1)
Standard solution: 0.052 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
Sample solution: Nominally 0.04 mg/mL of montelukast prepared as follows. Transfer one Tablet equivalent to 10 mg of montelukast to a suitable volumetric flask, add 25% of the flask volume of water, and let stand for 5–10 min until the Tablet has disintegrated. Add 60% of the flask volume of methanol, shake well, and sonicate for 70 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Pass a portion of the resulting solution through a suitable filter or centrifuge to obtain a clear solution.
Chromatographic system: Proceed as directed in *Dissolution Test 1*, except use an *Injection volume* of 10 µL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*
r_S = peak response of montelukast from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak with an area less than that of the *Sensitivity solution*.

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Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfoxide impurity[a, b]	0.45	1.0	2.0
Montelukast ketone impurity[c]	0.71	1.7	0.2
cis-Isomer[d]	0.92	1.0	0.2
Montelukast	1.0	—	—
Methylketone impurity[e, f]	1.04	—	—
Michael adduct 1[g, e]	1.16	—	—
Michael adduct 2[h, e]	1.18	—	—
Methylstyrene impurity[i, e]	1.55	—	—
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	3.0

[a] These two impurities are not resolved by the method and need to be integrated together to determine conformance.
[b] [1-[[[1-[3-[(*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfinyl]methyl]cyclopropyl]acetic acid.
[c] (*E*)-1-{3-[2-(7-Chloroquinolin-2-yl)vinyl]phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propan-1-one.
[d] [1-[[[(1*R*)-1-[3-[(*Z*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[e] This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.
[f] [1-[[[(1*R*)-3-(2-Acetylphenyl)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[g] 1-[[[(1*R*)-1-[3-[(1*R*)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[h] 1-[[[(1*R*)-1-[3-[(1*S*)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[i] [1-[[[(1*R*)-1-[3-[(*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-methylethenyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- **LABELING** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Montelukast Dicyclohexylamine RS
  $C_{35}H_{36}ClNO_3S \cdot C_{12}H_{23}N$ 767.50

Montelukast Sodium Chewable Tablets

DEFINITION

Montelukast Sodium Chewable Tablets contain Montelukast Sodium equivalent to NLT 92.5% and NMT 107.5% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$).
[NOTE—Avoid exposure of samples containing montelukast to light.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Diluent: Methanol and water (3:1)
  Standard solution (for 4-mg Chewable Tablets): 0.026 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
  Standard solution (for 5-mg Chewable Tablets): 0.033 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
  Sample solution: Nominally (*L*/200) mg/mL of montelukast, where *L* is the label claim of montelukast in mg/Chewable Tablet prepared as follows. Transfer 1 Chewable Tablet to a suitable volumetric flask, add 25% of the flask volume of water, and let stand for 5–10 min until the Chewable Tablet has disintegrated. Add 55% of the flask volume of methanol, shake well, and sonicate for 70 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Centrifuge a portion of the resulting solution to obtain a clear solution.
  Wavelength range: 210–400 nm
  Acceptance criteria: The *Sample solution* exhibits maxima only at the same wavelengths as the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Diluent: Methanol and water (3:1)
  Solution A: 0.2% (v/v) Trifluoroacetic acid in water
  Solution B: Methanol and acetonitrile (3:2)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	48	52
5	45	55
12	45	55
22	25	75
23	25	75
25	48	52
30	48	52

Standard solution: 0.33 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
System suitability solution: Transfer 10 mL of the *Standard solution* to a clear 10-mL volumetric flask, add 4 µL of hydrogen peroxide, and mix well. Expose the flask for at least 4 h to ambient light or 10 min to a 4 klx cool white light. [NOTE—Montelukast is partially converted to the *cis*-isomer under these conditions.]
Sensitivity solution: 0.33 µg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent* from the *Standard solution*
Sample solution (for 4-mg Chewable Tablets): Nominally 0.24 mg/mL of montelukast prepared as follows. Transfer 12 Chewable Tablets to a suitable volumetric flask, add 75% of the flask volume of *Diluent*, and shake vigorously for 60 min. Dilute with *Diluent* to volume. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size, discarding the first mL of filtrate. Use the filtrate.

USP Monographs

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Sample solution (for 5-mg Chewable Tablets): Nominally 0.25 mg/mL of montelukast prepared as follows. Transfer 10 Chewable Tablets to a suitable volumetric flask, add 75% of the flask volume of *Diluent*, and shake vigorously for 60 min. Dilute with *Diluent* to volume. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size, discarding the first mL of filtrate. Use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 255 nm
Columns
Guard: 3.0-mm × 4-mm; packing L11
Analytical: 4.6-mm × 10-cm; 3-µm packing L11
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of montelukast

System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
[NOTE—The relative retention times for the *cis*-isomer and montelukast are about 0.92 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the *cis*-isomer and montelukast, *System suitability solution*
Relative standard deviation: NMT 2% for five injections, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1
Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL. Do not deaerate.
Apparatus 2: 50 rpm
Time: 20 min
Solution A: 0.2% (v/v) Trifluoroacetic acid in water
Solution B: 0.2% (v/v) Trifluoroacetic acid in acetonitrile
Mobile phase: *Solution A* and *Solution B* (1:1)
Standard stock solution (for 4-mg Chewable Tablets): 0.30 mg/mL of USP Montelukast Dicyclohexylamine RS in methanol (equivalent to 0.23 mg/mL of montelukast)
Standard stock solution (for 5-mg Chewable Tablets): 0.35 mg/mL of USP Montelukast Dicyclohexylamine RS in methanol (equivalent to 0.27 mg/mL of montelukast)
Standard solution: (L/900) mg/mL of montelukast in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Chewable Tablet of montelukast
Sample solution: Pass a portion of the solution under test through a suitable filter or centrifuge to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 389 nm
Column: 3.0-mm × 10-cm; 5-µm packing L11
Column temperature: 50°
Flow rate: 0.9 mL/min
Injection volume: 50 µL
Run time: 1.5 times the retention time of montelukast
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of montelukast in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Chewable Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Solution A: 0.07 g/L of monobasic sodium phosphate
Solution B: Acetonitrile
Mobile phase: *Solution A* and *Solution B* (45:55). Add 1.33 mL/L of triethylamine and adjust with phosphoric acid to a pH of 6.7.
Standard stock solution: 0.1 mg/mL of montelukast from montelukast sodium hydrate prepared as follows. Transfer a suitable amount of montelukast sodium hydrate to an appropriate volumetric flask. Dissolve in 4% of the flask volume of methanol and dilute with *Medium* to volume. Determine the water content of montelukast sodium hydrate at the time of use.
Standard solution: 0.005 mg/mL of montelukast in *Medium* from the *Standard stock solution*
Sample solution: Centrifuge a portion of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 5-cm; 1.8-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: 1.5 times the retention time of montelukast
System suitability
Sample: *Standard solution*

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Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of montelukast in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Chewable Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Solution A, Solution B, Mobile phase, and **System suitability:** Proceed as directed in *Dissolution Test 1*.
Diluent: Methanol and water (3:1)
Standard solution (for 4-mg Chewable Tablets): 0.026 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
Standard solution (for 5-mg Chewable Tablets): 0.033 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
Sample solution: Nominally (L/200) mg/mL of montelukast, where L is the label claim of montelukast in mg/Chewable Tablet prepared as follows. Transfer 1 Chewable Tablet to a suitable volumetric flask, add 25% of the flask volume of water, and let stand for 5–10 min until the Chewable Tablet has disintegrated. Add 55% of the flask volume of methanol, shake well, and sonicate for 70 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Pass a portion of the resulting solution through a suitable filter or centrifuge to obtain a clear solution.
Chromatographic system: Proceed as directed in *Dissolution Test 1*, except use an *Injection volume* of 10 µL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the Chewable Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*
r_S = peak response of montelukast from the *Standard solution*
C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of montelukast, 586.18
M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak with an area less than that of the *Sensitivity solution*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfoxide impurity[a, b]	0.45	1.0	1.5
Montelukast ketone impurity[c]	0.71	1.7	0.2
cis-Isomer[d]	0.92	1.0	0.2
Montelukast	1.0	—	—
Methylketone impurity[e, f]	1.04	—	—
Michael adduct 1[g, e]	1.16	—	—
Michael adduct 2[h, e]	1.18	—	—
Methylstyrene impurity[i, e]	1.55	—	—
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	2.0

[a] These two impurities are not resolved by the method and need to be integrated together to determine conformance.
[b] [1-[[[1-[3-[(*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfinyl]methyl]cyclopropyl]acetic acid.
[c] (*E*)-1-(3-[2-(7-Chloroquinolin-2-yl)vinyl]phenyl)-3-[2-(2-hydroxypropan-2-yl) phenyl]propan-1-one.
[d] [1-[[[(1*R*)-1-[3-[(*Z*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[e] This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.
[f] [1-[[[(1*R*)-3-(2-Acetylphenyl)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl] phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[g] 1-[[[(1*R*)-1-[3-[(1*R*)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[h] 1-[[[(1*R*)-1-[3-[(1*S*)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propyl]sulfanyl]methyl]cyclopropyl]acetic acid.
[i] [1-[[[(1*R*)-1-[3-[(*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-methylethenyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

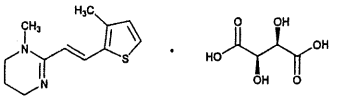
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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- **LABELING** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Montelukast Dicyclohexylamine RS
  $C_{35}H_{36}ClNO_3S \cdot C_{12}H_{23}N$ 767.50

Morantel Tartrate



$C_{12}H_{16}N_2S \cdot C_4H_6O_6$ 370.42

Pyrimidine, 1,4,5,6-tetrahydro-1-methyl-2-[2-(3-methyl-2-thienyl)ethenyl]-, (*E*)-,[*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1).

(*E*)-1,4,5,6-Tetrahydro-1-methyl-2-[2-(3-methyl-2-thienyl) vinyl]pyrimidine tartrate (1:1) [26155-31-7].

Morantel [20574-50-9].

» Morantel Tartrate contains not less than 96.4 percent and not more than 101.5 percent of $C_{12}H_{16}N_2S \cdot C_4H_6O_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Labeling—Label it to indicate it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Morantel Tartrate RS

Clarity and color of solution—Dissolve and dilute 0.25 g to 25.0 mL in carbon dioxide-free water. The solution is clear and yellow to greenish yellow in color.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: It meets the requirements of the test for *Tartrate* ⟨191⟩.

C: The retention time of the morantel peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of *Standard solution 1*, as obtained in the test for *Related compounds.*

Melting temperature ⟨741⟩: 167° to 172°.

pH ⟨791⟩: between 2.8 and 3.9.

Solution—Dissolve and dilute 0.25 g to 25.0 mL in carbon dioxide-free water.

Loss on drying ⟨731⟩—Dry it at 100° to 105° to constant weight: it loses not more than 1.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—

[NOTE—Conduct this test without exposure to daylight, and with the minimum necessary exposure to artificial light.]

Mobile phase—Mix 3.5 mL of triethylamine and 850 mL of water. Adjust with phosphoric acid to a pH of 2.5. Add 50 mL of tetrahydrofuran and 100 mL of methanol, and mix.

Tartrate solution—Prepare a solution containing about 0.15 mg of tartaric acid per mL in *Mobile phase.*

Standard solution 1—Dissolve an accurately weighed quantity of USP Morantel Tartrate RS in *Mobile phase* to obtain a solution having a known concentration of about 5.0 µg per mL.

Standard solution 2—Dilute 2.0 mL of *Standard solution 1* to 100.0 mL with *Mobile phase.*

System suitability solution—Expose 10 mL of *Standard solution 1* to daylight for 15 minutes before injection.

Test solution—Dissolve an accurately weighed quantity of Morantel Tartrate in *Mobile phase* to obtain a solution having a concentration of about 0.5 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 226-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 0.75 mL per minute. Chromatograph the *Tartrate solution, Standard solution 1,* and the *System suitability solution,* and record the peak areas as directed for *Procedure:* using the *System suitability solution,* the resolution, *R,* between morantel and its preceding peak ((*Z*)-isomer) is not less than 2. The relative retention times are about 0.8, 1.0, and 1.2 for the morantel (*Z*)-isomer, morantel, and the morantel 4-methyl isomer (1-methyl-2-[(*E*)-2-(4-methylthiophen-2-yl)ethenyl]-1,4,5,6 tet rahydropyrimidine), respectively.

Procedure—Separately inject equal volumes (about 20 µL) of the *Tartrate solution, Standard solution 1, Standard solution 2,* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Disregarding the tartrate peak and any peak in the chromatogram of the *Test solution* less than the area of the principal peak in the chromatogram of *Standard solution 2,* calculate the area percentage of each impurity, relative to morantel, in the portion of Morantel Tartrate taken by the formula:

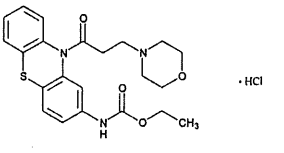
$$100(C_S/C_U)(r_i/r_S)$$

in which C_S and C_U are the concentrations of morantel tartrate, in mg per mL, of *Standard solution 1* and the *Test solution,* respectively; and r_i and r_S are the peak areas of each individual impurity and morantel obtained from the *Test solution* and *Standard solution 1,* respectively: not more than 3% of the morantel 4-methyl isomer is found; not more than 0.5% of any other individual impurity is found; and not more than 1% of total other individual impurities is found.

Assay—

Dissolve 0.280 g in 40 mL of anhydrous acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩). One mL of 0.1 N perchloric acid is equivalent to 37.04 mg of $C_{12}H_{16}N_2S \cdot C_4H_6O_6$.

Moricizine Hydrochloride



$C_{22}H_{25}N_3O_4S \cdot HCl$ 463.98

Carbamic acid, [10-[3-(4-morpholinyl)-l-oxopropyl]-10*H*-phenothiazin-2yl]-, ethyl ester, hydrochloride;

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Ethyl 10-(3-morpholinopropionyl)phenothiazine-2-carbamate, hydrochloride [29560-58-5].

DEFINITION

Moricizine Hydrochloride contains NLT 98.0% and NMT 102.0% of moricizine hydrochloride ($C_{22}H_{25}N_3O_4S \cdot HCl$), calculated on the anhydrous and alcohol-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS–GENERAL, *Chloride* ⟨191⟩:** Meets the requirements

ASSAY

- **PROCEDURE:** Use low-actinic glassware. Protect the solutions containing moricizine hydrochloride from light.

Mobile phase: A mixture of acetonitrile, triethylamine, glacial acetic acid, and water (420:1:20:580) containing 5 mM sodium 1-octane sulfonate

Diluent: Acetonitrile and 0.02 N hydrochloric acid (42:58)

Internal standard solution: 5 mg/mL of butamben in *Diluent*

Standard solution: 1 mg/mL of USP Moricizine Hydrochloride RS in *Diluent* prepared as follows. Transfer a suitable amount of USP Moricizine Hydrochloride RS to a suitable volumetric flask, add *Internal standard solution* to fill about 20% of the total volume, and dilute with *Diluent* to volume.

Sample solution: 1 mg/mL of Moricizine Hydrochloride prepared as follows. To a suitable amount of Moricizine Hydrochloride in a 50-mL volumetric flask add *Internal standard solution* to fill about 20% of the total volume, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 35°

Flow rate: 2.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for moricizine, butamben, the reverse Mannich product, and the amide hydrolysis product are about 0.6, 1.0, 1.7, and 2.0, respectively.]

Suitability requirements

Resolution: NLT 2 between moricizine and butamben

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of moricizine hydrochloride ($C_{22}H_{25}N_3O_4S \cdot HCl$) in the portion of Moricizine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the moricizine peak area response to the butamben peak area response from the *Sample solution*

R_S = ratio of the moricizine peak area response to the butamben peak area response from the *Standard solution*

C_S = concentration of USP Moricizine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Moricizine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and alcohol-free basis

OTHER COMPONENTS

- **CONTENT OF CHLORIDE**

Sample solution: Transfer 400 mg of Moricizine Hydrochloride to a conical flask and add 75 mL of methanol. Swirl to dissolve. Add 5 mL of glacial acetic acid and 3 drops of eosin Y TS.

Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.546 mg of chloride.

Acceptance criteria: 7.49%–7.80% on the anhydrous and alcohol-free basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase: A mixture of acetonitrile, triethylamine, and water (420:1:580) containing 5 mM sodium 1-octane sulfonate. Adjust with glacial acetic acid to a pH of 4.2.

Diluent: Acetonitrile and 0.02 N hydrochloric acid (42:58)

Internal standard solution: 0.1 mg/mL of butamben in *Diluent*

Standard stock solution: 0.10 mg/mL of USP Moricizine Hydrochloride RS in *Diluent*

Standard solution: 2.0 µg/mL of USP Moricizine Hydrochloride RS prepared as follows. Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask, add *Internal standard solution* to fill 5% of the total volume, and dilute with *Diluent* to volume. [NOTE—Protect the solution from light.]

Sample solution: 1 mg/mL of Moricizine Hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Moricizine Hydrochloride to a suitable volumetric flask, add *Internal standard solution* to fill 5% of the total volume, and dilute with *Diluent* to volume. [NOTE—Protect the solution from light.]

Chromatographic system: Proceed as directed in the *Assay* except for the following:

Run time: Five times the elution time of moricizine

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for moricizine and butamben are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2 between moricizine and butamben

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the responses for all the peaks except the solvent peak.

Calculate the percentage of each impurity in the portion of Moricizine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the peak area response of each impurity to the butamben peak area response from the *Sample solution*

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R_S = ratio of the peak area response of moricizine to the peak area response of butamben from the *Standard solution*
C_S = concentration of USP Moricizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: Disregard any impurity of less than 0.1%.
Any impurity eluting before moricizine: NMT 0.25%
Any impurity eluting after moricizine: NMT 0.20%
Total of all impurities: NMT 1.5%

• **LIMIT OF ALCOHOL**
Standard solution: 0.1184 mg/mL of dehydrated alcohol in water prepared as follows. Transfer 6.0 mL of dehydrated alcohol to a 100-mL volumetric flask and dilute with water to volume. Dilute 5.0 mL of this solution in a 100-mL volumetric flask with water to volume. Further dilute 5.0 mL of this solution in a third 100-mL volumetric flask with water to volume.
Sample solution: Transfer 1 g of Moricizine Hydrochloride to a 50-mL glass-stoppered centrifuge tube, add 19.0 mL of water, and sonicate to dissolve. Transfer 1.0 mL of 3 N ammonium hydroxide to the tube, insert the stopper, and shake the tube by mechanical means for 30 min. Centrifuge, draw off a portion of the clear supernatant, and pass through a suitable filter of 0.5-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 4-mm × 1.8-m glass; supporting S2
Temperatures
Column: 150°
Injector port: 170°
Detector block: 170°
Carrier gas: Helium
Flow rate: 50 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alcohol (C_2H_5OH) in the portion of Moricizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

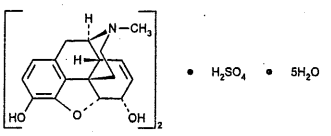
r_U = peak response of alcohol from the *Sample solution*
r_S = peak response of alcohol from the *Standard solution*
C_S = concentration of alcohol in the *Standard solution* (mg/mL)
C_U = concentration of Moricizine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.25%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 1.0%
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%
• **CLARITY OF SOLUTION**
Sample solution: Dissolve 1 g in 30 mL of methanol, sonicating for 5 min if necessary.
Acceptance criteria: The solution is not less clear than an equal volume of methanol contained in a similar vessel and examined similarly.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Moricizine Hydrochloride RS

Morphine Sulfate



$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ 758.83
Morphinan-3,6-diol, 7,8-didehydro-4,5-epoxy-17-methyl, (5α,6α)-, sulfate (2:1) (salt), pentahydrate.
7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol sulfate (2:1) (salt) pentahydrate [6211-15-0].
Anhydrous 668.77
[64-31-3].

» Morphine Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store up to 40° as permitted by the manufacturer.
USP Reference standards ⟨11⟩—
USP Morphine Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020) Dried at 145° for 1 hour.
B: To 1 mg in a porcelain crucible or small dish add 0.5 mL of sulfuric acid containing, in each mL, 1 drop of formaldehyde TS: an intense purple color is produced at once, and quickly changes to deep blue-violet *(distinction from codeine, which gives at once an intense violet-blue color, and from hydromorphone, which gives at first a yellow to brown color, changing to pink and then to purplish red).*
C: To a solution of 5 mg in 5 mL of sulfuric acid in a test tube add 1 drop of ferric chloride TS, mix, and heat in boiling water for 2 minutes: a blue color is produced, and when 1 drop of nitric acid is added, it changes to dark red-brown *(codeine and ethylmorphine give the same color reactions, but hydromorphone and papaverine do not produce this color change).*
D: A solution (1 in 50) responds to the tests for *Sulfate* ⟨191⟩.
Specific rotation ⟨781S⟩**:** between −107° and −109.5°.
Test solution: the equivalent of 20 mg per mL, in water.
Acidity—Dissolve 500 mg in 15 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.50 mL is required to produce a yellow color.
Water Determination, *Method I* ⟨921⟩ **:** between 10.4% and 13.4% is found.
Residue on ignition ⟨281⟩**:** not more than 0.1%, from 500 mg.

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Chloride—To 10 mL of a solution (1 in 100) add 1 mL of 2 N nitric acid and 1 mL of silver nitrate TS: no precipitate or turbidity is produced immediately.
Ammonium salts—Heat 200 mg with 5 mL of 1 N sodium hydroxide on a steam bath for 1 minute: no odor of ammonia is perceptible.
Limit of foreign alkaloids—Dissolve 1.00 g in 10 mL of 1 N sodium hydroxide in a separator, and shake the solution with three successive portions of 15, 10, and 10 mL of chloroform, passing the chloroform solutions through a small filter previously moistened with chloroform. Shake the combined chloroform solutions with 5 mL of water, separate the chloroform layer, and carefully evaporate on a steam bath to dryness. To the residue add 10.0 mL of 0.020 N sulfuric acid, and heat gently until dissolved. Cool, add 2 drops of methyl red TS, and titrate the excess acid with 0.020 N sodium hydroxide: not less than 7.5 mL is required (1.5%).

Assay—
Mobile phase—Dissolve 0.73 g of sodium 1-heptanesulfonate in 720 mL of water, add 280 mL of methanol and 10 mL of glacial acetic acid, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Morphine Sulfate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.24 mg per mL. Prepare a fresh solution daily.
System suitability preparation—Dissolve suitable quantities of USP Morphine Sulfate RS and phenol in *Mobile phase* to obtain a solution containing about 0.24 and 0.15 mg per mL, respectively.
Assay preparation—Transfer about 24 mg of Morphine Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 284-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for phenol and 1.0 for morphine sulfate; the resolution, *R,* between phenol and morphine sulfate is not less than 2.0; the tailing factor for the morphine sulfate peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$ in the portion of Morphine Sulfate taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of anhydrous morphine sulfate in the *Standard preparation,* as determined from the concentration of USP Morphine Sulfate RS corrected for moisture content by a titrimetric water determination; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Morphine Sulfate Extended-Release Capsules

DEFINITION
Morphine Sulfate Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$.

IDENTIFICATION
• **A.**
Standard solution and **Sample solution:** Prepare as directed in the *Assay.*
Analysis: Inject 10 µL each of the *Standard solution* and the *Sample solution* using the *Chromatographic system* except for the *Injection volume* in the *Assay.*
Acceptance criteria: The UV absorption spectrum of the morphine peak of the *Sample solution* and of the *Standard solution* exhibits maxima and minima at the same wavelengths, as obtained in the *Assay.*
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Diluent: Water. Adjust with phosphoric acid to a pH of 3.6.
Buffer solution: 13.8 mg/mL of monobasic sodium phosphate
Solution A: Acetonitrile, triethylamine, *Buffer solution,* and water (25: 0.5: 100: 874.5). Adjust with phosphoric acid to a pH of 3.6.
Solution B: Acetonitrile
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
33	100	0
44	85	15
54	85	15
55	100	0
65	100	0

System suitability solution: 400 µg/mL of USP Morphine Sulfate RS and 10 µg/mL each of USP Morphine Related Compound A RS and USP Morphine Related Compound B RS (pseudomorphine) in *Diluent*
Standard solution: 1.0 mg/mL of USP Morphine Sulfate RS in *Diluent*
Sample stock solution: Transfer a weighed portion of the contents from NLT 20 Capsules, nominally equivalent to 250 mg of morphine sulfate pentahydrate, to a 100-mL volumetric flask. Add 5 mL of methanol and mix well for NLT 30 min with gentle swirling about every 5 min. Add *Diluent* up to half of the flask volume and sonicate for NLT 5 min to dissolve. Dilute with *Diluent* to volume.
Sample solution: Nominally 1.0 mg/mL of morphine sulfate pentahydrate from the *Sample stock solution* in *Diluent.* Pass through a suitable filter and use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 245 nm. For *Identification A,* use a diode array detector in the range of 200–400 nm.

USP Monographs

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Columns
Guard: Packing L1
Analytical: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 40 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the morphine related compound A and morphine sulfate peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL), calculated on the anhydrous basis
C_U = nominal concentration of morphine sulfate pentahydrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of morphine sulfate pentahydrate, 758.83
M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
pH 7.5 phosphate buffer: 6.8 mg/mL of monobasic potassium phosphate and 1.6 mg/mL of sodium hydroxide. Adjust with phosphoric acid or 2 N sodium hydroxide to a pH of 7.5.
Medium: Prepare as directed in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*, observing the following exceptions. Perform *Acid Stage* testing, using 500 mL of 0.1 N hydrochloric acid for 1 h; and perform *Buffer Stage* testing, using 500 mL of *pH 7.5 phosphate buffer* for NLT 8 h.
Apparatus 1: 100 rpm
Times: 1, 4, 6, and 9 h
Mobile phase: Methanol, glacial acetic acid, and water (280:10:720), containing 0.73 g of sodium 1-heptanesulfonate for each 1.01 L of the solvent mixture
System suitability solution: 0.1 mg/mL each of phenol and USP Morphine Sulfate RS in *Mobile phase*
Standard solution: USP Morphine Sulfate RS in *pH 7.5 phosphate buffer* to obtain a solution with a known concentration corresponding to that of the *Sample solution*
Sample solution: Sample per *Dissolution* ⟨711⟩.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 284 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 25 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for phenol and morphine sulfate are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the phenol and morphine sulfate peaks
Tailing factor: NMT 2.0 for the morphine sulfate peak
Relative standard deviation: NMT 2.0% for the morphine sulfate peak
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	NMT 10
4	25–50
6	50–90
9	NLT 85

The percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ dissolved in 1 h conforms to *Dissolution* ⟨711⟩, *Acceptance Table 3*. The percentages of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ ▲ (ERR 1-Jul-2019)] dissolved at the other times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium
Acid stage: 0.1 N hydrochloric acid (HCl); 500 mL
Buffer stage: pH 7.5 phosphate buffer (dissolve 40.8 g of monobasic potassium phosphate and 9.6 g of sodium hydroxide in 6 L of water; adjust with phosphoric acid or 2 N sodium hydroxide to a pH of 7.5); 500 mL
Apparatus 1: 100 rpm
Times: 1, 4, 6, and 9 h
Solution A: 0.1% phosphoric acid and 0.1% triethylamine in water
Mobile phase: *Solution A* and methanol (93:7)
Standard stock solution: 2.0 mg/mL of USP Morphine Sulfate RS in water
Standard solution: 0.16 mg/mL of USP Morphine Sulfate RS in either the *Acid stage* under *Medium* or in the *Buffer stage* under *Medium*, from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size. Centrifuge the filtrate if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 25°
Flow rate: 1.5 mL/min
Injection volume: 5 µL
Run time: NLT 2 times the retention time of morphine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0

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Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Replace the *Acid stage* under *Medium* immediately after 1 h with the *Buffer stage* under *Medium*.

Calculate the concentration (C_i) of morphine sulfate pentahydrate [$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$] in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of morphine from the *Sample solution* at each time point (i)

r_S = peak response of morphine from the appropriate *Standard solution* at each time point (i)

C_S = concentration of USP Morphine Sulfate RS in the appropriate *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of morphine sulfate pentahydrate [$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$] dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = C_2 \times V \times (1/L) \times 100$$
$$\text{Result}_3 = \{[C_3 \times (V - V_S)] + (C_2 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of morphine sulfate pentahydrate [$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$] in the portion of the sample withdrawn at each time point (i) (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Capsule)

V_S = volume of *Medium* taken (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 10
2	4	10–35
3	6	50–70
4	9	NLT 80

The percentages of the labeled amount of morphine sulfate pentahydrate released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*. Proceed as directed in *Test 1*, except for *Tolerances*.

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (%)
1	NMT 10
4	36–56
6	74–94
9	NLT 85

The percentage of the labeled amount of morphine sulfate pentahydrate [$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$] dissolved in 1 h conforms to *Dissolution* ⟨711⟩, *Acceptance Table 3*. The percentages of the labeled amount of morphine sulfate pentahydrate [$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$▲ (ERR 1-Jul-2019)] dissolved at the other times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Diluent, Solution A, System suitability solution, Chromatographic system, and Sample solution: Proceed as directed in the *Assay*.

Sensitivity solution: 0.5 µg/mL of USP Morphine Sulfate RS in *Diluent*

Standard solution: 0.002 mg/mL of USP Morphine Sulfate RS and 0.005 mg/mL each of USP Morphine Related Compound A RS and USP Morphine Related Compound B RS (pseudomorphine) in *Diluent*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between the morphine related compound A and morphine sulfate peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10 for morphine sulfate, *Sensitivity solution*

Relative standard deviation: NMT 5% for morphine related compound A, morphine sulfate, and morphine related compound B, *Standard solution*

Analysis

Samples: *Diluent*, *Standard solution*, and *Sample solution*

Calculate the percentage of morphine related compound A and morphine related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of morphine related compound A or morphine related compound B from the *Sample solution*

r_S = peak response of USP Morphine Related Compound A RS or USP Morphine Related Compound B RS from the *Standard solution*

C_S = concentration of USP Morphine Related Compound A RS or USP Morphine Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of morphine sulfate pentahydrate in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*

r_T = peak response of morphine sulfate from the *Sample solution*

Acceptance criteria: See *Table 5*. Disregard any peaks below 0.05% and the peaks corresponding to those from the *Diluent*.

USP Monographs

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Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Morphine related compound A[a]	1.4	0.5
Morphine sulfate	1.0	—
Morphine related compound B (anhydrous)[b]	2.3	0.5
Any unspecified impurity	—	0.2
Total impurities	—	1.5

[a] 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol, *N*-oxide.
[b] 2,2′-Bimorphine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one test for *Dissolution* is given, the *Labeling* section states the test for *Dissolution* used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Morphine Related Compound A RS
  7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol, *N*-oxide.
  $C_{17}H_{19}NO_4$ 301.34
  USP Morphine Related Compound B RS
  2,2′-Bimorphine trihydrate.
  $C_{34}H_{36}N_2O_6 \cdot 3H_2O$ 622.72
  USP Morphine Sulfate RS

Morphine Sulfate Injection

DEFINITION

Morphine Sulfate Injection is a sterile solution of Morphine Sulfate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$. Injection intended for intramuscular or intravenous administration may contain sodium chloride as a tonicity-adjusting agent, and suitable antioxidants and antimicrobial agents. Injection intended for intrathecal or epidural use may contain sodium chloride as a tonicity-adjusting agent, but contains no other added substances.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS** ⟨191⟩**—GENERAL,** *Sulfate*: It meets the requirements of test *A*.

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 0.73 g of sodium 1-heptanesulfonate in 720 mL of water, and add 280 mL of methanol and 10 mL of glacial acetic acid.
System suitability solution: 0.24 mg/mL of USP Morphine Sulfate RS and 0.15 mg/mL of phenol in *Mobile phase*
Standard solution: 0.24 mg/mL of USP Morphine Sulfate RS (on the anhydrous basis) in *Mobile phase*. [NOTE—Prepare a fresh solution daily.]
Sample solution: Nominally 0.24 mg/mL of morphine sulfate from the Injection in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 284 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for phenol and morphine are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between phenol and morphine sulfate, *System suitability solution*
Tailing factor: NMT 2.0 for the morphine sulfate peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of morphine sulfate pentahydrate, 758.83
M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 2.5–6.5
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 17.0 USP Endotoxin Units/mg of morphine sulfate. If labeled for intrathecal use, it contains NMT 14.29 USP Endotoxin Units/mg of morphine sulfate.
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements under small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light. Preserve Injection labeled "Preservative-free" in single-dose containers.
- **LABELING:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. Label it also to state that the Injection is not to be used if its color is darker than pale yellow, if it is discolored in any other way, or if it contains a precipitate. Injection containing no antioxidant or antimicrobial agents prominently bears on its label the words "Preservative-free" and includes in its labeling its routes of administration and the statement that it is not to be heat-sterilized. Injection containing antioxidant or antimicrobial agents includes in its labeling its routes of administration and the statement that it is not for intrathecal or epidural use.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Morphine Sulfate RS

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Morphine Sulfate Compounded Injection

DEFINITION

Morphine Sulfate Compounded Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$.

Prepare Morphine Sulfate Compounded Injection, 10 or 50 mg/mL, as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

10-mg/mL Morphine Sulfate Compounded Injection

Morphine sulfate pentahydrate[a]	1 g
Sodium Chloride	760 mg
Sterile Water for Injection, a sufficient quantity to make	100 mL

[a] Morphine Sulfate, *USP*, is morphine sulfate pentahydrate; therefore no additional calculation is needed to account for the waters of hydration.

50-mg/mL Morphine Sulfate Compounded Injection

Morphine sulfate pentahydrate[a]	5 g
Sodium Chloride	450 mg
Sterile Water for Injection, a sufficient quantity to make	100 mL

[a] Morphine Sulfate, *USP*, is morphine sulfate pentahydrate; therefore no additional calculation is needed to account for the waters of hydration.

Dissolve the *Morphine sulfate pentahydrate* and *Sodium Chloride* in *Sterile Water for Injection* in a suitable calibrated container. Add sufficient *Sterile Water for Injection* to bring to final volume and mix well. Pass through a sterile filter of 0.22-µm pore size into sterile container(s).

ASSAY

• **PROCEDURE**

Solution A: Dissolve 5.44 g of dibasic potassium phosphate in 800 mL of water and add 200 mL of methanol.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Methanol (%)	Solution A (%)
0.0	0	100
3.0	45	55
8.0	45	55
13.0	45	55
13.1	0	100
20.0	0	100

Standard solution: 1 mg/mL of morphine sulfate pentahydrate prepared from USP Morphine Sulfate RS in water

Sample solution: Transfer 0.4 mL of the Injection into a 100-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for morphine sulfate is about 10.5 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of morphine sulfate pentahydrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 2.5–6.5

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 14.29 USP Endotoxin Units/mg of morphine sulfate

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** It meets the requirements.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in sterile syringes for single-use in one patient only, protected from light. Store at controlled room temperature.

Change to read:

• **BEYOND-USE DATE:** ▲In the absence of passing a sterility and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 90 days after the date on which it was compounded when stored at controlled room temperature▲ (CN 1-May-2020)

• **LABELING:** Label it to indicate that it is for use in a single patient only. Label it to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Morphine Sulfate RS

Morphine Sulfate Compounded Suppositories

DEFINITION

Morphine Sulfate Compounded Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$.

Prepare Morphine Sulfate Compounded Suppositories in Fatty Acid Base or Polyethylene Glycol Base as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Morphine Sulfate	50 mg
Silica Gel	25 mg

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Fatty Acid Base or Polyethylene Glycol Base, a sufficient quantity to make	1 suppository

Calibrate the actual molds with the *Base* that is used for preparing the Suppositories, and adjust the formula accordingly. Thoroughly mix the *Morphine Sulfate* and *Silica Gel* to obtain a uniform powder. Heat the *Base* slowly and evenly until melted. Slowly add the powder to the melted *Base* with stirring. Mix thoroughly, and pour into molds. Cool, trim, and wrap.

ASSAY

• **SUPPOSITORIES IN FATTY ACID BASE**

Mobile phase: Dissolve 5.5 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol and 10 mL of glacial acetic acid. Filter and degas.

System suitability solution: 0.24 mg/mL of USP Morphine Sulfate RS and 0.15 mg/mL of phenol in *Mobile phase*

Standard solution: 0.5 mg/mL of USP Morphine Sulfate RS in *Mobile phase*. Prepare a fresh solution daily.

Sample solution: Transfer 1 Suppository to a 60-mL separator containing 20 mL of chloroform and 20 mL of 0.01 N hydrochloric acid, and shake to dissolve the Suppository. Transfer the chloroform layer to a 250-mL separator. Extract the aqueous layer with a second 20-mL portion of chloroform, and combine the chloroform extracts in the 250-mL separator. Wash the chloroform extracts with two additional 20-mL portions of 0.01 N hydrochloric acid, combine the aqueous layers in a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass through a filter of 0.45-µm or finer pore size, discarding the first 4 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 284 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phenol and morphine sulfate are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between phenol and morphine sulfate, *System suitability solution*

Tailing factor: NMT 2.0 for the morphine sulfate peak

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the Suppository:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL) (corrected for moisture content by titrimetric determination)

C_U = nominal concentration of morphine sulfate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of morphine sulfate pentahydrate, 758.83

M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77

Acceptance criteria: 90.0%–110.0%

• **SUPPOSITORIES IN POLYETHYLENE GLYCOL BASE**

Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* for *Suppositories in Fatty Acid Base*.

Sample solution: Transfer 1 Suppository to a 100-mL volumetric flask, and add 70 mL of *Mobile phase*. Sonicate for 15 min to dissolve the Suppository, cool, dilute with *Mobile phase* to volume, and mix. Pass a 10-mL portion of the solution through a filter of 0.45-µm or finer pore size, discarding the first 4 mL of filtrate.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the Suppository:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL) (corrected for moisture content by titrimetric determination)

C_U = nominal concentration of morphine sulfate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of morphine sulfate pentahydrate, 758.83

M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Weight Variation*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight containers, and store in a refrigerator. Do not dispense or store Polyethylene Glycol Base Suppositories in polystyrene containers.

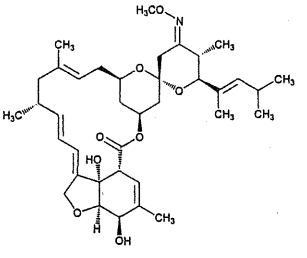
• **BEYOND-USE DATE:** NMT 90 days after the date on which they were compounded when stored in a refrigerator

• **LABELING:** Label Morphine Sulfate Compounded Suppositories to state whether they are in a Fatty Acid Base or in a Polyethylene Glycol Base. Label to indicate that they are for rectal use only. Label to state that they are to be stored in a refrigerator. Label it to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Morphine Sulfate RS

Moxidectin



$C_{37}H_{53}NO_8$ 639.82

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(6*R*,25*S*)-5-*O*-Demethyl-28-deoxy-25-[(*E*)-1,3-dimethyl-1-butenyl]-6,28-epoxy-23-oxomilbemycin B 23-(*E*)-(*O*-methyloxime);
(2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6′-[(*E*)-1,3-Dimethyl-1-butenyl]-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime) [113507-06-5].

DEFINITION

Moxidectin contains NLT 92.0% and NMT 102.0% of moxidectin ($C_{37}H_{53}NO_8$), calculated on the anhydrous basis. It may contain a suitable antioxidant.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 7.7 g of ammonium acetate in 400 mL of water, and adjust with glacial acetic acid to a pH of 4.8.
Mobile phase: Acetonitrile and *Buffer* (60:40)
Standard solution: 1.0 mg/mL of USP Moxidectin RS in acetonitrile. Sonicate if necessary to facilitate dissolution.
Sample solution: 1.0 mg/mL of Moxidectin in acetonitrile. Sonicate if necessary to facilitate dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Column temperature: 50°
Flow rate: 2.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1%, for 4 replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of moxidectin ($C_{37}H_{53}NO_8$) in the portion of Moxidectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Moxidectin RS in the *Standard solution* (mg/mL)
C_U = concentration of Moxidectin in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Delete the following:

- **▲HEAVY METALS,** *Method II* ⟨231⟩: NMT 20 ppm▲ (Official 1-Jan-2018)

- **ORGANIC IMPURITIES: EARLY-ELUTING IMPURITIES**

Buffer, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1.0 mg/mL of USP Moxidectin System Suitability Mixture RS in acetonitrile. Sonicate if necessary to facilitate dissolution.
Standard solution: 0.01 mg/mL of Moxidectin in acetonitrile from the *Sample solution*
System suitability
Sample: *System suitability solution*
Suitability requirements
Peak-to-valley ratio: NLT 3.0 between moxidectin 17a-epimer and moxidectin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each early-eluting impurity in the portion of Moxidectin taken:

$$\text{Result} = (r_U/r_S) \times F \times D \times 100$$

r_U = peak response of each early-eluting impurity from the *Sample solution*
r_S = peak response of moxidectin from the *Standard solution*
F = *Assay* value expressed as a decimal
D = dilution factor used to prepare the *Standard solution*, 0.01

Acceptance criteria: See *Table 1*. The reporting level for impurities is 0.1%. Disregard the peak due to the stabilizer (identify this peak, where applicable, by injecting a suitable reference solution).

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moxidectin butenyl analog[a]	0.5	1.5
5′-Demethyl moxidectin[b]	0.7	0.5
Moxidectin pentenyl analog[c]	0.75	1.5
Moxidectin 17a-epimer[d]	0.9	2.5
Moxidectin	1.0	—
Sum of moxidectin 19-*S*-17a-ene[e] and moxidectin ethyl isomers[f]	1.3–1.5	1.7[g]
Milbemycin B analog (moxidectin open ring)[h]	1.6	1.5

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Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other individual impurity eluting before milbemycin B analog (moxidectin open ring)	—	0.5

[a] (2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6′-[(*E*)-But-2-en-2-yl]-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime).

[b] (2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6′-[(*E*)-4-methylpent-2-en-2-yl]-6,8,19-trimethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime).

[c] (2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-5′,6,8,19-tetramethyl-6′-[(*E*)-pent-2-en-2-yl]spiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime).

[d] (2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,17a*S*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6′-[(*E*)-4-methylpent-2-en-2-yl]-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime).

[e] (2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,19*S*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,19,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6′-[(*E*)-4-methylpent-2-en-2-yl]-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime).

[f] Mixture of five possible isomers, where one methyl group in the analyte is replaced with an ethyl group.

[g] If present, moxidectin 19-*S*-17a-ene and the moxidectin ethyl isomers may not be completely resolved by the method. These peaks are integrated together to determine conformance.

[h] (2′*R*,3*S*,5′*S*,6′*S*,7*R*,9*E*,12*R*,13*E*,15*E*,16a*S*,18*S*,20a*R*)-16a,18-Dihydroxy-5′,10,12,16,19-pentamethyl-6′-[(*E*)-4-methylpent-2-en-2-yl]-3,4,5′,6′,7,8,11,12,16a,17,18,20a-dodecahydro-1*H*-spiro[3,7-methanobenzo[*g*][1,5]dioxacyclooctadecin-5,2′-[2*H*]pyran]-14′-dione (*E*)-(*O*-methyloxime).

• **Organic Impurities: Late-Eluting Impurities**

Buffer: Dissolve 3.8 g of ammonium acetate in 250 mL of water, and adjust with glacial acetic acid to a pH of 4.2.

Mobile phase: Acetonitrile and *Buffer* (75:25)

System suitability solution: 3.0 mg/mL of USP Moxidectin System Suitability Mixture RS in acetonitrile. Sonicate if necessary to facilitate dissolution.

Sample solution: 3.0 mg/mL of Moxidectin in acetonitrile. Sonicate if necessary to facilitate dissolution.

Standard solution: 0.03 mg/mL of Moxidectin in acetonitrile from the *Sample solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Column temperature: 35°

Flow rate: 2 mL/min

Injection volume: 10 µL

Run time: NLT 10 times the retention time of moxidectin

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between moxidectin deoxydiene/methylthiomethoxymoxidectin and 20b-methylthiomoxidectin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each late-eluting impurity in the portion of Moxidectin taken:

$$\text{Result} = (r_U/r_S) \times F \times D \times 100$$

r_U = peak response of each late-eluting impurity from the *Sample solution*

r_S = peak response of moxidectin from the *Standard solution*

F = *Assay* value expressed as a decimal

D = dilution factor used to prepare the *Standard solution*, 0.01

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.1%. Disregard the peak due to the stabilizer (identify this peak, where applicable, by injecting a suitable reference solution).

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moxidectin	1.0	—
Moxidectin deoxydiene[a] and 4′-methylthiomethoxymoxidectin[b]	2.0	1.0[c]
20b-Methylthiomoxidectin[d]	2.2	0.5
20-Nitrobenzoylmoxidectin[e]	3.4	0.5
Any other individual impurity eluting after the milbemycin B analog (moxidectin open ring) (≅1.4 RRT)	—	0.5

[a] (2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,20a,20b-Decahydro-20b-hydroxy-6′-[(*E*)-4-methylpent-2-en-2-yl]-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime).

[b] (2a*E*,4*E*,4′*S*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)- 3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-Tetradecahydro-20,20b-dihydroxy-6′-[(*E*)-4-methylpent-2-en-2-yl]-4′-methylthiomethoxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17-one.

[c] If present, impurities moxidectin deoxydiene and 4′-methylthiomethoxymoxidectin may not be completely resolved by the method. These peaks are integrated together to determine conformance.

[d] (2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20-hydroxy-6′-[(*E*)-4-methylpent-2-en-2-yl]-20b-methylthiomethoxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime).

[e] (2a*E*,4*E*,5′*R*,6*R*,6′*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20b-hydroxy-6′-[(*E*)-4-methylpent-2-en-2-yl]-20-(4-nitrobenzoyloxy)-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-4′,17(3′*H*)-dione 4′-(*E*)-(*O*-methyloxime).

• **Total Organic Impurities**

Analysis: Calculate the sum of all impurities found in the tests for *Organic Impurities: Early-Eluting Impurities* and *Organic Impurities: Late-Eluting Impurities* in the portion of Moxidectin taken.

Acceptance criteria: NMT 7.0%

SPECIFIC TESTS

• **Water Determination** ⟨921⟩, *Method I*: NMT 1.3%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers, and store in a refrigerator.

Change to read:

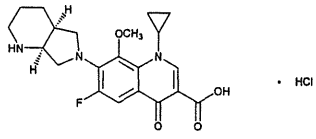
• **Labeling:** ▲If it is intended for use in animals, it is so labeled.▲ (RB 27-Jun-2018) Label it to state the name(s) and amount(s) of any added substance(s).

USP Monographs

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Moxidectin RS
USP Moxidectin System Suitability Mixture RS

Moxifloxacin Hydrochloride



$C_{21}H_{24}FN_3O_4 \cdot HCl$ 437.89

(4a*S*-*cis*)-1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)-4-oxo-3-quinolinecarboxylic acid, monohydrochloride;
1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid, monohydrochloride [186826-86-8].

DEFINITION
Moxifloxacin Hydrochloride contains NLT 98.0% and NMT 102.0% of moxifloxacin hydrochloride ($C_{21}H_{24}FN_3O_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
Sample solution: To a solution (1 in 160) add diluted nitric acid, and filter.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Buffer: Dissolve 0.5 g of tetrabutylammonium hydrogen sulfate and 1.0 g of monobasic potassium phosphate in water, add 2 mL of phosphoric acid, dilute with water to 1000 mL, mix, and pass through a filter of 0.45-µm pore size.
Mobile phase: Methanol and *Buffer* (28:72)
Diluent: Add 20 mg of anhydrous sodium sulfite to 1000 mL of *Buffer*, mix gently, and pass through a filter of 0.45-µm pore size.
System suitability solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS and 1 µg/mL of USP Moxifloxacin Related Compound A RS, in *Diluent*
Standard solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS in *Diluent*
Sample solution: 0.1 mg/mL of Moxifloxacin Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 293 nm
Column: 4.0-mm × 25-cm; 5-µm packing L11
Column temperature: 45°
Flow rate: 0.9 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for moxifloxacin and moxifloxacin related compound A are about 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.5 between moxifloxacin and moxifloxacin related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of moxifloxacin hydrochloride ($C_{21}H_{24}FN_3O_4 \cdot HCl$) in the portion of Moxifloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Moxifloxacin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*
Sample: 0.6 g
Acceptance criteria: 0.04%; the *Sample* shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid.
• **ORGANIC IMPURITIES**
Protect all solutions containing moxifloxacin from light.
Mobile phase, Diluent, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Standard solution: 2 µg/mL of USP Moxifloxacin Hydrochloride RS in *Diluent*
Sensitivity solution: 0.05 µg/mL of USP Moxifloxacin Hydrochloride RS from the *Standard solution* in *Diluent*. Store the *Sensitivity solution* under refrigeration and protected from light.
Chromatographic system: Proceed as directed in the *Assay* with a run time of two times the retention time of moxifloxacin.
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between moxifloxacin and moxifloxacin related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Moxifloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

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r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of moxifloxacin from the *Standard solution*
C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Moxifloxacin Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Moxifloxacin	1.0	—	—
Moxifloxacin related compound A[a]	1.15	1.0	0.1
Moxifloxacin related compound B[b]	1.32	0.71	0.1
Moxifloxacin related compound C[c]	1.48	1.0	0.1
Moxifloxacin related compound D[d]	1.71	1.0	0.1
Moxifloxacin related compound E[e]	1.83	0.29	0.1
Other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] 1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[b] 1-Cyclopropyl-6,8-dimethoxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[c] 1-Cyclopropyl-8-ethoxy-6-fluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[d] 1-Cyclopropyl-8-fluoro-6-methoxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[e] 1-Cyclopropyl-6-fluoro-8-hydroxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.

• **ENANTIOMERIC PURITY**
Protect all solutions containing moxifloxacin from light.
Buffer: 0.47 g/L of anhydrous cupric sulfate and 1.31 g/L of L-isoleucine in water. Adjust with 0.1 N sodium hydroxide to a pH of 4.50.
Solution A: Methanol and *Buffer* (500:1500)
Solution B: Methanol and *Buffer* (225:450)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
50	100	0
51	0	100
61	0	100
62	100	0
85	100	0

System suitability solution: 8 mg/mL of USP Moxifloxacin Hydrochloride RS and 8 µg/mL of USP Moxifloxacin Related Compound G RS in *Solution A*
Sensitivity solution: 0.8 µg/mL of USP Moxifloxacin Related Compound G RS in *Solution A*
Sample solution: 8 mg/mL of Moxifloxacin Hydrochloride in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 3.0-mm × 15-cm; 3-µm packing L1
Flow rate: 0.42 mL/min
Injection volume: 1.5 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for moxifloxacin related compound G and moxifloxacin are 0.78 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between moxifloxacin related compound G and moxifloxacin, *System suitability solution*
Signal-to-noise ratio: NLT 5, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of moxifloxacin related compound G in the portion of Moxifloxacin Hydrochloride taken:

$$\text{Result} = [r_U/(r_S + r_U)] \times 100$$

r_U = peak response of moxifloxacin related compound G from the *Sample solution*
r_S = peak response of moxifloxacin from the *Sample solution*

Acceptance criteria: NMT 0.15%

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩
Total aerobic microbial count: NMT 10^3 cfu/g
Total combined molds and yeasts count: NMT 10^2 cfu/g
• **pH** ⟨791⟩
Sample solution: 2 mg/mL
Acceptance criteria: 3.9–4.6
• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: NMT 4.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Moxifloxacin Hydrochloride RS
USP Moxifloxacin Related Compound A RS
1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
$C_{20}H_{21}F_2N_3O_3$ 389.40
USP Moxifloxacin Related Compound G RS
1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4a*R*,7a*R*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid, monohydrochloride.
$C_{21}H_{24}FN_3O_4 \cdot HCl$ 437.89

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Moxifloxacin Ophthalmic Solution

DEFINITION

Moxifloxacin Ophthalmic Solution is a sterile, self-preserved aqueous solution of Moxifloxacin Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of moxifloxacin ($C_{21}H_{24}FN_3O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 0.5 g of tetrabutylammonium hydrogen sulfate and 1.0 g of monobasic potassium phosphate in 1000 mL of water. Add 2 mL of phosphoric acid, filter, and degas.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Flow Rate (mL/min)	Solution A (%)	Solution B (%)
0	0.5	69	31
30	0.5	69	31
31	0.9	60	40
36	0.9	60	40
37	0.5	69	31
42	0.5	69	31

System suitability solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS and 1 µg/mL of USP Moxifloxacin Related Compound A RS in *Solution A*

Standard stock solution: 6 mg/mL of USP Moxifloxacin Hydrochloride RS in water

Standard solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS in *Solution A* from the *Standard stock solution*

Sample solution: Nominally 0.1 mg/mL of moxifloxacin from Ophthalmic Solution in *Solution A*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 293 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.0-mm × 25-cm; 5-µm packing L11

Column temperature: 45°

Flow rate: See *Table 1*.

Injection volume: 25 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the moxifloxacin and moxifloxacin related compound A peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of moxifloxacin ($C_{21}H_{24}FN_3O_4$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of moxifloxacin from the *Sample solution*

r_S = peak response of moxifloxacin from the *Standard solution*

C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moxifloxacin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of moxifloxacin, 401.43

M_{r2} = molecular weight of moxifloxacin hydrochloride, 437.89

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES: EARLY-ELUTING RELATED COMPOUNDS (RELATIVE RETENTION LESS THAN 1.8)**

Protect the *System suitability solution*, *Sample solution*, and *Standard solution* from light. Analyze the *Sample solution* immediately after preparation.

Solution A, Solution B, Mobile phase, System suitability solution, Standard stock solution, and **Sample solution:** Prepare as directed in the *Assay*.

Blank solution: Use *Solution A*.

Standard solution: 2 µg/mL of USP Moxifloxacin Hydrochloride RS in *Solution A* from the *Standard stock solution*

Sensitivity solution: 0.05 µg/mL of USP Moxifloxacin Hydrochloride RS from the *Standard solution* in *Solution A*. Store the *Sensitivity solution* under refrigeration and protected from light.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 293 nm

Column: 4.0-mm × 25-cm; 5-µm packing L11

Column temperature: 45°

Flow rate: See *Table 1*.

Injection volume: 25 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between the moxifloxacin and moxifloxacin related compound A peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Blank solution*, *Standard solution*, and *Sample solution*

[NOTE—Determine the relative retention values (r) for components listed in *Table 2*, using the time measured at the first baseline deflection of the *Standard solution* chromatogram as the void volume (t_M).]

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

USP Monographs

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r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of moxifloxacin from the *Standard solution*
C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of moxifloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of moxifloxacin, 401.43
M_{r2} = molecular weight of moxifloxacin hydrochloride, 437.89
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention (*r*)	Relative Response Factor	Acceptance Criteria, NMT (%)
Specified unknown impurity #1	0.3	1.0	0.2
Decarboxy moxifloxacin[a]	0.4	0.16	0.3
Specified unknown impurity #2	0.9	1.0	0.3
Moxifloxacin	1.0	—	—
Moxifloxacin related compound A[b]	1.1	—	—
8-Hydroxy[c, d]	1.8	—	—
Any other individual impurity	—	1.0	0.1
Any specified and identified impurity	—	1.0	1.0

[a] 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-pyrrolo[3,4-*b*]pyridin-6-yl]-1*H*-quinolin-4-one.
[b] Disregard this peak because this is a process impurity controlled for the drug substance.
[c] 1-Cyclopropyl-6-fluoro-8-hydroxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[d] Disregard this peak because it is quantitated using *Organic Impurities: Late-Eluting Related Compounds (Relative Retention Equal to More Than 1.8)*.

• **ORGANIC IMPURITIES: LATE-ELUTING RELATED COMPOUNDS (RELATIVE RETENTION EQUAL TO MORE THAN 1.8)**

Protect the *Standard solution* and *Sample solution* from light. Analyze the *Sample solution* immediately after preparation.

Solution A, Solution B, and **Standard stock solution:** Prepare as directed in the *Assay*.

Mobile phase: *Solution A* and *Solution B* (60:40)

Blank solution: Use *Solution A*.

Standard solution: 2 µg/mL of USP Moxifloxacin Hydrochloride RS in *Solution A* from the *Standard stock solution*

Sensitivity solution: 0.05 µg/mL of USP Moxifloxacin Hydrochloride RS from the *Standard solution* in *Solution A*. Store the *Sensitivity solution* under refrigeration and protected from light.

Sample solution: Nominally 0.1 mg/mL of moxifloxacin from Ophthalmic Solution in *Solution A*. The 8-hydroxy compound is unstable in dilute aqueous solutions.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 293 nm
Column: 4.0-mm × 25-cm; 5-µm packing L11
Column temperature: 45°
Flow rate: 0.9 mL/min
Injection volume: 25 µL
Run time: NLT 5 times the retention time of moxifloxacin peak

System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Blank solution*, *Standard solution*, and *Sample solution*
[NOTE—Determine the relative retention values (*r*) for components listed in *Table 3*, using the time measured at the first baseline deflection of the *Standard solution* chromatogram as the void volume (t_M).]
Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of moxifloxacin from the *Standard solution*
C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of moxifloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of moxifloxacin, 401.43
M_{r2} = molecular weight of moxifloxacin hydrochloride, 437.89
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention (*r*)	Relative Response Factor	Acceptance Criteria, NMT (%)
Moxifloxacin	1.0	—	—
8-Hydroxy	1.8	0.29	0.2
Specified unknown impurity #3	3.4	1.0	0.2
Floxacin amine[a]	3.9	0.42	0.2
Any other individual impurity	—	1.0	0.1

[a] 7-Amino-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.

Total impurities: NMT 1.5%, sum from both *Organic Impurities* tests

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
• **pH** ⟨791⟩: 6.3–7.9
• **OSMOLALITY AND OSMOLARITY** ⟨785⟩: 260–370 mOsmol/kg

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 2° and 25°.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Moxifloxacin Hydrochloride RS
USP Moxifloxacin Related Compound A RS
1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
$C_{20}H_{21}F_2N_3O_3$ 389.40

Moxifloxacin Tablets

DEFINITION
Moxifloxacin Tablets contain an amount of Moxifloxacin Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of moxifloxacin ($C_{21}H_{24}FN_3O_4$).

IDENTIFICATION
• **A.** The UV absorption spectrum of the *Sample solution* exhibits maxima at the same wavelength as that of the *Standard solution*, as obtained in the *Dissolution* test.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 1.36 g/L of monobasic potassium phosphate in water. Add 2 mL of triethylamine for each liter of the solution and adjust with phosphoric acid to a pH of 1.9.
Solution A: Methanol, *n*-propyl alcohol, and *Buffer* (300:34:666)
Mobile phase: Acetonitrile and *Solution A* (50:1000)
Diluent: Methanol and *Buffer* (20:80)
Standard solution: 0.09 mg/mL of USP Moxifloxacin Hydrochloride RS in *Diluent*. Sonicate, if necessary, to dissolve.
Sample stock solution: Nominally 4 mg/mL of moxifloxacin prepared as follows. Transfer NLT 5 Tablets to an appropriate volumetric flask. Add *Diluent* to about 40% of the final volume of the flask and sonicate for about 30 min with intermittent shaking. Dilute with *Diluent* to volume.
Sample solution: 0.08 mg/mL of moxifloxacin prepared as follows. Centrifuge a portion of the *Sample stock solution* and pipette 5 mL of the supernatant into a 250-mL volumetric flask. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 293 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 10 min
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of moxifloxacin ($C_{21}H_{24}FN_3O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of moxifloxacin from the *Sample solution*
r_S = peak response of moxifloxacin from the *Standard solution*
C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of moxifloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of moxifloxacin, 401.43
M_{r2} = molecular weight of moxifloxacin hydrochloride, 437.89

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL, degassed
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: 9 µg/mL of USP Moxifloxacin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Blank: *Medium*
Instrumental conditions
Mode: UV
Analytical wavelength: 296 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of moxifloxacin ($C_{21}H_{24}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of moxifloxacin, 401.43
M_{r2} = molecular weight of moxifloxacin hydrochloride, 437.89
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, if applicable
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of moxifloxacin ($C_{21}H_{24}FN_3O_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Buffer: 1.5 g/L of tetrabutyl ammonium hydrogen sulfate and 1 g/L of potassium dihydrogen phosphate in water. Add 2 mL of phosphoric acid for each liter of the solution and adjust with triethylamine to a pH of 2.5. Pass through a suitable filter of 0.45-µm pore size.
Solution A: Methanol and *Buffer* (20:80)
Solution B: Methanol and *Buffer* (80:20)
Solution C: 0.5 g/L of tetrabutyl ammonium hydrogen sulfate and 1 g/L of monobasic potassium phosphate in water. Add 2 mL of phosphoric acid for each liter of the solution. Pass through a suitable filter of 0.45-µm pore size.
Mobile phase: See *Table 1*.

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
22	95	5
39	30	70
52	30	70
53	0	100
58	0	100
58.1	95	5
70	95	5

Diluent: *Solution C* and 0.1 N sodium hydroxide (1000:10)
Impurity stock solution: 0.02 mg/mL each of USP Moxifloxacin Related Compound F RS and USP Moxifloxacin Related Compound A RS in acetonitrile
System suitability solution: 0.3 mg/mL of USP Moxifloxacin Hydrochloride RS and 0.3 µg/mL each of USP Moxifloxacin Related Compound F RS and USP Moxifloxacin Related Compound A RS in *Diluent* prepared as follows. Transfer 82 mg of USP Moxifloxacin Hydrochloride RS to a 250-mL volumetric flask and add 4.0 mL of *Impurity stock solution*. Dilute with *Diluent* to volume.
Standard solution: 3.3 µg/mL of USP Moxifloxacin Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.33 mg/mL of moxifloxacin hydrochloride in *Diluent* prepared as follows. Transfer an amount equivalent to 82 mg of moxifloxacin hydrochloride from finely powdered Tablets (NLT 10) to a 250-mL volumetric flask. Add about 150 mL of *Diluent* and sonicate for 30 min with intermittent shaking. Dilute with *Diluent* to volume. Centrifuge a portion of the solution and pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 293 and 317 nm
Column: 4.0-mm × 25-cm; 5-µm packing L11
Column temperature: 50°
Flow rate: 1.1 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between moxifloxacin related compound F and moxifloxacin, *System suitability solution* at 293 nm
Peak-to-valley ratio: NLT 1.5 between moxifloxacin and moxifloxacin related compound A, *System suitability solution* at 293 nm
Tailing factor: NMT 2.0, *Standard solution* at 293 nm
Relative standard deviation: NMT 10.0%, *Standard solution* at 293 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of moxifloxacin from the *Standard solution*
C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of moxifloxacin hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard the peaks eluting before 3 min, after the 8-methoxy quinolonic ethyl ester peak, and any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Wavelength (nm)	Acceptance Criteria, NMT (%)
Moxifloxacin related compound F	0.82	1.0	293	—[a]
Moxifloxacin	1.0	—	293/317	—
Moxifloxacin related compound A	1.1	0.53	293	—[a]
Moxifloxacin related compound B[b]	1.26	0.77	317	—[a]
Moxifloxacin related compound C[c]	1.33	1.0	293	—[a]
Moxifloxacin related compound D[d]	1.38	0.76	293	—[a]
Moxifloxacin related compound E[e]	1.49	0.26	293	—[a]
8-Hydroxy quinolonic acid derivative[f]	1.72	1.3	293	—[a]
8-Methoxy quinolonic acid derivative[g]	1.89	1.9	317	—[a]
8-Methoxy quinolonic ethyl ester[h]	1.93	1.6	317	—[a]
Any other individual impurity	—	1.0	293	0.2
Total impurities	—	—	293/317	0.75

[a] For identification only. These are process related impurities monitored in the drug substance and not included in the total impurities calculation.
[b] 1-Cyclopropyl-6,8-dimethoxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[c] 1-Cyclopropyl-8-ethoxy-6-fluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[d] 1-Cyclopropyl-8-fluoro-6-methoxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[e] 1-Cyclopropyl-6-fluoro-8-hydroxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
[f] 1-Cyclopropyl-6,7-difluoro-8-hydroxy-4-oxo-3-quinolinecarboxylic acid.
[g] 1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-3-quinolinecarboxylic acid.
[h] Ethyl 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-3-quinolinecarboxylate.

ADDITIONAL REQUIREMENTS

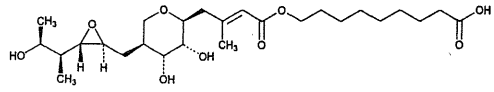
- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature. Avoid high humidity.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Moxifloxacin Hydrochloride RS

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USP Moxifloxacin Related Compound A RS
1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
$C_{20}H_{21}F_2N_3O_3$ 389.40

USP Moxifloxacin Related Compound F RS
1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-1-methylhexahydro-1*H*-pyrrolo[3,4-*b*]pyridin-6(2*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
$C_{22}H_{26}FN_3O_4$ 415.46

Mupirocin



$C_{26}H_{44}O_9$ 500.62

Nonanoic acid, 9-[[3-methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiran yl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy]-,[2*S*-2α(*E*),3β,4β,5α[2*R**, 3*R**(1*R**,[2*R**)]]]-.
(*E*)-(2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-Epoxy-5-hydroxy-4-methylhexyl]tetrahydro-3,4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid [12650-69-0].

» Mupirocin contains not less than 920 µg and not more than 1020 µg of mupirocin ($C_{26}H_{44}O_9$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Mupirocin RS
USP Mupirocin Lithium RS
Identification—The IR absorption spectrum of a mineral oil dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mupirocin RS.
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 3.5 and 4.5, in a saturated aqueous solution.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%.

Assay—
pH 6.3 phosphate buffer—Prepare 0.05 M monobasic sodium phosphate, and adjust with 10 N sodium hydroxide to a pH of 6.3 ± 0.2.
Mobile phase—Prepare a suitable mixture of *pH 6.3 phosphate buffer* and acetonitrile (750:250), pass through a suitable filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 11 mg of USP Mupirocin Lithium RS, accurately weighed, to a 100-mL volumetric flask, add 25 mL of acetonitrile, and swirl to dissolve. Dilute with *pH 6.3 phosphate buffer* to volume, and mix.
Resolution solution—Adjust 10 mL of *Standard preparation* with 6 N hydrochloric acid to a pH of 2.0, allow to stand for 2 hours, and adjust with 5 N sodium hydroxide to a pH of 6.3 ± 0.2.
Assay preparation—Transfer about 11 mg of Mupirocin, accurately weighed, to a 100-mL volumetric flask, add 25 mL of acetonitrile, and swirl to dissolve. Dilute with *pH 6.3 phosphate buffer* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm × 25-cm column that contains packing L1 based on spherical silica particles. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.9 for the mupirocin acid hydrolysis product and 1.0 for mupirocin, and the resolution, *R*, between the mupirocin acid hydrolysis product and mupirocin is not less than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2, the column efficiency is not less than 1500 theoretical plates when calculated by the formula:

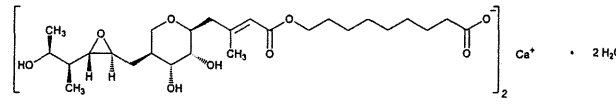
$$5.545(t_r/W_{h/2})^2$$

in which the terms are as defined therein. The relative standard deviation for replicate injections is not more than 2.0%.
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of mupirocin ($C_{26}H_{44}O_9$) in each mg of Mupirocin taken by the formula:

$$(M_S E/M_U)(r_U/r_S)$$

in which M_S is the weight, in mg, of USP Mupirocin Lithium RS taken to prepare the *Standard preparation; E* is the mupirocin equivalent, in µg per mg, of USP Mupirocin Lithium RS; M_U is the weight, in mg, of mupirocin taken to prepare the *Assay preparation;* and r_U and r_S are the mupirocin peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Mupirocin Calcium



$C_{52}H_{86}CaO_{18} \cdot 2H_2O$ 1075.34

Nonanoic acid, 9-[[3-methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy-, calcium salt (2:1), dihydrate, [2*S*-[2α(*E*),3β,4β,5α[2*R**,3*R**(1*R**,2*R**)]]]-;
(α*E*,2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-Epoxy-5-hydroxy-4-methylhexyl]tetrahydro-3,4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid, calcium salt (2:1), dihydrate [115074-43-6].

DEFINITION
Mupirocin Calcium contains the equivalent of NLT 865 µg/mg and NMT 936 µg/mg of mupirocin ($C_{26}H_{44}O_9$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: Do not grind extensively.
Acceptance criteria: Meets the requirements

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• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Calcium*: Meets the requirements

ASSAY

• **PROCEDURE**

Solution A: 7.7 g/L of ammonium acetate in water, adjusted with glacial acetic acid to a pH of 5.7 before diluting to the final volume
Mobile phase: Tetrahydrofuran and *Solution A* (32:68)
Standard solution: 125 µg/mL of USP Mupirocin Lithium RS prepared as follows. Transfer a suitable amount of USP Mupirocin Lithium RS to a suitable volumetric flask, dissolve in methanol, using 2.5% of the final volume, and dilute with *Solution A* to volume.
System suitability solution: Adjust 10 mL of the *Standard solution* with 6 N hydrochloric acid to a pH of 2.0, and allow to stand for 20 h.
Sample solution: Transfer 25 mg of Mupirocin Calcium to a 200-mL volumetric flask, dissolve in 5 mL of methanol, and dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 7.0 between the second of the two peaks corresponding to mupirocin rearrangement products and the peak corresponding to mupirocin, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of mupirocin ($C_{26}H_{44}O_9$) in the portion of Mupirocin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of mupirocin from the *Sample solution*
r_S = peak area of mupirocin from the *Standard solution*
C_S = concentration of USP Mupirocin Lithium RS in the *Standard solution* (mg/mL)
C_U = concentration of Mupirocin Calcium in the *Sample solution* (mg/mL)
P = potency of mupirocin in USP Mupirocin Lithium RS (µg/mg)

Acceptance criteria: 865–936 µg/mg

IMPURITIES

• **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*

Analysis: Dissolve 50 mg in a mixture of 1 mL of 2 N nitric acid and 15 mL of methanol. Add 1 mL of silver nitrate TS.
Acceptance criteria: The turbidity does not exceed that produced by 0.70 mL of 0.020 N hydrochloric acid (0.5%).

• **ORGANIC IMPURITIES**

Solution A: Prepare as directed in the *Assay*.
Solution B: 13.6 g/L of sodium acetate in water, adjusted with glacial acetic acid to a pH of 4.0 before diluting to the final volume
Mobile phase: Tetrahydrofuran and *Solution A* (30:70)
Diluent: Methanol and *Solution B* (1:1)
Standard solution: 125 µg/mL of USP Mupirocin Lithium RS in *Diluent*
System suitability solution: Adjust 10 mL of the *Standard solution* with 6 N hydrochloric acid to a pH of 2.0, allow to stand for 20 h, and adjust with 5 N sodium hydroxide to a pH of 4.0.
Sample solution: 5 mg/mL of Mupirocin Calcium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for two mupirocin rearrangement products and mupirocin in the *System suitability solution* are 0.63, 0.67, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 7.0 between the mupirocin rearrangement product, with a relative retention time of about 0.67, and mupirocin, *System suitability solution*
Column efficiency: NLT 3000 theoretical plates for the mupirocin peak, *Standard solution*
Tailing factor: NMT 2 for the mupirocin peak, *Standard solution*
Relative standard deviation: NMT 5% for the mupirocin peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each related compound in the portion of Mupirocin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of any impurity from the *Sample solution*
r_S = peak area of mupirocin from the *Standard solution*
C_S = concentration of USP Mupirocin Lithium RS in the *Standard solution* (mg/mL)
C_U = concentration of Mupirocin Calcium in the *Sample solution* (mg/mL)
P = potency of mupirocin in USP Mupirocin Lithium RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*. Disregard any peak with an area less than 0.05 times the area of the mupirocin peak in the *Standard solution*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pseudomonic acid D[a]	0.75	2.5
Mupirocin	1.0	—
Any other unspecified impurity	—	1
Total impurities	—	4.5

[a] (*E*)-9-{(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-({(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyloxy}non-4-enoic acid.

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SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Specific Rotation*
  Sample solution: 50 mg/mL in methanol
  Acceptance criteria: −16° to −20°
- **WATER DETERMINATION** ⟨921⟩, *Method I*: 3.0%–4.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mupirocin Calcium RS
  USP Mupirocin Lithium RS

Mupirocin Cream

» Mupirocin Cream contains a quantity of Mupirocin Calcium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of mupirocin ($C_{26}H_{44}O_9$). It may contain one or more suitable buffers, dispersants, and preservatives.

Packaging and storage—Preserve in collapsible tubes or well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
Labeling—Label it to indicate that it contains Mupirocin Calcium and its equivalent content of mupirocin.
USP Reference standards ⟨11⟩—
USP Mupirocin Lithium RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Minimum fill ⟨755⟩: meets the requirements.
pH ⟨791⟩: between 6.0 and 8.0.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩ —It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The total aerobic microbial count does not exceed 100 cfu per g.

Related compounds—
0.1 M Ammonium acetate, Solution A, Solution B, Mobile phase, and *pH 6.3 Phosphate buffer*— Proceed as directed in the *Assay.*
Sodium acetate solution—Add 5.8 mL of glacial acetic acid to 900 mL of water, adjust with sodium hydroxide TS to a pH of 4.0, dilute with water to 1000 mL, and mix.
Tetrahydrofuran solution—Mix 750 mL of tetrahydrofuran and 250 mL of water.
Sodium acetate and tetrahydrofuran solution—Prepare a mixture of *Sodium acetate solution* and *Tetrahydrofuran solution* (50:50).
System suitability solution—Use the *Standard preparation,* prepared as directed in the *Assay.*
Test stock solution —Transfer an accurately weighed quantity of Cream, equivalent to about 50 mg of mupirocin, to a screw-capped centrifuge tube. Add 5.0 mL of *Tetrahydrofuran solution,* cap, and disperse the Cream by mixing on a vortex mixer and shaking. Add 5.0 mL of *Sodium acetate solution,* cap, and mix. Centrifuge for about 15 minutes. Withdraw the lower layer from the tube, pass it through a filter having a 0.5-µm or finer porosity, and use the filtrate.
Test solution—Transfer 1.0 mL of the *Test stock solution* to a 50-mL volumetric flask, dilute with *Sodium acetate and tetrahydrofuran solution* to volume, mix, and pass through a filter having a 0.5-µm or finer porosity.
Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay.* Chromatograph the *Test stock solution,* and record the responses as directed for *Procedure.* Identify the peaks based on the relative retention times for mupirocin and related substances shown in *Table 1:* the resolution, *R,* between pseudomonic acid D and mupirocin is not less than 3. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the column efficiency for the mupirocin peak is not less than 7000 theoretical plates; the tailing factor for the mupirocin peak is not more than 1.75; and the relative standard deviation of the mupirocin peak for replicate injections is not more than 2%.
Procedure— [NOTE—Ensure that buffers, dispersants, or preservatives in the formulation do not interfere with quantification of either impurities or degradation products.] Separately inject equal volumes (about 20 µL) of the *Test stock solution* and the *Test solution* into the chromatograph, and measure the peak responses for all of the peaks that do not correspond to buffers, dispersants, or preservatives. Calculate the percentage of each related compound and degradation product relative to mupirocin in the portion of Cream taken by the formula:

$$(r_i/r_M)(100/50)$$

in which r_i is the peak response for each related compound or degradation product obtained from the *Test stock solution;* r_M is the peak response of the mupirocin peak obtained from the *Test solution;* and 50 is the dilution factor for the *Test solution.*

Table 1

Name	Relative Retention Time	Limit (%)
Pseudomonic acid F[1]	0.36	NMT 1.2
Mupirocin impurity 1[2]	0.6	NMT 8.5
Mupirocin impurity 2[3]	0.63	NMT 16
Pseudomonic acid D[4]	0.75	NMT 3.0
Pseudomonic acid B[5]	0.9	NMT 1.2
Mupirocin	1.0	—
Mupirocin impurity 3[6]	1.15	NMT 1.2
Mupirocin impurity 4[6]	1.23	NMT 1.2
Pseudomonic acid C[7]	2.03	NMT 1.2
Pseudomonic acid E[8]	2.24	NMT 1.2
Any other unspecified impurity	—	NMT 1.2

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Table 1 *(continued)*

Name	Relative Retention Time	Limit (%)
Total impurities	—	NMT 30

[1] 7-{(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-({(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyloxy}heptanoic acid.
[2] 9-{(*E*)-4-[(2*R*,3a*S*,6*S*,7*S*, 8a*RS*)-2-{(1*RS*,2*S*,3*S*)-1,3-Dihydroxy-2-methylbutyl}-7-hydroxyhexahydro-2*H*-furo[3,2-c]pyran-6-yl]-3-methylbut-2-enoyloxy} nonanoic acid.
[3] 9-{(*E*)-4-[(2*R*,3*RS*,4a*S*,7*S*,8*S*,8a*R*)-3,8-Dihydroxy-2-{(2*S*,3*S*)-3-hydroxybutan-2-yl}octahydropyrano[3,2-c]pyran-7-yl]-3-methylbut-2-enoyloxy}nonanoic acid.
[4] (*E*)-9-{(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-({(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyloxy}non-4-enoic acid.
[5] 9-{(*E*)-3-Methyl-4-[(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxy-5-({(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2*H*-pyran-2-yl]but-2-enoyloxy}nonanoic acid.
[6] 9-{(*E*)-4-{(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[(3-hydroxy-4,5-dimethyltetrahydrofuran-2-yl)methyl]tetrahydro-2*H*-pyran-2-yl}-3-methylbut-2-enoyloxy) nonanoic acid.
[7] 9-((*E*)-4-{(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[(4*R*,5*S*,*E*)-5-hydroxy-4-methylhex-2-enyl]tetrahydro-2*H*-pyran-2-yl}-3-methylbut-2-enoyloxy)nonanoic acid.
[8] 11-{(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-({(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyloxy}undecanoic acid.

Assay—

0.1 M Ammonium acetate—Dissolve about 7.7 g of ammonium acetate in about 900 mL of water in a 1000-mL volumetric flask, adjust with glacial acetic acid to a pH of 5.7, and dilute with water to volume.

Solution A—Prepare a filtered and degassed mixture of *0.1 M Ammonium acetate* and tetrahydrofuran (75:25).

Solution B—Prepare a filtered and degassed mixture of *0.1 M Ammonium acetate* and tetrahydrofuran (70:30).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

pH 6.3 Phosphate buffer—Dissolve 69 g of monobasic sodium phosphate in 800 mL of water, adjust with sodium hydroxide TS to a pH of 6.3, dilute with water to 1000 mL, and mix.

Standard preparation—Dissolve an accurately weighed portion of USP Mupirocin Lithium RS in *pH 6.3 Phosphate buffer.* Dilute an accurately measured volume of this solution quantitatively with the same solvent to obtain a solution having a known concentration of about 0.1 mg of mupirocin per mL.

Assay preparation—Transfer an accurately weighed quantity of Cream, equivalent to about 10 mg of mupirocin, to a 100-mL volumetric flask. Add 50 mL of *pH 6.3 Phosphate buffer* and 25 mL of tetrahydrofuran. Insert the stopper into the flask, mix on a vortex mixer, and shake for 1 to 3 minutes. Dilute with *pH 6.3 Phosphate buffer* to volume. Allow to stand until the oil layer separates out, then dilute the aqueous layer with *pH 6.3 Phosphate buffer* to volume. Repeat 2 to 3 times until as much of the oil layer has separated out as possible. After the final dilution, pass the final solution (bottom layer) through a filter having a 0.5-µm or finer porosity. This solution will have a nominal concentration of 0.1 mg per mL of mupirocin based on label claim.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. Maintain the column at a constant temperature up to 35°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–6	100	0	isocratic
6–35	100→0	0→100	linear gradient
35–55	0	100	isocratic
55–55.01	0→100	100→0	immediate
55.01–65	100	0	isocratic

Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure.* [NOTE—Pseudomonic acid D is a minor component that is always present in mupirocin calcium.] Identify the peaks by their retention times which are about 0.75 for pseudomonic acid D and 1.0 for mupirocin: the resolution, *R,* between pseudomonic acid D and mupirocin is not less than 3; the column efficiency for the mupirocin peak is not less than 7000 theoretical plates; the tailing factor for the mupirocin peak is not more than 1.75; and the relative standard deviation of the mupirocin peak for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak area responses for the major peaks. Calculate the percent label claim of mupirocin in the portion of Cream taken by the formula:

$$(P/1000)(C_S/C_U)(r_U/r_S)(100)$$

in which $P/1000$ is the potency of mupirocin, converted from µg per mg to mg per mg, in USP Mupirocin Lithium RS; C_S is the concentration, in mg per mL, of USP Mupirocin Lithium RS in the *Standard preparation;* C_U is the nominal concentration, in mg per mL, of Cream in the *Assay preparation;* and r_U and r_S are the mupirocin peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Mupirocin Ointment

» Mupirocin Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mupirocin ($C_{26}H_{44}O_9$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards ⟨11⟩—
USP Mupirocin Lithium RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for mupirocin, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay.*

Minimum fill ⟨755⟩: meets the requirements.

Assay—

pH 6.3 phosphate buffer, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Mupirocin.*

Assay preparation—Dissolve an accurately weighed quantity of Ointment, equivalent to about 10 mg of mupirocin, in 25 mL of acetonitrile. Transfer this solution, with

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the aid of *pH 6.3 phosphate buffer,* to a 100-mL volumetric flask, dilute with *pH 6.3 phosphate buffer* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Mupirocin*. Calculate the quantity, in mg, of mupirocin ($C_{26}H_{44}O_9$) in the portion of Ointment taken by the formula:

$$(M_S E/1000)(r_U/r_S)$$

in which the terms are as defined therein.

Mupirocin Nasal Ointment

» Mupirocin Nasal Ointment contains a quantity of Mupirocin Calcium equivalent to not less than 90.0 percent and not more than 105.0 percent of the labeled amount of mupirocin ($C_{26}H_{44}O_9$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Mupirocin Lithium RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—The total aerobic microbial count does not exceed 100 cfu per g, and the total combined molds and yeasts count does not exceed 10 cfu per g. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Minimum fill ⟨755⟩**:** meets the requirements.

Uniformity of dosage units ⟨905⟩**:** meets the requirements.

Related compounds—

Ammonium acetate buffer, Mobile phase, Sodium acetate buffer, Diluent A, Diluent B, and *Chromatographic system*—Prepare as directed in the *Assay.*

Test solution—Transfer an accurately weighed portion of Mupirocin Nasal Ointment, equivalent to about 50 mg of mupirocin, to a suitable stoppered conical flask, add 5 mL of *Diluent A,* and shake vigorously on a mechanical shaker at full speed for 1 hour to disperse the ointment. Add 5 mL of *Sodium acetate buffer,* and shake vigorously on a mechanical shaker at full speed for 15 minutes. Pass through a filter having a porosity of 0.45 µm.

Diluted test solution—Dilute a portion of the *Test solution* quantitatively, and stepwise if necessary, with *Diluent B* to obtain a solution having a nominal concentration of about 0.1 mg of mupirocin per mL, based on the label claim.

Procedure—Separately inject equal volumes (about 20 µL) of the *Test solution* and the *Diluted test solution* into the chromatograph, and measure the peak area responses for all the peaks. Identify the peaks by the relative retention times shown in *Table 1.*

Table 1

Name	Relative Retention Time	Limit (%)
Pseudomonic acid F[1]	0.32	1.0
Mupirocin impurity 1[2]	0.56	5.0
Mupirocin impurity 2[3]	0.60	5.0
Pseudomonic acid D[4]	0.72	4.0
Pseudomonic acid B[5]	0.88	1.0
Mupirocin	1.0	—
Mupirocin impurity 3[6]	1.24	1.0
Mupirocin impurity 4[6]	1.36	1.0
Mupirocin impurity 5[7] and pseudomonic acid C[8]	2.80	1.0
Any individual unspecified impurity	—	0.5
Total impurities	—	10.0

[1] 7-[(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-({(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyloxy}heptanoic acid.

[2] 9-[(*E*)-4-[(2*R*,3a*S*,6*S*,7*S*, 8a*RS*)-2-{(1*RS*,2*S*,3*S*)-1,3-Dihydroxy-2-methylbutyl}-7-hydroxyhexahydro-2*H*-furo[3,2-*c*]pyran-6-yl]-3-methylbut-2-enoyloxy} nonanoic acid.

[3] 9-[(*E*)-4-[(2*R*,3*RS*,4a*S*,7*S*,8*S*,8a*R*)-3,8-Dihydroxy-2-{(2*S*,3*S*)-3-hydroxybutan-2-yl}octahydropyrano[3,2-*c*]pyran-7-yl]-3-methylbut-2-enoyloxy}nonanoic acid.

[4] (*E*)-9-[(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-({(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyloxy}non-4-enoic acid.

[5] 9-[(*E*)-3-Methyl-4-[(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxy-5-({(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl}methyl)tetrahydro-2*H*-pyran-2-yl]but-2-enoyloxy}nonanoic acid.

[6] 9-((*E*)-4-{(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[(3-hydroxy-4,5-dimethyltetrahydrofuran-2-yl)methyl]tetrahydro-2*H*-pyran-2-yl}-3-methylbut-2-enoyloxy) nonanoic acid.

[7] 9-((*E*)-4-((2*S*,3*R*,4*R*,5*S*)-5-((4*S*,5*S*)-2-Chloro-3,5-dihydroxy-4-methylhexyl)-3,4-dihydroxytetrahydro-2*H*-pyran-2yl)-3-methylbut-2-enoyloxy)nonanoic acid.

[8] 9-((*E*)-4-{(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[(4*R*,5*S*,*E*)-5-hydroxy-4-methylhex-2-enyl]tetrahydro-2*H*-pyran-2-yl}-3-methylbut-2-enoyloxy)nonanoic acid.

Calculate the percentage of each related compound in the portion of Mupirocin Nasal Ointment taken by the formula:

$$(100 \times r_i)/(D \times r_S)$$

in which r_i is the peak response of any impurity in the *Test solution; D* is the dilution factor used to convert the *Test solution* to the *Diluted test solution;* and r_S is the peak response for the mupirocin peak in the *Diluted test solution.* The specified and unspecified impurities meet the limits listed in *Table 1.*

Assay—

Ammonium acetate buffer—Dissolve about 7.7 g of ammonium acetate in water, and dilute with water to 1000 mL. Mix, and adjust with acetic acid to a pH of 5.7. Filter this solution prior to preparation of the *Mobile phase.*

Mobile phase—Prepare a mixture of *Ammonium acetate buffer* and tetrahydrofuran (75:25). Make adjustments if necessary (see *System suitability* under *Chromatography* ⟨621⟩). The *Mobile phase* is extremely sensitive to changes in tetrahydrofuran concentration. Degas the *Mobile phase* by helium sparging or ultrasonication before use.

Sodium acetate buffer—Dissolve about 13.6 g of sodium acetate in water, and dilute with water to 1000 mL. Mix, and adjust with acetic acid to a pH of 4.0.

Diluent A: a mixture of tetrahydrofuran and water (75:25).

Diluent B: a mixture of *Diluent A* and *Sodium acetate buffer* (1:1).

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Standard preparation—Prepare a solution of USP Mupirocin Lithium RS in *Diluent B,* containing about 0.1 mg per mL of mupirocin.

Assay preparation—Transfer an accurately weighed portion of Mupirocin Nasal Ointment, equivalent to about 10 mg of mupirocin, to a 100-mL volumetric flask, add 50.0 mL of *Diluent A,* and shake vigorously on a mechanical shaker at full speed for 1 hour to disperse the ointment. Dilute with *Sodium acetate buffer* to volume, and shake vigorously on a mechanical shaker at full speed for 15 minutes. Prior to use, pass through a filter having a porosity of 0.45 µm.

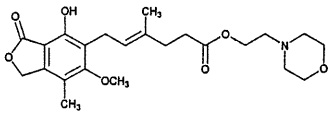
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm×25-cm column that contains 7-µm packing L7. The flow rate is about 1.5 mL per minute. [NOTE—The flow rate may be adjusted if needed to obtain a retention time of about 13 minutes for the mupirocin peak.] Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the peaks for pseudomonic acid D and mupirocin is not less than 3.5; the column efficiency for the mupirocin peak is not less than 3000 theoretical plates; the tailing factor for the mupirocin peak is not more than 2.0; and the relative standard deviation of the mupirocin peak for five replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Calculate the percentage of the label claim of mupirocin in the portion of Mupirocin Nasal Ointment taken by the formula:

$$(P/1000)(C_S/C_U)(r_U/r_S)(100)$$

in which $(P/1000)$ is the potency, converted from µg per mg to mg per mg, of mupirocin in USP Mupirocin Lithium RS; C_S is the concentration, in mg per mL, of mupirocin in the *Standard preparation;* C_U is the nominal concentration, in mg per mL, of mupirocin in the *Assay preparation;* and r_U and r_S are the peak responses for the mupirocin peak in the *Assay preparation* and the *Standard preparation,* respectively.

Mycophenolate Mofetil



$C_{23}H_{31}NO_7$ 433.49

4-Hexenoic acid, 6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-, 2-(4-morpholinyl)ethyl ester, (*E*)-;

2-Morpholinoethyl (*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate [128794-94-5].

DEFINITION

Mycophenolate Mofetil contains NLT 98.0% and NMT 102.0% of mycophenolate mofetil ($C_{23}H_{31}NO_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: Triethylamine and water (1:325). Adjust with phosphoric acid to a pH of 5.3.
Mobile phase: Acetonitrile and *Buffer* (7:13)
Standard solution: 0.4 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile
Sample solution: 0.4 mg/mL of Mycophenolate Mofetil in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mycophenolate mofetil ($C_{23}H_{31}NO_7$) in the portion of Mycophenolate Mofetil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = concentration of Mycophenolate Mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Prepare as directed in the *Assay.*
Sample solution: 2 mg/mL of Mycophenolate Mofetil in acetonitrile
System suitability solution: 10 µg/mL each of USP Mycophenolate Mofetil Related Compound A RS and USP Mycophenolate Mofetil Related Compound B RS in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B
Analysis
Sample: *Sample solution*

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Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.03%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mycophenolic acid[a]	0.33	0.50
Mycophenolate mofetil related compound A[b]	0.45	0.10
Mycophenolate mofetil related compound B[c]	0.49	0.10
N-Oxide analog[d]	0.60	0.10
1-Morpholinoethoxy analog[e]	0.86	0.10
Mycophenolate mofetil	1.0	—
Z-Mycophenolate mofetil[f]	1.1	0.10
O-Methyl analog[g]	1.2	0.10
Methyl mycophenolate[h]	1.5	0.10
Any single unspecified impurity	—	0.10
Total impurities	—	0.70

[a] (*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.
[b] 2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
[c] (*RS*)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuran-1-one.
[d] 2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate *N*-oxide.
[e] 2-Morpholinoethyl (*RS*)-(*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-1-(2-morpholinoethoxy)-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
[f] 2-Morpholinoethyl (*Z*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
[g] 2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dimethoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
[h] Methyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample under vacuum at 60° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Mycophenolate Mofetil RS
  USP Mycophenolate Mofetil Related Compound A RS
  2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
  $C_{22}H_{29}NO_7$ 419.47
  USP Mycophenolate Mofetil Related Compound B RS
  (*RS*)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuran-1-one.
  $C_{17}H_{20}O_6$ 320.34

Mycophenolate Mofetil Capsules

DEFINITION

Mycophenolate Mofetil Capsules contain NLT 94.0% and NMT 105.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Standard solution and **Sample solution:** Use the *Standard solution* and the *Sample solution* as prepared in *DissolutionTest 1*.
  Acceptance criteria: The UV absorption spectra of the *Standard solution* and the *Sample solution* exhibit maxima and minima at the same wavelength within ±3 nm.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Phosphoric acid solution: Phosphoric acid and water (3:50)
  Triethylamine solution: Transfer 3 mL of triethylamine to 1000 mL of water. Adjust with *Phosphoric acid solution* to a pH of 5.3.
  Mobile phase: Acetonitrile and *Triethylamine solution* (11:9)
  Standard solution: 0.125 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile
  Sample solution: Open Capsules, equivalent to 1.25 g of mycophenolate mofetil based on the label claim, and transfer the contents including Capsule shells into a 500-mL volumetric flask. Add 50 mL of water and shake mechanically for a minimum of 15 min. Add 350 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask and dilute with acetonitrile to volume. Pass through a nylon filter of 0.45-µm pore size and discard the first 5 mL of the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 250 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Temperatures
  Column: 45°
  Autosampler: 10 ± 5°
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

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r_S = peak response from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 94.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 40 rpm, with sinkers
Time: 20 min
Standard solution: 0.278 mg/mL of USP Mycophenolate Mofetil RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Detector: UV 250 nm
Path length: 0.1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 40 rpm, with sinker
Time: 30 min
Standard solution: 0.028 mg/mL of USP Mycophenolate Mofetil RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable nylon filter of 0.45-µm pore size. Discard the first 3–5 mL of the filtrate. Dilute 1 mL of the filtrate with *Medium* to 10 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 250 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor, 10
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **LIMIT OF DEGRADATION PRODUCTS**

Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.0625 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
[NOTE—The run time for the *Sample solution* is three times that of the retention time of the mycophenolate mofetil peak.]
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peaks at relative retention times of 1.45 and 2.15. Disregard any peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid[a]	0.6	1.4	1.0
Mycophenolate *N*-oxide analog[b]	0.8	1.0	0.2
Mycophenolate mofetil	1.0	—	—
Any single unspecified impurity	—	1.0	0.1
Total degradation products	—	—	1.5

[a] (*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.
[b] 2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate *N*-oxide.

• **LIMIT OF *Z*-MYCOPHENOLATE MOFETIL**

[NOTE—*Z*-Mycophenolate mofetil is 2-morpholinoethyl (*Z*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate.]
Triethylamine solution: Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile and *Triethylamine solution* (7:13)
Sensitivity solution: 1.25 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile

USP Monographs

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Standard solution: 0.025 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile
Sample solution: Open Capsules, equivalent to 1.25 g of mycophenolate mofetil based on the label claim, and transfer the contents including Capsule shells into a 500-mL volumetric flask. Add 50 mL of water and shake mechanically for a minimum of 15 min. Add 350 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Pass through a nylon filter of 0.45-µm pore size and discard the first 2 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Column temperature: 60°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 1.7 times the retention time of the mycophenolate mofetil peak
System suitability
Samples: *Sensitivity solution* and *Standard solution*
[NOTE—The relative retention times for mycophenolate mofetil and *Z*-mycophenolate mofetil are 1.0 and 1.1, respectively.]
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of *Z*-mycophenolate mofetil in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *Z*-mycophenolate mofetil from the *Sample solution*
r_S = peak response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.10%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Mycophenolate Mofetil RS

Mycophenolate Mofetil for Injection

DEFINITION
Mycophenolate Mofetil for Injection contains an amount of Mycophenolate Mofetil Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION

Change to read:

- **A.** ▲(USP 1-May-2019) The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

- **PROCEDURE**

Protect solutions from light.
Buffer 1: Transfer 10 mL of triethylamine to a 1000-mL volumetric flask containing about 950 mL of water and mix. Adjust with phosphoric acid to a pH of 7.2, and dilute with water to volume.
Buffer 2: Transfer 10 mL of triethylamine to a 1000-mL volumetric flask containing about 950 mL of water and mix. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.
Solution A: *Buffer 1* and water (4:9)
Diluent: Acetonitrile, *Buffer 2*, and water (7:4:9)
Mobile phase: Acetonitrile and *Solution A* (3:7)
Standard stock solution: 1.0 mg/mL of USP Mycophenolate Mofetil RS in *Diluent* prepared as follows. Transfer a known quantity of USP Mycophenolate Mofetil RS to a suitable volumetric flask, add acetonitrile equivalent to about 10% of the final volume, sonicate for about 5 min or until the solid dissolves, and dilute with *Diluent* to volume.
Standard solution: 0.4 mg/mL of USP Mycophenolate Mofetil RS in *Diluent* from *Standard stock solution*
Sample stock solution: Nominally equivalent to 10 mg/mL of mycophenolate mofetil prepared as follows. Constitute each of the containers of Mycophenolate Mofetil for Injection with 14 mL of 5% dextrose injection. Quantitatively transfer the contents of all vials, the combined contents of which are equivalent to about 2 g of mycophenolate mofetil, to a 200-mL volumetric flask, and dilute with water to volume.
Sample solution: Nominally equivalent to 0.4 mg/mL of mycophenolate mofetil in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 249 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
Column: 4.6-mm × 25-cm; 5-µm packing L11
Temperatures
Autosampler: 5°
Column: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) in the portion of Mycophenolate Mofetil for Injection taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Protect solutions from light.
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound A RS and 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound B RS in *Diluent*
Sensitivity solution: 0.2 µg/mL in *Diluent* from the *Standard solution*
System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
[NOTE—The relative retention times for mycophenolate mofetil related compound A and mycophenolate mofetil related compound B are 0.40 and 0.46, respectively, measured with respect to mycophenolate mofetil.]
Suitability requirements
Resolution: NLT 2.0 between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The run time for the *Sample solution* is NLT 1.5 times the retention time of the mycophenolate mofetil peak.]
Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. ▲The reporting threshold is 0.05%.▲ (USP 1-May-2019)

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid[a]	0.12	1.4	1.1
Mycophenolate mofetil	1.00	—	—
Any unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.35

[a] (*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.

SPECIFIC TESTS

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** ▲Meets the requirements▲ (USP 1-May-2019)

Change to read:

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements▲▲ (USP 1-May-2019)
• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: NMT 1.0%
• **pH** ⟨791⟩**:** 2.7–4.1, in a reconstituted solution
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
• **CONSTITUTED SOLUTION:** At the time of use, meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mycophenolate Mofetil RS
USP Mycophenolate Mofetil Related Compound A RS
2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
$C_{22}H_{29}NO_7$ 419.47
USP Mycophenolate Mofetil Related Compound B RS
(*RS*)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuran-1-one.
$C_{17}H_{20}O_6$ 320.34

Mycophenolate Mofetil for Oral Suspension

DEFINITION

Mycophenolate Mofetil for Oral Suspension is a dry mixture of mycophenolate mofetil and one or more suitable buffers, colors, diluents, and flavors. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

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IDENTIFICATION

Change to read:

▲• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U.**▲ (CN 1-May-2020)
[NOTE—Alternatively, the UV spectra of the major peaks of the *Sample solution* and the *Standard solution*, as obtained in the *Assay*, may also be used to meet the *Acceptance criteria*.]
Standard stock solution: 0.5 mg/mL of USP Mycophenolate Mofetil RS prepared as follows. Transfer a suitable amount of USP Mycophenolate Mofetil RS to a volumetric flask, add water equivalent to 15% of the flask volume, shake, and mix. Add acetonitrile equivalent to 70% of the flask volume, and sonicate to dissolve. Dilute with acetonitrile to volume.
Standard solution: 0.01 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile, from *Standard stock solution*
Sample stock solution: Nominally equivalent to 0.5 mg/mL of mycophenolate mofetil prepared as follows. Constitute the Mycophenolate Mofetil for Oral Suspension as directed in the labeling. Transfer a suitable amount of reconstituted oral suspension to a volumetric flask, add water equivalent to 15% of the flask volume, shake, and mix. Add acetonitrile equivalent to 70% of the flask volume and sonicate to dissolve. Dilute with acetonitrile to volume. Pass a portion of this solution through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally equivalent to 0.01 mg/mL of mycophenolate mofetil in acetonitrile, from *Sample stock solution*
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*.▲ (USP 1-May-2019)

Change to read:

• **▲B.**▲ (USP 1-May-2019) The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Protect solutions from light.
Buffer 1: Pipet 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water, and mix. Adjust with phosphoric acid to a pH of 7.2, and dilute with water to volume.
Buffer 2: Pipet 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water, and mix. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.
Solution A: *Buffer 1* and water (4:9)
Extraction solvent: Acetonitrile, *Buffer 2*, and water (13:4:9)
Diluent: Acetonitrile, *Buffer 2*, and water (7:4:9)
Mobile phase: Acetonitrile and *Solution A* (3:7)
Standard stock solution: 4 mg/mL of USP Mycophenolate Mofetil RS in *Extraction solvent*. Sonicate to aid the dissolution.
Standard solution: 0.4 mg/mL of USP Mycophenolate Mofetil RS in *Diluent*, from the *Standard stock solution*
Sample stock solution: Nominally equivalent to 4 mg/mL of mycophenolate mofetil prepared as follows. Constitute Mycophenolate Mofetil for Oral Suspension as directed on the label. Prepare a composite sample by mixing NLT 4 bottles of the constituted Mycophenolate Mofetil for Oral Suspension. Transfer a volume of the composite sample so obtained, equivalent to 800 mg of mycophenolate mofetil, to a 200-mL volumetric flask, and dilute with *Extraction solvent* to volume.
Sample solution: Nominally equivalent to 0.4 mg/mL of mycophenolate mofetil prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 249 nm. ▲If this procedure is used for *Identification A*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
Column: 4.6-mm × 25-cm; 5-µm packing L11
Temperatures
Autosampler: 5°
Column: 45°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) in the portion of Mycophenolate Mofetil for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of ▲USP Mycophenolate Mofetil RS▲ (USP 1-May-2019) in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
▲Protect solutions from light.▲ (USP 1-May-2019)
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 2: 40 rpm
Time: 20 min
Standard solution: 0.278 mg/mL of USP Mycophenolate Mofetil RS in *Medium*
Sample solution: Reconstitute Mycophenolate Mofetil for Oral Suspension according to the labeling instructions. Shake well. Use a separate 3-mL syringe for each vessel. Withdraw 2 mL of suspension. Remove air bubbles from the syringe. Adjust the volume to 1.2 mL and accurately weigh the filled syringe. Operate the apparatus, holding the syringe above the surface of the medium, at a location that is halfway between the paddle shaft and the vessel wall. Carefully introduce the sample to the vessel over a 5–10 s period. Weigh the empty syringe and determine the weight of the sample (g). At the time specified, withdraw an aliquot and immediately pass through a suitable filter of 10-µm pore size, discarding the first few milliliters.

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Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 304 nm
Cell: 0.2 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mycophenolate mofetil dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (V_1/V_2) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of ▲USP Mycophenolate Mofetil RS▲ (USP 1-May-2019) in the *Standard solution* (mg/mL)
L = suspension label claim of mycophenolate mofetil (mg/mL)
V_1 = volume of *Medium*, 900 (mL)
V_2 = volume of sample (mL), weight (g) of the sample divided by the density of the suspension (g/mL)

Tolerances: NLT 80% (Q) of the labeled amount of mycophenolate mofetil is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Protect solutions from light.
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound A RS and 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound B RS in *Diluent*. [NOTE—The relative retention times for mycophenolate mofetil related compound A and mycophenolate mofetil related compound B are 0.40 and 0.46, respectively, measured with respect to mycophenolate mofetil.]
Sensitivity solution: 0.2 µg/mL in *Diluent* from the *Standard solution*
System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The run time for the *Sample solution* is NLT 1.5 times the retention time of the mycophenolate mofetil peak.]
Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of ▲USP Mycophenolate Mofetil RS▲ (USP 1-May-2019) in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. ▲The reporting threshold is 0.05%.▲ (USP 1-May-2019)

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid[a]	0.12	1.4	3.3
▲Sorbitolyl▲ (USP 1-May-2019) ester of mycophenolic acid[b]	0.24	0.77	0.2
Mycophenolate mofetil	1.00	—	—
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	3.8

[a] (*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.
[b] Sorbitol (*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample: The suspension constituted as directed in the labeling
Acceptance criteria: 6.0–7.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mycophenolate Mofetil RS
USP Mycophenolate Mofetil Related Compound A RS
2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
$C_{22}H_{29}NO_7$ 419.47
USP Mycophenolate Mofetil Related Compound B RS
(*RS*)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuran-1-one.
$C_{17}H_{20}O_6$ 320.34

Mycophenolate Mofetil Tablets

DEFINITION
Mycophenolate Mofetil Tablets contain NLT 94.0% and NMT 105.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Standard solution and **Sample solution:** Use the *Standard solution* and *Sample solution* as prepared in the test for *Dissolution*.

Acceptance criteria: The UV absorption spectrum of the *Standard solution* and the *Sample solution* exhibit maxima and minima at the same wavelength within ±3 nm.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Phosphoric acid solution: Phosphoric acid and water (3:50)

Triethylamine solution: Transfer 3 mL of triethylamine to 1000 mL of water. Adjust with *Phosphoric acid solution* to a pH of 5.3.

Mobile phase: Acetonitrile and *Triethylamine solution* (11:9)

Standard solution: 0.125 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Sample solution: Nominally equivalent to 0.125 mg/mL of mycophenolate mofetil prepared as follows. Place Tablets, equivalent to 2.5 g of mycophenolate mofetil based on the label claim, into a 1000-mL volumetric flask. Add 100 mL of water, and shake mechanically for a minimum of 15 min. Add 700 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with acetonitrile to volume. Pass through a nylon filter of 0.45-µm pore size, and discard the first 5 mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Temperatures

Column: 45°

Autosampler: 10 ± 5°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 94.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times: 5 and 15 min

Standard solution: 0.55 mg/mL of USP Mycophenolate Mofetil RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Detector: UV 304 nm

Path length: 0.1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

C_i = concentration of mycophenolate mofetil in the portion of sample withdrawn at the specified time (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (i)	Time (min)	Tolerances (Q)
1	5	NLT 75%
2	15	NLT 85%

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated

Apparatus 2: 50 rpm

Times: 5 and 15 min

Diluted phosphoric acid: Transfer 5 mL of phosphoric acid to a 25-mL volumetric flask. Dilute with water to volume.

Buffer: 3.0 mL/L of triethylamine in water. Adjust with *Diluted phosphoric acid* to a pH of 5.3.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Diluent: Acetonitrile and *Buffer* (20:80)

Standard stock solution: 0.56 mg/mL of USP Mycophenolate Mofetil RS in *Medium*

Standard solution: 0.11 mg/mL of USP Mycophenolate Mofetil RS in *Diluent*, prepared from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Transfer

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5 mL of the filtrate to a 25-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*, 5

Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

C_i = concentration of mycophenolate mofetil in the portion of sample withdrawn at the specified time (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (min)	Tolerances (*Q*)
1	5	NLT 60%
2	15	NLT 80%

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Times: 5 and 15 min
Standard solution: 0.011 mg/mL of USP Mycophenolate Mofetil RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute 2 mL of the filtrate with *Medium* to 100 mL.
Detector: UV 250 nm
Path length: 1 cm
Blank: *Medium*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*, 50

Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

C_i = concentration of mycophenolate mofetil in the portion of sample withdrawn at the specified time (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (min)	Tolerances (*Q*)
1	5	NLT 70%
2	15	NLT 85%

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Limit of Degradation Products**
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.0625 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile

System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
[Note—The run time for the *Sample solution* is 3 times that of the retention time of the mycophenolate mofetil peak.]
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 4*)

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Acceptance criteria: See *Table 4*. [NOTE—Disregard peaks at relative retention times of 1.45 and 2.15. Disregard any peaks less than 0.05%.]

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid[a]	0.6	1.4	1.0
Mycophenolate *N*-oxide analog[b]	0.8	1.0	0.2
Mycophenolate mofetil	1.0	—	—
Any single unspecified impurity	—	1.0	0.1
Total degradation products	—	—	1.5

[a] (*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.
[b] 2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate *N*-oxide.

• **LIMIT OF *Z*-MYCOPHENOLATE MOFETIL**
[NOTE—*Z*-Mycophenolate mofetil is 2-morpholinoethyl (*Z*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate.]
Triethylamine solution: Proceed as directed in the *Assay*.
Mobile phase: Acetonitrile and *Triethylamine solution* (7:13)
Sensitivity solution: 1.25 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile
Standard solution: 0.025 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile
Sample solution: Place Tablets, equivalent to 2.5 g of mycophenolate mofetil based on the label claim, into a 1000-mL volumetric flask. Add 100 mL of water, and shake mechanically for a minimum of 15 min. Add 700 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Pass through a nylon filter of 0.45-µm pore size, and discard the first 2 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Column temperature: 60°
Flow rate: 1.5 mL/min
Run time: 1.7 times the retention time of the mycophenolate mofetil peak
Injection volume: 10 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
[NOTE—The relative retention times for mycophenolate mofetil and *Z*-mycophenolate mofetil are 1.0 and 1.1, respectively.]
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of *Z*-mycophenolate mofetil in the portion of Tablets taken:

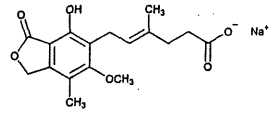
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *Z*-mycophenolate mofetil from the *Sample solution*
r_S = peak response of mycophenolate mofetil from the *Standard solution*
C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.10%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mycophenolate Mofetil RS

Mycophenolate Sodium



$C_{17}H_{19}NaO_6$ 342.32
4-Hexenoic acid, 6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-, monosodium salt, (*E*)-;
Sodium (*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate [37415-62-6].

DEFINITION
Mycophenolate Sodium contains NLT 98.0% and NMT 102.0% of mycophenolate sodium ($C_{17}H_{19}NaO_6$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Protect solutions from light.
Solution A: Acetonitrile, phosphoric acid, and water (100: 0.2: 900)
Solution B: Acetonitrile, phosphoric acid, and water (800: 0.2: 200)
Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
35	10	90

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
40	10	90

Diluent: Methanol and water (1:9)
Standard solution: 0.08 mg/mL of USP Mycophenolate Sodium RS in *Diluent*. Protect from light.
Sample solution: 0.08 mg/mL of Mycophenolate Sodium in *Diluent*. Protect from light.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 216 nm
Column: 3-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%
Tailing factor: 0.7–1.5
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mycophenolate sodium ($C_{17}H_{19}NaO_6$) in the portion of Mycophenolate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Mycophenolate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• ORGANIC IMPURITIES

Protect solutions from light.
Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability stock solution 1: 0.02 mg/mL of USP Mycophenolate Mofetil Related Compound B RS prepared as follows. Transfer USP Mycophenolate Mofetil Related Compound B RS to a suitable volumetric flask and dilute in methanol equivalent to 10% of the final volume, and then dilute with water to volume.
System suitability stock solution 2: Transfer 4.0 mL of *System suitability stock solution 1* to a 100-mL volumetric flask and dilute with *Diluent* to volume.
System suitability solution: 0.8 µg/mL of USP Mycophenolate Mofetil Related Compound B RS and 0.08 mg/mL of USP Mycophenolate Sodium RS prepared by dissolving a suitable amount of USP Mycophenolate Sodium RS in *System suitability stock solution 2*
Sensitivity solution: 0.024 µg/mL of USP Mycophenolate Sodium RS in *Diluent* from *Standard solution*
System suitability
Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between the mycophenolate mofetil related compound B and mycophenolate peaks, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Mycophenolate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of mycophenolate from the *Standard solution*
C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Mycophenolate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mycophenolate mofetil related compound B[a]	0.9	0.1
Mycophenolate	1.0	—
Any individual unspecified impurity	—	0.07
Total impurities	—	0.4

[a] (*RS*)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuran-1-one.

SPECIFIC TESTS

• SODIUM CONTENT

Standard stock solution: Use commercially available sodium atomic absorption spectroscopy standard solution of 1000 µg/mL of sodium in 0.5 M nitric acid.
Diluent: Transfer 3 mL of nitric acid to a 250-mL volumetric flask, and dilute with water to volume.
Standard solution A: 6.0 µg/mL of sodium in *Diluent* from *Standard stock solution*
Standard solution B: 9.0 µg/mL of sodium in *Diluent* from *Standard stock solution*
Standard solution C: 12.0 µg/mL of sodium in *Diluent* from *Standard stock solution*
Sample solution: 0.14 mg/mL of Mycophenolate Sodium prepared as follows. Weigh 30–40 mg of Mycophenolate Sodium into a digestion vessel, add 3 mL of nitric acid and digest at 150° for 5 h. Allow to cool, transfer the digestion solution to a 250-mL volumetric flask, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 589.0 nm
Lamp: Sodium hollow-cathode
Flame: Air–acetylene
Blank: *Diluent*
System suitability
Sample: *Standard solution B*

USP Monographs

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Suitability requirements
Relative standard deviation: NMT 5% for absorbance from three readings
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Blank* and *Standard solutions* versus their concentrations of sodium (0, 6.0, 9.0, and 12.0 µg/mL), and draw a calibration curve best fitting the four points. From the graph so obtained, determine the concentration, in µg/mL, of sodium in the *Sample solution*.
Calculate the percentage of sodium in the portion of Mycophenolate Sodium taken:

$$\text{Result} = F \times (C_S/C_U) \times 100$$

F = conversion factor (0.001 mg/µg)
C_S = concentration of sodium in the *Sample solution* (µg/mL)
C_U = concentration of Mycophenolate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 5.7%–7.7% on the anhydrous basis
- **X-RAY DIFFRACTION** ⟨941⟩**:** Its X-ray diffraction pattern conforms to that of USP Mycophenolate Sodium RS, similarly determined.
- **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 1.5%
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial limit does not exceed 10^3 cfu/g. The total yeasts and molds count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Mycophenolate Mofetil Related Compound B RS
(*RS*)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuran-1-one.
$C_{17}H_{20}O_6$ 320.34
USP Mycophenolate Sodium RS

Mycophenolic Acid Delayed-Release Tablets

DEFINITION
Mycophenolic Acid Delayed-Release Tablets contain an amount of mycophenolate sodium equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Protect solutions from light.
Solution A: Dissolve 21 g of citric acid in a suitable volume of water, add 200 mL of 1 M sodium hydroxide solution, and dilute with water to 1 L.
Buffer: *Solution A* and 0.1 M hydrochloric acid (399:601)
Solution B: Acetonitrile, *Buffer*, and water (40:15:45)
Solution C: Acetonitrile and *Buffer* (85:15)
Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
9	100	0
18	0	100
22	0	100

Diluent: *Solution B*
Standard solution: 0.385 mg/mL of USP Mycophenolate Sodium RS in *Diluent*. Stir magnetically for at least 60 min to aid dissolution.
Sample stock solution: Nominally equivalent to 9 mg/mL of mycophenolic acid in *Diluent* prepared as follows. Transfer NLT 25 Tablets to a volumetric flask and add *Diluent* to volume. Add a stirring bar and stir vigorously for at least 60 min. Centrifuge a portion of the suspension and use the clear supernatant.
Sample solution: Nominally equivalent to 0.36 mg/mL of mycophenolic acid in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 251 nm or diode array. [NOTE—Use a diode array detector to perform *Identification B*.]
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of mycophenolate from the *Sample solution*
r_S = peak response of mycophenolate from the *Standard solution*
C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolic acid in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of mycophenolic acid, 320.34
M_{r2} = molecular weight of mycophenolate sodium, 342.32

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
Protect solutions from light.
Acid stage
Acid stage medium: 0.1 N hydrochloric acid; 750 mL
Apparatus 2: 50 rpm
Time: 2 h

USP Monographs

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Determine the percentage of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) dissolved by using one of the following procedures.

Spectrophotometric procedure

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Standard solution: Transfer USP Mycophenolate Sodium RS to a suitable volumetric flask, dissolve in methanol equivalent to 5% of the final volume, and dilute with *Acid stage medium* to volume to obtain the solution having the following concentrations:

For Tablets labeled to contain 180 mg of mycophenolic acid: 0.0128 mg/mL of USP Mycophenolate Sodium RS

For Tablets labeled to contain 360 mg of mycophenolic acid: 0.0256 mg/mL of USP Mycophenolate Sodium RS

Sample solution: Centrifuge portions of the solution under test or pass through a suitable glass fiber filter of 1-µm pore size.

Instrumental conditions

Mode: UV

Analytical wavelength: 250 nm

Cell path length

For Tablets containing 180 mg of mycophenolic acid per Tablet: 0.2 cm

For Tablets containing 360 mg of mycophenolic acid per Tablet: 0.1 cm

Blank: *Acid stage medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Acid stage medium*, 750 mL
M_{r1} = molecular weight of mycophenolic acid, 320.34
M_{r2} = molecular weight of mycophenolate sodium, 342.32

Chromatographic procedure

Solution A: Solution of 4 mL of triethylamine in 1300 mL of water. Adjust with phosphoric acid to a pH of 5.3.

Mobile phase: Acetonitrile and *Solution A* (35:65)

Diluent: Mixture of 0.1 N hydrochloric acid and 0.2 M sodium phosphate (75:25), adjusted with either 2 M sodium hydroxide or hydrochloric acid to a pH of 6.8

Standard solution: Solution of USP Mycophenolate Sodium RS in *Diluent*, equivalent to (L/1000) mg/mL of mycophenolic acid, where L is the label claim in mg/Tablet

Sample solution: Mix 10 mL of the solution under test and 10 mL of 0.2 M sodium phosphate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-µm packing L11

Temperatures

Autosampler: 4°

Column: 45°

Flow rate: 1.5 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) dissolved:

$$(r_U/r_S) \times (C_S/L) \times V \times D \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Acid stage medium*, 750 mL
D = dilution factor for the *Sample solution*
M_{r1} = molecular weight of mycophenolic acid, 320.34
M_{r2} = molecular weight of mycophenolate sodium, 342.32

Tolerances: NMT 5% of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) is dissolved.

Buffer stage

Buffer stage medium: After 2 h, continue with a pH 6.8 phosphate buffer as follows. Add 250 mL of 0.2 M sodium phosphate and adjust, if necessary, with 2 M sodium hydroxide or hydrochloric acid to a pH of 6.8; 1000 mL. [NOTE—If the volume of the sample withdrawn is greater than 2 mL, replace it with *Acid stage medium*. Alternatively, increase the volume of 0.2 M sodium phosphate being added to obtain the final volume of 1000 mL and adjust, if necessary, with 2 M sodium hydroxide or hydrochloric acid to a pH of 6.8.]

Apparatus 2: 50 rpm

Time: 1 h. [NOTE—The total time for this analysis is 3 h, where 2 h is from the *Acid stage* and 1 h is from the *Buffer stage*.]

Determine the percentage of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) dissolved by using one of the following procedures.

Spectrophotometric procedure

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Standard solution: Solution of USP Mycophenolate Sodium RS, equivalent to (L/1000) mg/mL of mycophenolic acid, where L is the label claim, in mg/Tablet. Prepare the solution as follows. Transfer USP Mycophenolate Sodium RS to a suitable volumetric flask, dissolve in methanol equivalent to 5% of the final volume, and dilute with *Buffer stage medium* to volume.

Sample solution: Centrifuge portions of the solution under test or pass through a suitable glass fiber filter of 1-µm pore size.

Instrumental conditions

Mode: UV

Analytical wavelength: 250 nm

Cell path length

For Tablets containing 180 mg of mycophenolic acid per Tablet: 0.2 cm

For Tablets containing 360 mg of mycophenolic acid per Tablet: 0.1 cm

Blank: *Buffer stage medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) dissolved:

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$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Buffer stage medium*, 1000 mL
M_{r1} = molecular weight of mycophenolic acid, 320.34
M_{r2} = molecular weight of mycophenolate sodium, 342.32

Chromatographic procedure
Solution A, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed under *Chromatographic procedure* in *Acid stage.*
Sample solution: Pass the solution under test through a suitable polyethersulfone membrane filter of 0.45-µm pore size and discard a few milliliters of the filtrate.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) dissolved:

$$(r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Buffer stage medium*, 1000 mL
M_{r1} = molecular weight of mycophenolic acid, 320.34
M_{r2} = molecular weight of mycophenolate sodium, 342.32

Tolerances: NLT 80% (Q) of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩, *Weight Variation*: Meet the requirements

IMPURITIES

• **Organic Impurities**
Protect solutions from light.
Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*
System suitability solution: 0.36 µg/mL of USP Mycophenolate Mofetil Related Compound B RS and 0.385 mg/mL of USP Mycophenolate Sodium RS in *Diluent.* Stir magnetically for at least 60 min to aid dissolution.
Sensitivity solution: 0.18 µg/mL of USP Mycophenolate Sodium RS in *Diluent* from *Standard solution*
System suitability
Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between the mycophenolate mofetil related compound B and mycophenolate peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of mycophenolate from the *Standard solution*
C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of mycophenolic acid in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of mycophenolic acid, 320.34
M_{r2} = molecular weight of mycophenolate sodium, 342.32

Acceptance criteria: See *Table 2.* The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phthalic acid[a]	0.30	—
Phthalic acid monoethyl ester[a, b]	0.55	—
Mycophenolate mofetil related compound B	0.90	0.2
Mycophenolate	1.0	—
Ethyl ester of mycophenolate[c]	2.3	0.2
Any individual unspecified impurity	—	0.1
Total impurities	—	1.0

[a] It is a process impurity and is listed for identification only. It is controlled in the drug substance. It is not reported for the drug product and should not be included in the total impurities.
[b] 2-(Ethoxycarbonyl)benzoic acid.
[c] Ethyl (*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and protect from moisture. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Mycophenolate Mofetil Related Compound B RS
(*RS*)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuran-1-one.
$C_{17}H_{20}O_6$ 320.34
USP Mycophenolate Sodium RS

Myrrh

DEFINITION

Myrrh is the oleo-gum resin of stems and branches of *Commiphora molmol* Engler and other related species of *Commiphora* other than *Commiphora mukul* (Fam. Burseraceae).

IDENTIFICATION

• **A.**
Sample: 0.4 g of crushed Myrrh
Analysis: Triturate the *Sample* with 1 g of washed sand, shake for a few min with 10 mL of ethyl ether, and filter. Evaporate the filtrate to dryness in a porcelain dish, and add a few drops of nitric acid to the residue.

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Acceptance criteria: A purplish violet color is produced instantly.

• **B.**

Sample: 0.1 g of powdered Myrrh

Analysis: Transfer the *Sample* to a test tube, and add 1 mL of nitric acid.

Acceptance criteria: A red color is produced. Upon addition of a crystal of vanillin, the red color deepens and does not diminish when water is added.

• **C. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 7 µg/mL of (*E*)-anethole, 8 µg/mL of linalool, and 10 µg/mL each of (–)-bornyl acetate and (*R*)-(–)-carvone in toluene

Sample solution: 250 mg/mL of finely powdered Myrrh in alcohol. [NOTE—Shake for 1 min, centrifuge, and filter.]

Spray reagent: Dissolve 0.5 mL of *p*-anisaldehyde in 10 mL of glacial acetic acid. Add 85 mL of methanol, and then carefully add 5 mL of sulfuric acid. [NOTE—Prepare fresh immediately before use.]

Application volume: 2 µL for the *Sample solution* and 1 µL for the *Standard solution*

Developing solvent system: Toluene and ethyl acetate (93:7)

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Wash the plate in the *Developing solvent system*, and air-dry before use.]

Spray the plate with *Spray reagent*, heat in an oven at 100° for 5 min, and examine in white light.

Acceptance criteria: The chromatogram of the *Standard solution* exhibits four well-resolved spots: an olive-brown spot due to (*E*)-anethole at an R_F value of about 0.6; an orange-brown spot due to (–)-bornyl acetate at an R_F value of about 0.5; a reddish-brown spot due to (*R*)-(–)-carvone at an R_F value of about 0.4; and a deep gray spot due to linalool at an R_F value of about 0.2.

The chromatogram of the *Sample solution* exhibits an intense purplish-red spot at an R_F value of about 0.7 and two moderately intense purplish-red spots at R_F values of about 0.5 and 0.4. The chromatogram of the *Sample solution* may exhibit other spots of varying intensities, including a spot at the origin.

• **D. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 4 µg/mL of (*E*)-anethole and 1 mg/mL of thymol in alcohol

Sample solution: Transfer 0.5 g of finely powdered Myrrh to a test tube containing 5.0 mL of alcohol, and warm the mixture in a water bath for 2–3 min. Cool, and filter.

Developing solvent system: Toluene and ethyl acetate (49:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed. Allow the plate to air-dry, and examine under UV light at 365 nm.

Acceptance criteria: The chromatogram of the *Sample solution* shows no blue-to-violet fluorescent zones in the lower third of the chromatogram (absence of *Commiphora mukul*).

CONTAMINANTS

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Limits of Elemental Impurities*: Meets the requirements

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*: NMT 5.0%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Total Ash*: NMT 10.0%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*: NMT 2%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS

• **BOTANICAL CHARACTERISTICS:** Myrrh occurs in rounded or irregular tears, or bumps of agglutinated tears, of variable sizes; brownish yellow to reddish brown, covered with some grayish or yellowish dust, externally; rich brown or reddish brown internally, sometimes marked with white spots or lines; thin splinters, translucent or almost transparent; brittle; waxy, granular, conchoidal fracture; characteristic and aromatic odor; aromatic, bitter, and acrid taste.

• **LOSS ON DRYING** ⟨731⟩

Sample: 1.0 g of powdered Myrrh

Analysis: Dry the *Sample* at 100°–105° for 2 h.

Acceptance criteria: NMT 15.0%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Alcohol-Soluble Extractives, Method 2*: 40%–70%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Water-Soluble Extractives, Method 2*: NLT 50%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Volatile Oil Determination*: NLT 6.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature, in a dry place.

• **LABELING:** Label it to indicate the species of *Commiphora* from which the oleo-gum resin was obtained. Label it to indicate that it is intended for topical and oropharyngeal use only.

Myrrh Topical Solution

» Prepare Myrrh Topical Solution as follows:

Myrrh	200 g
A mixture of Alcohol and Water (85:15)	900 mL
Alcohol, a sufficient quantity, to make	1000 mL

Macerate about 200 g of coarsely ground Myrrh with an alcohol-water mixture for 48 hours at room temperature in a suitable vessel, which is fitted with a lid and a mechanical stirrer, agitating the mixture with the stirrer. Allow the resulting mixture to stand overnight. Decant the mixture, filter, dilute the filtrate with Alcohol to 1000 mL, and mix.

Labeling—Label it to indicate that it is intended for topical and oropharyngeal use only.

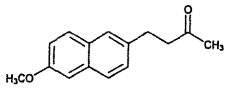
Identification—Using Topical Solution as the *Test solution*, proceed as directed for *Identification* tests *C* and *D* under *Myrrh*.

Alcohol Determination, *Method II* ⟨611⟩: between 90.0% and 110.0% of the labeled amount of C_2H_5OH.

Other requirements—It meets the requirements for *Packaging and Storage* and *Labeling* for *Tinctures* under *Botanical Extracts* ⟨565⟩.

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Nabumetone



$C_{15}H_{16}O_2$ 228.29

2-Butanone, 4-(6-methoxy-2-naphthalenyl)-;
4-(6-Methoxy-2-naphthyl)-2-butanone [42924-53-8].

DEFINITION

Nabumetone contains NLT 98.0% and NMT 101.0% of nabumetone ($C_{15}H_{16}O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Water and glacial acetic acid (999:1), filtered and degassed
Solution B: Acetonitrile and tetrahydrofuran (700:300), filtered and degassed
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
12	60	40
28	20	80
29	60	40
30	60	40

Standard solution: 1.0 mg/mL of USP Nabumetone RS in acetonitrile
Sample solution: 1.0 mg/mL of Nabumetone in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 4-µm packing L1
Flow rate: 1.3 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nabumetone ($C_{15}H_{16}O_2$) in the portion of Nabumetone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nabumetone RS in the *Standard solution* (mg/mL)
C_U = concentration of Nabumetone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1 mg/mL of USP Nabumetone RS and 1 µg/mL of USP Nabumetone Related Compound A RS in acetonitrile
Sample solution: Use the *Sample solution* from the *Assay*.
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times are about 0.9 for nabumetone related compound A and 1.0 for nabumetone; see *Table 2*.]
Suitability requirements
Resolution: NLT 1.5 between nabumetone related compound A and nabumetone
Column efficiency: NLT 3600 theoretical plates
Tailing factor: 0.8–2.0 for the nabumetone peak
Relative standard deviation: NMT 2.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Nabumetone taken:

$$\text{Result} = (F \times r_U)/\{r_N + [\Sigma(F \times r_U)]\} \times 100$$

F = relative response factor for each impurity (see *Table 2*)
r_U = peak area of each impurity from the *Sample solution*
r_N = peak area of nabumetone from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (w/w, %)
6-Methoxy-2-naphthaldehyde	0.73	0.12	0.1
4-(6′-Methoxy-2′-naphthyl)-butan-2-ol	0.85	0.94	0.1
1-(6′-Methoxy-2′-naphthyl)-but-1-en-3-one (nabumetone related compound A)	0.93	0.25	0.1
Nabumetone	1.0	—	—
5-(6′-Methoxy-2′-naphthyl)-3-methylcyclohex-2-en-1-one	1.2	0.42	0.1
5-(6′-Methoxy-2′-naphthyl)-3-methylcyclohexan-1-one	1.9	1.02	0.1
1,5-Di-(6′-methoxy-2′-naphthyl)-pentan-3-one	2.6	0.91	0.1
6,6-Dimethoxy-2,2′-binaphthyl	2.7	0.10	0.3

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (w/w, %)
Any individual unknown impurity	—	—	0.1
Total impurities	—	—	0.8

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩
  Sample: 1 g
  Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nabumetone RS
  USP Nabumetone Related Compound A RS
  1-(6′-Methoxy-2′-naphthyl)-but-1-en-3-one.
  $C_{15}H_{14}O_2$ 226.27

Nabumetone Tablets

DEFINITION
Nabumetone Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of nabumetone ($C_{15}H_{16}O_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: Prepare a solution of 6 mL of glacial acetic acid in 350 mL of water. Adjust with 1 N sodium hydroxide to a pH of 3.7, and dilute with water to 400 mL.
  Mobile phase: Acetonitrile and *Solution A* (300:200). Filter, and degas.
  Diluent: Methanol and water (900:100)
  Standard solution: 0.5 mg/mL of USP Nabumetone RS in *Diluent*
  Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 500 mg of nabumetone, to a 1000-mL volumetric flask. Add 100 mL of water, and stir with the aid of a magnetic stirrer for 5 min. Dilute with methanol to volume, stir for another 15 min, and filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 8-mm × 10-cm; 10-µm packing L1
  Flow rate: 2 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 1500 theoretical plates
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of nabumetone ($C_{15}H_{16}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

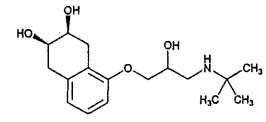
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nabumetone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nabumetone in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Sodium lauryl sulfate solution (2 in 100); 900 mL
  Apparatus 2: 50 rpm
  Time: 45 min
  Standard solution: A known concentration of USP Nabumetone RS in *Medium*
  Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium* if necessary.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Determine the amount of nabumetone ($C_{15}H_{16}O_2$) dissolved from the differences between the UV absorbances at the wavelengths of maximum and minimum absorbances at about 270 and 296 nm, respectively.
  Tolerances: NLT 75% (*Q*) of the labeled amount of nabumetone ($C_{15}H_{16}O_2$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nabumetone RS

Nadolol



$C_{17}H_{27}NO_4$ 309.40
2,3-Naphthalenediol, 5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydro-, *cis*-;
1-(*tert*-Butylamino)-3-[(5,6,7,8-tetrahydro-*cis*-6,7-dihydroxy-1-naphthyl)oxy]-2-propanol [42200-33-9].

DEFINITION
Nadolol contains NLT 98.0% and NMT 102.0% of nadolol ($C_{17}H_{27}NO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 0.1% Trifluoroacetic acid

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Mobile phase: *Acetonitrile* and *Solution A* (150:850)
Standard solution: 0.2 mg/mL of USP Nadolol RS in *Mobile phase*. Sonication and occasional hand shaking may be necessary for complete dissolution.
Sample solution: 0.2 mg/mL of Nadolol in *Mobile phase*. Sonication and occasional hand shaking may be necessary for complete dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.7 times the retention time of nadolol peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nadolol ($C_{17}H_{27}NO_4$) in the portion of Nadolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nadolol from the *Sample solution*
r_S = peak response of nadolol from the *Standard solution*
C_S = concentration of USP Nadolol RS in the *Standard solution* (mg/mL)
C_U = concentration of Nadolol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS

• **RACEMATE COMPOSITION**
Sample solution: Prepare a mineral oil dispersion of Nadolol, previously dried, adjusting the thickness of the mull to give an absorbance reading of 0.6 ± 0.1 at a wavelength of 6.3 µm.
Instrumental conditions
Mode: IR
Wavelength range: 6–9 µm
Analysis: Record the spectrum using mineral oil in the reference beam.
Sample: *Sample solution*
Calculate the percentage of racemate A in the portion of Nadolol taken:

$$\text{Result} = (A_A/A_B) \times (F/A_{av}) \times 100$$

A_A = uncorrected absorbance at the wavelength of maximum absorbance at about 7.90 µm, corresponding to racemate A
A_B = uncorrected absorbance at the wavelength of maximum absorbance at about 8.00 µm, corresponding to racemate B
F = fraction of racemate A in a (1:1) reference mixture of racemate A and racemate B, 0.5
A_{av} = average value of the absorbance of (A_A /A_B) in a (1:1) mixture of racemate A and racemate B, 0.9

Acceptance criteria: 40%–60%

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**
Solution A: Proceed as directed in the *Assay*.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)
0	85	15
3	85	15
7	50	50
10	50	50
10.5	85	15
15	85	15

Diluent: Acetonitrile and *Solution A* (15:85)
Standard solution: 0.007 mg/mL of USP Nadolol RS in *Diluent*. Sonication may be necessary for complete dissolution.
Sensitivity solution: 0.35 µg/mL of USP Nadolol RS in *Diluent* from *Standard solution*
Sample solution: 0.7 mg/mL of Nadolol in *Diluent*. Sonication and occasional hand shaking may be necessary for complete dissolution.
Chromatographic system: Proceed as directed in the *Assay* except for *Injection volume*.
Injection volume: 50 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Nadolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of nadolol from the *Standard solution*
C_S = concentration of USP Nadolol RS in the *Standard solution* (mg/mL)
C_U = concentration of Nadolol in the *Sample solution* (mg/mL)
F = relative response factor from *Table 2*

Acceptance criteria: See *Table 2*. Disregard peaks below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nadolol alcohol[a]	0.78	1.35	0.20
Nadolol	1.00	—	—
Nadolol methoxy analog[b]	1.53	1.07	0.20
Nadolol dimer[c]	1.76	1.11	0.20
Diaryl glycerol analog[d]	1.83	1.42	0.20
Naphthyl analog[e]	2.03	3.85	0.20

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dideoxy nadolol[f]	2.10	0.78	0.20
Any unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.50

[a] (2*RS*,3*SR*)-5-(2,3-Dihydroxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol.
[b] (2*RS*,3*SR*)-5-(2-Hydroxy-3-methoxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol.
[c] (2*SR*,3*RS*)-5-{3-[*tert*-Butyl(3-{[(6RS,7SR)-6,7-dihydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]oxy}-2-hydroxypropyl)amino]-2-hydroxypropoxy}-1,2,3,4-tetrahydronaphthalene-2,3-diol.
[d] (2*SR*,3*RS*)-5-(3-{[(6*RS*,7*SR*)-6,7-Dihydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]oxy}-2-hydroxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol and 1,3-Bis[(6*R*,7*S*)-6,7-dihydroxy-5,6,7,8-tetrahydronaphthalene-1-yloxy]propane-2-ol.
[e] 1-(*tert*-Butylamino)-3-(naphthalen-1-yloxy)propan-2-ol.
[f] 1-(*tert*-Butylamino)-3-(5,6,7,8-tetrahydronaphthalen-1-yloxy)propan-2-ol.

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum at 60° for 3 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Nadolol RS

Nadolol Tablets

DEFINITION

Nadolol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nadolol ($C_{17}H_{27}NO_4$).

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Standard solution: 5 mg/mL of USP Nadolol RS in 0.1 N hydrochloric acid
Sample solution: Nominally 5 mg/mL of nadolol from powdered Tablets in 0.1 N hydrochloric acid. Stir for 30 min, using a magnetic stirrer, and place in an ultrasonic bath for an additional 30 min. Centrifuge, and use the supernatant.
Chromatographic system
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 100 µL
Developing solvent system: Acetone, chloroform, and 2 N ammonium hydroxide (80:10:10)
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the *Samples* as streaks. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, allow the solvent to evaporate, and examine the chromatogram under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **Procedure**
Mobile phase: A mixture of 700 mL of methanol and 1300 mL of water containing 5.84 g of sodium chloride and 1.0 mL of 0.1 N hydrochloric acid
Standard solution: 0.2 mg/mL of USP Nadolol RS in *Mobile phase*
Sample solution: Equivalent to 0.2 mg/mL of nadolol from NLT 20 finely powdered Tablets in *Mobile phase*, prepared as follows. To a suitable amount of the powder in a suitable volumetric flask, add *Mobile phase* to fill 75% of the total volume. Place the flask in an ultrasonic bath for 15 min, shaking intermittently, and clarify the solution by filtration or centrifugation. Dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L16
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 3
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nadolol ($C_{17}H_{27}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nadolol from the *Sample solution*
r_S = peak response of nadolol from the *Standard solution*
C_S = concentration of USP Nadolol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nadolol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 50 min
Solution A: Dissolve 5.84 g of sodium chloride in 1440 mL of water.
Mobile phase: Methanol and *Solution A* (560:1440). Adjust with 0.1 N hydrochloric acid to a pH of 2.5.
Standard solution: USP Nadolol RS in *Medium*
Sample solution: Use filtered portions of the solution under test. Dilute with *Medium*, as necessary.
Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Determine the percentage of the labeled amount of nadolol ($C_{17}H_{27}NO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak response of nadolol from the *Sample solution*
r_S = peak response of nadolol from the *Standard solution*
C_S = concentration of USP Nadolol RS in the *Standard solution* (mg/mL)
L = label claim (mg/tablet)
V = volume of *Medium*, 900 mL

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D = dilution factor used in preparation of the *Sample solution*

Acceptance criteria: NLT 80% (Q) of the labeled amount of nadolol ($C_{17}H_{27}NO_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Nadolol RS

Nadolol and Bendroflumethiazide Tablets

DEFINITION

Nadolol and Bendroflumethiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of nadolol ($C_{17}H_{27}NO_4$) and bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$).

IDENTIFICATION

• **A.** The retention times of the two major peaks of the *Sample solution* correspond to the corresponding peaks of the *Standard solution*, obtained as directed in the *Assay*.

ASSAY

• **PROCEDURE**

Use low-actinic glassware for the *Sample solution* and the *Standard solution*.

Mobile phase: Transfer 5.62 g of sodium chloride and 1.97 g of anhydrous sodium acetate to 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol. Dilute with water to volume.

System suitability solution: 0.4 mg/mL each of USP Nadolol RS and USP 2,4-Disulfamyl-5-trifluoromethylaniline RS in methanol

Standard solution: 0.4 mg/mL of USP Nadolol RS and 0.4J mg/mL of USP Bendroflumethiazide RS in methanol, where J is the ratio of the labeled amount of bendroflumethiazide, in mg, to the labeled amount of nadolol, in mg/Tablet.

Sample solution: Nominally equivalent to 0.4 mg/mL of nadolol in methanol prepared as follows. Weigh and finely powder. Transfer a portion of the powder from NLT 20 Tablets, equivalent to 40 mg of nadolol, to a 100-mL volumetric flask. Add methanol, and sonicate for 15 min with occasional shaking. Dilute with methanol to volume, and centrifuge.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 30-cm; packing L11
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for nadolol and bendroflumethiazide are about 0.3 and 1.0, respectively.]

Suitability requirements
Resolution: NLT 1.4 between the solvent and 2,4-disulfamyl-5-trifluoromethylaniline peaks, NLT 1.4 between the 2,4-disulfamyl-5-trifluoromethylaniline and nadolol peaks, and NLT 1.7 between the nadolol and bendroflumethiazide peaks; *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nadolol ($C_{17}H_{27}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nadolol from the *Sample solution*
r_S = peak response of nadolol from the *Standard solution*
C_S = concentration of USP Nadolol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nadolol in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bendroflumethiazide from the *Sample solution*
r_S = peak response of bendroflumethiazide from the *Standard solution*
C_S = concentration of USP Bendroflumethiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bendroflumethiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Protect solutions from light throughout this test.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: Dissolve the USP Nadolol RS and USP Bendroflumethiazide RS in the minimal amount of methanol, and dilute with *Medium* to the desired concentrations.
Sample solutions: Sample per *Dissolution* ⟨711⟩.
Using the filtered portion of the solution under test, dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Chromatographic system, System suitability, and **Analysis:** Proceed as directed in the *Assay*.
Tolerances: NLT 80% (Q) of the labeled amounts of nadolol ($C_{17}H_{27}NO_4$) and bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Content Uniformity*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Bendroflumethiazide RS
USP 2,4-Disulfamyl-5-trifluoromethylaniline RS
$C_7H_8F_3N_3O_4S_2$ 319.29
USP Nadolol RS

USP Monographs

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Nafcillin Injection

» Nafcillin Injection is a sterile isoosmotic solution of Nafcillin Sodium and one or more buffer substances in Water for Injection. It contains dextrose as a tonicity-adjusting agent. It contains an amount of nafcillin sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of nafcillin ($C_{21}H_{22}N_2O_5S$). It contains no antimicrobial preservatives.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
USP Reference standards ⟨11⟩—
USP Nafcillin Sodium RS
Identification—The retention time of the major peak for nafcillin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.13 USP Endotoxin Unit per mg of nafcillin.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 6.0 and 8.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—
Acetic acid solution, 0.05 M Sodium acetate, Diluent, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Nafcillin Sodium*.
Assay preparation—Allow one container of Injection to thaw, and mix. Transfer an accurately measured volume of Injection, equivalent to about 40 mg of nafcillin, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Nafcillin Sodium*. Calculate the quantity, in mg, of nafcillin ($C_{21}H_{22}N_2O_5S$) in each mL of the Injection taken by the formula:

$$0.1(C/V)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of nafcillin in the *Standard preparation*; V is the volume, in mL, of Injection taken to prepare the *Assay preparation*; and r_U and r_S are the nafcillin peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Nafcillin for Injection

» Nafcillin for Injection contains an amount of Nafcillin Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of nafcillin ($C_{21}H_{22}N_2O_5S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
USP Reference standards ⟨11⟩—
USP Nafcillin Sodium RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
Identification—The retention time of the major peak for nafcillin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.13 USP Endotoxin Unit per mg of nafcillin.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 6.0 and 8.5, in the solution constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: between 3.5% and 5.3%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—
Acetic acid solution, 0.05 M Sodium acetate, Diluent, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Nafcillin Sodium*.
Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Nafcillin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Diluent* to obtain a solution having a concentration of about 0.4 mg of nafcillin ($C_{21}H_{22}N_2O_5S$) per mL.
Assay preparation 2 (where the label states the quantity of nafcillin in a given volume of constituted solution)—Constitute Nafcillin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Diluent* to obtain a solution having a concentration of about 0.4 mg of nafcillin ($C_{21}H_{22}N_2O_5S$) per mL.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Nafcillin Sodium*. Calculate the quantity, in mg, of nafcillin ($C_{21}H_{22}N_2O_5S$) in the portion of constituted Nafcillin for Injection taken by the formula:

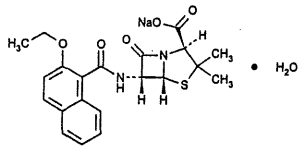
$$(C/1000)(L/D)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of nafcillin in the portion of Nafcillin for Injection taken; D is the concentration, in mg per mL, of nafcillin in *Assay preparation 1* or *Assay preparation 2*, as appropriate, based on the volume of constituted Nafcillin for Injection taken and the extent of dilution; and the other terms are as defined therein.
Perform the above procedure on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of nafcillin in a given volume of constituted solution. Use the

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individual results to determine the *Uniformity of dosage units* and the average thereof as the *Assay* value.

Nafcillin Sodium



$C_{21}H_{21}N_2NaO_5S \cdot H_2O$ 454.47
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[(2-ethoxy-1-naphthalenyl)carbonyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2*S*-(2α,5α,6β)].
Monosodium (2*S*,5*R*,6*R*)-6-(2-ethoxy-1-naphthamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [7177-50-6].
Anhydrous 436.47
[985-16-0].

» Nafcillin Sodium has a potency equivalent to not less than 820 µg of nafcillin ($C_{21}H_{22}N_2O_5S$) per mg.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Nafcillin Sodium RS

Identification—

Change to read:

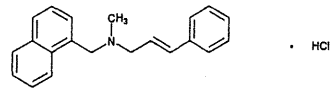
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 50 µg per mL.
Medium: water.
B: The retention time of the major peak for nafcillin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
C: It responds to the tests for *Sodium* ⟨191⟩.
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 5.0 and 7.0, in a solution containing 30 mg per mL.
Water Determination, *Method I* ⟨921⟩: between 3.5% and 5.3%.
Other requirements—Where the label states that Nafcillin Sodium is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and for *Bacterial endotoxins* under *Nafcillin for Injection*. Where the label states that Nafcillin Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Nafcillin for Injection*.

Assay—
Acetic acid solution—Prepare a 1 in 20 solution of glacial acetic acid and water.
0.05 M Sodium acetate—Dissolve 6.8 g of sodium acetate in about 800 mL of water, adjust with *Acetic acid solution* to a pH of 7.5, dilute with water to 1000 mL, and mix.
Mobile phase—Prepare a suitable filtered and degassed mixture of *0.05 M Sodium acetate* and acetonitrile (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Dissolve 6.9 g of sodium citrate in about 800 mL of water, adjust with 1 N hydrochloric acid to a pH of 7.0, dilute with water to 1000 mL, and mix.
Standard preparation—Dissolve an accurately weighed quantity of *USP Nafcillin Sodium RS* quantitatively in *Diluent* to obtain a solution having a known concentration of about 400 µg of nafcillin ($C_{21}H_{22}N_2O_5S$) per mL.
Resolution solution—Prepare a solution of orcinol in water containing about 35 mg per mL. Add 0.5 mL of this solution to 25 mL of *Standard preparation* to obtain a solution containing about 0.7 mg of orcinol and 400 µg of nafcillin per mL.
Assay preparation—Transfer about 88 mg of Nafcillin Sodium, accurately weighed, to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for orcinol and 1.0 for nafcillin; and the resolution between the orcinol and nafcillin peaks is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of nafcillin ($C_{21}H_{22}N_2O_5S$) in each mg of Nafcillin Sodium taken by the formula:

$$200(C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of nafcillin ($C_{21}H_{22}N_2O_5S$) in the *Standard preparation*; W is the weight, in mg, of the portion of Nafcillin Sodium taken; and r_U and r_S are the nafcillin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Naftifine Hydrochloride



$C_{21}H_{21}N \cdot HCl$ 323.86
1-Naphthalenemethanamine, *N*-methyl-*N*-(3-phenyl-2-propenyl)-, hydrochloride, (*E*)-;
(*E*)-*N*-Cinnamyl-*N*-methyl-1-naphthalenemethylamine hydrochloride [65473-14-5].

DEFINITION
Naftifine Hydrochloride contains NLT 99.0% and NMT 101.0% of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: *n*-Hexane, absolute alcohol, dimethylformamide, and formic acid (200:60:40:2). Cover tightly with a moisture-proof film, and allow to stand for 12 h at room temperature.

Standard solution: 0.2 mg/mL of USP Naftifine Hydrochloride RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Naftifine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L3

Flow rate: 2 mL/min

Injection volume: 15 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in the portion of Naftifine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Naftifine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Naftifine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria

Individual impurity: NMT 0.1%

Total impurities: NMT 1.0%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over phosphorus pentoxide at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Naftifine Hydrochloride RS

Naftifine Hydrochloride Cream

DEFINITION

Naftifine Hydrochloride Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in a water-miscible base.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: *n*-Hexane, alcohol, dimethylformamide, and formic acid (200:60:40:2). Cover tightly with a moisture-proof film, and allow to stand for 12 h at room temperature.

Standard solution: 0.2 mg/mL of USP Naftifine Hydrochloride RS in *Mobile phase*

Sample solution: Transfer 1000 mg of Cream to a 100-mL volumetric flask. Dissolve in 60 mL of methanol, mix vigorously for 2 min, and dilute with methanol to volume. Heat at 45° for 5 min, and cool to room temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L3

Flow rate: 2 mL/min

Injection volume: 15 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Naftifine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naftifine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED ORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **pH** ⟨791⟩: 4.0–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Naftifine Hydrochloride RS

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Naftifine Hydrochloride Gel

DEFINITION

Naftifine Hydrochloride Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in a water-miscible base.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: *n*-Hexane, alcohol, dimethylformamide, and formic acid (200:60:40:2). Cover tightly with a moisture-proof film, and allow to stand for 12 h at room temperature.

Standard solution: 0.2 mg/mL of USP Naftifine Hydrochloride RS in *Mobile phase*

Sample solution: Transfer 1000 mg of Gel to a 100-mL volumetric flask. Dissolve in 60 mL of methanol, mix vigorously for 2 min, and dilute with methanol to volume. Heat at 45° for 5 min, and cool to room temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L3

Flow rate: 2 mL/min

Injection volume: 20 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Naftifine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naftifine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS

• **CONTENT OF ALCOHOL**

Internal standard solution: Transfer 10.0 mL of *n*-propyl alcohol to a 200-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 10.0 mg/mL of alcohol

Standard solution: Transfer 3.0 mL of *Internal standard solution* to a 10-mL volumetric flask, and dilute with *Standard stock solution* to volume.

Sample solution: Transfer 250 mg of Gel to a suitable container. Add 14.0 mL of water and 6.0 mL of *Internal standard solution*, and shake for 15 min.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 3.2-mm × 1.5-m; 80- to 100-mesh support S3

Temperatures

Injector: 200°

Column: 170°

Detector: 200°

Carrier gas: Nitrogen

Flow rate: 45 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between alcohol and the internal standard

Capacity factor, *k'*: 2.0–3.5 for alcohol and 6.0–8.0 for the internal standard

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of alcohol (C_2H_5OH) in the portion of Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alcohol to the internal standard from the *Sample solution*

R_S = peak response ratio of alcohol to the internal standard from the *Standard solution*

C_S = concentration of alcohol in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 40%–45%

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED ORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

• **PH** ⟨791⟩**:** 5.5–7.5

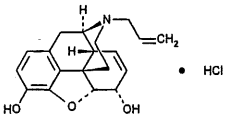
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Naftifine Hydrochloride RS

Nalorphine Hydrochloride



$C_{19}H_{21}NO_3 \cdot HCl$ 347.84

Morphinan-3,6-diol, 7,8-didehydro-4,5-epoxy-17-(2-propenyl)-(5α,6α)-, hydrochloride.

17-Allyl-7,8-didehydro-4,5α-epoxymorphinan-3,6α-diol hydrochloride [57-29-4].

» Nalorphine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{19}H_{21}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩**—**

USP Nalorphine Hydrochloride RS

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 100 µg per mL.
Medium: water.
C: A solution of it responds to the tests for *Chloride* ⟨191⟩.
Specific rotation ⟨781S⟩: between −122° and −125°.
Test solution: 20 mg per mL, in water.
Loss on drying ⟨731⟩—Dry it in vacuum at 100° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Assay—Transfer about 25 mg of Nalorphine Hydrochloride, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Nalorphine Hydrochloride RS in the same medium having a known concentration of about 100 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 285 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{19}H_{21}NO_3 \cdot HCl$ in the Nalorphine Hydrochloride taken by the formula:

$$0.25C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Nalorphine Hydrochloride RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Nalorphine Hydrochloride and the Standard solution, respectively.

Nalorphine Hydrochloride Injection

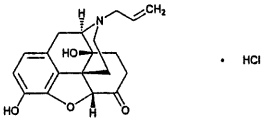
» Nalorphine Hydrochloride Injection is a suitably buffered, sterile solution of Nalorphine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nalorphine hydrochloride ($C_{19}H_{21}NO_3 \cdot HCl$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Nalorphine Hydrochloride RS
Identification—Apply 15 µL of Injection and 15 µL of a Standard solution of USP Nalorphine Hydrochloride RS in methanol containing 5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the applications to dry, and develop the chromatogram in an equilibrated chamber containing methanol until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short- and long-wavelength UV light: the R_F value of the principal spot obtained from the Injection corresponds to that obtained from the Standard solution.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 11.6 USP Endotoxin Units per mg of nalorphine hydrochloride.
pH ⟨791⟩: between 6.0 and 7.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of nalorphine hydrochloride, to a 25-mL centrifuge separator, add 1 mL of 3 N hydrochloric acid, and dilute with water to about 10 mL. Extract with five 5-mL portions of chloroform, separating the layers by centrifugation before drawing off each chloroform extract, and discard the chloroform extracts. Transfer the aqueous layer to a 100-mL volumetric flask with the aid of water, dilute with water to volume, and mix. Proceed as directed in the *Assay* under *Nalorphine Hydrochloride*, beginning with "Concomitantly determine the absorbances." Calculate the quantity, in mg, of $C_{19}H_{21}NO_3 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(0.1C/V)(A_U/A_S)$$

in which V is the volume, in mL, of Injection taken, and C, A_U, and A_S are as defined therein.

Naloxone Hydrochloride



$C_{19}H_{21}NO_4 \cdot HCl$ 363.84
Morphinan-6-one, 4,5-epoxy-3,14-dihydroxy-17-(2-propenyl)-, hydrochloride, (5α)-;
17-Allyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride [357-08-4].
Dihydrate 399.87
[51481-60-8].

DEFINITION
Naloxone Hydrochloride is anhydrous or contains two molecules of water of hydration. It contains NLT 98.0% and NMT 100.5% of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: About 150 mg
Analysis: Dissolve the *Sample* in 25 mL of water in a small separator, and add a few drops of 6 N ammonium hydroxide slowly until no more white precipitate is formed. Extract with three 5-mL portions of chloroform, and pass the extracts through a dry filter, collecting the filtrate in a small flask. Evaporate the filtrate on a steam bath to dryness, and dry the residue at 105° for 1 h.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Sample: About 300 mg

USP Monographs

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Analysis: Dissolve the *Sample*, previously dried, in a mixture of 40 mL of glacial acetic acid and 10 mL of acetic anhydride. Add 10 mL of mercuric acetate TS and 1 drop of methyl violet TS. Titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 36.38 mg of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$).
Acceptance criteria: 98.0%–100.5% on the dried basis

OTHER COMPONENTS

• **CONTENT OF CHLORIDE**
Sample: About 300 mg
Analysis: Dissolve the *Sample* in 50 mL of methanol contained in a 125-mL conical flask, and add 5 mL of glacial acetic acid and 2 drops of eosin Y TS. Titrate with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.
Acceptance criteria: 9.54%–9.94% on the dried basis

IMPURITIES

• **NOROXYMORPHONE HYDROCHLORIDE [(−)-4,5α-EPOXY-3,14-DIHYDROXYMORPHINAN-6-ONE HYDROCHLORIDE] AND OTHER IMPURITIES**
Standard solution A: 7.6 mg/mL of USP Naloxone RS in chloroform
Standard solution B: 0.084 mg/mL of USP Noroxymorphone Hydrochloride RS in dilute methanol (3 in 5)
Sample solution: Transfer about 40 mg to a 5-mL volumetric flask. Dissolve completely in 2.0 mL of water, add methanol to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel, previously activated by heating for 15 min at 105°
Application volume: 5 µL
Developing solvent system: A solution (1 in 20) of methanol in ammoniacal butanol, prepared by shaking 100 mL of butyl alcohol with 60 mL of ammonium hydroxide solution (1 in 100) and discarding the lower layer
Spray reagent: 5 mg/mL of potassium ferricyanide in ferric chloride solution (1 in 10). Prepare immediately before use.
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Develop the chromatogram, protected from light, until the solvent front has moved about 10 cm from the point of application. Remove the plate, dry thoroughly, and spray with *Spray reagent*.
Acceptance criteria: Other than the principal spot corresponding in R_F value to that of USP Naloxone RS and the spot at the origin (ammonium chloride), no other spot is more intense than the spot corresponding to that of USP Noroxymorphone Hydrochloride RS (1.0%).

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 25 mg/mL in water
Acceptance criteria: −170° to −181°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 0.5% for the anhydrous form and NMT 11.0% for the hydrous form

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Naloxone RS
USP Noroxymorphone Hydrochloride RS

Naloxone Hydrochloride Injection

DEFINITION

Naloxone Hydrochloride Injection is a sterile, isotonic solution of Naloxone Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$). It may contain suitable preservatives.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV absorption spectrum of the naloxone peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: A mixture of 1.36 g of sodium 1-octanesulfonate (anhydrous), 1.0 g of sodium chloride, 580 mL of water, 420 mL of methanol, and 1.0 mL of phosphoric acid
Diluent: Transfer 150 mg of edetate disodium to a 2000-mL volumetric flask, and add 0.9 mL of hydrochloric acid. Dilute with water to volume, and mix.
Standard solution: 10 µg/mL of USP Naloxone RS in *Diluent*
Sample solution: Nominally 10 µg/mL of naloxone hydrochloride prepared as follows. Transfer an adequate volume from NLT 5 Injections to a suitable volumetric flask. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 229 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of naloxone from the *Sample solution*
r_S = peak area of naloxone from the *Standard solution*
C_S = concentration of USP Naloxone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of naloxone hydrochloride in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of naloxone hydrochloride (anhydrous), 363.84
M_{r2} = molecular weight of naloxone (anhydrous), 327.38

USP Monographs

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Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **LIMIT OF 2,2′-BISNALOXONE**

Standard solution A: Prepare as directed for the *Standard solution* in the *Assay*.

Mobile phase, Diluent, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* by using *Standard solution A* in place of the *Standard solution*.

Standard solution B: 0.2 µg/mL of USP Naloxone RS in *Diluent* from *Standard solution A*

Ferric chloride solution: 4% (v/v) ferric chloride TS in water

Peak identification solution: Dissolve 10 mg of naloxone in 100 mL of 0.1 N hydrochloric acid. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, and add 0.5 mL of the *Ferric chloride solution*. Heat on a steam bath for 10 min, cool, dilute with water to volume, and mix.

Sample solution: Nominally 10 µg/mL of naloxone hydrochloride prepared as follows. Transfer an adequate volume from NLT 5 Injections to a suitable volumetric flask. Dilute with *Diluent* to volume.

Analysis

Samples: *Standard solution B, Peak identification solution,* and *Sample solution*

[NOTE—The relative retention times for naloxone and 2,2′-bisnaloxone (4,5′:4′,5″-diepoxy-3,3′,14,14′-tetrahydroxy-17,17′-bis(prop-2-enyl)-2,2′-bimorphinanyl-6,6′-dione) are 1.0 and 2.8, respectively.]

Calculate the percentage of 2,2′-bisnaloxone in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of 2,2′-bisnaloxone from the *Sample solution*

r_S = peak area of naloxone from *Standard solution B*

C_S = concentration of USP Naloxone RS in *Standard solution B* (µg/mL)

C_U = nominal concentration of naloxone hydrochloride in the *Sample solution* (µg/mL)

F = relative response factor of 2,2′-bisnaloxone to naloxone hydrochloride, 1.8

M_{r1} = molecular weight of naloxone hydrochloride (anhydrous), 363.84

M_{r2} = molecular weight of naloxone (anhydrous), 327.38

Acceptance criteria: NMT 4.0%

SPECIFIC TESTS

• **PH** ⟨791⟩: 3.0–6.5

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 500 USP Endotoxin Units/mg of naloxone hydrochloride

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

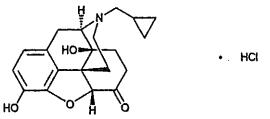
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers of Type I glass, protected from light, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Naloxone RS

Naltrexone Hydrochloride



$C_{20}H_{23}NO_4 \cdot HCl$ 377.86

Morphinan-6-one, 17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxy-, hydrochloride, (5α)-;
17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy morphinan-6-one hydrochloride [16676-29-2].

Free base

$C_{20}H_{23}NO_4$ 341.41

[16590-41-3].

Dihydrate

$C_{20}H_{23}NO_4 \cdot HCl \cdot 2H_2O$ 413.89

[850808-02-5].

DEFINITION

Naltrexone Hydrochloride contains NLT 98.0% and NMT 102.0% of naltrexone hydrochloride ($C_{20}H_{23}NO_4 \cdot HCl$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

Sample: Dissolve 150 mg of Naltrexone Hydrochloride in 25 mL of water in a small separator. Add a few drops of 6 N ammonium hydroxide slowly until no more white precipitate is formed. Extract with three 5-mL portions of chloroform, and pass the extracts through a dry filter, collecting the filtrate in a small flask. Evaporate the filtrate on a steam bath to dryness, and dry the residue at 105° for 1 h.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the naltrexone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Dissolve about 1.08 g of sodium 1-octanesulfonate and about 23.8 g of sodium acetate in 800 mL of water. Add 1.0 mL of triethylamine and 200 mL of methanol, and adjust with glacial acetic acid to a pH of 6.5 ± 0.1.

Solution B: Dissolve about 1.08 g of sodium 1-octanesulfonate and about 23.8 g of sodium acetate in 400 mL of water. Add 1.0 mL of triethylamine and 600 mL of methanol, and adjust with glacial acetic acid to a pH of 6.5 ± 0.1.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	0	100
36	100	0
53	100	0

USP Monographs

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Standard solution: 2.25 mg/mL of USP Naltrexone RS prepared as follows. Transfer an amount of USP Naltrexone RS to a suitable volumetric flask, add 15% of the final volume of methanol and 6% of the final volume of 0.1 N hydrochloric acid, and dissolve by swirling. Dilute with 0.1 M phosphoric acid to volume.

System suitability stock solution: 0.3 mg/mL of USP Naltrexone Related Compound A RS prepared as follows. Transfer a quantity of USP Naltrexone Related Compound A RS to a suitable volumetric flask, add 30% of the final volume of methanol, and dissolve by swirling. Dilute with 0.1 M phosphoric acid to volume.

System suitability solution: 1.125 mg/mL of USP Naltrexone RS and 0.015 mg/mL of USP Naltrexone Related Compound A RS prepared as follows. Transfer suitable volumes of *System suitability stock solution* and *Standard solution* to an adequate volumetric flask, and dilute with 0.1 M phosphoric acid to volume.

Sample solution: 2.5 mg/mL of Naltrexone Hydrochloride in 0.1 M phosphoric acid

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for naltrexone and naltrexone related compound A are 1.0 and 1.26, respectively.]
Suitability requirements
Resolution: NLT 2.0 between naltrexone and naltrexone related compound A, *System suitability solution*
Tailing factor: NMT 1.4, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of naltrexone hydrochloride ($C_{20}H_{23}NO_4 \cdot HCl$) in the portion of Naltrexone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of naltrexone from the *Sample solution*
r_S = peak response of naltrexone from the *Standard solution*
C_S = concentration of USP Naltrexone RS in the *Standard solution* (mg/mL)
C_U = concentration of Naltrexone Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of naltrexone hydrochloride, 377.86
M_{r2} = molecular weight of naltrexone, 341.41

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF TOTAL SOLVENTS:** The sum of water and total solvents (methanol and ethanol) is NMT 5.0% for the anhydrous form and NMT 11.0% for the dihydrate form.
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Standard solution, System suitability stock solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 2.25 µg/mL of USP Naltrexone RS in 0.1 M phosphoric acid from *Standard solution*

System suitability
Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between naltrexone and naltrexone related compound A, *System suitability solution*
Tailing factor: NMT 1.4, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Naltrexone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of naltrexone from the *Standard solution*
C_S = concentration of USP Naltrexone RS in the *Standard solution* (mg/mL)
C_U = concentration of Naltrexone Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Noroxymorphone[a]	0.55	1.0	0.5
10-Hydroxynaltrexone[b]	0.70	1.0	0.5
Naltrexone	1.00	1.0	—
Naltrexone related compound A	1.26	1.0	0.5
2,2′-Bisnaltrexone[c]	1.80	2.3	0.5
10-Ketonaltrexone[d]	1.99	4.0	0.5
Any unspecified impurity	—	1.0	0.5
Total impurities	—	—	1.5

[a] 4,5α-Epoxy-3,14-dihydroxymorphinan-6-one.
[b] 17-Cyclopropylmethyl-4,5α-epoxy-3,10,14-trihydroxymorphinan-6-one.
[c] 17,17′-Bis(cyclopropylmethyl)-4,5α:4′,5′α-diepoxy-3,3′,14,14′-tetrahydroxy-2,2′-bimorphinanyl-6,6′-dione.
[d] 17-Cyclopropylmethyl-4,5α-epoxy-3,14-dihydroxymorphinan-6,10-dione.

SPECIFIC TESTS

- **CONTENT OF CHLORIDE**

Sample solution: Transfer about 300 mg of Naltrexone Hydrochloride to a 250-mL conical flask, add 50 mL of methanol, 50 mL of water, and 3 mL of nitric acid. Mix to dissolve.

Analysis: Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each milliliter of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.

Acceptance criteria: 9.20%–9.58%, calculated on the anhydrous, solvent-free basis

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• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 25 mg/mL of Naltrexone Hydrochloride in water
Acceptance criteria: −187° to −197°, calculated on the anhydrous, solvent-free basis
• **WATER DETERMINATION** ⟨921⟩, *Method I*: Determine the water content as directed. [NOTE—The result of this test is used in the calculation in the test for *Limit of Total Solvents*.]
• **CLARITY OF SOLUTION**
Sample solution: Dissolve 1.0 g of Naltrexone Hydrochloride in 50 mL of water.
Analysis: Determine the turbidity of the *Sample solution* (see *Nephelometry, Turbidimetry, and Visual Comparison* ⟨855⟩).
Acceptance criteria: NMT 3 NTUs

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Naltrexone RS
USP Naltrexone Related Compound A RS
17-(But-3-en-1-yl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride.
$C_{20}H_{23}NO_4 \cdot HCl$ 377.86

Naltrexone Hydrochloride Tablets

DEFINITION
Naltrexone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of naltrexone hydrochloride ($C_{20}H_{23}NO_4 \cdot HCl$).

IDENTIFICATION

Change to read:

• **▲A.▲** (USP 1-Aug-2019) The retention time of the naltrexone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained by the *Assay*.

Add the following:

▲• B. The UV spectrum of the naltrexone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Dissolve about 1.08 g of sodium 1-octanesulfonate and about 23.8 g of sodium acetate in 800 mL of water. Add 1.0 mL of triethylamine and 200 mL of methanol, and adjust with glacial acetic acid to a pH of 6.5 ± 0.1.
Solution B: Dissolve about 1.08 g of sodium 1-octanesulfonate and about 23.8 g of sodium acetate in 400 mL of water. Add 1.0 mL of triethylamine and 600 mL of methanol, and adjust with glacial acetic acid to a pH of 6.5 ± 0.1.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	0	100
36	100	0
53	100	0

Standard solution: 2.25 mg/mL of USP Naltrexone RS prepared as follows. Transfer an amount of USP Naltrexone RS to a suitable volumetric flask, add 15% of the final volume of methanol and 6% of the final volume of 0.1 N hydrochloric acid, and dissolve by swirling. Dilute with 0.1 M phosphoric acid to volume.
System suitability stock solution: 0.3 mg/mL of USP Naltrexone Related Compound A RS prepared as follows. Transfer a quantity of USP Naltrexone Related Compound A RS to a suitable volumetric flask, add 30% of the final volume of methanol, and dissolve by swirling. Dilute with 0.1 M phosphoric acid to volume.
System suitability solution: 1.125 mg/mL of USP Naltrexone RS and 0.015 mg/mL of USP Naltrexone Related Compound A RS prepared as follows. Transfer suitable volumes of *System suitability stock solution* and *Standard solution* to an adequate volumetric flask, and dilute with 0.1 M phosphoric acid to volume.
Sample solution: Nominally 2.5 mg/mL of naltrexone hydrochloride in 0.1 M phosphoric acid prepared as follows. Transfer NLT 20 Tablets to a tared container, and determine the average Tablet weight. Grind the Tablets to a homogeneous mixture. Transfer a portion of the powdered Tablets, equivalent to 250 mg of naltrexone hydrochloride, to a 100-mL volumetric flask. Add 80 mL of 0.1 M phosphoric acid and shake or sonicate for at least 30 min. Dilute with 0.1 M phosphoric acid to volume, mix, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)
Column: 3.9-mm × 15-cm; ▲4-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: ▲*Standard solution* and▲ (USP 1-Aug-2019) *System suitability solution*
[NOTE—The relative retention times for ▲naltrexone and naltrexone related compound A are 1.0 and 1.26, respectively.▲ (USP 1-Aug-2019)]
Suitability requirements
Resolution: NLT 2.0 between naltrexone and naltrexone related compound A, ▲*System suitability solution*▲ (USP 1-Aug-2019)
Tailing factor: NMT 1.4, ▲*Standard solution*▲ (USP 1-Aug-2019)
Relative standard deviation: NMT 2.0%, ▲*Standard solution*▲ (USP 1-Aug-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naltrexone hydrochloride ($C_{20}H_{23}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of naltrexone from the *Sample solution*
r_S = peak response of naltrexone from the *Standard solution*

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C_S = concentration of USP Naltrexone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naltrexone hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of naltrexone hydrochloride, 377.86
M_{r2} = molecular weight of naltrexone, ▲341.41▲ (USP 1-Aug-2019)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Standard solution: A known concentration of USP Naltrexone RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Buffer: 0.05 M phosphate buffer (Dissolve 7.0 g of monobasic sodium phosphate in 1 L of water.)
Mobile phase: Mix 600 mL of *Buffer*, 1.1 g of sodium 1-octane sulfonate monohydrate, and 400 mL of methanol. Adjust with dilute sodium hydroxide to a pH of 6.7 ± 0.05.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 15-cm; packing L1
Temperature: 45°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of the labeled amount of naltrexone hydrochloride ($C_{20}H_{23}NO_4 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of naltrexone from the *Sample solution*
r_S = peak response of naltrexone from the *Standard solution*
C_S = concentration of USP Naltrexone RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of naltrexone hydrochloride, 377.86
M_{r2} = molecular weight of naltrexone, 341.41
L = label claim (mg/Tablet)

▲ (USP 1-Aug-2019)
Tolerances: NLT 80% (*Q*) of the labeled amount of naltrexone hydrochloride ($C_{20}H_{23}NO_4 \cdot HCl$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

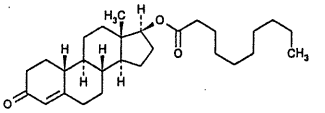
Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers.
▲Store at controlled room temperature.▲ (USP 1-Aug-2019)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Naltrexone RS
USP Naltrexone Related Compound A RS
▲17-(But-3-en-1-yl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride.▲ (USP 1-Aug-2019)
$C_{20}H_{23}NO_4 \cdot HCl$ ▲377.86▲ (USP 1-Aug-2019)

Nandrolone Decanoate



$C_{28}H_{44}O_3$ 428.65
Estr-4-en-3-one, 17-[(1-oxodecyl)oxy]-, (17β)-.
17β-Hydroxyestr-4-en-3-one decanoate [360-70-3].

» Nandrolone Decanoate contains not less than 97.0 percent and not more than 103.0 percent of $C_{28}H_{44}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a refrigerator.
USP Reference standards ⟨11⟩—
USP Nandrolone RS
USP Nandrolone Decanoate RS
Completeness and clarity of solution—A solution in dioxane (1 in 50) is clear.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: alcohol.
Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: Prepare a solution in acetone containing 5 mg per mL. Apply 10 µL of this solution and 10 µL of a solution of USP Nandrolone Decanoate RS in acetone containing 5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-heptane and acetone (3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the

USP Monographs

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solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with a solution of sulfuric acid in alcohol (1 in 50) and heating in an oven at 110° for 15 minutes: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Melting range ⟨741⟩: between 33° and 37°.

Specific rotation ⟨781S⟩: between +32° and +36°.

Test solution: 10 mg per mL, previously dried in dioxane.

Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.5% of its weight.

Chromatographic purity—

Mobile phase—Prepare a filtered and degassed mixture of chromatographic *n*-heptane and *n*-propyl alcohol (HPLC grade) (97:3). Make adjustments if necessary (see *System suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve accurately weighed quantities of USP Nandrolone Decanoate RS, dimethyl phthalate, and USP Nandrolone RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.25 mg per mL, 0.25 mg per mL, and 0.16 mg per mL, respectively.

Test solution—Transfer about 13 mg of Nandrolone Decanoate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.67 for dimethyl phthalate and 1.0 for nandrolone decanoate; the resolution, *R,* between dimethyl phthalate and nandrolone decanoate is not less than 9.0, and the nandrolone peak elutes before 4.5 times the elution time of nandrolone decanoate; the tailing factor is not more than 1.3 for the nandrolone decanoate and dimethyl phthalate peaks; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Nandrolone Decanoate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity, and r_s is the sum of the responses of all of the peaks: the sum of all impurities is not more than 3.0%.

Assay— [NOTE—Use low-actinic glassware throughout this procedure.]

Mobile phase—Prepare a filtered and degassed mixture of methanol and water (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer an accurately weighed quantity of USP Nandrolone Decanoate RS to a suitable volumetric flask, and dilute with methanol to volume to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of about 20 mg of Nandrolone Decanoate to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and an 8-mm × 10-cm analytical column containing packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, is not less than 1.3; the column efficiency is not less than 8000 theoretical plates; the tailing factor is not less than 0.9 and not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{28}H_{44}O_3$ in the portion of Nandrolone Decanoate taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Nandrolone Decanoate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nandrolone Decanoate Injection

» Nandrolone Decanoate Injection is a sterile solution of Nandrolone Decanoate in Sesame Oil, with a suitable preservative. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nandrolone decanoate ($C_{28}H_{44}O_3$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Nandrolone RS
USP Nandrolone Decanoate RS

Identification—Dilute a volume of Injection with acetone to provide a solution containing approximately 5 mg of nandrolone decanoate per mL. This solution responds to *Identification* test *C* under *Nandrolone Decanoate*, 5-µL portions of the test solution and the Standard solution being used.

Limit of nandrolone—

Standard preparation—Dissolve 25.0 mg of USP Nandrolone RS in 50.0 mL of acetone. Dilute 5.0 mL of this solution with acetone to 50.0 mL, and mix.

Test preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of nandrolone decanoate, to a 10-mL volumetric flask, dilute with acetone to volume, and mix.

Procedure—Apply 10 µL each of the *Standard preparation* and of the *Test preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-heptane and acetone (3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Return the dry plate to the developing chamber containing the same solvent system, and again develop the chromatogram until the solvent front has moved the same distance from the origin. Remove the plate from the developing chamber, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with a 4 in 10

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solution of sulfuric acid in methanol and heating at about 100° for 10 minutes. Cool, and examine under long-wavelength UV light: any yellow fluorescent spot from the *Test preparation* at an R_f value of about 0.2 is not greater in size or intensity than that produced by the *Standard preparation* at the same R_f value, corresponding to not more than 1.0% of nandrolone.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

0.02 M Ammonium acetate solution—Transfer about 1.6 g of ammonium acetate to a 1-liter volumetric flask. Dissolve in and dilute with water to volume.

Mobile phase—Prepare a filtered and degassed mixture of alcohol and *0.02 M Ammonium acetate solution* (66:34). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Nandrolone Decanoate RS with tetrahydrofuran, and dilute quantitatively and stepwise if necessary, with tetrahydrofuran to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 400 mg of nandrolone decanoate to a 200-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix.

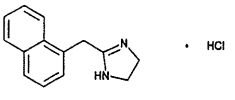
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column containing 5-µm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, for nandrolone decanoate is not less than 5.3; the tailing factor for the Nandrolone Decanoate peak is not more than 1.4; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{28}H_{44}O_3$ in each mL of the Injection taken by the formula:

$$2000(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Nandrolone Decanoate RS in the *Standard preparation; V* is the volume, in mL, of the injection taken to prepare the *Assay preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Naphazoline Hydrochloride



$C_{14}H_{14}N_2 \cdot HCl$ 246.74

1*H*-Imidazole, 4,5-dihydro-2-(1-naphthalenylmethyl)-, monohydrochloride;

2-(1-Naphthylmethyl)-2-imidazoline monohydrochloride [550-99-2].

DEFINITION

Naphazoline Hydrochloride contains NLT 98.0% and NMT 102.0% of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Buffer: In a 1000-mL volumetric flask, dissolve 3.0 g of monobasic potassium phosphate in 800 mL of water. Add 3.0 mL of triethylamine, adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.
  Mobile phase: Acetonitrile and *Buffer* (20:80)
  Standard solution: 0.05 mg/mL of USP Naphazoline Hydrochloride RS in water
  Sample solution: 0.05 mg/mL of Naphazoline Hydrochloride in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 4.0-mm × 25-cm; packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Naphazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
- **ORGANIC IMPURITIES**
  Solution A: Acetonitrile, glacial acetic acid, and water (30: 0.5: 70)
  Mobile phase: 1.1 g/L of anhydrous sodium 1-octanesulfonate in *Solution A*
  System suitability solution: 0.025 mg/mL of USP Naphazoline Hydrochloride RS and 0.05 mg/mL of 1-naphthylacetic acid in *Mobile phase*

USP Monographs

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Standard solution: 0.5 µg/mL each of USP Naphazoline Hydrochloride RS and USP Naphazoline Related Compound A RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Naphazoline Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 25-cm; 3-µm or 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of the naphazoline peak
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 5.0 between the naphazoline and 1-naphthylacetic acid peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of naphazoline related compound A in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naphazoline related compound A from the *Sample solution*
r_S = peak response of naphazoline related compound A from the *Standard solution*
C_S = concentration of USP Naphazoline Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Naphazoline Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of naphazoline from the *Standard solution*
C_S = concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Naphazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Naphazoline related compound A	0.76	0.1
Naphazoline	1.0	—
1-Naphthylacetic acid	1.4	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 10 mg/mL in carbon dioxide-free water
Acceptance criteria: 5.0–6.6. The *Sample solution* is clear and colorless.
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Naphazoline Hydrochloride RS
USP Naphazoline Related Compound A RS
N-(2-Aminoethyl)-2-(naphthalen-1-yl)acetamide.
$C_{14}H_{16}N_2O$ 228.29

Naphazoline Hydrochloride Nasal Solution

DEFINITION
Naphazoline Hydrochloride Nasal Solution is a solution of Naphazoline Hydrochloride in water adjusted to a suitable pH and tonicity. It contains NLT 90.0% and NMT 110.0% of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 400 mL of water. Add 250 mL of acetonitrile and 10 mL of glacial acetic acid, and dilute with water to 1000 mL. Sonicate for 10 min to obtain a solution having a pH of about 3.5.
Standard solution: 0.25 mg/mL of USP Naphazoline Hydrochloride RS in water
Sample solution: Equivalent to 0.25 mg/mL of naphazoline hydrochloride in water from Nasal Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4-mm × 30-cm; packing L11
Flow rate: 2 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Nasal Solution taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naphazoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Naphazoline Hydrochloride RS

Naphazoline Hydrochloride Ophthalmic Solution

DEFINITION
Naphazoline Hydrochloride Ophthalmic Solution is a sterile, buffered solution of Naphazoline Hydrochloride in water adjusted to a suitable tonicity. It contains NLT 90.0% and NMT 115.0% of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$). It contains a suitable preservative.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Dissolve 3 g of monobasic potassium phosphate in 1 L of water, and add 3 mL of triethylamine. Adjust with phosphoric acid to a pH of 3.
Mobile phase: Acetonitrile and *Buffer* (20:80)
Standard solution: 0.05 mg/mL of USP Naphazoline Hydrochloride RS in *Mobile phase*
Sample solution: Equivalent to 0.05 mg/mL of naphazoline hydrochloride in *Mobile phase* from Ophthalmic Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm or diode array. [NOTE—Use diode array detector to perform *Identification* test *B*.]
Column: 4.6-mm × 15-cm; packing L10
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naphazoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 5.5–7.0
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Naphazoline Hydrochloride RS

Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution

DEFINITION
Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution is a sterile, buffered solution of Naphazoline Hydrochloride and Pheniramine Maleate in water adjusted to a suitable tonicity. It contains NLT 90.0% and NMT 110.0% of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) and pheniramine maleate ($C_{16}H_{20}N_2 \cdot C_4H_4O_4$). It contains a suitable preservative.

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 1.5 mg/mL of USP Naphazoline Hydrochloride RS in water
Standard solution B: 6.0 mg/mL of USP Pheniramine Maleate RS in water
Sample solution: Equivalent to 0.25 mg/mL of naphazoline hydrochloride and 3 mg/mL of pheniramine maleate in water from Ophthalmic Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of silica gel on a 20-cm × 20-cm chromatographic plate
Application volume: 5 µL of *Standard solution A*, 10 µL of *Standard solution B*, and 30 µL of the *Sample solution*
Developing solvent system: Methanol, acetic acid, and water (8:1:1)
Spray reagent: Ninhydrin TS
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Allow the spots to dry, then place the plate in a saturated chromatographic chamber, and develop in the *Developing solvent system* until the solvent front has moved to 1.5 cm from the top of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray with *Spray reagent*, and place in an oven at 105° to visualize the spots. Both the naphazoline and pheniramine spots are purplish gray in color.
Acceptance criteria: The R_F values of the spots of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*.

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• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer solution: Dissolve 14.2 g of anhydrous dibasic sodium phosphate and 20 mL of triethylamine in 1900 mL of water. Adjust with phosphoric acid to a pH of 5.6 ± 0.1, and dilute with water to 2000 mL.

Mobile phase: Acetonitrile and *Buffer solution* (20:80)

Standard stock solution A: 0.75 mg/mL of USP Naphazoline Hydrochloride RS in *Mobile phase*

Standard stock solution B: 3.00 mg/mL of USP Pheniramine Maleate RS in *Mobile phase*

Standard solution: 0.03 mg/mL of naphazoline hydrochloride and 0.36 mg/mL of pheniramine maleate in *Mobile phase* prepared as follows. Transfer 1.0 mL of *Standard stock solution A* and 3.0 mL of *Standard stock solution B* to a 25-mL volumetric flask. Dilute with *Mobile phase* to volume.

Sample solution: Transfer a volume of Ophthalmic Solution, equivalent to 0.75 mg of naphazoline hydrochloride and 9.0 mg of pheniramine maleate, to a 25-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; packing L7

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2 between the naphazoline and pheniramine peaks

Column efficiency: NLT 750 theoretical plates for the naphazoline and pheniramine peaks

Tailing factor: NMT 2.5 for pheniramine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naphazoline from the *Sample solution*

r_S = peak response of naphazoline from the *Standard solution*

C_S = concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naphazoline hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of pheniramine maleate ($C_{16}H_{20}N_2 \cdot C_4H_4O_4$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pheniramine from the *Sample solution*

r_S = peak response of pheniramine from the *Standard solution*

C_S = concentration of USP Pheniramine Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pheniramine maleate in the *Sample solution* (mg/mL)

Acceptance criteria

Naphazoline hydrochloride: 90.0%–110.0%

Pheniramine maleate: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 5.7–6.3

• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

ADDITIONAL REQUIREMENTS

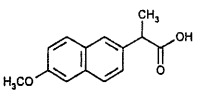
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature between 20° and 25°, protected from light.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Naphazoline Hydrochloride RS

USP Pheniramine Maleate RS

Naproxen



$C_{14}H_{14}O_3$ 230.26

2-Naphthaleneacetic acid, 6-methoxy-α-methyl-, (*S*)-.

(+)-(*S*)-6-Methoxy-α-methyl-2-naphthaleneacetic acid [22204-53-1].

» Naproxen contains not less than 98.5 percent and not more than 101.5 percent of $C_{14}H_{14}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**

USP Naproxen RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 25 µg per mL.

Medium:methanol.

Absorptivities at 271 nm, calculated on the dried basis, do not differ by more than 3%.

Specific rotation ⟨781S⟩**:** between +83.0° and +89.5°.

Test solution: 10 mg per mL, in methyl isobutyl ketone.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Chromatographic purity—Dissolve 100 mg of Naproxen in methanol, and dilute with methanol to 5.0 mL to obtain the *Test solution*. Dissolve a suitable quantity of USP Naproxen RS in methanol to obtain a *Standard solution* having a known concentration of about 20 mg per mL. Dilute a portion of this solution quantitatively and stepwise with methanol to obtain three *Comparison solutions* having concentrations of 20, 60,

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and 100 µg per mL (0.1%, 0.3%, and 0.5% of the *Standard solution*), respectively. Apply separate 10-µL portions of the five solutions to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of toluene, tetrahydrofuran, and glacial acetic acid (30:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, air-dry, and view under short-wavelength UV light: the R_f value of the principal spot in the chromatogram of the *Test solution* corresponds to that of the *Standard solution,* and any other spot obtained from the *Test solution* does not exceed, in size or intensity, the principal spot obtained from the 100-µg-per-mL *Comparison solution* (0.5%), and the sum of the intensities of any secondary spots, similarly compared, does not exceed 2.0%.

Assay—Dissolve about 500 mg of Naproxen, accurately weighed, in a mixture of 75 mL of methanol and 25 mL of water that has been previously neutralized to the phenolphthalein endpoint with 0.1 N sodium hydroxide. Dissolve by gentle warming, if necessary, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 23.03 mg of $C_{14}H_{14}O_3$.

Add the following:

▲Naproxen Compounded Oral Suspension

DEFINITION

Naproxen Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of naproxen ($C_{14}H_{14}O_3$). Prepare Naproxen Compounded Oral Suspension containing 25 mg/mL of naproxen as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Naproxen tablets[a] equivalent to	2500 mg of naproxen
Vehicle: Oral Mix[b] or Oral Mix SF[b], a sufficient quantity to make	100 mL

[a] Naproxen 250-mg tablets, Apotex Inc., Weston, Ontario.
[b] Medisca Pharmaceutique Inc., Montréal, Quebec.

Place the *Naproxen tablets* in a suitable container and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Buffer: 5 mM solution of ammonium formate
Mobile phase: Methanol and *Buffer* (82:18)
Internal standard solution: 1.0 mg/mL of USP Ranitidine Hydrochloride RS in water
Standard stock solution: 25 mg/mL of USP Naproxen RS in methanol
Standard solution: Transfer 0.1 mL of *Standard stock solution* to a 100-mL volumetric flask, add 10-mL of the *Internal standard solution,* and dilute with methanol to volume to obtain a solution containing 0.025 mg/mL of naproxen and 0.1 mg/mL of ranitidine hydrochloride. Pass through a hydrophilic propylene filter of 0.45-µm pore size.
Sample solution: Add 0.2 mL of Oral Suspension to 1.8 mL of methanol and centrifuge at 5200 rpm for 5 min. Transfer 0.1 mL of the supernatant to a 10-mL volumetric flask, add 1 mL of the *Internal standard solution* and dilute with methanol to volume to obtain a solution containing 0.025 mg/mL of naproxen and 0.1 mg/mL of ranitidine hydrochloride. Filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 232 nm
Columns
Guard: 3.9-mm × 2-cm; packing L1
Analytical: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: See *Table 1.*

Table 1

Time (min)	Flow Rate (mL/min)
0	0.8
1	0.85
2	0.9
3	1

Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention times for ranitidine hydrochloride and naproxen are about 1.4 and 2.4 min, respectively.]
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of naproxen to the internal standard from the *Sample solution*
R_S = peak response ratio of naproxen to the internal standard from the *Standard solution*
C_S = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
• **LABELING:** Label it to state the *Beyond-Use Date.*

USP Monographs

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Naproxen RS
USP Ranitidine Hydrochloride RS▲ (USP 1-Aug-2019)

Naproxen Oral Suspension

DEFINITION
Naproxen Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of naproxen ($C_{14}H_{14}O_3$).

IDENTIFICATION
• **A.**
Sample solution: *Standard solution* and *Sample solution* (1:1), prepared as directed in the *Assay*
Analysis: Chromatograph as directed in the *Assay*.
Acceptance criteria: The chromatogram of the *Sample solution* exhibits two main peaks corresponding to naproxen and the internal standard.

ASSAY
• **PROCEDURE**
Mobile phase: Prepare a mixture of 500 mL of methanol, 500 mL of water, and 2.46 g of anhydrous sodium acetate, and mix until dissolved. Adjust with glacial acetic acid to a pH of 5.8.
Internal standard solution: 1.1 mg/mL of ethylparaben in methanol
Standard stock solution: Transfer about 62.5 mg of USP Naproxen RS, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of methanol, and sonicate to dissolve. Add 5.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.
Standard solution: 0.05 mg/mL of USP Naproxen RS and 0.0044 mg/mL of ethylparaben in *Mobile phase* from *Standard stock solution*
Sample stock solution: Transfer an accurately measured volume of Oral Suspension, previously well-mixed and free from air bubbles, nominally equivalent to about 125 mg of naproxen, to a 100-mL volumetric flask, using a "to contain" pipet. Rinse the pipet several times with methanol, and add the rinsings to the volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.
Sample solution: Nominally equivalent to 0.05 mg/mL of naproxen and 0.0044 mg/mL of ethylparaben in *Mobile phase* from *Sample stock solution*. Filter if necessary to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 35 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethylparaben and naproxen are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between ethylparaben and naproxen
Tailing factor: NMT 2.0 for the naproxen peak
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of naproxen to ethylparaben from the *Sample solution*
R_S = peak response ratio of naproxen to ethylparaben from the *Standard solution*
C_S = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for oral suspension packaged in multiple-unit containers
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for oral suspension packaged in single-unit containers

SPECIFIC TESTS
• **PH** ⟨791⟩**:** 2.2–3.7

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Naproxen RS

Naproxen Tablets

DEFINITION
Naproxen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of naproxen ($C_{14}H_{14}O_3$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, water, and glacial acetic acid (450:540:10)
Standard solution: 0.1 mg/mL of USP Naproxen RS in *Mobile phase*
Sample stock solution: Nominally equivalent to 1 mg/mL of naproxen in *Mobile phase*. Transfer an amount equivalent to about 500 mg of naproxen, from NLT 10 finely powdered Tablets, to a 500-mL volumetric flask. Add about 300 mL of *Mobile phase*, and sonicate for 30 min. Cool to room temperature, and dilute with *Mobile phase* to volume.
Sample solution: Nominally equivalent to 0.1 mg/mL of naproxen in *Mobile phase* from *Sample stock solution*. Pass through a suitable filter of 0.45-µm of pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254-nm diode array
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.2 mL/min

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Injection volume: 20 µL
Run time: NLT 2 times the retention time of naproxen
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naproxen from the *Sample solution*
r_S = peak response of naproxen from the *Standard solution*
C_S = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Buffer: 0.1 M of pH 7.4 phosphate buffer prepared as follows. Dissolve 2.62 g of monobasic sodium phosphate and 11.50 g of anhydrous dibasic sodium phosphate in 1000 mL of water, and mix.
Medium: *Buffer*; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: A known concentration of USP Naproxen RS in *Buffer*
Sample solution: Filter portions of the solution under test, and suitably dilute with *Buffer*.
Instrumental conditions
Mode: UV
Analytical wavelength: About 332 nm (maximum absorbance)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of naproxen ($C_{14}H_{14}O_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Buffer: Dissolve 1.36 g of monobasic potassium phosphate in 1 L of water. Adjust with triethylamine to a pH of 6.5. Pass through a suitable filter of 0.45-µm pore size.
Diluent: Acetonitrile and *Buffer* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	85	15
5	85	15
25	60	40
45	50	50
50	85	15
60	85	15

Standard stock solution 1: Prepare 5 mg/mL of USP Naproxen RS in *Diluent*. Further dilute this solution with *Diluent* to obtain 0.05 mg/mL of USP Naproxen RS in *Diluent*.
Standard stock solution 2: 0.01 mg/mL of USP Naproxen Related Compound A RS in methanol
Standard stock solution 3: 0.01 mg/mL of USP Naproxen Related Compound L RS in methanol
System suitability solution: ▲0.5 µg/mL of USP Naproxen Related Compound A RS from *Standard stock solution 2* and 0.5 mg/mL of USP Naproxen RS in *Diluent*▲ (ERR 1-Oct-2018)
Standard solution: 1.0 µg/mL of USP Naproxen RS, 0.5 µg/mL each of USP Naproxen Related Compound A RS and USP Naproxen Related Compound L RS in *Diluent*, from *Standard stock solution 1*, *Standard stock solution 2*, and *Standard stock solution 3*, respectively
Sample solution: Nominally equivalent to 0.5 mg/mL of naproxen from NLT 10 finely powdered Tablets. Transfer nominally equivalent to about 500 mg of naproxen to a 1000-mL volumetric flask. Add 600 mL of *Diluent*, and sonicate for 30 min with intermittent shaking. Cool to room temperature, and dilute with *Diluent* to volume. Mix, and allow to settle for 5 min. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 236 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 6.0 between naproxen related compound A and naproxen, *System suitability solution*
Relative standard deviation: NMT 5.0% for naproxen, naproxen related compound A, and naproxen related compound L, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of naproxen related compound A and naproxen related compound L in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naproxen related compound A or naproxen related compound L from the *Sample solution*
r_S = peak response of naproxen related compound A or naproxen related compound L from the *Standard solution*
C_S = concentration of USP Naproxen Related Compound A RS or USP Naproxen Related Compound L RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)

USP Monographs

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Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of naproxen from the *Standard solution*
C_S = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)
F = relative response factor of each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Naproxen related compound A[a]	0.63	—	0.10
Naproxen	1.00	—	—
Naproxen related compound L[b]	2.32	—	0.10
Naproxen methyl ester[c]	3.19	1.0	0.10
Any other individual impurity	—	1.0	0.10
Total impurities[d]	—	—	0.50

[a] 6-Methoxy-2-naphthoic acid.
[b] 1-(6-Methoxynaphthalen-2-yl)ethanone.
[c] (*S*)-Methyl 2-(6-methoxynaphthalen-2-yl)propanoate.
[d] Disregard any peaks below LOQ (0.004% for any other individual impurity and naproxen methyl ester, 0.002% for naproxen related compound A, and 0.006% for naproxen related compound L).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Naproxen RS
  USP Naproxen Related Compound A RS
  6-Methoxy-2-naphthoic acid.
  $C_{12}H_{10}O_3$ 202.21
  USP Naproxen Related Compound L RS
  1-(6-Methoxynaphthalen-2-yl)ethanone.
  $C_{13}H_{12}O_2$ 200.23

Naproxen Delayed-Release Tablets

DEFINITION

Naproxen Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of naproxen ($C_{14}H_{14}O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Standard solution and **Sample solution:** Prepare as directed in the *Buffer stage* of the *Dissolution* test.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile and 1% acetic acid solution (900:1100). Filter and degas.
  Diluent A: Acetonitrile and water (9:1)
  Diluent B: Acetonitrile and water (1:1)
  Standard stock solution: 0.5 mg/mL of USP Naproxen RS in *Diluent A*
  Standard solution: 0.1 mg/mL of USP Naproxen RS in *Mobile phase* from the *Standard stock solution*
  Sample solution: Transfer an amount nominally equivalent to 250 mg of naproxen from 20 powdered Tablets into a 100-mL volumetric flask, and add about 70 mL of *Diluent B*. Shake by mechanical means for 15 min, sonicate for 15 min, dilute with *Diluent B* to volume, and mix. Pass this solution through a suitable filter of 0.45-µm pore size. Transfer 2.0 mL of the filtrate into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.0 mL/min
  Injection volume: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naproxen from the *Sample solution*
r_S = peak response of naproxen from the *Standard solution*
C_S = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION,** *Delayed-Release Dosage Forms, Method B* ⟨711⟩
  Acid stage
  Medium: 0.1 N hydrochloric acid; 1000 mL
  Apparatus 2: 50 rpm
  Time: 2 h
  Standard solution: A known concentration of USP Naproxen RS in *Medium*

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Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* if necessary.
Analysis: Determine the amount of naproxen ($C_{14}H_{14}O_3$) dissolved by UV absorption at the wavelength of maximum absorbance at about 332 nm with the *Sample solution* in comparison with the *Standard solution*.
Tolerances: NMT 10% (*Q*) of the labeled amount of naproxen ($C_{14}H_{14}O_3$) is dissolved.

Buffer stage
Buffer: 0.2 M phosphate buffer, pH 6.8
Medium: *Buffer*, 1000 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: A known concentration of USP Naproxen RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* if necessary.
Analysis: Determine the amount of naproxen ($C_{14}H_{14}O_3$) dissolved by UV absorption at the wavelength of maximum absorbance at about 332 nm with the *Sample solution* in comparison with the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of naproxen ($C_{14}H_{14}O_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Mobile phase, Diluent A, Diluent B, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard stock solution: 0.25 mg/mL of USP Naproxen RS in *Diluent A*
Standard solution: 0.1 mg/mL of USP Naproxen RS in *Diluent B* from *Standard stock solution*
Sample solution: Transfer 1 Tablet to a 200-mL volumetric flask, and add 140 mL of *Diluent B*. Shake by mechanical means for 15 min, sonicate for 15 min, and dilute with *Diluent B* to volume. Pass a portion of this solution through a suitable filter of 0.45-µm pore size, pipet 2.0 mL of the filtrate for a 500-mg tablet and 2.5 mL for a 375-mg tablet into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Naproxen RS

Naproxen Sodium

$C_{14}H_{13}NaO_3$ 252.24
2-Naphthaleneacetic acid, 6-methoxy-α-methyl-, sodium salt, (*S*)-.
(–)-Sodium (*S*)-6-methoxy-α-methyl-2-naphthaleneacetate [26159-34-2].

» Naproxen Sodium contains not less than 98.0 percent and not more than 102.0 percent of $C_{14}H_{13}NaO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Naproxen Sodium RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 25 µg per mL.
Medium: methanol.
Absorptivities at 272 nm, calculated on the dried basis, do not differ by more than 3%.
Specific rotation ⟨781S⟩: between –15.3° and –17.0°.
Test solution: 50 mg per mL, in 0.1 N sodium hydroxide.
Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 3 hours: it loses not more than 1.0% of its weight.
Free naproxen—Dissolve about 5.0 g in 25 mL of water in a separator, and extract the solution with three 15-mL portions of chloroform. Evaporate the combined extracts on a steam bath to dryness. Dissolve the residue in 10 mL of a mixture of methanol and water (3:1) previously neutralized with 0.1 N sodium hydroxide to the phenolphthalein endpoint. Add phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 2.2 mL is consumed (1.0%).
Chromatographic purity—Dissolve 100 mg in 5 mL of methanol. Dissolve a suitable quantity of USP Naproxen Sodium RS in methanol to obtain a *Standard solution* having a known concentration of about 20 mg per mL. Dilute a portion of this solution quantitatively with methanol to obtain three *Comparison solutions* having concentrations of 20, 60, and 100 µg per mL (0.1%, 0.3%, and 0.5% of the *Standard solution*), respectively. Apply separate 10-µL portions of the five solutions on the starting line to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of toluene, tetrahydrofuran, and glacial acetic acid (30:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, air-dry, and view under short-wavelength UV light: the R_F value of the principal spot in the chromatogram of the solution under test corresponds to that of the *Standard solution*, the intensity of any individual secondary spot does not exceed that of the 100-µg-per-mL *Comparison solution* (0.5%), and the sum of the intensities of any secondary spots, similarly compared, does not exceed 2.0%.
Assay—Dissolve about 200 mg of Naproxen Sodium, accurately weighed, in 50 mL of glacial acetic acid containing 2 drops of *p*-naphtholbenzein TS previously neutralized with 0.1 N perchloric acid if necessary. Titrate with 0.1 N perchloric acid VS. Each mL of 0.1 N perchloric acid is equivalent to 25.22 mg of $C_{14}H_{13}NaO_3$.

Naproxen Sodium Tablets

DEFINITION
Naproxen Sodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$).

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IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*

Sample: Transfer an amount nominally equivalent to about 250 mg of naproxen sodium from finely powdered Tablets to a centrifuge tube. Add 12 mL of water and 1 mL of hydrochloric acid. A dense white precipitate is formed. Centrifuge the mixture. Use the clear supernatant for the test.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, water, and glacial acetic acid (450:540:10)

Standard solution: 0.1 mg/mL of USP Naproxen Sodium RS in *Mobile phase*

Sample stock solution: Nominally 1.0 mg/mL of naproxen sodium from Tablets prepared as follows. Transfer an appropriate amount of naproxen sodium from NLT 20 Tablets, finely powdered, to a suitable volumetric flask. Add 15% of the volume of water and sonicate for 5 min. Add 50% of the volume of *Mobile phase* and sonicate for an additional 30 min, shaking intermittently. Allow the solution to cool to room temperature and then dilute with *Mobile phase* to volume. Centrifuge or pass a portion of this solution through a suitable filter.

Sample solution: Nominally equivalent to 0.1 mg/mL of naproxen sodium in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm, diode array

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.2 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of naproxen

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naproxen from the *Sample solution*

r_S = peak response of naproxen from the *Standard solution*

C_S = concentration of USP Naproxen Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naproxen sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Buffer: 0.1 M of a phosphate buffer with a pH of 7.4, containing 2.62 g/L of monobasic sodium phosphate and 11.50 g/L of anhydrous dibasic sodium phosphate in water

Medium: *Buffer*; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: 50 µg/mL of USP Naproxen Sodium RS in *Medium*

Sample solution: Dilute a filtered portion of the solution under test with *Medium* as necessary to obtain a nominal concentration of 50 µg/mL of naproxen sodium ($C_{14}H_{13}NaO_3$).

Instrumental conditions

Mode: UV

Analytical wavelength: About 332 nm (maximum absorbance)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) dissolved.

Tolerances: NLT 80% (*Q*) of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Solution A: Dissolve 1.36 g of monobasic potassium phosphate in 1 L of water. Adjust with triethylamine to a pH of 6.5. Pass through a suitable filter of 0.45-µm pore size.

Solution B: Acetonitrile

Diluent: Acetonitrile and *Solution A* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
5	85	15
25	60	40
45	50	50
50	85	15
60	85	15

Standard stock solution 1: 0.05 mg/mL of USP Naproxen Sodium RS in *Diluent*

Standard stock solution 2: 0.01 mg/mL of USP Naproxen Related Compound A RS in methanol

Standard stock solution 3: 0.01 mg/mL of USP Naproxen Related Compound L RS in methanol

System suitability solution: ▲0.5 µg/mL of USP Naproxen Related Compound A RS from *Standard stock solution 2* and 0.5 mg/mL of USP Naproxen Sodium RS in *Diluent*▲ (ERR 1-Dec-2018)

Standard solution: 1.0 µg/mL of USP Naproxen Sodium RS, and 0.5 µg/mL each of USP Naproxen Related Compound A RS and USP Naproxen Related Compound L RS in *Diluent*, from *Standard stock solution 1*, *Standard stock solution 2*, and *Standard stock solution 3*, respectively

USP Monographs

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Sample stock solution: Nominally 1.0 mg/mL of naproxen sodium from Tablets prepared as follows. Transfer an appropriate amount of naproxen sodium from NLT 20 Tablets, finely powdered, to a suitable volumetric flask. Add 15% of the volume of water and sonicate for 5 min. Add 50% of the volume of *Mobile phase* described in the *Assay* and sonicate for an additional 30 min, shaking intermittently. Allow the solution to cool to room temperature and then dilute with *Mobile phase* described in the *Assay* to volume. Centrifuge or pass a portion of this solution through a suitable filter.

Sample solution: Nominally equivalent to 0.55 mg/mL of naproxen sodium in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 236 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 6.0 between naproxen related compound A and naproxen, *System suitability solution*

Relative standard deviation: NMT 5.0% for naproxen, naproxen related compound A, and naproxen related compound L, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of naproxen related compound A and naproxen related compound L in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naproxen related compound A or naproxen related compound L from the *Sample solution*

r_S = peak response of naproxen related compound A or naproxen related compound L from the *Standard solution*

C_S = concentration of USP Naproxen Related Compound A RS or USP Naproxen Related Compound L RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naproxen sodium in the *Sample solution* (mg/mL)

Calculate the percentage of naproxen methyl ester and any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naproxen methyl ester or any individual unspecified degradation product from the *Sample solution*

r_S = peak response of naproxen from the *Standard solution*

C_S = concentration of USP Naproxen Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naproxen sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peaks below LOQ (0.004% for naproxen methyl ester and any individual unspecified degradation product, 0.002% for naproxen related compound A, and 0.006% for naproxen related compound L).

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Naproxen related compound A[a]	0.63	0.2
Naproxen	1.00	—
Naproxen related compound L[b]	2.32	0.2
Naproxen methyl ester[c]	3.19	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	1.5

[a] 6-Methoxy-2-naphthoic acid.
[b] 1-(6-Methoxynaphthalen-2-yl)ethanone.
[c] (*S*)-Methyl 2-(6-methoxynaphthalen-2-yl)propanoate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Naproxen Sodium RS

USP Naproxen Related Compound A RS
6-Methoxy-2-naphthoic acid.
$C_{12}H_{10}O_3$ 202.21

USP Naproxen Related Compound L RS
1-(6-Methoxynaphthalen-2-yl)ethanone.
$C_{13}H_{12}O_2$ 200.23

Add the following:

▲Naproxen Sodium and Pseudoephedrine Hydrochloride Extended-Release Tablets

DEFINITION

Naproxen Sodium and Pseudoephedrine Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

- **A.** The retention times of the naproxen and pseudoephedrine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the naproxen and pseudoephedrine peaks of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peaks of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 400 mg of sodium lauryl sulfate in 1 L of water. Add 5 mL of triethylamine and adjust with glacial acetic acid to a pH of 4.1.

USP Monographs

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Mobile phase: Acetonitrile, methanol, and *Buffer* (25:25:50)
Standard solution: 0.22 mg/mL of USP Naproxen Sodium RS and 0.12 mg/mL of USP Pseudoephedrine Hydrochloride RS in methanol
Sample stock solution: Nominally 2.2 mg/mL of naproxen sodium and 1.2 mg/mL of pseudoephedrine hydrochloride in methanol prepared as follows. Transfer NLT 5 whole Tablets to an appropriate volumetric flask, add 70% of the final volume of methanol, and shake to disintegrate the Tablets. Sonicate for 30 min with intermittent shaking. Allow the solution to cool to room temperature and dilute with methanol to volume. Centrifuge 10 mL of the solution for 10 min, and use the clear supernatant to prepare the *Sample solution*.
Sample solution: Nominally 0.22 mg/mL of naproxen sodium and 0.12 mg/mL of pseudoephedrine hydrochloride in methanol from the *Sample stock solution*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Run time: NLT 6 times the retention time of pseudoephedrine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the naproxen and pseudoephedrine peaks
Relative standard deviation: NMT 2.0% for the naproxen and pseudoephedrine peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naproxen or pseudoephedrine from the *Sample solution*
r_S = peak response of naproxen or pseudoephedrine from the *Standard solution*
C_S = concentration of USP Naproxen Sodium RS or USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naproxen sodium or pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Test 1
Medium: 0.01% sodium lauryl sulfate in water, degassed; 900 mL
Apparatus 1: 75 rpm
Times
Naproxen sodium: 1 h
Pseudoephedrine hydrochloride: 1, 3, and 8 h
Buffer: Add 5 mL of triethylamine to 1 L of 0.4 g/L of sodium lauryl sulfate in water. Adjust with glacial acetic acid to a pH of 4.1.
Mobile phase: Acetonitrile, methanol, and *Buffer* (25:25:50)
Standard stock solution: 1.22 mg/mL of USP Naproxen Sodium RS and 0.66 mg/mL of USP Pseudoephedrine Hydrochloride RS in methanol
Standard solution: 0.24 mg/mL of USP Naproxen Sodium RS and 0.13 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: At the times specified, withdraw 10 mL of the solution under test, and pass through a suitable filter of 0.45-µm pore size. Replace the aliquots withdrawn for analysis with equal volumes of fresh portions of *Medium* maintained at 37°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 40 µL
Run time: NLT 2.5 times the retention time of pseudoephedrine
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for pseudoephedrine and naproxen are 0.5 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for the naproxen and pseudoephedrine peaks
Relative standard deviation: NMT 2.0% for the naproxen and pseudoephedrine peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of naproxen from the *Sample solution*
r_S = peak response of naproxen from the *Standard solution*
C_S = concentration of USP Naproxen Sodium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of naproxen sodium (mg/Tablet)

Calculate the concentration (C_i), in mg/mL, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

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Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at each time point (*i*):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$
$$Result_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$Result_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of pseudoephedrine hydrochloride in *Medium* in the portion of the sample withdrawn at each time point (*i*) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of pseudoephedrine hydrochloride (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances

Naproxen sodium: NLT 80% (*Q*) of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) is dissolved.

Pseudoephedrine hydrochloride: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount of Pseudoephedrine Hydrochloride Dissolved (%)
1	1	35–55
2	3	75–95
3	8	NLT 85

The percentages of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.01% sodium lauryl sulfate in water; 900 mL

Apparatus 1: 75 rpm

Times

Naproxen sodium: 1 h

Pseudoephedrine hydrochloride: 1, 3, and 8 h

Solution A: Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 4.0.

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
10.0	45	55
11.0	90	10
14.0	90	10

Standard solution: 0.24 mg/mL of USP Naproxen Sodium RS and 0.13 mg/mL of USP Pseudoephedrine Hydrochloride RS prepared as follows. Dissolve suitable quantities of USP Naproxen Sodium RS and USP Pseudoephedrine Hydrochloride RS with 2% of the final volume of methanol and sonicate if necessary. Dilute with *Medium* to volume.

Sample solution: At the times specified, withdraw 10 mL of the solution under test, and pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm for the pseudoephedrine peak (before relative retention time of 0.5 in relation to the naproxen peak); UV 290 nm for the naproxen peak (at relative retention time of 0.5 and after relative retention time of 0.5 in relation to the naproxen peak)

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.6 mL/min

Injection volume: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the naproxen and pseudoephedrine peaks

Relative standard deviation: NMT 2.0% for the naproxen and pseudoephedrine peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage (*Q*) of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) dissolved:

$$Result = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of naproxen from the *Sample solution*
r_S = peak response of naproxen from the *Standard solution*
C_S = concentration of USP Naproxen Sodium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of naproxen sodium (mg/Tablet)

Calculate the concentration (C_i), in mg/mL, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$Result_i = (r_U/r_S) \times C_S$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at each time point (*i*):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$
$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$Result_3 = \{C_3 \times [V - (2 \times V_S)] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of pseudoephedrine hydrochloride in *Medium* in the portion of the

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sample withdrawn at each time point (*i*) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of pseudoephedrine hydrochloride (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the vessel, 10 mL

Tolerances

Naproxen sodium: NLT 80% (*Q*) of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) is dissolved.

Pseudoephedrine hydrochloride: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount of Pseudoephedrine Hydrochloride Dissolved (%)
1	1	40–65
2	3	75–100
3	8	NLT 85

The percentages of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**

Naproxen sodium related impurities

Mobile phase: Acetonitrile, water, and glacial acetic acid (50:50:1)

Diluent: Acetonitrile and water (90:10)

System suitability solution: 3.1 mg/mL of USP Naproxen Sodium RS and 8 µg/mL each of USP Naproxen Related Compound K RS and USP Naproxen Related Compound L RS in *Diluent*

Standard solution: 0.006 mg/mL of USP Naproxen Sodium RS in *Diluent*

Sample solution: Nominally 3.1 mg/mL of naproxen sodium in *Diluent* prepared as follows. Transfer a suitable amount of naproxen sodium from NLT 20 finely powdered Tablets to an appropriate volumetric flask, add 70% of the final volume of *Diluent*, and sonicate for 20 min with intermittent shaking. Allow the solution to cool to room temperature and dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Run time: NLT 5.6 times the retention time of naproxen

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between naproxen related compound K and naproxen; NLT 2 between naproxen and naproxen related compound L, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each naproxen sodium related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each naproxen related impurity from the *Sample solution*
r_S = peak response of naproxen from the *Standard solution*
C_S = concentration of USP Naproxen Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naproxen sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Naproxen related compound K	0.84	1.2	0.2
Naproxen	1.00	—	—
Naproxen related compound L	1.33	6.8	0.2
Naproxen methyl ester[a]	2.23	0.95	0.2
Naproxen isopropyl ester[b]	4.61	0.83	0.2
Any unspecified impurity	—	1.0	▲0.2▲ (RB 1-Mar-2019)
Total impurities[c]	—	—	1.0

[a] Methyl 2-(6-methoxynaphthalen-2-yl)propionate.
[b] Isopropyl 2-(6-methoxynaphthalen-2-yl)propionate.
[c] Exclude pseudoephedrine related peaks before the relative retention time of 0.4.

Pseudoephedrine hydrochloride related impurities

Solution A: 5 mL/L of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile and water (90:10)

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	100	0
40	0	100
50	0	100
55	100	0
90	100	0

Diluent: 5 mL/L of triethylamine in water. Adjust with phosphoric acid to a pH of 6.8.

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System suitability solution: 0.5 mg/mL of USP Pseudoephedrine Hydrochloride RS and 1.5 µg/mL of USP Ephedrine Hydrochloride RS in *Diluent*
Standard solution: 0.001 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of pseudoephedrine hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of pseudoephedrine hydrochloride from NLT 20 finely powdered Tablets to an appropriate volumetric flask, add 70% of the total volume of *Diluent*, and sonicate for 30 min with intermittent shaking. Allow the solution to cool to room temperature and dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between ephedrine and pseudoephedrine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each pseudoephedrine hydrochloride related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each pseudoephedrine hydrochloride related impurity from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 6*)

Acceptance criteria: See *Table 6*.

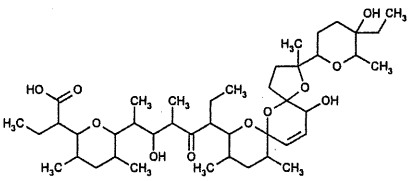
Table 6

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Norephedrine hydrochloride[a]	0.62	1.0	0.2
Norpseudoephedrine hydrochloride[b]	0.72	1.3	0.2
Ephedrine hydrochloride	0.90	0.94	0.2
Pseudoephedrine hydrochloride	1.00	—	—
Any unspecified impurity	—	1.0	▲0.2▲ (RB 1-Mar-2019)
Total impurities[c]	—	—	1.0

[a] (1*R*,2*S*)-2-Amino-1-phenylpropan-1-ol hydrochloride.
[b] (1*S*,2*S*)-2-Amino-1-phenylpropan-1-ol hydrochloride.
[c] Exclude naproxen related peaks after a relative retention time of 1.8 and blank peaks before a relative retention time of 0.2.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in a dry place. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ephedrine Hydrochloride RS
  (1*R*,2*S*)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride.
  $C_{10}H_{15}NO \cdot HCl$ 201.69
  USP Naproxen Sodium RS
  USP Naproxen Related Compound K RS
  1-(6-Methoxynaphthalen-2-yl)ethanol.
  $C_{13}H_{14}O_2$ 202.25
  USP Naproxen Related Compound L RS
  1-(6-Methoxynaphthalen-2-yl)ethanone.
  $C_{13}H_{12}O_2$ 200.23
  USP Pseudoephedrine Hydrochloride RS▲ 2S (USP41)

Narasin Granular



$C_{43}H_{72}O_{11}$ (narasin A) 765.03
$C_{43}H_{70}O_{11}$ (narasin B) 763.01
$C_{44}H_{74}O_{11}$ (narasin D) 779.05
$C_{44}H_{74}O_{11}$ (narasin I) 779.05
Narasin.
2*H*-Pyran-2-acetic acid, α-ethyl-6-[5-[2-(5-ethyltetrahydro-5-hydroxy-6-methyl-2*H*-pyran-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5.3]pentadec-13-en-9-yl]-2-hydroxy-1,3-dimethyl-4-oxoheptyl]tet rahydro-3,5-dimethyl-.
α-Ethyl-6-[5-[2-(5-ethyltetrahydro-5-hydroxy-6-methyl-2*H*-pyran-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5.3]pentadec-13-en-9-yl]-2-hydroxy-1,3-dimethyl-4-oxoheptyl]tetrahydro-3,5-dimethyl-2*H*-pyran-2-acetic acid [55134-13-9].

» Narasin Granular contains narasin mixed with suitable carriers and inactive ingredients prepared in a granular form that is free-flowing and free of

USP Monographs

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aggregates. It contains not less than 100 mg and not more than 160 mg of narasin per g.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.
Labeling—Label it to indicate that it is for animal use only. Label it also to indicate that it is for manufacturing, processing, or repackaging.
USP Reference standards ⟨11⟩—
USP Monensin Sodium RS
USP Narasin RS
Identification—The retention time of the major peak for narasin A in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 10% of its weight.
Powder fineness ⟨811⟩—Not less than 99% passes a No. 30 sieve, and not more than 15% passes a No. 140 sieve.
Content of narasin A—Using the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of narasin A by the formula:

$$100A/[A + (D + I)]$$

in which A is the narasin A biopotency and $D + I$ is the narasin $D + I$ biopotency. Not less than 85% of narasin A is found.

Assay—
Diluent—Prepare a mixture of methanol and water (9:1).
Mobile phase—Prepare a degassed mixture of methanol, water, and glacial acetic acid (94: 6: 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Neutralized methanol—Add 1 g of sodium bicarbonate to 4 L of methanol, mix, and filter.
Derivatizing reagent—Dissolve 30 g of vanillin in a mixture of methanol and sulfuric acid (950:20) in a container protected from light. [**CAUTION**—To avoid splattering, add the sulfuric acid carefully and slowly with a pipet; do not pour. Allow the mixture of methanol and sulfuric acid to cool before adding the vanillin.] Do not filter.
Resolution solution—Prepare a solution in *Neutralized methanol* containing about 3 mg of USP Narasin RS and 1 mg of USP Monensin Sodium RS per mL. Transfer 2 mL of this solution to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Standard preparations—Dissolve an accurately weighed quantity of USP Narasin RS in *Neutralized methanol* to obtain a solution having a known concentration of about 1 mg per mL. Transfer 1.0 mL of this stock solution to a 200-mL volumetric flask, and transfer 2.0 mL and 4.0 mL of the stock solution to two separate 100-mL volumetric flasks, dilute each with *Diluent* to volume, and mix. These solutions contain about 5, 20, and 40 µg of USP Narasin RS per mL. Using the designated percentage of narasin A in the USP Narasin RS, calculate the exact narasin A concentration, in µg per mL, in each of the *Standard preparations*.
Assay preparation—Transfer about 5 g of Narasin Granular, accurately weighed, to a suitable container, add 200.0 mL of *Diluent*, and shake by mechanical means for 1 hour. Allow the solids to settle, and quantitatively dilute an accurately measured volume of the supernatant with *Diluent* to obtain a solution containing about 20 µg of narasin per mL. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, and use the filtrate as the *Assay preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 4.6-mm × 25-cm column that contains packing L1. The column outlet is attached to a tee, the opposing arm is attached to a tube from which is pumped the *Derivatizing reagent*, and the outlet is connected to a 2-mL postcolumn reaction coil maintained at 98°. The outlet of the reaction coil is connected to a detector set at 520 nm. The *Mobile phase* and the *Derivatizing reagent* flow at the rate of about 0.7 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for monensin B, 0.75 for monensin A, 1.0 for narasin A, and 1.1 for narasin D + I; and the resolution, R, between the monensin B peak and the monensin A peak is not less than 1.25, and between the monensin A peak and the narasin A peak not less than 3.5. Chromatograph the *Standard preparations*, and record the peak responses as directed for *Procedure:* the tailing factor for the narasin A peak is not more than 1.4 when calculated by the formula:

$$W_{0.1}/2f$$

in which $W_{0.1}$ is the width of the peak at 10% of peak height; and f is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point on the baseline at which 10% peak height is reached. The relative standard deviation for replicate injections is not more than 10%. [NOTE—After use, flush the system with methanol.]
Procedure—Separately inject equal volumes (about 200 µL) of the *Standard preparations* and the *Assay preparation* into the chromatograph, and measure the areas of the peak responses for the narasin A and narasin D + I peaks [NOTE—Narasin D and narasin I will co-elute under this chromatographic system.]
Plot the three narasin peak responses in the chromatograms obtained from the *Standard preparations* versus the concentration, in µg per mL, of narasin A, and draw the straight line best fitting the three plotted points. From the graph so obtained, and the narasin A peak response in the chromatogram obtained from the *Assay preparation*, determine the concentration, C_A, in µg per mL, of narasin A in the *Assay preparation*. From the same graph and the narasin D + I peak response in the chromatogram obtained from the *Assay preparation*, determine the concentration, C_{D+I}, in µg per mL, of narasin D + I in the *Assay preparation*. Calculate the biopotency, in mg per g, in the portion of Narasin Granular taken by the formula:

$$(0.001)(C_A F_A + C_{D+I} F_{D+I})(VE/M)$$

in which F_A is 1.077 representing the biopotency conversion factor for narasin A; F_{D+I} is the biopotency conversion factor for narasin D + I; V is the extraction volume, in mL; E is the dilution factor used in diluting the extract to the final estimated concentration of 20 µg per mL; and M is the weight, in g, of Narasin Granular taken to prepare the *Assay preparation*. Calculate the bioconversion factor, F_{D+I}, for narasin D + I by the formula:

$$(1.510D + 0.012I)/(D + I)$$

in which D and I are the specified percentages of narasin D and narasin I, respectively, in USP Narasin RS; 1.510 is the factor for converting narasin D to narasin D biopotency; and 0.012 is the factor for converting narasin I to narasin I biopotency.

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Narasin Type A Medicated Article

(Title for this monograph not to change until December 1, 2017)
(Prior to December 1, 2017, the current practice of labeling the article of commerce with the name Narasin Premix may be continued. Use of the name Narasin Type A Medicated Article will be permitted as of June 1, 2017; however, the use of this name will not be mandatory until December 1, 2017.)

DEFINITION

Narasin Type A Medicated Article contains Narasin Granular mixed with suitable diluents and inactive ingredients. It contains NLT 90% and NMT 110% of the labeled amount of narasin.

IDENTIFICATION

• **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, water, and glacial acetic acid (94:6:0.1)

Diluent: Methanol and water (9:1)

Neutralized methanol: Add 1 g of sodium bicarbonate to 4 L of methanol, mix, and filter.

Derivatizing reagent: 30 g of vanillin in a mixture of methanol and sulfuric acid (950:20), in a container protected from light. [**CAUTION**—To avoid splattering, add the sulfuric acid carefully and slowly with a pipet; do not pour. Allow the mixture of methanol and sulfuric acid to cool before adding the vanillin. Do not filter.]

System suitability solution: Prepare a solution containing 3 mg/mL of USP Narasin RS and 1 mg/mL of USP Monensin Sodium RS in *Neutralized methanol*. Dilute 2.0 mL of this solution with *Diluent* to 200 mL.

Standard stock solution: 1 mg/mL of USP Narasin RS in *Neutralized methanol*

Standard solution A: 5 µg/mL of USP Narasin RS from *Standard stock solution*, in *Diluent*

Standard solution B: 20 µg/mL of USP Narasin RS from *Standard stock solution*, in *Diluent*

Standard solution C: 40 µg/mL of USP Narasin RS from *Standard stock solution*, in *Diluent*

Sample solution: Transfer 5 g of Narasin Type A Medicated Article to a suitable container, add 200.0 mL of *Diluent*, and shake by mechanical means for 1 h. Allow the solids to settle, and quantitatively dilute a volume of the supernatant with *Diluent* to obtain a solution with a nominal concentration of 20 µg/mL of narasin. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 520 nm

Column: 4.6-mm × 25-cm; packing L1. The column outlet is attached to a tee, the opposing arm is attached to a tube from which is pumped the *Derivatizing reagent*, and the outlet is connected to a 2-mL postcolumn reaction coil maintained at 98°. The outlet of the reaction coil is connected to the *Detector*.

Flow rate: 0.7 mL/min for the *Mobile phase* and the *Derivatizing reagent*

Injection volume: 200 µL

System suitability

Samples: *System suitability solution*, *Standard solution A*, *Standard solution B*, and *Standard solution C*

[NOTE—The relative retention times for monensin B, monensin A, narasin A, and narasin D+I are 0.7, 0.75, 1.0, and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.25 between the monensin B peak and the monensin A peak; NLT 3.5 between the monensin A peak and the narasin A peak, *System suitability solution*

Tailing factor: NMT 1.4 for the narasin A peak, *Standard solution A*, *Standard solution B*, and *Standard solution C*, when calculated:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$ = width of the peak at 10% of peak height
f = distance from the peak maximum to the leading edge of the peak, the distance being measured at a point on the baseline at which 10% peak height is reached

Relative standard deviation: NMT 10.0%, *Standard solution A*, *Standard solution B*, and *Standard solution C*

[NOTE—After use, flush the system with methanol.]

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

[NOTE—Narasin D and narasin I will coelute under this chromatographic system.]

Plot the three narasin peak responses from the *Standard solutions* versus the concentration (µg/mL) of narasin A, and draw the straight line best fitting the three plotted points. From the graph and the narasin A peak response from the *Sample solution*, determine the concentration, C_A, in µg/mL, of narasin A in the *Sample solution*. From the same graph and the narasin D+I peak response from the *Sample solution*, determine the concentration, C_{D+I}, in µg/mL, of narasin D+I in the *Sample solution*.

Calculate the biopotency conversion factor, F_{D+I}, for narasin D+I:

$$\text{Result} = [(F_D \times D) + (F_I \times I)]/(D + I)$$

F_D = biopotency conversion factor for narasin D, 1.510
D = specified percentage of narasin D in USP Narasin RS
F_I = biopotency conversion factor for narasin I, 0.012
I = specified percentage of narasin I in USP Narasin RS

Calculate the biopotency, in mg/g, in the portion of Narasin Type A Medicated Article taken:

$$\text{Result} = 0.001 \times [(C_A \times F_A) + (C_{D+I} \times F_{D+I})] \times (V \times E/M)$$

C_A = concentration of narasin A in the *Sample solution* (µg/mL)
F_A = biopotency conversion factor for narasin A, 1.077
C_{D+I} = concentration of narasin D+I in the *Sample solution* (µg/mL)
F_{D+I} = biopotency conversion factor for narasin D+I, calculated previously
V = extraction volume (mL)
E = dilution factor to prepare the final estimated *Sample solution* concentration of 20 µg/mL
M = weight of Narasin Type A Medicated Article taken to prepare the *Sample solution* (g)

Acceptance criteria: 90%–110%

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SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at 60° for 3 h.
  Acceptance criteria: NMT 12%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Avoid moisture and excessive heat.
- **LABELING:** Label it to indicate that it is for animal use only. The label bears the statement "Do not feed undiluted".
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Monensin Sodium RS
  USP Narasin RS

Naratriptan Tablets

DEFINITION
Naratriptan Tablets contain an amount of Naratriptan Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$).

IDENTIFICATION

Delete the following:

▲• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Diluent: Methylene chloride and methanol (1:1)
Standard solution: 1.0 mg/mL of USP Naratriptan Hydrochloride RS prepared as follows. Transfer 5.5 mg of USP Naratriptan Hydrochloride RS to a 25-mL flask, add 1 mL of water and gently shake. Add 4.5 mL of *Diluent*, and shake for 5 min. Centrifuge at 3000 rpm for 10 min, and pass through a suitable filter of a 0.45-µm pore size.
Sample solution: Transfer a number of Tablets equivalent to 5 mg of naratriptan to a 25-mL flask, add 1.0 mL of water to wet the tablets, and gently shake to remove the Tablet film coating. Add 4.5 mL of *Diluent*, and shake for 5 min or until the Tablets have dispersed. Centrifuge at 3000 rpm for 10 min, and pass through a suitable filter of a 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Developing solvent system: Methylene chloride, alcohol, and triethylamine (10:2:1)
Analysis
Sample: *Standard solution* and *Sample solution*
Acceptance criteria: The R_F value of the principal spot of the Sample solution corresponds to that of the Standard solution. ▲2S (USP41)

Change to read:

• ▲**A.**▲2S (USP41) The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Dilute 0.6 mL of phosphoric acid with water to 900 mL, and adjust with triethylamine to a pH of 2.5.
Mobile phase: Isopropyl alcohol and *Solution A* (10:90)
System suitability solution: 0.7 mg/mL of USP Naratriptan Resolution Mixture RS in *Mobile phase*
Standard stock solution: 0.2 mg/mL of USP Naratriptan Hydrochloride RS in 0.1 N sodium hydroxide
Standard solution: 0.02 mg/mL of USP Naratriptan Hydrochloride RS in *Solution A* from *Standard stock solution*
Sample solution: Nominally (L/50) mg/mL of naratriptan, where L is the label claim in mg/Tablet, prepared as follows. Transfer 5 Tablets into an amber 250-mL volumetric flask, add 30 mL of 0.1 N sodium hydroxide, and shake on a wrist-action shaker for at least 30 min. Sonicate for 10 min with regular vigorous swirling of the flask. Add about 170 mL of *Solution A*. Allow to cool to room temperature and dilute with *Solution A* to volume. Centrifuge a portion of this solution at 3500 rpm for about 10 min, and pass through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 224 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲2S (USP41)
Column: 4.6-mm × 15-cm; 5-µm packing L11
Flow rate: 1.3 mL/min
Injection volumes
System suitability solution: 10 µL
Standard solution: 50 µL
Tablets labeled to contain 1 mg of naratriptan: 50 µL
Tablets labeled to contain 2.5 mg of naratriptan: 20 µL
▲**Run time:** NLT 1.5 times of the retention time of naratriptan▲2S (USP41)
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for 3-(1-methylpiperidin-4-yl)-1*H*-indole (naratriptan related compound A), naratriptan, and naratriptan related compound B are 0.9, 1.0, and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between naratriptan related compound A and naratriptan and between naratriptan related compound B and naratriptan, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Naratriptan Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naratriptan in the *Sample solution* (mg/mL)

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M_{r1} = molecular weight of naratriptan, 335.47
M_{r2} = molecular weight of naratriptan hydrochloride, 371.93

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid; 500 mL, deaerated
  Apparatus 1: 100 rpm
  Time: 15 min
  Standard solution: USP Naratriptan Hydrochloride RS in *Medium*
  [NOTE—Do not sonicate the *Standard solution* to dissolve. Dissolve USP Naratriptan Hydrochloride RS with *Medium* at about 37°.]
  Sample solution: Filter portions of the solution under test and dilute with *Medium*, if necessary.
  Instrumental conditions
  ▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ 2S (USP41)
  Mode: UV
  Analytical wavelength: 226–236 nm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Determine the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$) dissolved from the difference between the first derivative absorbance values at the wavelengths of maximum and minimum in the range from 226 to 236 nm on the *Sample solution*, suitably diluted with *Medium*, if necessary, in comparison with the *Standard solution*.
  Tolerances: NLT 80% (*Q*) of the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**
  Solution A: 5.75 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
35	80	20
40	80	20
41	97	3
51	97	3

System suitability solution: 0.11 mg/mL of USP Naratriptan Resolution Mixture RS
Sample solution: Nominally (*L*/10) mg/mL of naratriptan, where *L* is the label claim in mg/Tablet, prepared as follows. Transfer 5 Tablets into a suitable amber flask. Add 20.0 mL of 0.1 N sodium hydroxide, and allow to stand for 10 min. Sonicate for 10 min with regular vigorous swirling of the flask. Add 30.0 mL of *Solution A*, and mix well. Centrifuge a portion of this solution at 3500 rpm for about 10 min and pass through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; ▲4-µm▲ 2S (USP41) packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volumes
System suitability solution: 50 µL
Tablets labeled to contain 1 mg of naratriptan: 50 µL
Tablets labeled to contain 2.5 mg of naratriptan: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between naratriptan and naratriptan related compound B
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/F)/[r_N + \Sigma(r_U/F)] \times 100$$

r_U = peak response of each impurity from the *Sample solution*
F = relative response factor for each impurity (see *Table 2*)
r_N = peak response of naratriptan from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Naratriptan	1.0	—	—
Naratriptan related compound B[a]	1.07	0.6	0.2
Bisaryl naratriptan[b]	1.26	0.6	0.2
Naratriptan pyridinium salt[c]	1.33	0.4	0.3
N-Sulfamoylethyl naratriptan[d]	1.44	0.6	0.2
N-Sulfamoylethyl naratriptan pyridinium salt[e]	1.62	0.5	0.2
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.5

[a] 2-[3-(1-Methyl-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indol-5-yl]ethanesulfonic acid methylamide oxalate.
[b] 2,2-Bis-[3-(1-methylpiperidin-4-yl)-1*H*-indol-5-yl]ethanesulfonic acid methylamide.
[c] 1-Methyl-4-[5-(2-methylsulfamoyl-ethyl)-1*H*-indol-3-yl]-pyridinium chloride.
[d] 2-[3-(1-Methylpiperidin-4-yl)-5-(2-methylsulfamoyl-ethyl)-indol-1-yl] ethanesulfonic acid methylamide.
[e] 4-[1,5-Bis-(2-methylsulfamoyl-ethyl)-1*H*-indol-3-yl]-1-methylpyridinium chloride.

ADDITIONAL REQUIREMENTS

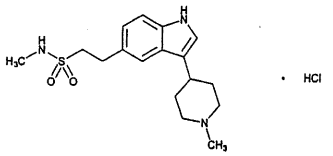
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Naratriptan Hydrochloride RS
USP Naratriptan Resolution Mixture RS
A mixture of naratriptan hydrochloride with approximately 0.1% each of naratriptan related compound A [3-(1-methylpiperidin-4-yl)-1*H*-indole hydrochloride] and naratriptan related compound B [2-[3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole-5-yl]ethanesulfonic acid methylamide oxalate].

Naratriptan Hydrochloride



$C_{17}H_{25}N_3O_2S \cdot HCl$ 371.93
1*H*-Indole-5-ethanesulfonamide, *N*-methyl-3-(1-methyl-4-piperidinyl)-, monohydrochloride.
N-Methyl-3-(1-methyl-4-piperidyl)indole-5-ethanesulfonamide monohydrochloride [143388-64-1].

» Naratriptan Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of $C_{17}H_{25}N_3O_2S \cdot HCl$, calculated on the anhydrous and solvent free-basis.

Packaging and storage—Preserve in tight containers, and store below 30°.
USP Reference standards ⟨11⟩—
USP Naratriptan Hydrochloride RS
USP Naratriptan Resolution Mixture RS
A mixture of naratriptan hydrochloride with approximately 0.1% each of naratriptan related compound A [3-(1-methylpiperidin-4-yl)-1*H*-indole hydrochloride] and naratriptan related compound B [2-[3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole-5-yl]ethanesulfonic acid methylamide oxalate].
[NOTE—When performing assays and tests, store all standard, system suitability, and sample solutions in a cool place, protected from light.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: It meets the requirements of test C under *Chloride* ⟨191⟩.

Water Determination, *Method I* ⟨921⟩: not more than 0.5%.

Chromatographic purity—

0.05 M Ammonium phosphate buffer—Dissolve 5.75 g of monobasic ammonium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.00 ± 0.05.

Solution A—Prepare a filtered and degassed mixture of *0.05 M Ammonium phosphate buffer* and acetonitrile (97:3).

Solution B—Prepare a filtered and degassed mixture of *0.05 M Ammonium phosphate buffer* and acetonitrile (4:1).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Dissolve an accurately weighed quantity of USP Naratriptan Resolution Mixture RS in water to obtain a solution having a known concentration of about 0.11 mg per mL.

Test solution—Dissolve an accurately weighed quantity of Naratriptan Hydrochloride in water to obtain a solution having a known concentration of about 0.11 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 15-cm column that contains 4-µm packing L1. The column temperature is maintained at 40°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–35.0	100→0	0→100	linear gradient
35.0–40.0	0	100	isocratic
40.0–41.0	0→100	100→0	linear gradient
41.0–51.0	100	0	re-equilibration

Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.04 for 2-[3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indol-5-yl]ethanesulfonic acid methylamide (naratriptan related compound B) and 1.0 for naratriptan; and the resolution, *R,* between naratriptan and naratriptan related compound B is not less than 1.5.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for all of the peaks. Calculate the percentage of each impurity in the portion of Naratriptan Hydrochloride taken by the formula:

$$100(r_i/F)/[r_N + \Sigma(r_i/F)]$$

in which F is the relative response factor (see the accompanying table for values) for each impurity; r_i is the peak response for each impurity; and r_N is the naratriptan peak response (see the accompanying table for limits).
In addition to not exceeding the limits listed in the accompanying table, not more than 0.1% of any other individual impurity is found; and not more than 1.5% of total impurities is found.

Assay—

0.01 M Triethylamine phosphate buffer—Dilute 0.6 mL of phosphoric acid with water to 900 mL, and adjust with triethylamine to a pH of 2.5.

Mobile phase—Prepare a filtered and degassed mixture of *0.01 M Triethylamine phosphate buffer* and isopropyl alcohol (9:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability preparation—Dissolve an accurately weighed quantity of USP Naratriptan Resolution Mixture RS in *Mobile phase* to obtain a solution having a concentration of about 0.7 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Naratriptan Hydrochloride RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with

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Compound Name	Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
3-(1-Methylpiperidin-4-yl)-1*H*-indole	about 0.93	1.0	0.2
2-[3-(1-Methyl-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indol-5-yl]ethanesulfonic acid methylamide	about 1.04	0.6	0.1
2,2-Bis-[3-(1-methylpiperidin-4-yl)-1*H*-indol-5-yl]ethanesulfonic acid methylamide	about 1.18	0.6	0.2
1-Methyl-4-[5-(2-methylsulfamoyl-ethyl)-1*H*-indol-3-yl]-pyridinium chloride	about 1.25	0.4	0.2
2-[3-(1-Methylpiperidin-4-yl)-5-(2-methylsulfamoyl-ethyl)-indol-1-yl]ethanesulfonic acid methylamide	about 1.36	0.6	0.3
4-[1,5-Bis-(2-methylsulfamoyl-ethyl)-1*H*-indol-3-yl]-1-methylpyridinium chloride	about 1.44	0.5	0.1
2-[3-(1-Methylpiperidin-4-yl)-1*H*-indol-5-yl]ethane-sulfonic acid methyl-(2-methylsulfamoyl-ethyl)amide	about 1.48	1.0	0.2
5-Ethyl-3-(1-methylpiperidin-4-yl)-1*H*-indole	about 1.90	1.00	0.2

Mobile phase to obtain a solution having a known concentration of about 0.11 mg per mL.

Assay preparation—Transfer about 11 mg of Naratriptan Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 282-nm detector and a 4.6-mm × 15-cm column that contains 3-µm packing L11. The column temperature is maintained at 35°. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between naratriptan related compound A and naraptriptan and between naratriptan related compound B and naratriptan is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%. [NOTE—For identification purposes, the approximate relative retention times are about 0.9 for naratriptan related compound A, 1.0 for naratriptan, and 1.1 for naratriptan related compound B.]

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{25}N_3O_2S \cdot HCl$ in the portion of Naratriptan Hydrochloride taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Naratriptan Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Naratriptan Compounded Oral Suspension

DEFINITION

Naratriptan Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$). Prepare Naratriptan Compounded Oral Suspension containing 0.5 mg/mL of *Naratriptan* as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Naratriptan (as Naratriptan Hydrochloride)	50 mg (55.43 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, *NF* (regular or sugar-free), and Vehicle for Oral Suspension, *NF,* a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder or add *Naratriptan Hydrochloride* powder. Add the *Vehicle* in small portions and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a naratriptan suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: 2-Propanol and 12 mM triethylamine phosphate buffer (1:10). Make adjustments if necessary.

Standard stock solution: 0.5 mg/mL of USP Naratriptan Hydrochloride RS in *Mobile phase*

Standard solution: Transfer 1.0 mL of *Standard stock solution* to a 25-mL volumetric flask, dilute with *Mobile phase* to volume to obtain a solution containing 20 µg/mL of naratriptan hydrochloride, and pass through a suitable filter of 0.22-µm pore size.

Sample solution: Shake the Oral Suspension thoroughly by hand. Pipet 0.4 mL into a 10-mL volumetric flask. Add 1 mL of 0.1 N sodium hydroxide solution by pipet, and sonicate for 5 min. Dilute with *Mobile phase* to volume, and mix to obtain a nominal concentration of 20 µg/mL of naratriptan. Centrifuge, and pass the naratriptan solution through a suitable filter of 0.22-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; 5-µm packing L11

Flow rate: 1.4 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Retention time: 9.7 min for the naratriptan peak

Relative standard deviation: NMT 4.9% for the replicate injections

USP Monographs

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

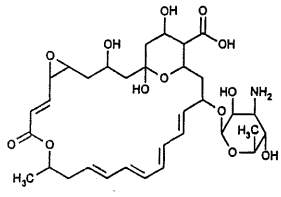
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Naratriptan Hydrochloride RS in the *Standard solution* (µg/mL), on the anhydrous basis
C_U = nominal concentration of naratriptan in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of naratriptan, 335.47
M_{r2} = molecular weight of naratriptan hydrochloride, 371.93

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–4.5

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **Beyond-Use Date:** NMT 7 days after the date on which it was compounded when stored at controlled room temperature, and NMT 90 days after the date on which it was compounded when stored in a refrigerator
- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP Reference Standards** ⟨11⟩
USP Naratriptan Hydrochloride RS

Natamycin



$C_{33}H_{47}NO_{13}$ 665.73

Stereoisomer of 22-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo [22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid [7681-93-8].

» Natamycin contains not less than 90.0 percent and not more than 102.0 percent of $C_{33}H_{47}NO_{13}$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Natamycin RS
Identification—Transfer 50 mg, accurately weighed, to a 200-mL volumetric flask, add 5.0 mL of water, and moisten the specimen. Add 100 mL of a 1 in 1000 solution of glacial acetic acid in methanol, and shake by mechanical means in the dark until dissolved. Dilute with the acetic acid-methanol solution to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with the acetic acid-methanol solution to volume, and mix: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Natamycin RS, concomitantly measured.
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 5.0 and 7.5, in an aqueous suspension containing 10 mg per mL.
Water Determination, *Method I* ⟨921⟩: between 6.0% and 9.0%.
Assay— [NOTE—Throughout the *Assay*, protect from direct light all solutions containing natamycin.]
Mobile phase—Dissolve 3.0 g of ammonium acetate and 1.0 g of ammonium chloride in 760 mL of water, and mix. Add 5.0 mL of tetrahydrofuran and 240 mL of acetonitrile, and mix. Pass this solution through a 0.5 µm or finer porosity filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 20 mg of USP Natamycin RS, accurately weighed, to a 100-mL volumetric flask. Add 5.0 mL of tetrahydrofuran, and sonicate for 10 minutes. Add 60 mL of methanol, and swirl to dissolve. Add 25 mL of water, and mix. Allow to cool to room temperature. Dilute with water to volume, and mix. Pass this solution through a suitable membrane filter of 0.5 µm or finer porosity.
Resolution solution—Dissolve 20 mg of Natamycin in a mixture of 99 mL of methanol and 1 mL of 0.1 N hydrochloric acid, and allow to stand for 2 hours. [NOTE—Use this solution within 1 hour.]
Assay preparation—Transfer about 20 mg of Natamycin, accurately weighed, to a 100-mL volumetric flask. Proceed as directed under *Standard preparation*, beginning with "add 5.0 mL of tetrahydrofuran."
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 303-nm detector and a 4.6-mm × 25-cm analytical column that contains packing L1. [NOTE—A 3.9-mm × 20-mm pre-column may be used to extend the useful life of the analytical column.] The flow rate is about 3 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*:the column efficiency determined from the analyte peak is not less than 3000 theoretical plates, the tailing factor is not less than 0.8 and not more than 1.3, and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between natamycin and its methyl ester is not less than 2.5. The relative retention times are about 0.7 for natamycin and 1.0 for its methyl ester.
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of natamycin ($C_{33}H_{47}NO_{13}$) in the portion of Natamycin taken by the formula:

$$0.1(W_S P_S/W_U)(r_U/r_S)$$

in which W_S is the weight, in mg, of USP Natamycin RS taken to prepare the *Standard preparation*; P_S is the stated content, in µg per mg, of USP Natamycin RS; W_U is the weight, in mg, of Natamycin taken to prepare the *Assay preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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Natamycin Ophthalmic Suspension

» Natamycin Ophthalmic Suspension is a sterile suspension of Natamycin in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{33}H_{47}NO_{13}$. It contains one or more suitable preservatives.

Packaging and storage—Preserve in tight, light-resistant containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—
USP Natamycin RS

Identification—Transfer a volume of Ophthalmic Suspension, equivalent to about 50 mg of natamycin, to a 200-mL volumetric flask, and add water to make a volume of 5 mL. Proceed as directed in the *Identification* test under *Natamycin*, beginning with "Add 100 mL of a 1 in 1000 solution of glacial acetic acid in methanol:" the specified result is obtained.

Sterility Tests ⟨71⟩ —It meets the requirements as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, using 0.25 mL of the Ophthalmic Suspension taken from each container.

pH ⟨791⟩: between 5.0 and 7.5.

Assay— [NOTE—Throughout the *Assay* protect from direct light all solutions containing natamycin.]

Mobile phase , *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Natamycin*.

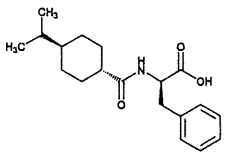
Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, equivalent to about 50 mg of natamycin, to a 250-mL volumetric flask. Add 12.5 mL of tetrahydrofuran, and sonicate for 10 minutes. Add 150 mL of methanol, and swirl to dissolve. Add 60 mL of water, and mix. Allow to cool to room temperature. Dilute with water to volume, and mix. Filter this solution through a suitable membrane filter of 0.5-µm or finer porosity.

Procedure—Proceed as directed in the *Assay* under *Natamycin*. Calculate the quantity, in mg, of $C_{33}H_{47}NO_{13}$ in each mL of the Ophthalmic Suspension taken by the formula:

$$0.25C(P_S/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Natamycin RS in the *Standard preparation*, P_S is the stated content, in µg per mg, of USP Natamycin RS, V is the volume, in mL, of Ophthalmic Suspension taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nateglinide



$C_{19}H_{27}NO_3$ 317.42

D-Phenylalanine, *N*-[[*trans*-4-(1-methylethyl)cyclohexyl] carbonyl]-;
(–)-*N*-[(*trans*-4-Isopropylcyclohexyl)carbonyl-D-phenylalanine [105816-04-4].

DEFINITION

Nateglinide contains NLT 98.0% and NMT 102.0% of $C_{19}H_{27}NO_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 8.5 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 7.5.

Mobile phase: Methanol and *Buffer* (1:1)

System suitability stock solution: 0.2 mg/mL each of USP Nateglinide Related Compound C RS and DL-phenylalanine in methanol. [NOTE—Sonicate, if necessary.]

System suitability solution: Transfer USP Nateglinide RS to a suitable volumetric flask, dissolve first in methanol, using 45% of the final volume, add *System suitability stock solution* equal to 5% of the final volume, and then dilute with *Buffer* to volume to obtain a solution containing about 1.0 mg/mL of nateglinide and about 0.01 mg/mL each of nateglinide related compound C and DL-phenylalanine.

Standard solution: 1.0 mg/mL of nateglinide prepared as follows: transfer USP Nateglinide RS to a suitable volumetric flask, dissolve first in methanol, using 50% of the final volume, and then dilute with *Buffer* to volume.

Sample solution: Transfer about 100 mg of Nateglinide to a 100-mL volumetric flask, dissolve in 50 mL of methanol, and dilute with *Buffer* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 6-mm × 15-cm; 6-µm packing L71 (see *Chromatographic Reagents* under *Reagents, Indicators, and Solutions*)
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 0.9 between nateglinide related compound C and nateglinide, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nateglinide ($C_{19}H_{27}NO_3$) in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nateglinide RS in the *Standard solution* (mg/mL)
C_U = concentration of Nateglinide in the *Sample solution* (mg/mL)

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Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF NATEGLINIDE RELATED COMPOUND A AND OTHER IMPURITIES**

Buffer: 7.8 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Buffer* (7:13)

System suitability stock solution: Dissolve USP Nateglinide Related Compound A RS in acetonitrile to obtain a solution containing about 0.6 mg/mL. Further dilute this solution with *Mobile phase* to obtain a solution containing about 0.12 mg/mL.

System suitability solution: Transfer an amount of USP Nateglinide RS to a suitable volumetric flask, dissolve first in acetonitrile using 10% of the final volume, then add *System suitability stock solution* equal to 10% of the final volume, and dilute with *Mobile phase* to volume to obtain a solution containing about 6 mg/mL of nateglinide and about 0.012 mg/mL of nateglinide related compound A.

Standard solution: Dissolve USP Nateglinide RS in acetonitrile to obtain a solution having a known concentration of about 0.3 mg/mL. Further dilute this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.06 mg/mL.

Sample solution: Transfer 60 mg of Nateglinide to a 10-mL volumetric flask, dissolve in a minimal amount of acetonitrile, and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 5-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 2 mL/min
Injection size: 100 µL
Run time: 5 times the retention time of nateglinide

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between nateglinide related compound A and nateglinide, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of nateglinide from the *Standard solution*
C_S = concentration of USP Nateglinide RS in the *Standard solution* (mg/mL)
C_U = concentration of Nateglinide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria
Individual impurities: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nateglinide related compound A[a]	0.5	0.015	0.2
Ethyl analog[b]	0.6	1.0	0.1
Nateglinide	1.0	—	—
IPP impurity[c]	3.1	1.0	0.1
Ester impurity[d]	4.1	0.94	0.1
Any other individual impurity	—	1.0	0.1

[a] *trans*-4-Isopropylcyclohexylcarboxylic acid.
[b] *N*-(*trans*-4-Ethylcyclohexylcarbonyl)-D-phenylalanine.
[c] *N*-(*trans*-4-Isopropylcyclohexylcarbonyl)-D-phenylalanine-D-phenylalanine.
[d] *N*-(*trans*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine-ethyl ester.

- **LIMIT OF NATEGLINIDE RELATED COMPOUND B**

Mobile phase: 0.77 g/L of ammonium acetate in methanol. [NOTE—The following solutions are stable for up to 48 h when stored in a refrigerator.]

System suitability solution: 10 mg/mL of USP Nateglinide RS and 0.02 mg/mL of USP Nateglinide Related Compound B RS in methanol

Standard solution: 0.02 mg/mL of USP Nateglinide Related Compound B RS in methanol. [NOTE—Nateglinide related compound B is *N*-(*trans*-4-isopropyl-cyclohexylcarbonyl)-L-phenylalanine.]

Sample solution: 10 mg/mL of Nateglinide in methanol

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 25-cm or 4.6-mm × 25-cm; 5-µm packing L72 (see *Chromatographic Reagents* under *Reagents, Indicators, and Solutions*)
Column temperature: 40°
Flow rate: 0.8 mL/min. [NOTE—The flow rate can be adjusted as needed to achieve a recommended retention time of nateglinide related compound B at about 25 min.]
Injection size: 10 µL

System suitability
[NOTE—The elution order is nateglinide related compound B, followed by the nateglinide peak.]
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 0.8 between nateglinide related compound B and nateglinide, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nateglinide related compound B in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nateglinide related compound B from the *Sample solution*
r_S = peak response of nateglinide related compound B from the *Standard solution*
C_S = concentration of USP Nateglinide Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Nateglinide in the *Sample solution* (mg/mL)

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Acceptance criteria: NMT 0.2%

• **LIMIT OF NATEGLINIDE RELATED COMPOUND C AND PHENYLALANINE**

Mobile phase, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*

Diluted standard solution: Dilute the *Standard solution* with *Mobile phase* to obtain a solution having a known concentration of about 0.01 mg/mL of nateglinide.

Analysis

Samples: *Sample solution* and *Diluted standard solution*

Calculate the percentage of each specified impurity listed in *Table 2* in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of nateglinide from the *Diluted standard solution*

C_S = concentration of nateglinide in the *Diluted standard solution* (mg/mL)

C_U = concentration of Nateglinide in the *Sample solution* (mg/mL)

F = relative response factor of each individual impurity (see *Table 2*)

Acceptance criteria

Individual impurities: See *Table 2.*

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylalanine	0.2	1.5	0.2
Nateglinide *cis*-isomer[a] (related compound C)	0.9	0.97	0.2
Nateglinide	1.0	—	—

[a] *N*-(*cis*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine.

Total impurities: The sum of all impurities found in the tests for *Limit of Nateglinide Related Compound A and Other Impurities, Limit of Nateglinide Related Compound B,* and *Limit of Nateglinide Related Compound C and Phenylalanine* is NMT 0.5%.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Nateglinide RS
(–)-*N*-[(*trans*-4-Isopropylcyclohexyl)carbonyl-D-phenylalanine.
$C_{19}H_{27}NO_3$ 317.42

USP Nateglinide Related Compound A RS
trans-4-Isopropylcyclohexylcarboxylic acid.
$C_{10}H_{18}O_2$ 170.2

USP Nateglinide Related Compound B RS
N-(*trans*-4-Isopropylcyclohexylcarbonyl)-L-phenylalanine.
$C_{19}H_{27}NO_3$ 317.4

USP Nateglinide Related Compound C RS
Nateglinide *cis*-isomer, *N*-(*cis*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine.
$C_{19}H_{27}NO_3$ 317.4

Nateglinide Tablets

DEFINITION

Nateglinide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of nateglinide ($C_{19}H_{27}NO_3$).

IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Diluent: Acetonitrile and water (11:9)

Mobile phase: Acetonitrile and 0.05% solution of trifluoroacetic acid (23:27)

Standard solution: 0.72 mg/mL of USP Nateglinide RS prepared as follows. Transfer USP Nateglinide RS to a suitable volumetric flask, and add acetonitrile to 40% of the volume of the flask. [NOTE—Sonicate to dissolve.] Add water equivalent to 30% of the final volume, mix, cool the solution to room temperature, and dilute with *Diluent* to volume.

Sample solution: Place 20 Tablets into a 500-mL volumetric flask, and add 60 mL of water to disintegrate the Tablets. [NOTE—Sonicate with cooling, if necessary.] Add 280 mL of acetonitrile, and shake by mechanical means for at least 30 min. Dilute with *Diluent* to volume. Pass a portion through a 0.45-µm glass microfiber filter, discarding the first 10 mL of the filtrate, or use centrifugation to obtain a clear solution. Dilute an aliquot of this solution with *Diluent* to obtain a solution having a concentration of 0.72 mg/mL based on the label claim.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability.*)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled quantity of $C_{19}H_{27}NO_3$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

USP Monographs

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PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.01 N hydrochloric acid containing 0.5% (w/v) of sodium lauryl sulfate; 1000 mL
Apparatus 2: 50 rpm
Time: 30 min
Determine the quantity of $C_{19}H_{27}NO_3$ dissolved by employing the following method.
Solution A: 6.9 mg/mL of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Solution A* (45:55)
Standard stock solution: 0.3 mg/mL of USP Nateglinide RS prepared as follows. Transfer USP Nateglinide RS to a suitable volumetric flask, dissolve in a small volume of acetonitrile not exceeding 5% of the final volume, and dilute with *Medium* to volume.
Standard solution: 0.12 mg/mL of USP Nateglinide RS in *Medium*, from the *Standard stock solution*
Sample solution: Pass through a suitable filter with pore size of 0.7 µm.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{27}NO_3$ dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V) \times (100/L)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)

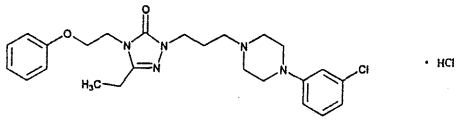
Tolerances: NLT 80% (Q) of the labeled amount of $C_{19}H_{27}NO_3$ is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Nateglinide RS

Nefazodone Hydrochloride



$C_{25}H_{32}ClN_5O_2 \cdot HCl$ 506.47

3*H*-1,2,4-Triazol-3-one, 2-[3-[4-(3-chlorophenyl)-1-piperazinyl)]propyl]-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-, monohydrochloride;
1-[3-[4-(*m*-Chlorophenyl)-1-piperazinyl]propyl]-3-ethyl-4-(2-phenoxyethyl)-Δ^2-1,2,4-triazolin-5-one monohydrochloride [82752-99-6].

DEFINITION

Nefazodone Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{25}H_{32}ClN_5O_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B. Identification Tests—General,** *Chloride* ⟨191⟩: Meets the requirements
Sample solution: 10 mg/mL in methanol

ASSAY

• **Procedure**
Sample solution: 800 mg of Nefazodone Hydrochloride in 50 mL of glacial acetic acid. Add 15 mL of 3% mercuric acetate in glacial acetic acid.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis
Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Carry out a blank titration.
Calculate the percentage of $C_{25}H_{32}ClN_5O_2 \cdot HCl$ in the portion of Nefazodone Hydrochloride taken:

$$\text{Result} = ((V - B) \times N \times F/W) \times 100$$

V = sample titrant volume (mL)
B = blank titrant volume (mL)
N = titrant normality (mEq/mL)
F = 506.5 (mg/mEq)
W = sample weight (mg)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

Organic Impurities

• **Procedure**
Diluent: Acetonitrile and water (1:1)
Solution A: 0.77 g/L of ammonium acetate in water. Adjust with triethylamine to a pH of 7.10 ± 0.05, and degas.
Solution B: Acetonitrile (degassed)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	50	50
10	45	55
16	35	65
25	35	65
26	50	50
35	50	50

Impurities stock solution: 0.1 mg/mL of USP Nefazodone Related Compound A RS and USP Nefazodone Related Compound B RS in *Diluent*
Standard stock solution: 0.1 mg/mL of USP Nefazodone Hydrochloride RS in *Diluent*

USP Monographs

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System suitability solution: 5 µg/mL each of USP Nefazodone Related Compound A RS and USP Nefazodone Related Compound B RS from the *Impurities stock solution* in the *Standard stock solution*
Standard solution: 1 µg/mL each of Nefazodone Hydrochloride, USP Nefazodone Related Compound A RS, and USP Nefazodone Related Compound B RS from the *Standard stock solution* and the *Impurities stock solution* in *Diluent*
Sample solution: 1 mg/mL of Nefazodone Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.7 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between nefazodone related compound A and nefazodone hydrochloride; NLT 1.5 between nefazodone hydrochloride and nefazodone related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0% for nefazodone related compound A and nefazodone related compound B, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each nefazodone related compound in the portion of Nefazodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the corresponding nefazodone related compound from the *Sample solution*
r_S = peak area of the corresponding nefazodone related compound from the *Standard solution*
C_S = concentration of the relevant USP RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

[NOTE—Use the peak area for nefazodone hydrochloride in the *Standard solution* as r_S to calculate any unknown impurity.]
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nefazodone related compound A	1.2	0.2
Nefazodone related compound B	0.94	0.2
Nefazodone	1.0	—
Any other individual, unidentified impurity	—	0.1

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample in a vacuum at 105° for 3 h: it loses NMT 0.5% of its weight.
• **COMPLETENESS OF SOLUTION** ⟨641⟩**:** Meets the requirements
Sample solution: 25 mg/mL in methanol

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Nefazodone Hydrochloride RS
USP Nefazodone Related Compound A RS
1-(3-Chloropropyl)-4-(chlorophenyl)piperazine.
$C_{13}H_{18}Cl_2N_2$ 273.20
USP Nefazodone Related Compound B RS
2-(3-(4-(Chlorophenyl)-1-piperazinyl)propyl)-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3*H*-1,2,4-triazol-3-one.
$C_{25}H_{32}ClN_5O_2$ 470.01

Nefazodone Hydrochloride Tablets

» Nefazodone Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nefazodone hydrochloride ($C_{25}H_{32}ClN_5O_2 \cdot HCl$).

Packaging and storage—Preserve in tight containers. Store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Nefazodone Hydrochloride RS
USP Nefazodone Related Compound A RS
1-(3-Chloropropyl)-4-(chlorophenyl)piperazine.
$C_{13}H_{18}Cl_2N_2$ 273.20
USP Nefazodone Related Compound B RS
2-(3-(4-(Chlorophenyl)-1-piperazinyl)propyl)-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3*H*-1,2,4-triazol-3-one.
$C_{25}H_{32}ClN_5O_2$ 470.01

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020)
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Standard stock solution—Transfer about 70 mg of USP Nefazodone Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask. Add 2.5 mL of methanol, dilute with *Medium* to volume, and mix.
Standard solution—Dilute the *Standard stock solution* with *Medium* in such a way that the final concentration is similar to the one expected in the *Test solution*.
Test solution—Use portions of the solution under test passed through a 0.45-µm PVDF filter, discarding the first 5 mL.
Procedure—Determine the percentage of the labeled amount of nefazodone hydrochloride dissolved by employing UV absorption, using a suitable spectrophotometer, at the wavelength of maximum absorbance at about 246 nm, on the *Test solution* in comparison with the *Standard solution*, using *Medium* as the blank. Calculate the percentage of nefazodone hydrochloride ($C_{25}H_{32}ClN_5O_2 \cdot HCl$) dissolved by the formula:

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$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times LC}$$

in which A_U and A_S are the absorbances obtained from the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of USP Nefazodone Hydrochloride RS in the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and *LC* is the tablet label claim, in mg.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{25}H_{32}ClN_5O_2 \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Related compounds—

Dilute acetic acid, Buffer solution, and *Mobile phase*—Proceed as directed in the *Assay.*

Nefazodone related compound A stock solution—Prepare a solution of USP Nefazodone Related Compound A RS in *Mobile phase* having a known concentration of about 80 µg per mL.

Nefazodone related compound B stock solution—Prepare a solution of USP Nefazodone Related Compound B RS in *Mobile phase* having a known concentration of about 80 µg per mL.

System suitability solution—Transfer about 10 mg of USP Nefazodone Hydrochloride RS into a 10-mL volumetric flask. Add 2.0 mL of *Nefazodone related compound A stock solution* and 2.0 mL of *Nefazodone related compound B stock solution,* and mix to dissolve the nefazodone hydrochloride. Dilute with *Mobile phase* to volume, and mix.

Standard solution—Use the *Standard preparation,* prepared as directed in the *Assay.*

Test solution—Use the *Assay stock preparation,* prepared as directed in the *Assay.*

Chromatographic system—Prepare as directed in the *Assay.* Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure.* Identify the peaks using the relative retention times given in *Table 1*: the resolution, *R,* between nefazodone related compound A and nefazodone hydrochloride is not less than 2.0; and the resolution, *R,* between nefazodone related compound B and nefazodone hydrochloride is not less than 1.5.

[NOTE—Approximate relative retention times are provided in *Table 1* for informational purposes only.]

Procedure—Inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of individual nefazodone related compounds in the portion of Tablets taken by the formula:

$$100(r_U/r_S)(C_S/C_T)(1/F)$$

in which r_U is the individual peak response for each nefazodone related compound obtained from the *Test solution;* r_S is the response of the corresponding peak in the *Standard solution,* respectively; C_S and C_T are the concentrations, in mg per mL, of nefazodone hydrochloride in the *Standard solution* and the *Test solution,* respectively; and *F* is the relative response factor obtained from *Table 1*. The related compound requirements are listed in *Table 1*.

Table 1

Related Compound	Relative Retention Time	Relative Response Factor *(F)*	Limit (%)
Nefazodone related compound A	1.4	1.2	0.2
Nefazodone related compound B	0.9	1.0	0.2
Any individual unknown impurity	—	1.0	0.2 each
Total known and unknown	—	—	0.5

Assay—

Dilute acetic acid—Prepare a mixture of acetic acid and water (1:1).

Buffer solution—Dissolve 0.77 g of ammonium acetate in 1 L of water. Add 1.0 mL of triethylamine, and mix well. Adjust with *Dilute acetic acid* to a pH of 7.10 ± 0.05, and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (58:42). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Prepare a solution of USP Nefazodone Hydrochloride RS in *Mobile phase* having a known concentration of about 0.1 mg per mL.

Assay stock preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of nefazodone hydrochloride, based on the label claim, to a 250-mL volumetric flask, add about 125 mL of *Mobile phase,* and sonicate for about 10 minutes with occasional shaking. Dilute with *Mobile phase* to volume, and mix to obtain a solution having a concentration of about 1 mg per mL of nefazodone hydrochloride. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, and use the filtrate, which has a concentration of about 1 mg per mL of nefazodone hydrochloride.

Assay preparation—Transfer 5.0 mL of *Assay stock preparation* into a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a solution having a concentration of 0.1 mg per mL of nefazodone hydrochloride.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 250-nm detector and a 4.6-mm × 25-cm column containing 5-µm L1 pack ing. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 30°. Inject the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in percent of label claim, of nefazodone hydrochloride ($C_{25}H_{32}ClN_5O_2 \cdot HCl$) in the portion of Tablets taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of nefazodone hydrochloride in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Neomycin for Injection

» Neomycin for Injection contains an amount of Neomycin Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.30 USP Endotoxin Units per mg of neomycin.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
Other requirements—It meets the requirements for *pH* and *Loss on drying* under *Neomycin Sulfate* and for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Assay preparation 1 (where it is packaged for dispensing) —Constitute Neomycin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer B.3* to obtain a solution having a convenient concentration.
Assay preparation 2 (where it is packaged for dispensing and where the labeling states the quantity of neomycin in a given volume of constituted solution)—Constitute Neomycin for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer B.3* to obtain a solution having a convenient concentration.
Procedure—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Boluses

» Neomycin Boluses contain an amount of Neomycin Sulfate equivalent to not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve in tight containers.
Labeling—Label Boluses to indicate that they are for veterinary use only.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
Identification—Blend a Bolus with 250 mL of water. Filter a portion of the suspension obtained. If necessary, dilute a portion of the filtrate with water to obtain a test solution containing about 2 mg of neomycin per mL. Dissolve a quantity of USP Neomycin Sulfate RS in water to obtain a Standard solution containing about 2 mg of neomycin per mL. Separately apply 1 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of water, butyl alcohol, glacial acetic acid, and pyridine (35:30:22:6) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry at about 110° for about 5 minutes. Spray the plate evenly with a solution of ninhydrin (2 mg per mL), and dry the plate at about 100° for about 5 minutes. Locate the spots on the plate: the R_F value of the principal spot in the chromatogram obtained from the test solution corresponds to that of the principal spot in the chromatogram obtained from the Standard solution.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation.*
Disintegration ⟨701⟩: 60 minutes.
Assay—Proceed as directed for the assay of neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, the *Test Dilution* being prepared as follows. Blend an accurately counted number of Boluses (not less than 2) at high speed in a blender jar with a sufficient accurately measured volume of *Buffer B.3* to obtain a stock solution having a convenient concentration. Dilute this stock solution quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate

Neomycin sulfate.
Neomycins sulfate [1405-10-3].

» Neomycin Sulfate is the sulfate salt of a kind of neomycin, an antibacterial substance produced by the growth of *Streptomyces fradiae* Waksman (Fam. Streptomycetaceae), or a mixture of two or more such salts. It has a potency equivalent to not less than 600 µg of neomycin per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: Dissolve about 10 mg in 1 mL of water, add 5 mL of 15 N sulfuric acid, and heat at 100° for 100 minutes. Allow to cool, add 10 mL of xylene, and shake for 10 minutes. Allow to separate, and decant the xylene layer. To the xylene layer add 10 mL of *p*-bromoaniline TS, and shake: a vivid pink-red color develops upon standing.
C: A solution (1 in 20) responds to the tests for *Sulfate* ⟨191⟩.
pH ⟨791⟩: between 5.0 and 7.5, in a solution containing 33 mg of neomycin per mL.
Loss on drying ⟨731⟩—Dry about 100 mg in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 8.0% of its weight.

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Other requirements—Where the label states that Neomycin Sulfate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Neomycin for Injection*. Where the label states that Neomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Neomycin for Injection*. Where it is intended for use in preparing nonparenteral sterile dosage forms, it is exempt from the requirements for *Bacterial endotoxins*.
Assay—Proceed with Neomycin Sulfate as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Neomycin Sulfate Cream

» Neomycin Sulfate Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Minimum fill ⟨755⟩: meets the requirements.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Cream, equivalent to about 1.75 mg of neomycin, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to an appropriate volume to obtain a stock solution of convenient concentration. Dilute this stock solution quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate Ointment

» Neomycin Sulfate Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, Method I ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Ointment, equivalent to about 3.5 mg of neomycin, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to an appropriate volume to obtain a stock solution of convenient concentration. Dilute this stock solution quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate Ophthalmic Ointment

DEFINITION
Neomycin Sulfate Ophthalmic Ointment is a sterile preparation of Neomycin Sulfate in a suitable ointment base. It contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of neomycin.

IDENTIFICATION
- **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩**:** Meets the requirements

ASSAY
- **Procedure**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of the Ophthalmic Ointment containing nominally 3.5 mg of neomycin in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–135.0%

SPECIFIC TESTS
- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Other Requirements:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in collapsible ophthalmic ointment tubes.
- **USP Reference Standards** ⟨11⟩
USP Neomycin Sulfate RS

Neomycin Sulfate Oral Solution

» Neomycin Sulfate Oral Solution contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin. It may contain one or more suitable colors, flavors, and preservatives.

Packaging and storage—Preserve in tight, light-resistant containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩**:** meets the requirements.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

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Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.
pH ⟨791⟩: between 5.0 and 7.5.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Oral Solution quantitatively diluted with *Buffer B.3* to yield a solution having a convenient concentration of neomycin. Quantitatively dilute this stock solution with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate Tablets

» Neomycin Sulfate Tablets contain the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS

Thin-layer chromatographic identification test ⟨201BNP⟩
—
Test solution—Shake a portion of ground Tablet powder, equivalent to about 70 mg of neomycin (base), with 5 mL of water, and filter. Dilute a portion of this solution with 0.1 N hydrochloric acid to obtain a solution containing the equivalent of 3.5 mg of neomycin (base) per mL. It meets the requirements.
Disintegration ⟨701⟩: 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Loss on drying ⟨731⟩—Dry about 100 mg of powdered Tablets, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 10.0% of its weight.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using not less than 5 Tablets blended at high-speed in a blender jar for 3 to 5 minutes with a sufficient accurately measured volume of *Buffer B.3* to obtain a stock solution having a convenient concentration. Dilute this stock solution quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate and Bacitracin Ointment

» Neomycin Sulfate and Bacitracin Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and bacitracin.

Packaging and storage—Preserve in tight, light-resistant containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Neomycin Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for bacitracin—Proceed with Ointment as directed in the *Assay* under *Bacitracin Ointment*

Neomycin Sulfate and Bacitracin Zinc Ointment

» Neomycin Sulfate and Bacitracin Zinc Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and bacitracin.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Neomycin Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin and Assay for bacitracin—Proceed with Ointment as directed in the *Assay for neomycin* and in the *Assay for bacitracin* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*

Neomycin Sulfate and Dexamethasone Sodium Phosphate Cream

DEFINITION
Neomycin Sulfate and Dexamethasone Sodium Phosphate Cream contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of neomycin, and the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$).

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩: Meets the requirements for neomycin
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay* for *Dexamethasone Phosphate.*

ASSAY
- **NEOMYCIN**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Cream, containing nominally 1.75 mg of neomycin, with 50 mL of ether, and extract with four 20-mL portions of *Buffer B.3.* Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* with a neomycin concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–135.0%

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• **DEXAMETHASONE PHOSPHATE**
Solution A: 6.9 g/L of monobasic sodium phosphate
Mobile phase: Methanol and *Solution A* (52:48)
Diluent: Dissolve 0.29 g of dibasic sodium phosphate in 450 mL of water, and add 550 mL of alcohol.
Standard solution: 33 µg/mL of USP Dexamethasone Sodium Phosphate RS in *Diluent*. Prepare this solution freshly.
Sample solution: Nominally 30 µg/mL of dexamethasone phosphate from Cream in *Diluent*, prepared as follows. Transfer a portion of Cream, containing nominally 3 mg of dexamethasone phosphate, to a suitable beaker. Add 65 mL of *Diluent*, and heat just to boiling. Pour the contents of the beaker into a separatory funnel containing 45 mL of isooctane. After shaking for 1 min, decant the lower layer into a 100-mL volumetric flask. Rinse the beaker with two 15-mL portions of *Diluent*, add each to the separatory funnel, shake, and decant the lower layer from each extraction into the 100-mL volumetric flask. Dilute with *Diluent* to volume, and mix. Pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for dexamethasone phosphate is about 8.5 min.]
Suitability requirements
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dexamethasone phosphate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of dexamethasone phosphate, 472.44
M_{r2} = molecular weight of dexamethasone sodium phosphate, 516.40

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dexamethasone Sodium Phosphate RS
USP Neomycin Sulfate RS

Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment

DEFINITION
Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate and Dexamethasone Sodium Phosphate. It contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of neomycin, and the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$).
[NOTE—Where Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment is prescribed without reference to the quantity of neomycin or dexamethasone phosphate contained therein, a product containing 3.5 mg of neomycin and 0.5 mg of dexamethasone phosphate per g shall be dispensed.]

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩**:** Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Dexamethasone Phosphate*.

ASSAY
• **NEOMYCIN**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a weighed portion of Ophthalmic Ointment in a separator with about 50 mL of ether, and extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–135.0%
• **DEXAMETHASONE PHOSPHATE**
Buffer: 6.9 g/L of monobasic sodium phosphate
Mobile phase: Methanol and *Buffer* (52:48)
Diluent: Dissolve 0.29 g of dibasic sodium phosphate in 450 mL of water, and add 550 mL of alcohol.
Standard solution: 33 µg/mL of USP Dexamethasone Sodium Phosphate RS in *Diluent*. Prepare this solution freshly.
Sample solution: Nominally 30 µg/mL of dexamethasone phosphate, prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 3 mg of dexamethasone phosphate to a suitable beaker. Add 65 mL of *Diluent*, and heat just to boiling. Pour the contents of the beaker into a separator containing 45 mL of isooctane. After shaking for 1 min, decant the lower layer into a 100-mL volumetric flask. Rinse the beaker with two 15-mL portions of *Diluent*, extracting the remaining isooctane in the separator with each portion, and decanting the lower layer from each extraction into the 100-mL volumetric flask. Dilute with *Diluent* to volume, and mix. Pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*

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[NOTE—The retention time for dexamethasone phosphate is about 8.5 min.]
Suitability requirements
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dexamethasone phosphate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of dexamethasone phosphate, 472.44
M_{r2} = molecular weight of dexamethasone sodium phosphate, 516.40

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dexamethasone Sodium Phosphate RS
  USP Neomycin Sulfate RS

Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution

DEFINITION
Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution is a sterile, aqueous solution of Neomycin Sulfate and Dexamethasone Sodium Phosphate. It contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amount of neomycin, and the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$). It may contain one or more suitable buffers, dispersants, and preservatives.
[NOTE—Where Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution is prescribed, without reference to the amount of neomycin or dexamethasone phosphate contained therein, a product containing 3.5 mg/mL of neomycin and 1.0 mg/mL of dexamethasone phosphate shall be dispensed.]

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩**:** Meets the requirements for neomycin
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Dexamethasone Phosphate*.

ASSAY
- **NEOMYCIN**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Analysis: Dilute an aliquot of Ophthalmic Solution with *Buffer B.3* to obtain a *Test Dilution* with a neomycin concentration that is nominally equivalent to the median level of the standard (1.0 µg/mL).
Acceptance criteria: 90.0%–130.0%
- **DEXAMETHASONE PHOSPHATE**
Solution A: 0.29 g/L of dibasic sodium phosphate
Solution B: 13.80 g/L of monobasic sodium phosphate
Mobile phase: Acetonitrile and *Solution B* (31:69)
Standard solution: 27 µg/mL of USP Dexamethasone Sodium Phosphate RS in *Solution A*. Pass through a filter of 1-µm or finer pore size.
Sample solution: Nominally 25 µg/mL of dexamethasone phosphate from Ophthalmic Solution in *Solution A* prepared as follows. Slowly dilute a portion of Ophthalmic Solution with *Solution A* to volume, mix, and pass through a suitable filter of 1-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.3 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for dexamethasone phosphate is about 8.5 min.]
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in each mL of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dexamethasone phosphate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of dexamethasone phosphate, 472.44
M_{r2} = molecular weight of dexamethasone sodium phosphate, 516.40

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **PH** ⟨791⟩**:** 6.0–8.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dexamethasone Sodium Phosphate RS
  USP Neomycin Sulfate RS

USP Monographs

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Neomycin Sulfate and Fluocinolone Acetonide Cream

» Neomycin Sulfate and Fluocinolone Acetonide Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers.
USP Reference standards ⟨11⟩—
USP Fluocinolone Acetonide RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: It meets the requirements for the *Identification* test under *Fluocinolone Acetonide Cream.*
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Cream.*
Assay for fluocinolone acetonide—Proceed with Cream as directed in the *Assay* under *Fluocinolone Acetonide Cream.*

Neomycin Sulfate and Fluorometholone Ointment

» Neomycin Sulfate and Fluorometholone Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluorometholone ($C_{22}H_{29}FO_4$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.
USP Reference standards ⟨11⟩—
USP Fluorometholone RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The ratios of the retention time of the main peak to that of the internal standard peak obtained from the *Standard preparation* and the *Assay preparation* as directed in the *Assay for fluorometholone* do not differ by more than 2.0%.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*

Assay for fluorometholone—
Internal standard solution, Mobile solvent, and *Standard preparation*—Prepare as directed in the *Assay* under *Fluorometholone Cream.*
Assay preparation—Transfer an accurately weighed quantity of Ointment, equivalent to about 1 mg of fluorometholone, to a suitable container, add 20.0 mL of *Internal standard solution,* and mix.
Procedure—Treat 20.0 mL each of the *Standard preparation* and the *Assay preparation* in the following manner. To each add 10.0 mL of hexane, shake for about 15 minutes, then allow the layers to separate, and centrifuge, if necessary. Using the lower (acetonitrile) layer, proceed as directed for *Procedure* in the *Assay* under *Fluorometholone Cream,* beginning with "Using a suitable microsyringe." Calculate the quantity, in mg, of $C_{22}H_{29}FO_4$ in the portion of Ointment taken by the formula:

$$20C(R_U/R_S)$$

in which the terms are as defined therein.

Neomycin Sulfate and Flurandrenolide Cream

» Neomycin Sulfate and Flurandrenolide Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Flurandrenolide RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: It meets the requirements for the *Identification* test under *Flurandrenolide Cream.*
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for flurandrenolide—Proceed with Cream as directed in the *Assay* under *Flurandrenolide Cream.* Calculate the quantity, in mg, of $C_{24}H_{33}FO_6$ in the portion of Cream taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Neomycin Sulfate and Flurandrenolide Lotion

» Neomycin Sulfate and Flurandrenolide Lotion contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Flurandrenolide RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: It meets the requirements for the *Identification* test under *Flurandrenolide Cream.*
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Lotion, equivalent to about 3.5 mg of neomycin, blended for 3 to 5 minutes in a high-speed glass blender jar containing an accurately measured volume of *Buffer B.3* sufficient to obtain a stock solution having a convenient concentration of neomycin. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median dose level of the Standard.
Assay for flurandrenolide—Proceed with Neomycin Sulfate and Flurandrenolide Lotion as directed in the *Assay* under *Flurandrenolide Cream.* Calculate the quantity, in mg, of $C_{24}H_{33}FO_6$ in the portion of Lotion taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin Sulfate and Flurandrenolide Ointment

» Neomycin Sulfate and Flurandrenolide Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Flurandrenolide RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: It meets the requirements for the *Identification* test under *Flurandrenolide Cream.*
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for flurandrenolide—Proceed with Ointment as directed in the *Assay* under *Flurandrenolide Cream.* Calculate the quantity, in mg, of $C_{24}H_{33}FO_6$ in the portion of Ointment taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin Sulfate and Gramicidin Ointment

» Neomycin Sulfate and Gramicidin Ointment contains the equivalent of not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of neomycin and gramicidin.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.
USP Reference standards ⟨11⟩—
USP Gramicidin RS
USP Neomycin Sulfate RS
Thin-Layer Chromatographic Identification Test ⟨201BNP⟩**:** meets the requirements.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for gramicidin—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Ointment dissolved in 50 mL of hexanes in a separator, and extracted with four 20-mL portions of 80 percent alcohol. Combine the extracts in a suitable volumetric flask, dilute with alcohol to volume, and mix. Dilute this solution quantitatively and stepwise with alcohol to obtain a *Test Dilution* having a concentration of gramicidin assumed to be equal to the median dose level of the Standard.

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Neomycin Sulfate and Hydrocortisone Cream

» Neomycin Sulfate and Hydrocortisone Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peaks for hydrocortisone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone.*
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for hydrocortisone—Proceed with Cream as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment.*

Neomycin Sulfate and Hydrocortisone Ointment

» Neomycin Sulfate and Hydrocortisone Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for hydrocortisone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone.*
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for hydrocortisone—Proceed with Ointment as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment.*

Neomycin Sulfate and Hydrocortisone Otic Suspension

» Neomycin Sulfate and Hydrocortisone Otic Suspension is a sterile suspension containing not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone. It contains Acetic Acid, and may contain one or more suitable buffers, dispersants, and preservatives.
[NOTE—Where Neomycin Sulfate and Hydrocortisone Otic Suspension is prescribed, without reference to the quantity of neomycin or hydrocortisone contained therein, a product containing 3.5 mg of neomycin and 10 mg of hydrocortisone per mL shall be dispensed.]

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Neomycin Sulfate RS
Sterility Tests ⟨71⟩ **:** meets the requirements.
pH ⟨791⟩: between 4.5 and 6.0.
Assay for neomycin—Using an accurately measured volume of Otic Suspension, freshly mixed and free from entrapped air, proceed as directed in the *Assay for neomycin* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution.*

Assay for hydrocortisone—
Mobile phase and *Standard preparation*—Prepare as directed in the *Assay for hydrocortisone content* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment.*
Assay preparation—Transfer 3.0 mL of Otic Suspension, freshly mixed and free from entrapped air, to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Filter the solution, rejecting the first 10 mL of the filtrate.
Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone content* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment.* Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Suspension taken by the formula:

$$(66.67C)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Neomycin Sulfate and Hydrocortisone Acetate Cream

» Neomycin Sulfate and Hydrocortisone Acetate Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation,* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for hydrocortisone acetate.*
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for hydrocortisone acetate—Proceed with Cream as directed in the *Assay* under *Hydrocortisone Acetate Lotion.*

Neomycin Sulfate and Hydrocortisone Acetate Lotion

» Neomycin Sulfate and Hydrocortisone Acetate Lotion contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for hydrocortisone acetate.*
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, blending an accurately measured volume of Lotion for 3 to 5 minutes in a high-speed glass blender jar containing an accurately measured volume of *Buffer B.3.* Dilute an accurately measured volume of the solution so obtained quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median dose level of the Standard.
Assay for hydrocortisone acetate—Proceed with Lotion as directed in the *Assay* under *Hydrocortisone Acetate Lotion.*

Neomycin Sulfate and Hydrocortisone Acetate Ointment

» Neomycin Sulfate and Hydrocortisone Acetate Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for hydrocortisone acetate.*
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin—Proceed with the Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for hydrocortisone acetate—Proceed with the Ointment as directed in the *Assay* under *Hydrocortisone Acetate Lotion.*

Neomycin Sulfate and Hydrocortisone Acetate Ophthalmic Suspension

» Neomycin Sulfate and Hydrocortisone Acetate Ophthalmic Suspension is a sterile, aqueous suspension containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.
USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS

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Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay for hydrocortisone acetate* exhibits a major peak for hydrocortisone acetate, the retention time of which corresponds with that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for hydrocortisone acetate.*
Sterility Tests ⟨71⟩ **:** meets the requirements.
pH ⟨791⟩: between 5.5 and 7.5.
Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone acetate—
Mobile phase—Prepare a solution containing *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6).
Internal standard solution—Prepare a solution of fluoxymesterone in chloroform containing 0.8 mg per mL.
Standard preparation—Dissolve about 10 mg of USP Hydrocortisone Acetate RS, accurately weighed, in 10.0 mL of *Internal standard solution*, dilute with about 40 mL of chloroform, and mix.
Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 10 mg of hydrocortisone acetate, to a suitable container. Add 10.0 mL of *Internal standard solution* and about 40 mL of chloroform, shake vigorously for about 5 minutes, and allow the phases to separate. Use the clear chloroform layer as the *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the analyte and internal standard peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 and 1.0 for hydrocortisone acetate and fluoxymesterone, respectively. Calculate the quantity, in mg, of hydrocortisone acetate ($C_{23}H_{32}O_6$) in each mL of the Ophthalmic Suspension taken by the formula:

$$(W/V)(R_U/R_S)$$

in which W is the quantity, in mg, of USP Hydrocortisone Acetate RS taken to prepare the *Standard preparation*, V is the volume, in mL, of Ophthalmic Suspension taken, and R_U and R_S are the peak response ratios of the hydrocortisone acetate peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Ointment

» Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Ointment contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of neomycin, and not less than 92.5 percent and not more than 117.5 percent of the labeled amounts of isoflupredone acetate ($C_{23}H_{29}FO_6$) and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in a suitable ointment base.

Packaging and storage—Preserve in collapsible tubes or well-closed containers.
Labeling—Label it to indicate that it is intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Isoflupredone Acetate RS
USP Neomycin Sulfate RS
USP Tetracaine Hydrochloride RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—To 2 g of Ointment in a centrifuge tube add 25 mL of chloroform, and heat at 60° for 5 minutes, with occasional shaking. Centrifuge, discard the chloroform layer, add 5 mL of water, shake, and filter. Use the filtrate.
Standard solution—Prepare a solution containing 2 mg of USP Neomycin Sulfate RS per mL.
Application volume: 1 µL.
Developing solvent system: a mixture of water, butyl alcohol, glacial acetic acid, and pyridine (35:30:22:6).
Spray reagent: a solution of triketohydrindene hydrate in butyl alcohol (2 in 1000).
Procedure—Proceed as directed in the chapter, except to locate the spots by spraying with *Spray reagent* and heating at 100° for 5 minutes.
B: The retention time of the major peak for isoflupredone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for isoflupredone acetate.*
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, a mixture of methanol and chloroform (3:2) being used instead of methanol in the titration vessel and the titration vessel being heated to between 45° and 55°.
Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩. Place an accurately weighed portion of Ointment in a centrifuge tube with 25 mL of chloroform. Heat at 60° for 3 minutes, shake until the Ointment is dissolved, centrifuge, and remove and discard the chloroform. Add 15 mL of chloroform, shake, centrifuge, and remove and discard the chloroform. Add 5.0 mL of water and 15 mL of chromatographic *n*-heptane, shake, centrifuge, and remove and discard the *n*-heptane layer. Dilute an accurately measured volume of the water layer quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median dose level of the Standard.

Assay for isoflupredone acetate—
Mobile phase, Diluent, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Isoflupredone Acetate.*

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Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 4 mg of isoflupredone acetate, to a suitable container. Add 8.0 mL of *Internal standard solution*, 32.0 mL of *Diluent*, and about 10 glass beads. Shake for about 15 minutes, centrifuge, and use the clear chloroform portion.

Procedure—Proceed as directed in the *Assay* under *Isoflupredone Acetate*. Calculate the quantity, in mg, of isoflupredone acetate ($C_{23}H_{29}FO_6$) in the portion of Ointment taken by the formula:

$$W_S(R_U/R_S)$$

in which the terms are as defined therein.

Assay for tetracaine hydrochloride—

Standard preparation—Prepare a solution in chloroform having a known concentration of about 5.0 µg of USP Tetracaine Hydrochloride RS per mL.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 1.25 mg of tetracaine hydrochloride, to a 250-mL volumetric flask, add about 100 mL of chloroform, and warm on a steam bath for about 3 minutes to dissolve the Ointment. Cool to room temperature, dilute with chloroform to volume, and mix.

Blank solution—Transfer an accurately weighed portion of the ointment base, equivalent to the weight used in the *Assay preparation*, to a 250-mL volumetric flask. Add 100 mL of chloroform, warm on a steam bath for about 3 minutes to dissolve, and allow to stand until the solution has equilibrated to room temperature. Dilute with chloroform to volume, and mix well.

Procedure—Concomitantly determine the absorbances of the *Standard preparation*, the *Blank solution*, and the *Assay preparation* with a suitable spectrophotometer at the wavelength of maximum absorbance at about 310 nm, using chloroform to zero the instrument. Calculate the absorbance of the *Blank solution*, A_B, adjusted for weight difference between the *Assay preparation* and the *Blank solution*, by the formula:

$$A(W_T/W_B)$$

in which A is the absorbance of the *Blank solution*; W_T is the weight, in mg, of Ointment taken to prepare the *Assay preparation*; and W_B is the weight, in mg, of the ointment base taken to prepare the *Blank solution*. Calculate the quantity, in mg, of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Ointment taken by the formula:

$$250C\,[(A_U - A_B)/A_S]$$

in which C is the concentration, in mg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation*; A_B is as obtained above; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Topical Powder

» Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Topical Powder contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of isoflupredone acetate ($C_{23}H_{29}FO_6$) and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards ⟨11⟩—
USP Isoflupredone Acetate RS
USP Neomycin Sulfate RS
USP Tetracaine Hydrochloride RS

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—*Test solution*—Add 20 mL of chloroform to 1 g of Topical Powder, shake for 5 to 10 minutes, and centrifuge. Evaporate a 10-mL portion of the clear solution to dryness, and dissolve the residue in 1 mL of a mixture of chloroform and alcohol (1:1).

Standard solution: 0.5 mg of USP Isoflupredone Acetate RS per mL, in a mixture of chloroform and alcohol (1:1).

Application volume: 30 µL.

Developing solvent system: a mixture of methylene chloride and methanol (180:16), in a paper-lined chromatographic chamber.

Spray reagent: a solution of sulfuric acid in methanol (70 in 100).

Procedure—Proceed as directed in the chapter, except to use a plate that has been activated by heating in an oven at 105° for 60 minutes. Allow the plate to cool before using. Locate the spots under short- and long-wavelength UV light. Spray the plate with *Spray reagent*, heat at 90° for 30 minutes, and locate the spots under short- and long-wavelength UV light (*presence of isoflupredone acetate*).

B: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—*Test solution*—To 1 g of Topical Powder in a centrifuge tube add 5 mL of water, and shake until dissolved. Prepare a suspension of 10 g of cation-exchange resin in 10 mL of water, add 5 mL of a solution of sodium hydroxide (1 in 2), mix, and wash the resin with water until the pH of the wash is about 9. Add 0.3 g of this suspension to the solution of Topical Powder, and shake for 10 seconds. Centrifuge for 1 minute, and discard the supernatant. Wash the resin in the tube with 10 mL of water, centrifuge, and discard the supernatant. Add 2 mL of 1 M ammonium hydroxide, shake for 10 seconds, and filter. Use the filtrate.

Standard solution, *Application volume*, *Developing solvent system*, *Spray reagent*, and *Procedure*—Proceed as directed in *Identification* test *A* under *Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Ointment* (*presence of neomycin*).

Minimum fill ⟨755⟩: meets the requirements.

Loss on drying ⟨731⟩—Dry about 2 g in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 8.0% of its weight.

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, the *Test Dilution* being prepared as follows. Use an accurately weighed quantity of Topical Powder diluted quantitatively with *Buffer B.3* to obtain a solution having a suitable concentration of neomycin. Dilute this stock solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

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Assay for isoflupredone acetate—

Mobile phase, Diluent, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Isoflupredone Acetate.*

Assay preparation—Transfer an accurately weighed portion of Topical Powder, equivalent to about 4 mg of isoflupredone acetate, to a suitable container. Add 8.0 mL of *Internal standard solution,* 32.0 mL of *Diluent,* and about 10 glass beads. Shake for about 15 minutes, centrifuge, and use the clear chloroform portion.

Procedure—Proceed as directed in the *Assay* under *Isoflupredone Acetate.* Calculate the quantity, in mg, of isoflupredone acetate ($C_{23}H_{29}FO_6$) in the portion of Topical Powder taken by the formula:

$$W_S(R_U/R_S)$$

in which the terms are as defined therein.

Assay for tetracaine hydrochloride—

Standard preparation—Prepare a solution having a known concentration of 5.5 µg of USP Tetracaine Hydrochloride RS per mL.

Assay preparation—Transfer an accurately weighed portion of Topical Powder, equivalent to about 5.5 mg of tetracaine hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pass about 30 mL through a fine, sintered-glass filter. Transfer 10.0 mL of the clear filtrate to a second 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 310 nm, with a suitable spectrophotometer, using water to zero the instrument. Calculate the quantity, in mg, of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Topical Powder taken by the formula:

$$1000C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin Sulfate and Methylprednisolone Acetate Cream

DEFINITION

Neomycin Sulfate and Methylprednisolone Acetate Cream contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of neomycin, and NLT 90.0% and NMT 110.0% of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$).

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩**:** Meets the requirements for neomycin

• **B.**

Solution A, Solution B, Standard solution, Sample solution, Adsorbent, Application volume, and **Developing solvent system:** Proceed as directed in the *Assay* for *Methylprednisolone Acetate.*

Analysis: Proceed with thin-layer chromatography as directed in the *Assay* for *Methylprednisolone Acetate.*

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY

• **Neomycin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Shake a portion of Cream containing nominally 3.5 mg of neomycin in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3.* Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–135.0%

• **Methylprednisolone Acetate**

Solution A: Alcohol and chloroform (1:1)

Solution B: Alcohol and tetramethylammonium hydroxide TS (9:1)

Standard solution: 500 µg/mL of USP Methylprednisolone Acetate RS in *Solution A*

Sample solution: Transfer a portion of Cream containing nominally 5 mg of methylprednisolone acetate to a 125-mL separator, and add 50 mL of solvent hexane. Extract with three 10-mL portions of acetonitrile, and evaporate the combined extracts on a steam bath with the aid of a current of air nearly to dryness. Transfer the residue to a 10-mL volumetric flask with the aid of one 5-mL portion and two 2-mL portions of *Solution A.* Dilute with *Solution A* to volume.

Adsorbent: 0.5-mm layer of chromatographic silica gel mixture (see *Chromatography* ⟨621⟩)

Application volume: 250 µL

Developing solvent system: Ethyl acetate and chloroform (7:5)

Analysis

Samples: *Standard solution* and *Sample solution*

Divide the plate into three equal sections, the left and right sections to be used for the *Sample solution* and *Standard solution,* respectively, and the center section for the blank. Apply the solutions as streaks 2.5 cm from the bottom of the designated section of the plate, and dry the streaks with the aid of a current of air. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Locate the principal bands from the *Standard solution* and the *Sample solution* (see also *Identification* test *B*) by viewing under short-wavelength UV light. Mark these bands and the corresponding band in the section of the plate representing the blank. Quantitatively remove the silica gel containing these bands, and transfer to separate glass-stoppered, 50-mL centrifuge tubes. Add 25.0 mL of alcohol to each tube, shake for 2 min, and centrifuge at about 1500 rpm for 5 min. Transfer 20.0 mL of each supernatant to separate glass-stoppered, 50-mL conical flasks. Add 2.0 mL of blue tetrazolium TS to each solution, and to each flask, add 2.0 mL of *Solution B.* Mix, and allow the solutions to stand in the dark for 90 min.

Instrumental conditions

Mode: Vis

Analytical wavelength: Maximum at about 525 nm

Cell: 1 cm

Calculate the percentage of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Cream taken:

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$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methylprednisolone acetate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers, protected from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methylprednisolone Acetate RS
USP Neomycin Sulfate RS

Neomycin and Polymyxin B Sulfates Cream

» Neomycin and Polymyxin B Sulfates Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B. It may contain a suitable local anesthetic.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩**:** meets the requirements.
Minimum fill ⟨755⟩**:** meets the requirements.
Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Cream, equivalent to about 1.75 mg of neomycin, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to an appropriate volume to obtain a stock solution of convenient concentration. Dilute this stock solution quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Assay for polymyxin B—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Cream shaken with about 50 mL of ether in a separator, and extracted with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to an appropriate volume to obtain a stock solution. Dilute this stock solution quantitatively and stepwise with *Buffer B.6* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (10 Polymyxin B Units per mL). Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin present in the *Test Dilution*.

Neomycin and Polymyxin B Sulfates Solution for Irrigation

» Neomycin and Polymyxin B Sulfates Solution for Irrigation is a sterile, aqueous solution containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and of polymyxin B. It may contain a suitable preservative.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is to be diluted for use in a urinary bladder irrigation and is not intended for injection.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩**:** meets the requirements.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩**:** between 4.5 and 6.0.
Assay for neomycin and Assay for polymyxin B—Proceed with Neomycin and Polymyxin B Sulfates Solution for Irrigation as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution.*

Neomycin and Polymyxin B Sulfates Ophthalmic Ointment

DEFINITION
Neomycin and Polymyxin B Sulfates Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate and Polymyxin B Sulfate. It contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amounts of neomycin and polymyxin B.

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩**:** Meets the requirements

ASSAY
• **NEOMYCIN**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–130.0%
• **POLYMYXIN B**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 Polymyxin B Units/mL).

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Add to each *Test Dilution* of the standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.
Acceptance criteria: 90.0%–130.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Neomycin Sulfate RS
  USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates Ophthalmic Solution

» Neomycin and Polymyxin B Sulfates Ophthalmic Solution contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B. It may contain one or more suitable buffers, dispersants, irrigants, and preservatives.

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 5.0 and 7.0.
Assay for neomycin and Assay for polymyxin B— Proceed with Ophthalmic Solution as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution.*

Neomycin and Polymyxin B Sulfates and Bacitracin Ointment

» Neomycin and Polymyxin B Sulfates and Bacitracin Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin. It may contain a suitable local anesthetic.

Packaging and storage—Preserve in tight, light-resistant containers, preferably at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin and Assay for polymyxin B— Proceed with Ointment as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*
Assay for bacitracin—Proceed with Ointment as directed in the *Assay* under *Bacitracin Ointment.*

Neomycin and Polymyxin B Sulfates and Bacitracin Ophthalmic Ointment

DEFINITION
Neomycin and Polymyxin B Sulfates and Bacitracin Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate, Polymyxin B Sulfate, and Bacitracin. It contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin.

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩**:** Meets the requirements

ASSAY
- **NEOMYCIN**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
  Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.
  Acceptance criteria: 90.0%–140.0%
- **POLYMYXIN B**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.
  Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 Polymyxin B Units/mL). Add to each *Test Dilution* of the standard, a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.
  Acceptance criteria: 90.0%–140.0%
- **BACITRACIN**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

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Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (1.0 Bacitracin Unit/mL). If the *Sample solution* has a concentration of less than 100 Bacitracin Units/mL, add hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.
Acceptance criteria: 90.0%–140.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bacitracin Zinc RS
  USP Neomycin Sulfate RS
  USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin, and Hydrocortisone Acetate Ointment

» Neomycin and Polymyxin B Sulfates, Bacitracin, and Hydrocortisone Acetate Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate in a suitable ointment base.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Identification—
A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone acetate*.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin and Assay for polymyxin B— Proceed with Ointment as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.
Assay for bacitracin—Proceed with Ointment as directed in the *Assay* under *Bacitracin Ointment*.
Assay for hydrocortisone acetate—Proceed with Ointment as directed in the *Assay* under *Hydrocortisone Acetate Lotion*.

Neomycin and Polymyxin B Sulfates, Bacitracin, and Hydrocortisone Acetate Ophthalmic Ointment

DEFINITION
Neomycin and Polymyxin B Sulfates, Bacitracin, and Hydrocortisone Acetate Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin, and NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone acetate in a suitable ointment base.

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩: Meets the requirements
- **B.** The retention time of the hydrocortisone acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Hydrocortisone Acetate*.

ASSAY
- **NEOMYCIN**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
  Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.
  Acceptance criteria: 90.0%–140.0%
- **POLYMYXIN B**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.
  Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 polymyxin B units/mL). Add to each *Test Dilution* of the standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.
  Acceptance criteria: 90.0%–140.0%
- **BACITRACIN**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 20-mL portions of *Buffer B.1*. Combine the buffer extracts, and dilute with *Buffer B.1* to a suitable volume.
  Analysis: Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to a portion of the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.
  Acceptance criteria: 90.0%–140.0%

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• **HYDROCORTISONE ACETATE**
Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30)
Standard solution: 0.10 mg/mL of USP Hydrocortisone Acetate RS in water-saturated chloroform
Sample solution: Nominally 0.10 mg/mL of hydrocortisone acetate from Ophthalmic Ointment prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 2.5 mg of hydrocortisone acetate to a closable container. Add 25.0 mL of water-saturated chloroform and about 10 glass beads. Securely close the container, and shake vigorously for approximately 15 min. Centrifuge, and use the clear, lower chloroform layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L3
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
• **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Bacitracin Zinc RS
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment

» Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Lidocaine RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Identification—
A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for lidocaine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lidocaine*.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin and Assay for polymyxin B— Proceed with Ointment as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.
Assay for bacitracin—Proceed with Ointment as directed in the *Assay* under *Bacitracin Ointment*.

Assay for lidocaine—
Mobile phase—Dissolve 4.44 g of docusate sodium in 1000 mL of a mixture of methanol and water (4:1), add 1 mL of 0.1 N sulfuric acid, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve a suitable quantity of USP Lidocaine RS, accurately weighed, in *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL.
Assay preparation—Transfer an accurately weighed quantity of Ointment, equivalent to about 40 mg of lidocaine, to a separator, add 50 mL of *n*-hexane, and shake until the specimen is in solution. Add 30 mL of *Mobile phase*, shake for 1 minute, and allow the layers to separate. Drain the lower layer into a 100-mL volumetric flask, and extract the *n*-hexane layer remaining in the separator with two 30-mL portions of *Mobile phase*, combining the lower layers in the volumetric flask. Dilute the combined extracts in the 100-mL volumetric flask with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Ointment taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Lidocaine RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ointment

» Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin. It may contain a suitable local anesthetic.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin, Assay for polymyxin B, and Assay for bacitracin—Proceed with Ointment as directed in the *Assay for neomycin,* in the *Assay for polymyxin B,* and in the *Assay for bacitracin* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*

Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment

DEFINITION
Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin.

IDENTIFICATION
- **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩**:** Meets the requirements

ASSAY
- **Neomycin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3.* Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–140.0%

- **Polymyxin B**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6.* Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 Polymyxin B Units/mL). Add to each *Test Dilution* of the standard, a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6,* to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.
Acceptance criteria: 90.0%–140.0%

- **Bacitracin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (1.0 Bacitracin Unit/mL). If the *Sample solution* has a concentration of less than 100 Bacitracin Units/mL, add hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.
Acceptance criteria: 90.0%–140.0%

SPECIFIC TESTS
- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Other Requirements:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents.*

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Bacitracin Zinc RS
  USP Neomycin Sulfate RS
  USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ointment

» Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Hydrocortisone RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Identification—
A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for hydrocortisone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for hydrocortisone.*

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Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin, Assay for polymyxin B, and Assay for bacitracin—Proceed with Ointment as directed in the *Assay for neomycin*, in the *Assay for polymyxin B*, and in the *Assay for bacitracin* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*
Assay for hydrocortisone—Proceed with the Ointment as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment.*

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment

DEFINITION
Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate, Polymyxin B Sulfate, Bacitracin Zinc, and Hydrocortisone. It contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin, and NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

IDENTIFICATION
- **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩**:** Meets the requirements
- **B.** The retention time of the hydrocortisone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Hydrocortisone*.

ASSAY
- **Neomycin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–140.0%

- **Polymyxin B**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 polymyxin B Units/mL). Add to each *Test Dilution* of the standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.
Acceptance criteria: 90.0%–140.0%

- **Bacitracin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (1.0 bacitracin Unit/mL). If the *Sample solution* has a concentration of less than 100 bacitracin Units/mL, add hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.
Acceptance criteria: 90.0%–140.0%

- **Hydrocortisone**

Mobile phase: Methanol, glacial acetic acid, and water (500:1:500)
Diluent: Methanol and water (1:1)
Standard solution: 0.15 mg/mL of USP Hydrocortisone RS in *Diluent*
Sample solution: Transfer 1.5 g of Ophthalmic Ointment to a separator. Add 3 mL of *n*-hexane, and warm gently on a steam bath with mild agitation until dissolved. Add 7 mL of *n*-hexane, mix by swirling, and extract with four 15-mL portions of *Diluent*. Collect the extracts in a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Filter the solution, rejecting the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Other Requirements:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in collapsible ophthalmic ointment tubes.
- **USP Reference Standards** ⟨11⟩

USP Bacitracin Zinc RS
USP Hydrocortisone RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

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Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Acetate Ophthalmic Ointment

DEFINITION

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Acetate Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate, Polymyxin B Sulfate, Bacitracin Zinc, and Hydrocortisone Acetate. It contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin, and NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

IDENTIFICATION

- **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩: Meets the requirements
- **B.** The retention time of the hydrocortisone acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Hydrocortisone Acetate*.

ASSAY

- **Neomycin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

- **Polymyxin B**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 polymyxin B Units/mL). Add to each *Test Dilution* of the standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–140.0%

- **Bacitracin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (1.0 bacitracin Unit/mL). If the *Sample solution* has a concentration of less than 100 bacitracin Units/mL, add hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–140.0%

- **Hydrocortisone Acetate**

Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30)

Standard solution: 0.10 mg/mL of USP Hydrocortisone Acetate RS in water-saturated chloroform

Sample solution: Nominally 0.10 mg/mL of hydrocortisone acetate from Ophthalmic Ointment prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 2.5 mg of hydrocortisone acetate to a closable container. Add 25.0 mL of water-saturated chloroform and about 10 glass beads. Securely close the container, and shake vigorously for approximately 15 min. Centrifuge, and use the clear, lower chloroform layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L3

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrocortisone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Other Requirements:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩

USP Bacitracin Zinc RS
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Lidocaine Ointment

» Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Lidocaine Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin, and not less than 90.0 percent and not more than 110.0

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percent of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Lidocaine RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Identification—
A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for lidocaine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for lidocaine.*
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin—Proceed with Ointment as directed in the *Assay for neomycin* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*
Assay for polymyxin B—Proceed with Ointment as directed in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*
Assay for bacitracin—Proceed with Ointment as directed in the *Assay for bacitracin* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*

Assay for lidocaine—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment.*
Assay preparation—Using the Ointment, proceed as directed for *Assay preparation* in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment.*
Procedure—Proceed as directed for *Procedure* in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment.* Calculate the quantity, in mg, of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Ointment taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Lidocaine RS in the *Standard preparation;* and r_U and r_S are the lidocaine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment

DEFINITION
Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amounts of neomycin and polymyxin B, and NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩**:** Meets the requirements
• **B.** The retention time of the dexamethasone peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay* for *Dexamethasone.*

ASSAY
• **Neomycin**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3.* Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–130.0%
• **Polymyxin B**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6.* Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 polymyxin B units/mL). Add to each *Test Dilution* of the standard, a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6,* to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.
Acceptance criteria: 90.0%–130.0%
• **Dexamethasone**
Mobile phase: Acetonitrile and water (1 in 3)
Diluent: Acetonitrile and methanol (1:1)
Standard solution: 60 µg/mL of USP Dexamethasone RS in *Diluent*
Sample solution: Nominally 60 µg/mL of dexamethasone from Ophthalmic Ointment in *Diluent* prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 3 mg of dexamethasone to a suitable test tube, and add 15 mL of cyclohexane. Heat in a water bath at 75 ± 5° for 10 min. If the Ophthalmic Ointment is not fully dissolved, heat on a steam bath for about 30 s, place a cap on the test tube, and place on a vortex mixer until all solid material is dissolved. Pass with suction through a medium-porosity, sintered-glass filter. Rinse the test tube twice with 10-mL portions of cyclohexane, passing the rinsings through the filter, and discard the filtrate. Wash the filter with about 10 mL of a mixture of *Diluent,* and collect the filtrate in a 50-mL beaker. Wash the test tube and the filter with several 10-mL portions of *Diluent,* and combine the washings in the 50-mL beaker. Transfer the contents of the beaker to a 50-mL volumetric flask with the aid of *Diluent,* and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5- to 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Ophthalmic Ointment taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dexamethasone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dexamethasone RS
  USP Neomycin Sulfate RS
  USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Suspension contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone. It may contain one or more suitable buffers, stabilizers, preservatives, and suspending agents.

Packaging and storage—Preserve in tight, light-resistant containers in a cool place or at controlled room temperature. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.
USP Reference standards ⟨11⟩—
USP Dexamethasone RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Identification—Transfer a quantity of Ophthalmic Suspension, equivalent to about 2.5 mg of dexamethasone, to a suitable test tube, add 5 mL of chloroform, mix, and centrifuge. Apply 25 µL of the lower chloroform layer and 25 µL of a Standard solution of USP Dexamethasone RS in chloroform containing 500 µg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and diethylamine (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 3.5 and 6.0.
Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as is present in the *Test Dilution.*

Assay for dexamethasone—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for dexamethasone* under *Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment.*
Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone RS in *Mobile phase* to obtain a solution having a known concentration of about 0.12 mg per mL.
Assay preparation—Dilute an accurately measured volume of freshly mixed Ophthalmic Suspension quantitatively with *Mobile phase* to obtain a solution containing about 0.12 mg of dexamethasone per mL.
Procedure—Proceed as directed for *Procedure* in the *Assay for dexamethasone* under *Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment.* Calculate the quantity, in mg per mL, of $C_{22}H_{29}FO_5$ in the Ophthalmic Suspension taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which L is the labeled quantity, in mg per mL, of dexamethasone in the Ophthalmic Suspension, D is the concentration, in mg per mL, of dexamethasone in the *Assay preparation* based on the labeled quantity in the Ophthalmic Suspension and the extent of dilution, and the other terms are as defined therein.

Neomycin and Polymyxin B Sulfates and Gramicidin Cream

» Neomycin and Polymyxin B Sulfates and Gramicidin Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.
USP Reference standards ⟨11⟩—
USP Gramicidin RS

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USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin and Assay for polymyxin B— Proceed with Cream as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*
Assay for gramicidin—Proceed with Cream as directed in the *Assay for gramicidin* under *Neomycin Sulfate and Gramicidin Ointment.*

Neomycin and Polymyxin B Sulfates and Gramicidin Ophthalmic Solution

» Neomycin and Polymyxin B Sulfates and Gramicidin Ophthalmic Solution is a sterile, isotonic aqueous solution of Neomycin Sulfate, Polymyxin B Sulfate, and Gramicidin. It contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.
USP Reference standards ⟨11⟩—
USP Gramicidin RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 4.7 and 6.0.
Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as is present in the *Test Dilution.*
Assay for gramicidin—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with alcohol to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin and Polymyxin B Sulfates, Gramicidin, and Hydrocortisone Acetate Cream

» Neomycin and Polymyxin B Sulfates, Gramicidin, and Hydrocortisone Acetate Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Gramicidin RS
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin and Assay for polymyxin B— Proceed with Cream as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*
Assay for gramicidin—Proceed with Cream as directed in the *Assay for gramicidin* under *Neomycin Sulfate and Gramicidin Ointment.*
Assay for hydrocortisone acetate—Proceed with Cream as directed in the *Assay* under *Hydrocortisone Acetate Lotion.*

Neomycin and Polymyxin B Sulfates and Hydrocortisone Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Ophthalmic Suspension is a sterile, aqueous suspension containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and of polymyxin B. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.
USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.
Sterility Tests ⟨71⟩ **:** meets the requirements.
pH ⟨791⟩: between 4.1 and 7.0.
Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a

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concentration assumed to be equal to the median dose level of the Standard.

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of Neomycin Sulfate RS, dissolved in *Buffer B.6*, to yield the same concentration of neomycin as is present in the *Test Dilution*.

Assay for hydrocortisone—

Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment.*

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 30 mg of hydrocortisone, to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Filter the solution, rejecting the first 10 mL of the filtrate.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment.* Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Ophthalmic Suspension taken by the formula:

$$200(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation; V* is the volume, in mL, of Ophthalmic Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution is a sterile solution containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone. It may contain one or more suitable buffers, dispersants, and solvents.

Packaging and storage—Preserve in tight, light-resistant containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Sterility Tests ⟨71⟩ : meets the requirements.

pH ⟨791⟩: between 2.0 and 4.5.

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Otic Solution diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (1.0 µg of neomycin per mL).

Assay for polymyxin B—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Otic Solution diluted quantitatively and stepwise with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (10 Polymyxin B Units per mL). Add to each test dilution of the Standard a quantity of Neomycin Standard, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin present in the *Test Dilution.*

Assay for hydrocortisone—

Mobile phase , Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment.*

Assay preparation—Transfer 3.0 mL of Otic Solution to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment.* Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Solution taken by the formula:

$$(66.67C)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Suspension

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Suspension is a sterile suspension containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and of polymyxin B. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone. It may contain one or more suitable buffers, dispersants, and preservatives.

Packaging and storage—Preserve in tight, light-resistant containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

Sterility Tests ⟨71⟩ : meets the requirements.

pH ⟨791⟩: between 3.0 and 7.0.

Assay for neomycin and Assay for polymyxin B—Using an accurately measured volume of Otic Suspension, freshly mixed and free from air bubbles, proceed as directed in the *Assay for neomycin* and the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution.*

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Assay for hydrocortisone—
Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment.*

Assay preparation—Transfer 3.0 mL of Otic Suspension, freshly mixed and free from air bubbles, to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Filter the solution, rejecting the first 10 mL of the filtrate.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment.* Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Suspension taken by the formula:

$$(66.67C)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of *USP Hydrocortisone RS* in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Acetate Cream

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Acetate Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Identification—
A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.

B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for hydrocortisone acetate.*
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin and Assay for polymyxin B—
Proceed with Cream as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates Cream.*
Assay for hydrocortisone acetate—Proceed with Cream as directed in the *Assay* under *Hydrocortisone Acetate Lotion.*

Neomycin and Polymyxin B Sulfates and Hydrocortisone Acetate Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Acetate Ophthalmic Suspension is a sterile suspension of Hydrocortisone Acetate in an aqueous solution of Neomycin Sulfate and Polymyxin B Sulfate. It contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$). It may contain suitable buffers, preservatives, and suspending agents.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.
USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 5.0 and 7.0.
Assay for neomycin and Assay for polymyxin B—
Proceed with Ophthalmic Suspension as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*

Change to read:

Assay for hydrocortisone acetate—
▲*Standard preparation*—Prepare as directed for *Assay for Steroids* ⟨351⟩, *Standard Preparation,* using USP Hydrocortisone Acetate RS.

Assay preparation—Transfer to a separator an accurately measured volume of Ophthalmic Suspension, equivalent to about 50 mg of hydrocortisone acetate, and dilute with water to about 15 mL. Extract with four 25-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 250-mL volumetric flask. Add chloroform to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, add chloroform to volume, and mix. Pipet 10 mL of the resulting solution into a glass-stoppered, 50-mL conical flask, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 20.0 mL of alcohol..

Procedure—Proceed as directed for *Assay for Steroids* ⟨351⟩, *Procedure.* Calculate the quantity, in mg, of hydrocortisone acetate ($C_{23}H_{32}O_6$) in each mL of Ophthalmic Suspension taken by the formula:

$$5(C/V)(A_U/A_S)$$

in which V is the volume, in mL, of Ophthalmic Suspension taken; and the other terms are defined therein. ▲ (ERR 1-Nov-2018)

USP Monographs

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Neomycin and Polymyxin B Sulfates and Lidocaine Cream

» Neomycin and Polymyxin B Sulfates and Lidocaine Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Lidocaine RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Identification—
A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for lidocaine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lidocaine*.
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin—Proceed with Cream as directed in the *Assay for neomycin* under *Neomycin and Polymyxin B Sulfates Cream*.
Assay for polymyxin B—Proceed with Cream as directed in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates Cream*.

Assay for lidocaine—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment*.
Assay preparation—Using Cream, proceed as directed for the *Assay preparation* in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment*.
Procedure—Proceed as directed for *Procedure* in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment*. Calculate the quantity, in mg, of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Cream taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Lidocaine RS in the *Standard preparation;* and r_U and r_S are the lidocaine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Pramoxine Hydrochloride Cream

» Neomycin and Polymyxin B Sulfates and Pramoxine Hydrochloride Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
USP Pramoxine Hydrochloride RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—Disperse a quantity of Cream, equivalent to about 25 mg of neomycin, with 20 mL of chloroform in a 60-mL separator. Add 0.2 mL of 2.5 N hydrochloric acid, and shake. Allow the layers to separate for about 30 minutes. Discard the lower chloroform layer, and centrifuge the upper aqueous layer. Use a portion of the centrifuged aqueous layer. *Standard solution*—Dissolve suitable quantities of USP Neomycin Sulfate RS and USP Polymyxin B Sulfate RS in 0.1 N hydrochloric acid to obtain a solution containing the equivalent of about 3.5 mg of neomycin and 10,000 USP Polymyxin B Units per mL.
Developing solvent system—Dissolve 0.1 g of benzalkonium chloride in a mixture of isopropyl alcohol, water, and ammonium hydroxide (60:40:10).
Procedure—Proceed as directed in the chapter. Place the plate in a chromatographic chamber saturated with *Developing solvent system,* and develop the chromatogram. Dry the plate at 105° for about 10 minutes, spray with a solution of ninhydrin in butyl alcohol (1 in 200), and heat the plate at 105° for about 15 minutes. The R_f values of the two principal spots in the chromatogram obtained from the *Test solution* correspond to those of the two principal spots in the chromatogram obtained from the *Standard solution*.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for pramoxine hydrochloride*.
pH ⟨791⟩—Transfer 1 g of Cream to a small beaker, add 10 mL of carbon dioxide-free water, and mix: the pH is between 3.3 and 6.0.
Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Cream, equivalent to about 3.5 mg of neomycin, blended for 3 to 5 minutes in a high-speed blender with 249 mL of *Buffer B.3* and 1 mL of polysorbate 80. Quantitatively dilute an accurately measured volume of this solution with *Buffer B.3* to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median level of the Standard (1 µg of neomycin per mL).
Assay for polymyxin—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Cream, equivalent to about 10,000 USP Polymyxin B Units, blended for 3 to 5 minutes in a high-speed blender with 199 mL of *Buffer B.6* and 1 mL of polysorbate 80. Quantitatively dilute an accurately measured volume of this solution with*Buffer B.6* to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard (10 USP Polymyxin B Units per mL).

Assay for pramoxine hydrochloride—
Mobile phase—Dissolve 3.5 g of dibasic potassium phosphate in 1000 mL of water. Prepare a mixture of this solution, acetonitrile, and triethylamine (700:300:2), and adjust with phosphoric acid to a pH of 4.0 ± 0.1. Filter and

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degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Prepare a solution of USP Pramoxine Hydrochloride RS in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 10 mg of pramoxine hydrochloride, to a 50-mL volumetric flask, add about 5 mL of chloroform, and sonicate at about 40° to disperse the Cream. Allow to cool to room temperature, dilute with methanol to volume, and mix. Pass a portion of this solution through a glass fiber filter and a PTFE filter having a 0.45-µm porosity, discarding the first few mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector, a guard column that contains packing L7, and a 4.6-mm × 25-cm analytical column that contains packing L7. The column is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$) in each g of Cream taken by the formula:

$$50(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pramoxine Hydrochloride RS in the *Standard preparation; W* is the weight, in g, of Cream taken to prepare the *Assay preparation;* and r_U and r_S are the peak areas for pramoxine obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin and Polymyxin B Sulfates and Prednisolone Acetate Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Prednisolone Acetate Ophthalmic Suspension is a sterile suspension of Prednisolone Acetate in an aqueous solution of Neomycin Sulfate and Polymyxin B Sulfate. It contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$). It may contain suitable buffers, preservatives, and suspending agents.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
USP Prednisolone Acetate RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay for prednisolone acetate* exhibits a major peak for prednisolone acetate, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for prednisolone acetate.*

Sterility Tests ⟨71⟩ **:** meets the requirements.

pH ⟨791⟩: between 5.0 and 7.0.

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of Neomycin Sulfate RS, dissolved in *Buffer B.6,* to obtain the same concentration of neomycin as is present in the *Test Dilution.*

Assay for prednisolone acetate—

Mobile phase , Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay for prednisolone acetate* under *Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension.*

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 2.5 mg of prednisolone acetate, to a suitable container, add 5.0 mL of *Internal standard solution* and about 100 mL of water-saturated chloroform, and shake by mechanical means for about 15 minutes. Allow to separate for about 15 minutes, and use the clear chloroform layer as the *Assay preparation.*

Procedure—Proceed as directed in the *Assay for prednisolone acetate* under *Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension.* Calculate the quantity, in mg, of prednisolone acetate ($C_{23}H_{30}O_6$) in each mL of the Ophthalmic Suspension taken by the formula:

$$0.1(C/V)(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Prednisolone Acetate RS in the *Standard preparation, V* is the volume, in mL, of Ophthalmic Suspension taken, and R_U and R_S are the peak response ratios of prednisolone acetate to betamethasone obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin Sulfate and Prednisolone Acetate Ointment

» Neomycin Sulfate and Prednisolone Acetate Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$).

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Packaging and storage—Preserve in collapsible tubes or in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
USP Prednisolone Acetate RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the major peak for prednisolone acetate in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* as obtained in the *Assay for prednisolone acetate.*
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*

Assay for prednisolone acetate—
Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay for prednisolone acetate* under *Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension.*
Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 1 mg of prednisolone acetate, to a suitable container, add 2.0 mL of *Internal standard solution,* dilute with water-saturated chloroform to about 35 mL, and shake to dissolve the ointment. Transfer about 5 mL of this solution to a suitable container, and evaporate to dryness. Add about 5 mL of water-saturated chloroform, and sonicate for 5 minutes. Filter, and use the clear solution as the *Assay preparation.*
Procedure—Proceed as directed for *Procedure* in the *Assay for prednisolone acetate* under *Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension.* Calculate the quantity, in mg, of prednisolone acetate ($C_{23}H_{30}O_6$) in the portion of Ointment taken by the formula:

$$0.04C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Prednisolone Acetate RS in the *Standard preparation,* and R_U and R_S are the peak response ratios of prednisolone acetate to betamethasone obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension

» Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$).

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
USP Prednisolone Acetate RS

Identification—
A: Filter a portion of Ophthalmic Suspension, freshly mixed but free from air bubbles, equivalent to about 60 mg of prednisolone acetate, discarding the filtrate. Wash the filter with about 10 mL of water, and dry at 105° for 3 hours: the IR absorption spectrum of a potassium bromide dispersion of the dried residue on the filter so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Prednisolone Acetate RS.
B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay for prednisolone acetate* exhibits a major peak for prednisolone acetate, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for prednisolone acetate.*
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 5.5 and 7.5.
Assay for neomycin—Proceed with Ophthalmic Suspension as directed in the *Assay for neomycin* under *Neomycin and Polymyxin B Sulfates and Prednisolone Acetate Ophthalmic Suspension.*

Assay for prednisolone acetate—
Mobile phase—Prepare a solution containing *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6).
Internal standard solution—Prepare a solution of betamethasone in tetrahydrofuran containing 10 mg per mL. Dilute this solution with water-saturated chloroform, and mix to obtain a solution having a concentration of about 1 mg per mL.
Standard preparation—Dissolve about 5 mg of USP Prednisolone Acetate RS, accurately weighed, in 10.0 mL of *Internal standard solution.* Use sonication, if necessary, dilute with water-saturated chloroform to 200.0 mL, and mix to obtain a solution having a known concentration of about 25 µg per mL.
Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 2.5 mg of prednisolone acetate, to a suitable container, add 5.0 mL of *Internal standard solution* and about 100 mL of water-saturated chloroform, and shake by mechanical means for about 15 minutes. Allow to separate for about 15 minutes, and use the clear chloroform layer as the *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the analyte and internal standard peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.6 for betamethasone and 1.0 for prednisolone acetate. Calculate the quantity, in mg, of prednisolone acetate ($C_{23}H_{30}O_6$) in each mL of the Ophthalmic Suspension taken by the formula:

$$0.1(C/V)(R_U/R_S)$$

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in which C is the concentration, in µg per mL, of USP Prednisolone Acetate RS in the *Standard preparation*, V is the volume, in mL, of Ophthalmic Suspension taken, and R_U and R_S are the peak response ratios of prednisolone acetate to betamethasone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

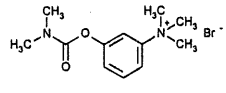
Neomycin Sulfate and Triamcinolone Acetonide Cream

» Neomycin Sulfate and Triamcinolone Acetonide Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{34}FO_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers.
USP Reference standards ⟨11⟩—
USP Neomycin Sulfate RS
USP Triamcinolone Acetonide RS

Identification—
A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: Place 2 g of Cream in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.
Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Cream*.
Assay for triamcinolone acetonide—Proceed with Cream as directed in the *Assay* under *Triamcinolone Acetonide Cream*.

Neostigmine Bromide



$C_{12}H_{19}BrN_2O_2$ 303.20
Benzenaminium, 3-[[(dimethylamino)carbonyl]oxy]-*N*,*N*,*N*-trimethyl-, bromide.
(*m*-Hydroxyphenyl)trimethylammonium bromide dimethylcarbamate [114-80-7].

» Neostigmine Bromide contains not less than 98.0 percent and not more than 102.0 percent of $C_{12}H_{19}BrN_2O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Neostigmine Bromide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: A solution (1 in 50) responds to the tests for *Bromide* ⟨191⟩.
Melting range ⟨741⟩: between 171° and 176°, with decomposition.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 2.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.15%.
Sulfate—Dissolve 250 mg in 10 mL of water, and add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.
Assay—Dissolve about 750 mg of Neostigmine Bromide, accurately weighed, in a mixture of 70 mL of glacial acetic acid and 20 mL of mercuric acetate TS, add 4 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.32 mg of $C_{12}H_{19}BrN_2O_2$.

Neostigmine Bromide Tablets

» Neostigmine Bromide Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{12}H_{19}BrN_2O_2$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Neostigmine Bromide RS
Identification—Extract a quantity of powdered Tablets, equivalent to about 300 mg of neostigmine bromide, with three 10-mL portions of alcohol, filtering after each extraction. Evaporate the combined filtrates under a stream of nitrogen to dryness. Dissolve the residue in 10 mL of water, transfer to a 125-mL separator with the aid of 5 mL of water, extract with 15 mL of ether, and proceed with the following tests.
A: Evaporate 3 mL of the aqueous layer on a steam bath, under a stream of nitrogen, to dryness. Dissolve the residue, warming if necessary, in 1 mL of alcohol. Add 5 mL of chloroform, filter, evaporate the filtrate under a stream of nitrogen to dryness, and dry the residue at 105° for 30 minutes: the IR absorption spectrum of a potassium bromide dispersion of the residue of neostigmine bromide so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Neostigmine Bromide RS.
B: A portion of the aqueous layer responds to the tests for *Bromide* ⟨191⟩.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—At the specified time interval, withdraw 30 mL of the solution under test, and filter. Pipet 10 mL each of the filtered test solution, a Standard solution having a known concentration of USP Neostigmine Bromide RS, and water to provide a blank, into respective 125-mL separators. Proceed as directed for *Procedure* in the *Assay*, beginning with "Add 15 mL of a solution."

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Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{12}H_{19}BrN_2O_2$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Standard preparation—Dissolve a suitable quantity of USP Neostigmine Bromide RS, accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution having a concentration of about 40 µg per mL.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of neostigmine bromide, to a 100-mL volumetric flask, add about 50 mL of water, shake by mechanical means for about 30 minutes, add water to volume, mix, and filter. Pipet 4 mL of the clear filtrate into a 50-mL volumetric flask, add water to volume, and mix.
Procedure—Pipet 10 mL each of *Assay preparation* and *Standard preparation* into respective 125-mL separators, and treat each solution as follows. Add 15 mL of a solution prepared by dissolving 25 mg of hexanitrodiphenylamine in methylene chloride to make 250 mL, without grinding the solid or heating the solution. Then add 10 mL of 5 N sodium hydroxide, and shake vigorously for 30 seconds. Collect the methylene chloride layer in a 100-mL volumetric flask, and extract the aqueous layer with three 15-mL portions of methylene chloride, collecting the methylene chloride extracts in each respective flask. Add methylene chloride to volume, and mix. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 420 nm, with a suitable spectrophotometer, using methylene chloride as the blank. Calculate the quantity, in mg, of $C_{12}H_{19}BrN_2O_2$ in the portion of Tablets taken by the formula:

$$1.25C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Neostigmine Bromide RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Neostigmine Methylsulfate

$C_{13}H_{22}N_2O_6S$ 334.39
Benzenaminium, 3-[[(dimethylamino)carbonyl]oxy]-*N*,*N*,*N*-trimethyl-, methyl sulfate.
(*m*-Hydroxyphenyl)trimethylammonium methyl sulfate dimethylcarbamate [51-60-5].

» Neostigmine Methylsulfate contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{22}N_2O_6S$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Neostigmine Methylsulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Place about 1 mg in a small porcelain dish, add 2 mL of water and 0.5 mL of sodium hydroxide solution (2 in 5), and evaporate on a steam bath to dryness. Transfer the residue to a small test tube, and quickly heat in a suitable liquid bath to 250°, continuing at that temperature for about 30 seconds. Cool, dissolve the residue in 0.5 mL of water, cool in ice water, and add 1 mL of diazobenzenesulfonic acid TS: a cherry-red color is produced.

C: Mix about 20 mg with 500 mg of sodium carbonate, and heat the mixture to fusion in a small crucible. Boil the fused mass with 10 mL of water until disintegrated, and filter. Add a few drops of bromine TS to the filtrate, heat to boiling, acidify with hydrochloric acid, and expel the excess bromine by boiling: the resulting solution responds to the tests for *Sulfate* ⟨191⟩.
Melting range ⟨741⟩: between 144° and 149°, determined after drying at 105° for 3 hours.
Loss on drying ⟨731⟩: Dry about 300 mg, accurately weighed, at 105° for 3 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chloride—To 10 mL of a solution (1 in 50) add 1 mL of 2 N nitric acid and 1 mL of silver nitrate TS: no opalescence is produced immediately.
Sulfate ion—To 10 mL of a solution (1 in 50) add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.
Assay—Place about 100 mg of Neostigmine Methylsulfate, accurately weighed, in a 500-mL Kjeldahl flask, dissolve in 150 mL of water, and add 40 mL of 2.5 N sodium hydroxide. Connect the flask by means of a distillation trap to a well-cooled condenser that dips into 25 mL of boric acid solution (1 in 25), distill about 150 mL of the contents of the flask, add methyl purple TS to the solution in the receiver, and titrate with 0.02 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N sulfuric acid is equivalent to 6.688 mg of $C_{13}H_{22}N_2O_6S$.

Neostigmine Methylsulfate Injection

» Neostigmine Methylsulfate Injection is a sterile solution of Neostigmine Methylsulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{13}H_{22}N_2O_6S$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, protected from light.
USP Reference standards ⟨11⟩—
USP Neostigmine Methylsulfate RS
Identification—Transfer a volume of Injection, containing the equivalent of 1 mg of neostigmine methylsulfate, to a small porcelain dish. Evaporate, if necessary, to 2 mL, add 0.5 mL of sodium hydroxide solution (2 in 5), and proceed as directed in *Identification* test *B* under *Neostigmine Methylsulfate*, beginning with "evaporate on a steam bath."
pH ⟨791⟩: between 5.0 and 6.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Standard preparation—Dissolve a suitable quantity of USP Neostigmine Methylsulfate RS, accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 40 µg per mL.
Assay preparation—Pipet an accurately measured volume of Injection, equivalent to about 2 mg of neostigmine

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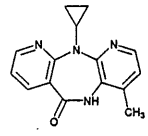
methylsulfate, into a 50-mL volumetric flask, add water to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Neostigmine Bromide Tablets*. Calculate the quantity, in mg, of $C_{13}H_{22}N_2O_6S$ in each mL of the Injection taken by the formula:

$$0.05(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Neostigmine Methylsulfate RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Nevirapine



$C_{15}H_{14}N_4O$ 266.30
6*H*-Dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one, 11-cyclopropyl-5,11-dihydro-4-methyl-;
11-Cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one [129618-40-2].
Hemihydrate 275.31

DEFINITION

Nevirapine is anhydrous or contains one-half molecule of water of hydration. It contains NLT 98.0% and NMT 102.0% of $C_{15}H_{14}N_4O$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K.▲** (CN 1-May-2020) Do not dry the specimens.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.025 M of monobasic ammonium phosphate in water prepared as follows. Dissolve 2.9 g of monobasic ammonium phosphate in 800 mL of water. Adjust with 1 N sodium hydroxide to a pH of 5.0, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (1:4)

Standard stock solution A: 0.24 mg/mL of USP Nevirapine Anhydrous RS prepared as follows. Dissolve a quantity of USP Nevirapine Anhydrous RS in Acetonitrile and *Mobile phase* (1:20). Sonicate for at least 15 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume. [NOTE—Do not use after 78 h.]

Standard stock solution B: 0.24 mg/mL of USP Nevirapine Related Compound A RS prepared as follows. Dissolve a quantity of USP Nevirapine Related Compound A RS in a volume of a mixture of acetonitrile and *Mobile phase* (1:3). Sonicate for at least 15 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume.

Standard stock solution C: 0.06 mg/mL of USP Nevirapine Related Compound B RS prepared as follows. Dissolve a quantity of USP Nevirapine Related Compound B RS in a volume of a mixture of acetonitrile and *Mobile phase* (10:22). Sonicate for at least 30 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume.

System suitability solution: 0.03 mg/mL each of USP Nevirapine Anhydrous RS and USP Nevirapine Related Compound A RS and 0.015 mg/mL of USP Nevirapine Related Compound B RS from suitable volumes of *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution C*, respectively, in *Mobile phase*. [NOTE—Do not use after 78 h.]

Standard solution: 0.03 mg/mL of USP Nevirapine Anhydrous RS in *Mobile phase* from *Standard stock solution A*. [NOTE—Do not use after 78 h.]

Sample stock solution: 0.24 mg/mL of Nevirapine in *Mobile phase* prepared as follows. Transfer the required amount of Nevirapine to a suitable volumetric flask, and add 4% of the final volume with acetonitrile and 80% of the final volume with *Mobile phase*. Sonicate for at least 15 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume.

Sample solution: 0.03 mg/mL of nevirapine anhydrous in *Mobile phase* from the *Sample stock solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L60
Column temperature: 35°
Flow rate: 1 mL/min
Injection size: 25 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times are shown in *Table 1*.]
Suitability requirements
Resolution: NLT 5.0 between nevirapine related compound B and nevirapine, and NLT 7.4 between nevirapine and nevirapine related compound A; *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nevirapine ($C_{15}H_{14}N_4O$) in the portion of Nevirapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
C_U = concentration of Nevirapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, and **Standard stock solution A:** Proceed as directed in the *Assay*.

Standard solution: 0.2 µg/mL of USP Nevirapine Anhydrous RS in *Mobile phase* from *Standard stock solution A*

Sample solution: 0.24 mg/mL of Nevirapine in *Mobile phase* prepared as follows. Transfer the required amount of Nevirapine to a suitable volumetric flask, and

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add 4% of the final volume with acetonitrile and 80% of the final volume with *Mobile phase*. Sonicate for at least 15 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume.

Chromatographic system: Proceed as directed in the *Assay*, except for *Run time* and *Injection size*.

Run time: At least 80 min

Injection size: 25 µL for *System suitability*; 50 µL for *Analysis*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are shown in *Table 1*.]

Suitability requirements

Resolution: NLT 5.0 between nevirapine related compound B and nevirapine, and NLT 7.4 between nevirapine and nevirapine related compound A; *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Nevirapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of nevirapine from the *Standard solution*

C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (µg/mL)

C_U = concentration of nevirapine anhydrous in the *Sample solution* (µg/mL)

F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nevirapine related compound B	0.7	1.3	0.2
Nevirapine	1.0	1.0	—
Nevirapine related compound A	1.5	1.0	0.2
Nevirapine impurity C	2.8	1.0	0.2
Any other individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.6

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩

For Nevirapine anhydrous: NMT 0.2%

For Nevirapine hemihydrate: 3.1%–3.9%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **LABELING:** Label to indicate whether it is anhydrous or the hemihydrate.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Nevirapine Anhydrous RS

USP Nevirapine Hemihydrate RS

USP Nevirapine Related Compound A RS

5,11-Dihydro-6*H*-11-ethyl-4-methyl- dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.

$C_{14}H_{14}N_4O$ 254.29

USP Nevirapine Related Compound B RS

5,11-Dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4] diazepin-6-one.

$C_{12}H_{10}N_4O$ 226.23

Nevirapine Oral Suspension

DEFINITION

Nevirapine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY** ⟨201⟩

Standard solution: 5 mg/mL of USP Nevirapine Anhydrous RS in chloroform

Sample solution: Transfer a volume of Oral Suspension, equivalent to 10 mg of nevirapine, to an 8-mL glass stoppered tube. Pipet 2.0 mL of chloroform into the tube. Shake the solution and allow the two phases to separate; then, using a disposable glass Pasteur pipet, remove some of the organic layer from the bottom, and transfer it to another container.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel 60 F254

Application volume: 5 µL

Developing solvent system: Ethyl acetate, isopropanol, and concentrated ammonium hydroxide (18: 2: 0.1)

Spray reagent: 1.35 g of ferric chloride in 25 mL of water and 1.64 g of potassium ferricyanide in 25 mL of water. Mix the two solutions immediately before use.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop in a chamber saturated with a solvent system until the solvent front has moved 6–7 cm from the point of application. Remove the plate from the chamber, mark the solvent front, and dry. Examine under UV light at 254 nm, and outline the spots with a soft pencil. Spray the plate with *Spray reagent*.

Acceptance criteria: The R_F value (approximately 0.4–0.5) of the principal blue spot, under UV and after spraying, from the *Sample solution*, corresponds to that from the *Standard solution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Methanol and water (1:4)

Solution A: 13.6 g of monobasic potassium phosphate in 1900 mL of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.

Solution B: Acetonitrile and *Solution A* (3:97)

Solution C: Acetonitrile and *Solution A* (24:76)

Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0

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Time (min)	Solution B (%)	Solution C (%)
1	100	0
31	0	100
32	100	0
42	100	0

Standard stock solution: Dissolve 50 mg of USP Nevirapine Anhydrous RS in 20 mL of methanol in a 50-mL volumetric flask. Sonicate with intermittent swirling until the sample dissolves. Add water to 1 cm below the meniscus, cool to room temperature, and dilute with water to volume. The concentration is 1 mg/mL of nevirapine.

Standard solution: 0.3 mg/mL of nevirapine from the *Standard stock solution* diluted with *Diluent*

Stock impurity solution: 3 mg of USP Nevirapine Related Compound A RS and 3 mg of USP Nevirapine Related Compound B RS in 20 mL of methanol in a 100-mL volumetric flask. Sonicate to dissolve. Add water to 1 cm below the meniscus, cool to room temperature, and dilute with water to volume.

System suitability solution: Transfer 15.0 mL of *Standard stock solution* and 2.0 mL of *Stock impurity solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Weight determination: Using a 1- to 10-mL suitable pipet and a positive displacement tip, withdraw 5.0 mL of Oral Suspension. The sample should be free of air bubbles. Dispense into a tared vial, and record the weight of the Oral Suspension to ±0.1 mg.

Sample solution: Using a 1- to 10-mL suitable pipet and a positive displacement tip, withdraw Oral Suspension equivalent to 60 mg of nevirapine. The sample should be free of air bubbles. Remove the excess Oral Suspension by wiping the outside of the tip carefully so as not to touch the opening of the tip, and deliver the sample into a 200-mL tared volumetric flask. Record the sample weight to the nearest ±0.1 mg. Add 40 mL of methanol, and sonicate for 5 min with intermittent swirling. Add water to 1 cm below the meniscus. Do not shake the flask. Allow the solution to attain room temperature, and dilute with water to volume. Shake the flask gently, and allow to stand for 5 min.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L10
Guard column: 4.6-mm × 12.5-mm; 5-µm packing L10
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between nevirapine and nevirapine related compound A; NLT 1.7 between nevirapine and nevirapine related compound B, *System suitability solution*
Tailing factor: NMT 1.5 for the nevirapine peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Measure the responses for the nevirapine peak. Calculate the percentage of $C_{15}H_{14}N_4O$ in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 25 rpm
Time: 45 min
Analysis: Determine the amount of $C_{15}H_{14}N_4O$ dissolved by using the following method.
Diluent: Dehydrated alcohol and water (1:1)
Mobile phase: Acetonitrile and water (23:77)
System suitability solution: Transfer 10 mg of USP Nevirapine Anhydrous RS and 15 mg of methylparaben to a 250-mL volumetric flask, dissolve with 2 mL of *Diluent*, and dilute with *Medium* to volume.
Standard solution: Transfer 28 mg of USP Nevirapine Anhydrous RS to a 500-mL volumetric flask, add 2 mL of *Diluent*, and sonicate for 1 min. The Standard will not be completely dissolved at this point. Dilute with *Medium* to volume, and visually examine the solution to ensure that the Standard is completely dissolved. The final concentration is 0.056 mg/mL of nevirapine.
Sample solution: For sample mixing, gently shake the bottle for approximately 10 s by inverting it slowly and rotating it from side to side. The sample should be free of air bubbles. Do not sonicate the sample. Using a 1- to 10-mL suitable positive displacement pipet set at 5 mL, withdraw the equivalent of 50 mg of nevirapine. Remove excess Oral Suspension by wiping the outside of the tip carefully so as not to touch the opening of the tip. Introduce the sample into the dissolution vessel over a period of 1–2 s by immersing the tip of the pipet midway between the paddle and the side of the vessel, approximately 1 cm below the meniscus. Similarly dispense the Oral Suspension into the other vessels. At 45 min, withdraw 5 mL of the solution under test, and pass through a nylon filter of 0.45-µm pore size, discarding the first 2 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Guard column: 3.9-mm × 20-mm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between nevirapine and methylparaben, *System suitability solution*
Tailing factor: NMT 1.8, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for at least 14 min, and measure the responses for the nevirapine peaks.
Calculate the percentage of $C_{15}H_{14}N_4O$ dissolved:

$$\text{Result} = (r_U \times C_S \times V_1)/(r_S \times V_2 \times L) \times 100$$

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r_U = peak response from the *Sample solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
V_1 = volume of the *Medium*, 900 mL
r_S = peak response from the *Standard solution*
V_2 = volume of Oral Suspension taken (mL)
L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{15}H_{14}N_4O$ is dissolved.

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Diluent, Solution A, Solution B, Solution C, and **Mobile phase:** Prepare as directed in the *Assay.*
Standard stock solution: Use the *Standard stock solution,* prepared as directed in the *Assay.*
Standard solution: 0.3 µg/mL of nevirapine from the *Standard stock solution* diluted with *Diluent*
System suitability solution: Prepare as directed in the *Assay.*
Weight determination: Use the weight obtained as directed for *Weight determination* in the *Assay.*
Sample solution: Prepare as directed in the *Assay.*
Chromatographic system
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L10
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between nevirapine and nevirapine related compound A and NLT 1.7 between nevirapine and nevirapine related compound B, *System suitability solution*
Tailing factor: NMT 1.5 for nevirapine, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unknown impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for nevirapine from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nevirapine in the *Sample solution* (mg/mL)

Acceptance criteria
Individual unknown impurities: NMT 0.1%
Total unknown impurities: NMT 0.2%
[NOTE—The excipients and their degradation products should not be included in the determination of impurities.]

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Escherichia coli.* The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeasts count does not exceed 50 cfu/mL.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Nevirapine Anhydrous RS
USP Nevirapine Related Compound A RS
5,11-Dihydro-6*H*-11-ethyl-4-methyl- dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.
$C_{14}H_{14}N_4O$ 254.29
USP Nevirapine Related Compound B RS
5,11-Dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.
$C_{12}H_{10}N_4O$ 226.23

Nevirapine Tablets

DEFINITION

Nevirapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer a portion of powdered Tablets equivalent to 25 mg of nevirapine to a 50-mL volumetric flask. Dissolve in 10 mL of methylene chloride. Swirl the solution for 30–60 s, and pass through a medium sintered-glass, fritted vacuum funnel. Using a glass syringe, pass the filtrate through a Teflon filter of 0.45-µm pore size. Dry the extract at 105° for a minimum of 1 h.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**
Mobile phase: Acetonitrile and water (23:77)
Diluent: Dehydrated alcohol and water (1:1)
System suitability solution: 0.025 mg/mL of USP Nevirapine Anhydrous RS and 0.025 mg/mL of USP Nevirapine Related Compound A RS in *Diluent*
Standard solution: 0.025 mg/mL of USP Nevirapine Anhydrous RS in *Diluent*
Sample stock solution: Nominally 1 mg/mL of nevirapine in *Diluent* prepared as follows. Transfer nevirapine, from finely powdered Tablets (NLT 20), to a suitable size volumetric flask, and add 75% of the final volume with *Diluent.* Sonicate the solution for 20 min, then shake for 20 min. Cool to room temperature, and dilute with *Diluent* to volume. Centrifuge a portion of the resulting solution at 1500 rpm for 5 min.
Sample solution: Nominally 0.025 mg/mL of nevirapine in *Diluent* from the *Sample stock solution.* Filter a portion of the resulting solution, and discard the first 2 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 214 nm
Column: 3.9-mm × 15-cm; packing L1
Flow rate: 1 mL/min

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Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between nevirapine and nevirapine related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nevirapine from the *Sample solution*
r_S = peak response of nevirapine from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
C_U = nominal concentration nevirapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 M phosphate buffer, pH 2.0 (transferring 3.9 mL/L of concentrated phosphoric acid and 5.73 g/L of monobasic sodium phosphate monohydrate in water, adjust with phosphoric acid to a pH of 2.0 ± 0.02); 900 mL
Apparatus 2: 50 rpm. [Note—Use stainless steel paddles only. Do not use paddles coated with polytetrafluoroethylene.]
Time: 60 min
Mobile phase, Diluent, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Standard stock solution A: 0.054 mg/mL of USP Nevirapine Anhydrous RS. Add 10% of the final volume with alcohol and 50% of the final volume of *Medium*. Sonicate for 20 min to dissolve, allow to cool to room temperature, and dilute with *Medium* to volume.
Standard stock solution B: 0.028 mg/mL of USP Nevirapine Related Compound A RS. Add 0.8% of the final volume of *Diluent*, sonicate until completely dissolved, and dilute with *Medium* to volume.
Standard solution: 0.014 mg/mL of USP Nevirapine Anhydrous RS from *Standard stock solution A* in *Medium*
System suitability solution: 0.014 mg/mL of USP Nevirapine Anhydrous RS from *Standard stock solution A* and 0.014 mg/mL of USP Nevirapine Related Compound A RS from *Standard stock solution B* in *Medium*
Sample solution: Pass 20 mL of the solution under test through a suitable nylon or glass fiber filter of 0.45-µm pore size, and dilute with *Medium* to obtain a solution having a final concentration of 0.014 mg/mL of nevirapine.
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/D_U) \times V \times (100/L)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
D_U = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase, Diluent, System suitability solution, Sample stock solution, and Sample solution: Proceed as directed in the *Assay*.
Standard solution: 0.125 µg/mL of USP Nevirapine Anhydrous RS in *Diluent*
Chromatographic system: Proceed as directed in the *Assay*, except use a run time of at least 13 min.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between nevirapine and nevirapine related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unknown impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unknown impurity from the *Sample solution*
r_S = peak response of nevirapine from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
C_U = nominal concentration nevirapine in the *Sample solution* (mg/mL)

[Note—Disregard all peaks due to the solvent or excipients and impurity peaks less than 0.1%.]
Acceptance criteria
Individual unknown impurity: NMT 0.1%
Total unknown impurities: NMT 0.2%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Nevirapine Anhydrous RS
USP Nevirapine Related Compound A RS
5,11-Dihydro-6*H*-11-ethyl-4-methyl- dipyrido[3,2-*b*:2′,3′- *e*][1,4]diazepin-6-one
$C_{14}H_{14}N_4O$ 254.29

Nevirapine Extended-Release Tablets

DEFINITION

Nevirapine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$).

IDENTIFICATION

• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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• **B.** The retention time of the nevirapine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Acetonitrile and water (15:85)
Solution B: Acetonitrile and water (40:60)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
23	0	100
33	0	100
33.1	100	0
48	100	0

[NOTE—These gradient elution times are established on an HPLC system with a hold time of approximately 7 min based on the dwell volume of 0.7 mL. Adjust the start of the gradient to be approximately 2 min after the retention time of nevirapine and adjust the remaining gradient accordingly. The hold time (X) can be calculated using the formula: X = (11.2 mL – dwell volume)/flow rate.]

Diluent: Acetonitrile and water (30:70)
Standard solution: 0.5 mg/mL of USP Nevirapine Anhydrous RS in *Solution B*. Sonicate if necessary to dissolve, before final dilution.
Sample stock solution: Nominally 2 mg/mL of nevirapine prepared as follows. Transfer an appropriate number of Tablets to a suitable volumetric flask. Add 50% of the flask volume of acetonitrile and shake for 30 min. Add 30% of the flask volume of water and continue shaking for another 30 min. Allow it to stand for at least 1 h and dilute with water to volume. Centrifuge a portion and use the supernatant.
Sample solution: Nominally 0.5 mg/mL of nevirapine in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm. For *Identification A*, use a diode array detector in the range of 210–400 nm.
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nevirapine from the *Sample solution*
r_S = peak response of nevirapine from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nevirapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
▲Test 1▲ (RB 1-May-2018)
Medium: 0.04 M sodium phosphate buffer pH 6.8 containing 2% sodium dodecyl sulfate prepared as follows. Dissolve 6.0 g of monobasic sodium phosphate in 800 mL of water and adjust with a 50% sodium hydroxide solution to a pH of 6.8. Add 20 g of sodium dodecyl sulfate and dilute with water to 1000 mL; 900 mL.
Apparatus 1: 75 rpm
Times
For Tablets labeled to contain 100 mg: 1, 5, and 12 h
For Tablets labeled to contain 400 mg: 2, 8, and 20 h
Mobile phase: Acetonitrile and water (18:82)
Standard solution: (L/900) mg/mL of USP Nevirapine Anhydrous RS in *Medium*, where L is the label claim in mg/Tablet prepared as follows. Transfer a weighed quantity of USP Nevirapine Anhydrous RS to a suitable volumetric flask and add ethyl alcohol to fill 12% of the flask volume. Sonicate for 15 min or until dissolved. Allow to cool to room temperature and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: About 1.6 times the retention time of nevirapine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of nevirapine ($C_{15}H_{14}N_4O$) dissolved in the portion of the sample withdrawn at each time point (i) (mg/mL):

$$C_i = (r_U/r_S) \times C_S$$

r_U = peak response of nevirapine from the *Sample solution*
r_S = peak response of nevirapine from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = \{C_3 \times [V - (2 \times V_S)] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

Supplement 3

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C_i = concentration of nevirapine in the portion of sample withdrawn at the time point (*i*)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances
For Tablets labeled to contain 100 mg: See *Table 2*.

Table 2

Time Point	Time	Amount Dissolved (%)
1	1	4–24
2	5	53–73
3	12	NLT 80

The percentages of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
For Tablets labeled to contain 400 mg: See *Table 3*.

Table 3

Time Point	Time	Amount Dissolved (%)
1	2	NMT 30
2	8	40–70
3	20	NLT 80

The percentages of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.04 M sodium phosphate buffer pH 6.8 containing 2% sodium dodecyl sulfate prepared as follows. Dissolve 6.24 g of sodium dihydrogen phosphate dihydrate and 0.89 g of sodium hydroxide in 1000 mL of water. Adjust with 0.2 N hydrochloric acid or with 0.2 N sodium hydroxide solution to a pH of 6.8. Add 20 g of sodium dodecyl sulfate and sonicate for about 30 min to dissolve; 900 mL.
Apparatus 1: 75 rpm
Times
For Tablets labeled to contain 100 mg: 1, 4, and 8 h
For Tablets labeled to contain 400 mg: 1, 6, and 20 h
Solution A: 0.2% (v/v) of triethylamine in water. Adjust with phosphoric acid to a pH of 5.0.
Mobile phase: Acetonitrile and *Solution A* (30:70)
Standard stock solution: 0.9 mg/mL of USP Nevirapine Anhydrous RS in methanol prepared as follows. Transfer a weighed quantity of USP Nevirapine Anhydrous RS to a suitable volumetric flask, add methanol to fill 60% of the flask volume, and sonicate to dissolve. Dilute with methanol to volume.
Standard solution: 0.036 mg/mL of USP Nevirapine Anhydrous RS in *Medium* from *Standard stock solution*. Pass through a suitable filter of 0.45-µm pore size.
Sample solution: Dilute a filtered portion of the solution under test with *Medium* to a concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25°
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of nevirapine ($C_{15}H_{14}N_4O$) dissolved in the portion of the sample withdrawn at each time point (*i*) (mg/mL):

$$C_i = (r_U/r_S) \times C_S \times D$$

r_U = peak response of nevirapine from the *Sample solution*
r_S = peak response of nevirapine from the *Standard solution*
C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*

Calculate the percentage of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of nevirapine in the portion of sample withdrawn at the time point (*i*)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances
For Tablets labeled to contain 100 mg: See *Table 4*.

Table 4

Time Point	Time	Amount Dissolved (%)
1	1	NMT 20
2	4	43–63
3	8	NLT 80

The percentages of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
For Tablets labeled to contain 400 mg: See *Table 5*.

Table 5

Time Point	Time	Amount Dissolved (%)
1	1	NMT 15
2	6	33–53
3	20	NLT 85

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The percentages of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2.* ▲ (RB 1-May-2018)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirement

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*

System suitability solution: 0.5 mg/mL of USP Nevirapine Anhydrous RS and 0.005 mg/mL of USP Nevirapine Related Compound A RS in *Solution B.* Sonicate if necessary to dissolve, before final dilution.

Standard stock solution: Use the *Standard solution* from the *Assay.*

Standard solution: 0.0005 mg/mL of USP Nevirapine Anhydrous RS in *Solution B* from the *Standard stock solution*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between the nevirapine and nevirapine related compound A peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of nevirapine from the *Standard solution*

C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nevirapine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table* ▲6. ▲ (RB 1-May-2018) Disregard any impurity peak less than 0.1%.

Table ▲6 ▲ (RB 1-May-2018)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nevirapine related compound B[a,b]	0.64	—
Nevirapine	1.00	—
Nevirapine related compound A[b]	1.34	—
Nevirapine related compound C[b,c]	1.73	—
Any individual unspecified impurity	—	0.2
Total impurities	—	0.4

[a] 5,11-Dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.

[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

[c] 5,11-Dihydro-6*H*-11-propyl-4-methyl-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

Add the following:

▲• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. ▲ (RB 1-May-2018)

• **USP REFERENCE STANDARDS** ⟨11⟩

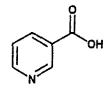
USP Nevirapine Anhydrous RS

USP Nevirapine Related Compound A RS

5,11-Dihydro-6*H*-11-ethyl-4-methyl-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.

$C_{14}H_{14}N_4O$ 254.29

Niacin



$C_6H_5NO_2$ 123.11

3-Pyridinecarboxylic acid;

Nicotinic acid [59-67-6].

DEFINITION

Niacin contains NLT 98.0% and NMT 102.0% of niacin ($C_6H_5NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M** ▲ (CN 1-May-2020)

• **B. ULTRAVIOLET ABSORPTION** ⟨197U⟩

Wavelength range: 200–300 nm

Buffer solution: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water. Adjust with 50% sodium hydroxide solution to a pH of 7.0.

Sample solution: 20 µg/mL in *Buffer solution*

Acceptance criteria: Meets the requirements. The A_{239}/A_{263} ratio is 0.46–0.52.

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Diluent: Methanol and water (82:18)

Mobile phase: Methanol and water (82:18), adjusted with glacial acetic acid to a pH of 3.15 ± 0.05

System suitability solution: 0.25 mg/mL of USP Niacin RS, 0.050 mg/mL of USP 6-Hydroxynicotinic Acid RS, and 0.10 mg/mL of pyridine in *Diluent*

Standard solution: 0.25 mg/mL of USP Niacin RS in *Diluent*

Sample solution: 0.25 mg/mL of Niacin in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-µm packing L8

Flow rate: 1.0 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

USP Monographs

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[NOTE—The relative retention times for pyridine, 6-hydroxynicotinic acid, and niacin are about 0.14, 0.64, and 1.0, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.5 between pyridine and 6-hydroxynicotinic acid and NLT 1.5 between 6-hydroxynicotinic acid and niacin, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of niacin ($C_6H_5NO_2$) in the portion of Niacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of niacin from the *Sample solution*

r_S = peak response of niacin from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

C_U = concentration of Niacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Standard: 0.15 mL of 0.020 N hydrochloric acid

Sample: 0.50 g of Niacin

Acceptance criteria: NMT 0.02%

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Standard: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.50 g of Niacin

Acceptance criteria: NMT 0.02%

- **RELATED COMPOUNDS**

Solution A: Dissolve 0.6 g of glacial acetic acid in 1 L of water, and adjust with 10% ammonium hydroxide solution to a pH of 5.6.

Solution B: Acetonitrile and methanol (1:1)

Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10[a]	100	0
30	20	80
35	20	80
36	100	0
48	100	0

[a] The gradient start time may be adjusted to achieve the required resolution between the 6-methylnicotinic acid and 6,6′-dinicotinic acid peaks of the *System suitability solution*.

System suitability solution: Transfer 3 mg each of USP 6-Methylnicotinic Acid RS, USP 6,6′-Dinicotinic Acid RS, and pyridine to a 100-mL volumetric flask, dissolve, and dilute with *Solution A* to volume. Transfer 2.0 mL of the resultant solution to a 5-mL volumetric flask, and dilute with *Solution A* to volume.

Standard solution: 0.012 mg/mL of USP Niacin RS in *Solution A*

Sample solution: Transfer 120 mg of Niacin to a 10-mL volumetric flask, add 200 µL of 10% ammonium hydroxide solution, and dilute with *Solution A* to volume. Shake the flask until the Niacin is completely dissolved.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Column temperature: 15°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 6-methylnicotinic acid, 6,6′-dinicotinic acid, and pyridine are about 1.0, 1.03, and 1.4, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.5 between 6-methylnicotinic acid and 6,6′-dinicotinic acid peaks, *System suitability solution*

Relative standard deviation: NMT 10.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Niacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of niacin from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

C_U = concentration of Niacin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Isocinchomeronic acid	0.38	0.05
6-Hydroxynicotinic acid	0.63	0.05
Isonicotinic acid	0.92	0.05
Niacin	1.00	—
6-Methylnicotinic acid	2.61	0.05
6,6′-Dinicotinic acid	2.68	0.05
5-Nitronicotinic acid	2.76	0.05
Pyridine	3.76	0.05
3-Nitropyridine	3.83	0.05
3,5-Dinitropyridine	4.03	0.05
3-Ethylpyridine	4.72	0.05
5-Ethyl-2-methylpyridine	5.00	0.05
Any individual unspecified impurity	—	0.05
Total impurities	—	0.20

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SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 1 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP 6,6′-Dinicotinic Acid RS
  USP 6-Hydroxynicotinic Acid RS
  USP 6-Methylnicotinic Acid RS
  USP Niacin RS

Niacin Injection

» Niacin Injection is a sterile solution of Niacin and niacin sodium in Water for Injection, made with the aid of Sodium Carbonate or Sodium Hydroxide. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_5NO_2$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Niacin RS
Identification—To a volume of Injection, equivalent to about 100 mg of niacin, add 0.3 mL of 3 N hydrochloric acid, evaporate, if necessary, on a steam bath to about 2 mL, and allow to stand for 1 hour in a cool place. Filter by suction, wash with small volumes of ice-cold water until the last washing does not give a reaction for chloride, and dry at 105° for 1 hour: the niacin so obtained responds to *Identification* tests *A* and *B* under *Niacin*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.5 USP Endotoxin Units per mg of niacin.
pH ⟨791⟩: between 4.0 and 6.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed with Injection as directed for *Niacin or Niacinamide Assay* ⟨441⟩, *Chemical Method*, using *Standard Niacin Preparation* as the *Standard Preparation* in the *Assay Procedure*, and the following as the *Assay Preparation*. Transfer an accurately measured volume of Injection, equivalent to about 50 mg of niacin, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, dilute with water to volume, and mix. Calculate the quantity, in mg, of $C_6H_5NO_2$ in each mL of the Injection taken by the formula:

$$(50/V)(A_U/A_S)$$

in which V is the volume, in mL, of Injection taken.

Niacin Tablets

DEFINITION
Niacin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of niacin ($C_6H_5NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
  Sample: Heat a portion of finely powdered Tablets, equivalent to 500 mg of niacin, with 25 mL of alcohol on a steam bath for a few min. Filter, and wash the residue with a few mL of hot alcohol. To the filtrate add 30 mL of water, and evaporate to 25 mL on the steam bath. Cool, filter if insoluble matter separates, and evaporate the filtrate to 10 mL. Cool, and place in a refrigerator for 1 h. Filter the separated niacin with suction, wash it with a few mL of cold alcohol, and dry at 105° for 1 h.
  Acceptance criteria: Meet the requirements

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Medium: 6.8 mg/mL of monobasic potassium phosphate in water, adjusted to a pH of 7.0 with 50% sodium hydroxide solution
  Sample solution: 20 µg/mL in *Medium* from the *Sample* obtained in the *Identification* test *A*
  Acceptance criteria: Meets the requirements in the chapter. The A_{237}/A_{262} ratio is 0.46–0.50.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 5-mM solution of sodium 1-hexanesulfonate in water
  Mobile phase: Methanol, acetonitrile, glacial acetic acid, and *Solution A* (14:7:1:78)
  Standard solution: 0.050 mg/mL of USP Niacin RS in water. Dissolve with the aid of heat in a steam bath.
  Sample solution: Transfer an equivalent to 500 mg of Niacin from NLT 20 finely powdered Tablets to a suitable flask. Add 50 mL of water, and heat on a steam bath for 30 min. Sonicate for 2 min, shake by mechanical means for 15 min, and cool to room temperature. Dilute with water to 0.050 mg/mL, and filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 262 nm
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 1.3 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 1000 theoretical plates for the analyte peak
  Tailing factor: NMT 2.0 for the analyte peak
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*

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C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of niacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Standard solution: 0.02 mg/mL of USP Niacin RS in the *Medium*
Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Maximum at about 260 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the concentration of niacin in the *Sample solution* in comparison with the *Standard solution*.
Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved:

$$\text{Result} = (C \times D \times V/L) \times 100$$

C = determined concentration of niacin in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 65% (Q) of the labeled amount of niacin ($C_6H_5NO_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Niacin RS

Niacin Extended-Release Tablets

DEFINITION
Niacin Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of niacin ($C_6H_5NO_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Diluent: Methanol and water (82:18)
Mobile phase: Methanol and water (82:18), adjusted with glacial acetic acid to a pH of 3.15 ± 0.05
Standard solution: 250 µg/mL of USP Niacin RS, 50 µg/mL of USP 6-Hydroxynicotinic Acid RS, and 97.8 µg/mL of pyridine in *Diluent*
Sample solution: Transfer a quantity of powder, equivalent to 50 mg of niacin from NLT 20 finely powdered Tablets, to a suitable flask, add *Diluent*, and stir for 2 h. Dilute with *Diluent* to a final concentration of 250 µg/mL of niacin.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L8
Flow rate: 1.0 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 13* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between pyridine and 6-hydroxynicotinic acid, and NLT 1.5 between 6-hydroxynicotinic acid and niacin
Relative standard deviation: NMT 3.0% for each of the peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of niacin from the *Sample solution*
r_S = peak area of niacin from the *Standard solution*
C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of niacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 1, 3, 6, 9, 12, and 20 h; without *Medium* replacement. [NOTE—Withdraw the same volume at each time point.]
Solution A: Solution of sodium heptanesulfonate in acetic acid, methanol, and water (4:44:33:19), w/w[1]
Mobile phase: Mixture of methanol, water, and *Solution A* (560:440:25)
Standard solution: USP Niacin RS at a known concentration in water in the range of 75–750 µg/mL
Sample solution: Filtered portion of the solution under test suitably diluted with *Medium* if necessary
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; 10-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine, in mg/mL, the content of niacin ($C_6H_5NO_2$) in the *Medium* at each time point:

[1] Commercially available from Waters Corporation as PIC B7 Reagent (Part #85103).

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$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak area of niacin from the *Sample solution*
r_S = peak area of niacin from the *Standard solution*
C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at each time point: At 1 h:

$$\text{Result}_1 = (C_1 \times V/L) \times 100$$

At 3 h:

$$\text{Result}_2 = [C_2 \times (V - V_S) + C_1 \times V_S] \times 100/L$$

At 6 h:

$$\text{Result}_3 = [C_3 \times (V - 2 \times V_S) + (C_1 + C_2) \times V_S] \times 100/L$$

At 9 h:

$$\text{Result}_4 = [C_4 \times (V - 3 \times V_S) + (C_1 + C_2 + C_3) \times V_S] \times 100/L$$

At 12 h:

$$\text{Result}_5 = [C_5 \times (V - 4 \times V_S) + (C_1 + C_2 + C_3 + C_4) \times V_S] \times 100/L$$

At 20 h:

$$\text{Result}_6 = [C_6 \times (V - 5 \times V_S) + (C_1 + C_2 + C_3 + C_4 + C_5) \times V_S] \times 100/L$$

C = as C_1, C_2, ..., C_6, the content of niacin in the *Medium* at each time point (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of sample withdrawn at each time point (mL)
L = label claim (mg/Tablet)

Tolerances: The percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at the times specified in *Table 1*, *Table 2*, and *Table 3* conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 1. For Tablets Labeled to Contain 500 mg or Less/ Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
3	17–32
6	33–48
9	43–63
12	52–77
20	NLT 75

Table 2. For Tablets Labeled to Contain 750 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
3	16–31
6	31–46

Table 2. For Tablets Labeled to Contain 750 mg/Tablet *(continued)*

Time (h)	Amount Dissolved (%)
9	42–62
12	51–76
20	NLT 75

Table 3. For Tablets Labeled to Contain 1000 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
3	15–30
6	30–45
9	40–60
12	50–75
20	NLT 75

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Acid stage medium: 0.1 N hydrochloric acid; 900 mL
Buffer stage medium: 6.8 g of monobasic potassium phosphate and 0.89 g of sodium hydroxide pellets in 1000 mL of water. Adjust with diluted sodium hydroxide or phosphoric acid to a pH of 6.8; 900 mL.
Apparatus 1: 100 rpm
Times: 1, 4, 12, and 24 h: 1 and 4 h in the *Acid stage medium*; 12 and 24 h in the *Buffer stage medium*. Replace the volume withdrawn with the equal volume of medium preheated to 37 ± 0.5°.
Procedure: After 4 h replace the *Acid stage medium* with the *Buffer stage medium*, and run the test for the times specified (additional 20 h for a total of 24 h). [NOTE—Withdraw the same volume at each time point. Pass a portion of the solution through a suitable filter.]
Standard stock solution: 0.2 mg/mL of USP Niacin RS in water
Standard solution 1: Dilute *Standard stock solution* with *Acid stage medium* to a final concentration of 0.01 mg/mL of USP Niacin RS.
Standard solution 2: Dilute *Standard stock solution* with *Buffer stage medium* to a final concentration of 0.01 mg/mL of USP Niacin RS.
Sample solution
For Tablets labeled to contain 500 mg: Dilute a filtered portion of the solution under test with appropriate dissolution medium 25-fold.
For Tablets labeled to contain 750 mg: Dilute a filtered portion of the solution under test with appropriate dissolution medium 33-fold.
For Tablets labeled to contain 1000 mg: Dilute a filtered portion of the solution under test with appropriate dissolution medium 50-fold.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 262 nm
Path length: 1 cm
Blank: *Acid stage medium* or *Buffer stage medium*
Analysis
Samples: *Standard solution 1* or *Standard solution 2* and *Sample solution*

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Determine the concentration, in mg/mL, of niacin ($C_6H_5NO_2$) in the sample withdrawn from the vessel at each time point:

$$\text{Result} = [(A_U - A_B)/A_S] \times C_S \times D$$

A_U = absorbance of the *Sample solution*
A_B = absorbance of the *Blank*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at each time point: At 1 h:

$$\text{Result}_1 = (C_1 \times V) \times 100/L$$

At 4 h:

$$\text{Result}_2 = (C_2 \times V + C_1 \times V_S) \times 100/L$$

At 12 h:

$$\text{Result}_3 = [(C_3 + C_2) \times V + C_1 \times V_S] \times 100/L$$

At 24 h:

$$\text{Result}_4 = [(C_4 + C_2) \times V + (C_1 + C_3) \times V_S] \times 100/L$$

C = as C_1, ..., C_4, the content of niacin in the related dissolution medium at each time point (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of sample withdrawn at each time point (mL)
L = label claim (mg/Tablet)

Tolerances: The percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at the times specified in *Table 4* and *Table 5* conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 4. For Tablets Labeled to Contain 500 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 25
4	30–50
12	65–85
24	NLT 80

Table 5. For Tablets Labeled to Contain 750 and 1000 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 25
4	30–50
12	55–75
24	NLT 80

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 1, 3, 6, 9, 12, and 20 h; without *Medium* replacement
[NOTE—Withdraw the same volume at each time point. Pass a portion of the solution through a stainless steel filter.]
Solution A: 1 mg/mL of sodium 1-hexanesulfonate monohydrate in water
Mobile phase: Mixture of *Solution A*, methanol, and glacial acetic acid (840:150:10)
Standard solution: (L/900) mg/mL of USP Niacin RS in *Medium*, where L is the label claim in mg/Tablet
[NOTE—Use sonication for complete dissolution, if necessary.]
Sample solution: Filtered portion of the solution under test
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Sample cooler: 10°
Detector: UV 262 nm
Column: 3.9-mm × 15-cm; 10-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Theoretical plates: NLT 1000
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the concentration, in mg/mL, of niacin ($C_6H_5NO_2$) in the *Medium* at each time point:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak area of niacin from the *Sample solution*
r_S = peak area of niacin from the *Standard solution*
C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at each time point: At 1 h:

$$\text{Result}_1 = (C_1 \times V/L) \times 100$$

At 3 h:

$$\text{Result}_2 = [C_2 \times (V - V_S) + C_1 \times V_S] \times 100/L$$

At 6 h:

$$\text{Result}_3 = [C_3 \times (V - 2 \times V_S) + (C_1 + C_2) \times V_S] \times 100/L$$

At 9 h:

$$\text{Result}_4 = [C_4 \times (V - 3 \times V_S) + (C_1 + C_2 + C_3) \times V_S] \times 100/L$$

At 12 h:

$$\text{Result}_5 = [C_5 \times (V - 4 \times V_S) + (C_1 + C_2 + C_3 + C_4) \times V_S] \times 100/L$$

At 20 h:

$$\text{Result}_6 = [C_6 \times (V - 5 \times V_S) + (C_1 + C_2 + C_3 + C_4 + C_5) \times V_S] \times 100/L$$

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C = as C_1, C_2, ..., C_6, the content of niacin in the *Medium* at each time point (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of sample withdrawn at each time point (mL)
L = label claim (mg/Tablet)

Tolerances: The percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at the times specified in *Table 6* and *Table 7* conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 6. For Tablets Labeled to Contain 500 and 1000 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 20
3	15–35
6	30–50
9	40–65
12	50–80
20	NLT 70

Table 7. For Tablets Labeled to Contain 750 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 16
3	15–35
6	30–50
9	40–65
12	50–75
20	NLT 75

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 1, 3, 6, 9, 12, and 24 h for Tablets labeled to contain 500 and 1000 mg/Tablet, and 1, 6, 12, and 24 h for Tablets labeled to contain 750 mg/Tablet; without *Medium* replacement
[NOTE—Withdraw the same volume at each time point. Pass a portion of the solution through a 0.45-µm PVDF membrane filter, discarding the first 2 mL of the filtrate.]
Standard stock solution: 0.5 mg/mL of USP Niacin RS in water
Standard solution: Dilute *Standard stock solution* with *Medium* to a final concentration of 0.02 mg/mL of USP Niacin RS.
Sample solution
For Tablets labeled to contain 500 mg: Dilute a filtered portion of the solution under test with *Medium* 25-fold.
For Tablets labeled to contain 750 mg: Dilute a filtered portion of the solution under test with *Medium* 40-fold.
For Tablets labeled to contain 1000 mg: Dilute a filtered portion of the solution under test with *Medium* 50-fold.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 262 nm
Path length: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the concentration, in mg/mL, of niacin ($C_6H_5NO_2$) in the sample withdrawn from the vessel at each time point:

$$\text{Result} = [(A_U - A_B)/A_S] \times C_S \times D$$

A_U = absorbance of the *Sample solution*
A_B = absorbance of the *Blank*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*

For Tablets labeled to contain 500 and 1000 mg: Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at each time point: At 1 h:

$$\text{Result}_1 = (C_1 \times V/L) \times 100$$

At 3 h:

$$\text{Result}_2 = [C_2 \times (V - V_S) + C_1 \times V_S] \times 100/L$$

At 6 h:

$$\text{Result}_3 = [C_3 \times (V - 2 \times V_S) + (C_1 + C_2) \times V_S] \times 100/L$$

At 9 h:

$$\text{Result}_4 = [C_4 \times (V - 3 \times V_S) + (C_1 + C_2 + C_3) \times V_S] \times 100/L$$

At 12 h:

$$\text{Result}_5 = [C_5 \times (V - 4 \times V_S) + (C_1 + C_2 + C_3 + C_4) \times V_S] \times 100/L$$

At 24 h:

$$\text{Result}_6 = [C_6 \times (V - 5 \times V_S) + (C_1 + C_2 + C_3 + C_4 + C_5) \times V_S] \times 100/L$$

For Tablets labeled to contain 750 mg: Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at each time point: At 1 h:

$$\text{Result}_1 = (C_1 \times V/L) \times 100$$

At 6 h:

$$\text{Result}_2 = [C_2 \times (V - V_S) + C_1 \times V_S] \times 100/L$$

At 12 h:

$$\text{Result}_3 = [C_3 \times (V - 2 \times V_S) + (C_1 + C_2) \times V_S] \times 100/L$$

At 24 h:

$$\text{Result}_4 = [C_4 \times (V - 3 \times V_S) + (C_1 + C_2 + C_3) \times V_S] \times 100/L$$

C = as C_1, C_2, ..., C_6, the content of niacin in the *Medium* at each time point (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of sample withdrawn at each time point (mL)
L = label claim (mg/Tablet)

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Tolerances: The percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at the times specified in *Table 8*, *Table 9*, and *Table 10* conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 8. For Tablets Labeled to Contain 500 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
3	17–32
6	33–48
9	48–68
12	60–80
24	NLT 80

Table 9. For Tablets Labeled to Contain 750 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
6	20–40
12	48–68
24	NLT 80

Table 10. For Tablets Labeled to Contain 1000 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
3	12–27
6	25–45
9	35–55
12	50–70
24	NLT 80

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 1, 6, 12, and 24 h; without *Medium* replacement
[NOTE—Withdraw the same volume at each time point. Pass a portion of the solution through a 0.45-µm nylon membrane filter, discarding the first 2 mL of the filtrate.]
Solution A: 1.1 mg/mL of sodium 1-heptanesulfonate in water
Mobile phase: Mixture of *Solution A* and methanol (70:30)
Standard solution: 0.84 mg/mL of USP Niacin RS in water
[NOTE—Use sonication for complete dissolution, if necessary.]
Sample solution: Filtered portion of the solution under test
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 262 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the concentration, in mg/mL, of niacin ($C_6H_5NO_2$) in the *Medium* at each time point:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak area of niacin from the *Sample solution*
r_S = peak area of niacin from the *Standard solution*
C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at each time point: At 1 h:

$$\text{Result}_1 = (C_1 \times V/L) \times 100$$

At 6 h:

$$\text{Result}_2 = [C_2 \times (V - V_S) + C_1 \times V_S] \times 100/L$$

At 12 h:

$$\text{Result}_3 = [C_3 \times (V - 2 \times V_S) + (C_1 + C_2) \times V_S] \times 100/L$$

At 24 h:

$$\text{Result}_4 = [C_4 \times (V - 3 \times V_S) + (C_1 + C_2 + C_3) \times V_S] \times 100/L$$

C = as C_1, …, C_4, the content of niacin in the *Medium* at each time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of sample withdrawn at each time point (mL)

Tolerances: The percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at the times specified in *Table 11* and *Table 12* conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 11. For Tablets Labeled to Contain 500 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 20
6	30–50
12	50–75
24	NLT 80

Table 12. For Tablets Labeled to Contain 1000 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 20
6	20–40
12	45–65
24	NLT 80

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

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IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 6-hydroxynicotinic acid or pyridine in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of 6-hydroxynicotinic acid or pyridine from the *Sample solution*

r_S = peak area of 6-hydroxynicotinic acid or pyridine from the *Standard solution*

C_S = concentration of USP 6-Hydroxynicotinic Acid RS or pyridine in the *Standard solution* (µg/mL)

C_U = nominal concentration of niacin in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of niacin from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of niacin in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 13*.

Table 13

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyridine	0.14	0.2
6-Hydroxynicotinic acid	0.64	0.2
Niacin	1.0	—
Any unspecified impurity	—	0.1
Total impurities	—	1.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

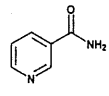
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP 6-Hydroxynicotinic Acid RS

USP Niacin RS

Niacinamide



$C_6H_6N_2O$ 122.12

3-Pyridinecarboxamide;
Nicotinamide [98-92-0].

DEFINITION

Niacinamide contains NLT 98.5% and NMT 101.5% of niacinamide ($C_6H_6N_2O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 20 µg/mL in water

Acceptance criteria: It meets the requirements in the chapter, and the A_{245}/A_{262} ratio is 0.63–0.67.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and 0.005 M sodium 1-heptanesulfonate (30:70)

Standard solution: Transfer 50 mg of USP Niacinamide RS to a 100-mL volumetric flask. Add 3 mL of water to dissolve, and dilute with *Mobile phase* to volume. Dilute with *Mobile phase* to 0.04 mg/mL.

Niacin standard solution Prepare as directed in the *Standard solution*, using USP Niacin RS instead of USP Niacinamide RS.

Sample solution: Prepare as directed in the *Standard solution*, using Niacinamide instead of USP Niacinamide RS.

System suitability solution: Mix equal volumes of the *Standard solution* and *Niacin standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between niacin and niacinamide, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of niacinamide ($C_6H_6N_2O$) in the portion of Niacinamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Niacinamide RS in the *Standard solution* (mg/mL)

C_U = concentration of Niacinamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **READILY CARBONIZABLE SUBSTANCES** ⟨271⟩

Sample solution: Dissolve 200 mg of Niacinamide in 5 mL of sulfuric acid.

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Acceptance criteria: The *Sample solution* has no more color than *Matching Fluid A*.

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 128°–131°
- **LOSS ON DRYING** ⟨731⟩: Dry a sample over silica gel for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Niacin RS
  USP Niacinamide RS

Niacinamide Injection

» Niacinamide Injection is a sterile solution of Niacinamide in Water for Injection. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_6N_2O$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Niacinamide RS
Identification—Dilute a quantity of the Injection, equivalent to about 200 mg of niacinamide, with water to about 10 mL. Add 1 mL of 2.5 N sodium hydroxide, evaporate on a steam bath to dryness, add 5 mL of water, and similarly evaporate to about 1 mL: during the initial evaporation, the odor of ammonia is perceptible. Neutralize to litmus paper with 3 N hydrochloric acid, add 1 mL of the acid in excess, and place the solution in a refrigerator for 2 hours. Then filter, wash the precipitated niacin with small portions of ice-cold water until free from chloride, and dry at 105° for 1 hour: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Niacinamide RS.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.5 USP Endotoxin Units per mg of niacinamide.
pH ⟨791⟩: between 5.0 and 7.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed with Injection as directed for under *Niacin or Niacinamide Assay* ⟨441⟩, *Chemical Method*, using *Standard Niacinamide Preparation* as the *Standard Preparation* in the *Assay Procedure*, and the following as the *Assay Preparation*. Dilute an accurately measured volume of Injection, equivalent to about 50 mg of niacinamide, with water to 500 mL in a volumetric flask, and mix. Pipet 10 mL of the solution into a 100-mL volumetric flask, dilute with water to volume, and mix. Calculate the quantity, in mg, of $C_6H_6N_2O$ in each mL of the Injection taken by the formula:

$$(50/V)(A_U/A_S)$$

in which V is the volume, in mL, of Injection taken.

Niacinamide Tablets

DEFINITION
Niacinamide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of niacinamide ($C_6H_6N_2O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Extract a quantity of powdered Tablets, equivalent to 500 mg of niacinamide, with two 10-mL portions of alcohol, evaporate the filtered alcohol extracts on a steam bath, and dry at 80° for 2 h.
  Acceptance criteria: Meet the requirements

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: 20 µg/mL of niacinamide in water from the *Sample* obtained in *Identification* test *A*
  Acceptance criteria: Meets the requirements in the chapter. The ratio A_{245}/A_{262} is 0.63–0.67.

ASSAY
- **NIACIN OR NIACINAMIDE ASSAY,** *Chemical Method* ⟨441⟩
  Standard niacinamide preparation: Prepare as directed in the chapter.
  Assay preparation: Transfer an equivalent of 25 mg of niacinamide, from NLT 10 finely powdered Tablets, to a suitable flask. Add 50 mL of water and heat, if necessary, until no more dissolves. Cool, dilute with water to 10 µg/mL, mix, and filter.
  Analysis: Proceed as directed in the chapter for *Procedure*. Calculate the percentage of the labeled amount of niacinamide ($C_6H_6N_2O$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Assay preparation*
A_S = absorbance of the *Standard niacinamide preparation*
C_S = concentration of the *Standard niacinamide preparation* (µg/mL)
C_U = nominal concentration of niacinamide in the *Assay preparation* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm
  Time: 45 min
  Standard solution: Known concentration of USP Niacinamide RS in the *Medium*
  Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary
  Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 1 mL/min

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Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of niacinamide ($C_6H_6N_2O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

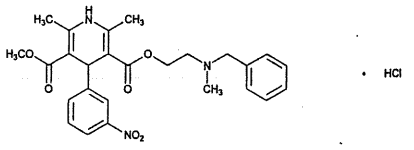
r_U = peak area of niacinamide from the *Sample solution*
r_S = peak area of niacinamide from the *Standard solution*
C_S = concentration of USP Niacinamide RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of niacinamide ($C_6H_6N_2O$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Niacinamide RS

Nicardipine Hydrochloride



$C_{26}H_{29}N_3O_6 \cdot HCl$ 515.99
3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-, methyl 2-[methyl(phenylmethyl)amino]ethyl ester, monohydrochloride;
2-(Benzylmethylamino)ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate monohydrochloride [54527-84-3].
Nicardipine free base
$C_{26}H_{29}N_3O_6$ 479.52
[55985-32-5].

DEFINITION

Nicardipine Hydrochloride contains NLT 98.0% and NMT 102.0% of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: 2.5 mg/mL in methanol
Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**
Protect all solutions from light.
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust the solution with potassium hydroxide to a pH of 4.8.
Solution A: Acetonitrile and methanol (82:18)
Mobile phase: *Solution A* and *Buffer* (40:60)
System suitability solution: 0.6 mg/mL of USP Nicardipine Hydrochloride RS in *Mobile phase* prepared as follows. To a suitable amount of USP Nicardipine Hydrochloride RS in a suitable volumetric flask add *Mobile phase* to fill about 10% of the volume of the flask, and sonicate for 2 min. Then add 10% hydrogen peroxide solution to fill an additional 10% of the volume of the flask. Allow it to stand for 30 min, then dilute with *Mobile phase* to volume.
Nicardipine Hydrochloride degrades to produce nicardipine pyridine analog, nicardipine dimethyl ester analog, and nicardipine bis analog. Use a freshly prepared sample to avoid further degradation for analysis.
Standard solution: 0.1 mg/mL of USP Nicardipine Hydrochloride RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Nicardipine Hydrochloride in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 35°–40°. [NOTE—To attain resolution, the *Column temperature* may be adjusted instead of the *Mobile phase* composition.]
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$) in the portion of Nicardipine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nicardipine from the *Sample solution*
r_S = peak response of nicardipine from the *Standard solution*
C_S = concentration of USP Nicardipine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Nicardipine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
Protect all solutions from light.
Buffer, Solution A, Mobile phase, and **System suitability solution:** Proceed as directed in the *Assay*.
Standard solution: 3 µg/mL of USP Nicardipine Hydrochloride RS in *Mobile phase*

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Sample solution: 0.6 mg/mL of Nicardipine Hydrochloride in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]
Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.
Run time: NLT 4 times the retention time of nicardipine
System suitability
Samples: *System suitability solution* and *Standard solution* [NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between nicardipine and nicardipine dimethyl ester analog and NLT 1.5 between nicardipine bis analog and nicardipine dimethyl ester analog, *System suitability solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of nicardipine from the *Standard solution*
C_S = concentration of USP Nicardipine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Nicardipine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.01%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nicardipine pyridine analog[a]	0.67	0.1
Nicardipine	1.00	—
Nicardipine dimethyl ester analog[b]	1.20	0.5
Nicardipine bis analog[c]	1.33	0.5
Any other individual unidentified impurity	—	0.1
Total impurities	—	1.0

[a] 3-{2-[Benzyl(methyl)amino]ethyl} 5-methyl 2,6-dimethyl-4-(3-nitrophenyl) pyridine-3,5-dicarboxylate dihydrochloride.
[b] Dimethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.
[c] Bis{2-[benzyl(methyl)amino]ethyl} 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate dihydrochloride.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105°, protected from light, to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tightly closed containers, protected from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Nicardipine Hydrochloride RS

Nicardipine Hydrochloride Injection

DEFINITION

Change to read:

Nicardipine Hydrochloride Injection is a sterile solution of Nicardipine Hydrochloride. It contains NTL 90.0% and NMT 110.0% each of the labeled amount of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$) and sorbitol ▲($C_6H_{14}O_6$).▲ (USP 1-May-2020)

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**
Buffer: 1.36 g/L of monobasic potassium phosphate in water
Mobile phase: Methanol and *Buffer* (80:20)
Diluent: Acetonitrile and *Buffer* (50:50)
Standard solution: 0.1 mg/mL of USP Nicardipine Hydrochloride RS in *Diluent*. Sonication may be used to aid in dissolution. Pass through a suitable filter of 0.45-µm pore size. Discard the first 2–3 mL of the filtrate.
Sample solution: Nominally ▲▲ (USP 1-May-2020) 0.1 mg/mL of nicardipine hydrochloride in *Diluent* from a suitable volume of Injection. Pass through a suitable filter of 0.45-µm pore size. Discard the first 2–3 mL of filtrate. [NOTE—The *Sample solution* is stable for about 26 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2020)
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of nicardipine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nicardipine from the *Sample solution*
r_S = peak response of nicardipine from the *Standard solution*

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C_S = concentration of USP Nicardipine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nicardipine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

• **LIMIT OF *N*-BENZYL-*N*-METHYL-ETHANOLAMINE**
Solution A: Dissolve 2.80 g of sodium perchlorate monohydrate in 1 L of water. Adjust with perchloric acid to a pH of 2.5.
Solution B: Acetonitrile and methanol (50:50)
Diluent: Acetonitrile and water (20:80)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
10	82	18
12	20	80
22	20	80
24	95	5
32	95	5

Standard solution: 2.5 µg/mL of USP *N*-Benzyl-*N*-methyl-ethanolamine RS in *Diluent* prepared as follows. To a suitable amount of USP *N*-Benzyl-*N*-methyl-ethanolamine RS, add *Diluent* to 70% of the final volume. Sonicate to dissolve. Cool, and dilute with *Diluent* to volume. Pass the solution through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally ▲▲ (USP 1-May-2020) 0.5 mg/mL of nicardipine hydrochloride in *Diluent* from a suitable volume of Injection. Pass the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
▲Autosampler:▲ (USP 1-May-2020) 10°
Column: 30°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of *N*-benzyl-*N*-methyl-ethanolamine in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *N*-benzyl-*N*-methyl-ethanolamine from the *Sample solution*
r_S = peak response of *N*-benzyl-*N*-methyl-ethanolamine from the *Standard solution*
C_S = concentration of USP *N*-Benzyl-*N*-methyl-ethanolamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nicardipine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.7%

Change to read:

• **ORGANIC IMPURITIES**
Solution A: 3.5 g/L of sodium perchlorate monohydrate in water. Add 1 mL/L of triethylamine, and adjust with perchloric acid to a pH of 2.0.
Solution B: Acetonitrile and methanol (70:30)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	70	30
15	70	30
55	35	65
60	35	65
62	70	30
70	70	30

Standard solution: 0.02 mg/mL of USP Nicardipine Hydrochloride RS in methanol prepared as follows. To a suitable amount of USP Nicardipine Hydrochloride RS add methanol to 60% of the final volume. Sonicate to dissolve. Cool, and dilute with methanol to volume. Pass the solution through a suitable filter of 0.45-µm pore size.
▲Sensitivity solution: 0.002 mg/mL of USP Nicardipine Hydrochloride RS in methanol from *Standard solution*▲ (USP 1-May-2020)
Sample solution: Nominally ▲▲ (USP 1-May-2020) 2 mg/mL of nicardipine hydrochloride in methanol from a suitable volume of Injection. Pass the solution through a suitable filter of 0.45-µm pore size. [NOTE—The *Sample solution* is stable for about 42 h at 10°.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
▲Autosampler:▲ (USP 1-May-2020) 10°
Column: 50°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* ▲and *Sensitivity solution*▲ (USP 1-May-2020)
Suitability requirements
Tailing factor: NMT 2.0, ▲*Standard solution*▲ (USP 1-May-2020)
Relative standard deviation: NMT 5.0%, ▲*Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*▲ (USP 1-May-2020)

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each ▲specified impurity and any unspecified degradation product▲ (USP 1-May-2020) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each ▲specified impurity or any unspecified degradation product▲ (USP 1-May-2020) from the *Sample solution*
r_S = peak response of nicardipine from the *Standard solution*
C_S = concentration of USP Nicardipine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nicardipine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nicardipine monoacid ▲(nicardipine related compound A)▲ (USP 1-May-2020)[a]	0.72	1.00	0.2
Nicardipine pyridine analog ▲(nicardipine related compound B)▲ (USP 1-May-2020)[b]	0.94	0.42	0.9
Nicardipine	1.00	1.00	—
Any unspecified degradation ▲product▲ (USP 1-May-2020)	—	—	0.2
Total impurities[c]	—	—	3.5

[a] 5-(Methoxycarbonyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid.
[b] 3-{2-[Benzyl(methyl)amino]ethyl} 5-methyl 2,6-dimethyl-4-(3-nitrophenyl) pyridine-3,5-dicarboxylate.
[c] Total impurities include the sum of all organic impurities and *N*-benzyl-*N*-methyl-ethanolamine.

OTHER COMPONENTS

Change to read:

- **CONTENT OF SORBITOL**
Buffer: 1 g/L of tetrabutylammonium hydrogen sulfate in water
Mobile phase: Acetonitrile and *Buffer* (70:30)
Standard solution: 4.8 mg/mL of USP Sorbitol RS in *Mobile phase*. Pass the solution through a suitable filter of 0.45-µm pore size. Sonication may be necessary to aid in dissolution.
Sample solution: Nominally ▲▲ (USP 1-May-2020) 4.8 mg/mL of sorbitol in *Mobile phase* from the contents of NLT 3 Injection vials. Pass the solution through a suitable filter of 0.45-µm pore size. [NOTE—*Sample solution* is stable for about 24 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 25-cm; 5-µm packing L8
Temperatures
Column: 40°
Detector: 50°
Flow rate: 1 mL/min
Injection volume: 25 µL
Run time: NLT 2 times the retention time of sorbitol
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sorbitol ▲($C_6H_{14}O_6$)▲ (USP 1-May-2020) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sorbitol from the *Sample solution*
r_S = peak response of sorbitol from the *Standard solution*
C_S = concentration of USP Sorbitol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sorbitol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

Change to read:

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements▲ (USP 1-May-2020)
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 3.0–3.9
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

Change to read:

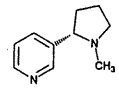
- **PACKAGING AND STORAGE:** Preserve in single-dose amber glass vials. ▲Store at controlled room temperature.▲ (USP 1-May-2020)
- **LABELING:** Label it to indicate that it is to be diluted to the appropriate strength with a suitable intravenous fluid prior to administration.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP *N*-Benzyl-*N*-methyl-ethanolamine RS
2-[Benzyl(methyl)amino]ethanol.
$C_{10}H_{15}NO$ 165.23
USP Nicardipine Hydrochloride RS
USP Sorbitol RS
D-Glucitol.
▲▲ (USP 1-May-2020)
$C_6H_{14}O_6$ 182.17

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Nicotine



$C_{10}H_{14}N_2$ 162.23
3-(1-Methyl-2-pyrrolidinyl)pyridine;
β-Pyridyl-α-*N*-methyl pyrrolidine [54-11-5].

DEFINITION
Nicotine contains NLT 99.0% and NMT 101.0% of nicotine ($C_{10}H_{14}N_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
  Standard: Transfer an amount of USP Nicotine Bitartrate Dihydrate RS equivalent to 100 mg of nicotine to a 100-mL glass-stoppered tube. Add 20 mL of 1 M ammonium hydroxide, 5 mL of 10 M sodium hydroxide, and 20 mL of *n*-hexane. Shake for 5 min, and allow the phases to separate. Transfer the upper hexane phase to an evaporating dish, and evaporate on a steam bath.
  Sample: Nicotine
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**
  Sample solution: Dissolve 60 mg of Nicotine in 40 mL of glacial acetic acid.
  Blank: 40 mL of glacial acetic acid
  Titrimetric system
  (see *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Endpoint detection: Potentiometric
  Analysis
  Samples: *Sample solution* and *Blank*
  Titrate the *Sample solution* with *Titrant*.
  Calculate the percentage of nicotine ($C_{10}H_{14}N_2$) in the portion of Nicotine taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = normality of the *Titrant* (mEq/mL)
F = equivalency factor, 81.1 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 99.0%–101.0% on the anhydrous basis

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A: Add 25 mL of 1 M acetic acid to 900 mL of water, then add 6 mL of ammonium hydroxide. Adjust with either 2 M acetic acid or 2 M ammonium hydroxide to a pH of 10.0, and dilute with water to 1000 mL.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.01	95	5
28	74	26
32	60	40

System suitability solution: 1.5 mg/mL of USP Nicotine Bitartrate Dihydrate RS and 6 µg/mL each of USP Nicotine Related Compound A RS, USP Nicotine Related Compound B RS, USP Nicotine Related Compound C RS, USP Nicotine Related Compound D RS, USP Nicotine Related Compound E RS, USP Nicotine Related Compound F RS, and USP Nicotine Related Compound G RS in water. [NOTE—The concentration of each related compound is in terms of the free base.]
Standard solution: 2.5 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water
Sensitivity solution: 1.2 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water from the *Standard solution*
Sample solution: 0.8 mg/mL of Nicotine in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.5 between nicotine and nicotine related compound G, *System suitability solution*
Tailing factor: NMT 2.0 for nicotine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Nicotine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of nicotine from the *Standard solution*
C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)
C_U = concentration of Nicotine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of nicotine, 162.23
M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05% of the nicotine peak.

USP Monographs

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Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nicotine related compound E	0.3	0.3
Nicotine related compound C	0.55	0.3
Nicotine related compound F	0.7	0.3
Nicotine related compound A	0.8	0.3
Nicotine related compound D	0.86	0.3
Nicotine related compound G	0.9	0.3
Nicotine	1.00	—
Nicotine related compound B	1.6	0.3
Any other unspecified impurity	—	0.10
Total impurities	—	0.8

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 20 mg/mL in alcohol
  Acceptance criteria: −130° to −143°
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store under nitrogen in well-closed containers below 25°, protected from light and moisture.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nicotine Bitartrate Dihydrate RS
  USP Nicotine Related Compound A RS
  Anatabine;
  1,2,3,6-Tetrahydro-2,3′-bipyridine.
  $C_{10}H_{12}N_2$ 160.22
  USP Nicotine Related Compound B RS
  Nicotyrine;
  3-(1-Methyl-1*H*-pyrrol-2-yl)pyridine.
  $C_{10}H_{10}N_2$ 158.20
  USP Nicotine Related Compound C RS
  Cotinine;
  (*S*)-1-Methyl-5-(pyridin-3-yl)pyrrolidin-2-one.
  $C_{10}H_{12}N_2O$ 176.22
  USP Nicotine Related Compound D RS
  Myosmine;
  3-(4,5-Dihydro-3*H*-pyrrol-2-yl)pyridine fumarate.
  $C_9H_{10}N_2 \cdot C_4H_4O_4$ 262.26
  USP Nicotine Related Compound E RS
  Nicotine *N*-oxide;
  (1*RS*,2*S*)-1-Methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide oxalate.
  $C_{10}H_{14}N_2O \cdot C_2H_2O_4$ 268.27
  USP Nicotine Related Compound F RS
  Nornicotine;
  3-(Pyrrolidin-2-yl)pyridine.
  $C_9H_{12}N_2$ 148.20
  USP Nicotine Related Compound G RS
  Anabasine;
  (*S*)-3-(Piperidin-2-yl)pyridine.
  $C_{10}H_{14}N_2$ 162.23

Nicotine Transdermal System

DEFINITION

Nicotine Transdermal System contains NLT 85% and NMT 115% of the labeled amount of nicotine ($C_{10}H_{14}N_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Solution A: Dissolve 1.1 g of monobasic potassium phosphate and 7.3 g of dibasic potassium phosphate in 1 L of water. [NOTE—The pH of this solution is between 7.5 and 7.6.]
  Solution B: Methanol
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	52.5	47.5
20.1	95	5
25	95	5

Extraction solvent: Methanol and tetrahydrofuran (90:10)
Standard stock solution: 3.0 mg/mL of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis (equivalent to about 1 mg/mL of nicotine) in *Extraction solvent*
Standard solution: 0.15 mg/mL of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis (equivalent to about 0.05 mg/mL of nicotine) in *Solution A* from the *Standard stock solution*
System suitability stock solution: 1 mg/mL each of USP Nicotine Related Compound E RS and USP Nicotine Related Compound D RS in *Extraction solvent*
System suitability solution: 0.15 mg/mL of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis and 0.1 mg/mL each of USP Nicotine Related Compound D RS and USP Nicotine Related Compound E RS in *Solution A* from the *Standard stock solution* and the *System suitability stock solution*
Sample stock solution: Nominally 1 mg/mL of nicotine from NLT 10 Transdermal System units prepared as follows. Transfer the required number of units to a suitable flask carefully ensuring that no adhesive contacts the walls. Add a suitable volume of *Extraction solvent*. Stopper the flask and seal to minimize the loss of the *Extraction solvent*. Shake the flask using a suitable shaker for 2 h.
Sample solution: Nominally 0.05 mg/mL of nicotine in *Solution A* from the *Sample stock solution*. Pass the solution through a suitable filter of 0.45-µm pore size.
Alternative sample preparation: If necessary, the *Sample stock solution* and the *Sample solution* may be prepared using the following alternative procedure.
Sample stock solution: Transfer one Transdermal System without the liner to a suitable 100-mL centrifuge tube with stopper. [NOTE—Cut Transdermal Systems larger than 2.5 cm^2 into 3–5-mm strips.] Add 10 mL of methylene chloride and a stirrer bar. Stopper the flask and stir the contents for NLT 4 h at room temperature.

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[NOTE—500 rpm for stirring speed is suitable.] Add 50 mL of 0.5% (v/v) phosphoric acid solution and continue extraction for another 1 h. Centrifuge at about 1000 rpm for NLT 5 min. Prepare separately the required number of extracts from the required number of Transdermal Systems following the same procedure described above. Combine equal volumes of the aqueous layer from each extract to obtain the *Sample stock solution.*

Sample solution: Nominally 0.03–0.05 mg/mL of nicotine from a suitable volume of the *Sample stock solution* and water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Column temperature: 45 ± 2°

Flow rate: 1.0 mL/min

Injection volume: 80 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between nicotine related compound D and nicotine, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nicotine ($C_{10}H_{14}N_2$) in the portion of Transdermal System taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of nicotine from the *Sample solution*

r_S = peak response of nicotine from the *Standard solution*

C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)

C_U = nominal concentration of nicotine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of nicotine, 162.23

M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

Acceptance criteria: 85%–115%

PERFORMANCE TESTS

• **DRUG RELEASE** ⟨724⟩

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 1.*

Medium: Phosphoric acid solution (1 in 1000); 250 mL, in a tall-form beaker

Apparatus 7: Proceed as directed in the chapter, using the transdermal system holder–cylinder (see *Drug Release* ⟨724⟩, *Figure 5b*).

Sample solution: Center the Transdermal System onto a dry, unused 10-cm × 10-cm piece of Cuprophan dialysis membrane with the adhesive side against the membrane, taking care to eliminate air bubbles between the membrane and the release surface. Attach the membrane to the cylinder using two Parker O-rings, such that one of the borders of the Transdermal System is aligned to the groove and it is wrapped around the cylinder. The filled beakers are weighed and pre-equilibrated to 32.0 ± 0.3°, before immersing the test sample. Reciprocate at a frequency of about 30 cycles/min with an amplitude of 2.0 ± 0.1 cm. At the end of each time interval, transfer the test sample to a fresh beaker containing the appropriate volume of *Medium*, weighed and pre-equilibrated to 32.0 ± 0.3°. At the end of each release interval, allow the beakers to cool to room temperature, make up for evaporative losses by adding water to obtain the original weight, and mix.

Times: 2, 12, and 24 h

Mobile phase: Transfer 0.2 mL of *N,N*-dimethyloctylamine to a 1-L volumetric flask, add 220 mL of acetonitrile, and mix. Add 300 mL of water, 0.2 mL of glacial acetic acid, 0.20 g of anhydrous sodium acetate, and 0.55 g of sodium 1-dodecanesulfonate, and dilute with water to volume. Mix for 1 h until clear. [NOTE—Equilibration of the column may take as long as 3 h.]

Standard solution: 0.15 mg/mL of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis (or 0.05 mg/mL of nicotine as free base) in *Medium*

System suitability solution: Transfer an amount of USP Nicotine Bitartrate Dihydrate RS equivalent to about 8 mg of nicotine to a 100-mL volumetric flask, and dissolve in 10 mL of acetonitrile. Add 5 mL of 30% hydrogen peroxide, and allow 15 min to react. Dilute with *Medium* to volume, and mix. Transfer 20 mL of this solution to a 100-mL volumetric flask, and dilute with *Standard solution* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.1 between nicotine and any degradation peaks

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: See *Table 2.*

Table 2

Time (h)	Amount Released (%)
0–2	31–87
2–12	62–191
12–24	85–261

The percentages of the labeled amount of nicotine ($C_{10}H_{14}N_2$) released at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 1.*

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 2.*

Buffer: 8 g/L of sodium chloride, 0.2 g/L of potassium chloride, 1.7 g/L of dibasic sodium phosphate, and 0.2 g/L of monobasic potassium phosphate in water

Medium: *Buffer*, 500 mL

Apparatus 6: 50 rpm, double-sided tape being used to attach the Transdermal System to the cylinder

Times: 6 and 24 h

Mobile phase: Acetonitrile, triethylamine, and water (300:1:700)

Standard solution: 0.6 mg/mL of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis (equivalent to about 0.2 mg/mL of nicotine) in *Medium*

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Sample solution: At each of the test times, withdraw a 2-mL aliquot of the solution under test. Replace the aliquots withdrawn for analysis with fresh portions of *Medium*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 12.5-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Released (%)
6	71–157
24	156–224

The percentages of the labeled amount of nicotine ($C_{10}H_{14}N_2$) released at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 1*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 3*.

Medium: Water; 900 mL

Apparatus 5: 50 rpm, the stainless steel disk assembly being replaced with a 5-cm watch glass for an 11-mg Transdermal System and an 8-cm watch glass for a 22-mg Transdermal System

Times: 1, 2, and 4 h

Standard solution: USP Nicotine Bitartrate Dihydrate RS in water having a known concentration of nicotine similar to that of the *Sample solution*.

Sample solution: At the specified times, withdraw a suitable volume of the solution under test.

Instrumental conditions
Mode: UV
Analytical wavelength: 259 nm
Blank: Water

Analysis
Samples: *Standard solution* and *Sample solution*

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Released (%)
1	35–75
2	55–95
4	NLT 73

The percentages of the labeled amount of nicotine ($C_{10}H_{14}N_2$) released at the times specified, conform to *Table 5*.

Table 5

Level	Tested	Criteria
L_1	6	No individual value lies outside each of the stated ranges and no individual value is less than the stated amount at the final test time.
L_2	6	The average value of the 12 units ($L_1 + L_2$) lies within each of the stated ranges and is NLT the stated amount at the final test time; none is more than 5% of the labeled content outside each of the stated ranges; and none is more than 5% of the labeled content below the stated amount at the final test time.
L_3	12	The average value of the 24 units ($L_1 + L_2 + L_3$) lies within each of the stated ranges and is NLT the stated amount at the final test time; NMT 2 of the 24 units are more than 5% of labeled content outside each of the stated ranges; NMT 2 of the 24 units are more than 5% of the labeled content below the stated amount at the final test time; and none of the units is more than 10% of the labeled content outside each of the stated ranges or more than 10% of the labeled content below the stated amount at the final test time.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 4*.

Medium: 0.025 N hydrochloric acid; 600 mL

Apparatus 5: 50 rpm, a convex screen being used to hold the Transdermal System in position during testing

Times: 4 and 16 h

Standard solution: USP Nicotine Bitartrate Dihydrate RS in water having a known concentration of nicotine similar to that of the *Sample solution*

Sample solution: At the specified times, withdraw a suitable volume of the solution under test.

Instrumental conditions
Mode: UV
Analytical wavelength: 259 nm
Blank: Water

Analysis
Samples: *Standard solution* and *Sample solution*

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Released (%)
4	36–66
16	72–112

The percentages of the labeled amount of nicotine ($C_{10}H_{14}N_2$) released at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 1*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 5*.

Buffer, Medium, and **Apparatus 6:** Proceed as directed in *Test 2*.

Times: 3, 6, and 24 h

Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in *Test 2*, except to use an *Injection volume* of 30 µL.

Tolerances: See *Table 7*.

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Table 7

Time (h)	Amount Released (%)
3	79–112
6	108–141
24	156–202

The percentages of the labeled amount of nicotine ($C_{10}H_{14}N_2$) released at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 1*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Diluent: Tetrahydrofuran, methanol, and *Solution A* (0.5: 4.5: 95)

Sensitivity solution: 0.15 µg/mL of USP Nicotine Bitartrate Dihydrate RS (equivalent to about 0.05 µg/mL of nicotine) in *Diluent* from the *Standard solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—See *Table 8* for the relative retention times. The 1*R*,2*S* isomer of nicotine related compound E is a small peak that precedes the 1*S*,2*S* isomer, which is the major peak.]

Suitability requirements

Resolution: NLT 3 between nicotine related compound D and nicotine, *System suitability solution*; NLT 1.2 between the (1*R*,2*S*) and (1*S*,2*S*) isomers of nicotine related compound E, *System suitability solution*

Signal-to-noise ratio: NLT 10 for nicotine, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Transdermal System taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor (see *Table 8*)

Acceptance criteria: See *Table 8*. Disregard peaks that are less than 0.05% of the nicotine peak.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in the hermetic, light-resistant, unit-dose pouch.

• **LABELING:** The labeling indicates the *Drug Release Test* with which the product complies.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Nicotine Bitartrate Dihydrate RS

$C_{10}H_{14}N_2 \cdot (C_4H_6O_6)_2 \cdot 2H_2O$ 498.44

USP Nicotine Related Compound D RS

3-(4,5-Dihydro-3*H*-pyrrol-2-yl)pyridine fumarate; Also known as Myosmine.

$C_9H_{10}N_2 \cdot C_4H_4O_4$ 262.26

USP Nicotine Related Compound E RS

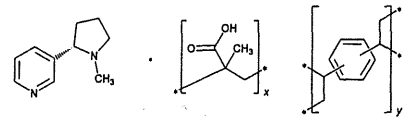
(1*RS*,2*S*)-1-Methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide oxalate;

Also known as Nicotine *N*-oxide.

[NOTE—This may be a mixture of (1*R*,2*S*) and (1*S*,2*S*) isomers.]

$C_{10}H_{14}N_2O \cdot C_2H_2O_4$ 268.27

Nicotine Polacrilex



$[(C_4H_6O_2)_x(C_{10}H_{10})_y](C_{10}H_{14}N_2)$

2-Propenoic acid, 2-methyl-, polymer with diethenylbenzene, complex with (*S*)-3-(1-methyl-2-pyrrolidinyl)pyridine; Methacrylic acid polymer with divinylbenzene, complex with nicotine [96055-45-7].

DEFINITION

Nicotine Polacrilex is a weak carboxylic cation-exchange resin prepared from methacrylic acid and divinylbenzene, in complex with nicotine. It may contain glycerin. Glycerin-free Nicotine Polacrilex contains NLT 95.0% and NMT 115.0% of the labeled amount of nicotine ($C_{10}H_{14}N_2$), calculated on the dried basis. Glycerin-containing Nicotine Polacrilex contains NLT 95.0% and NMT 115.0% of the labeled amount of nicotine ($C_{10}H_{14}N_2$), calculated on the anhydrous basis.

[NOTE—Nicotine Polacrilex is also known as Nicotine Resinate.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy (for Nicotine)*: 197K or 197A▲ (CN 1-May-2020)

Sample: Transfer an amount of Nicotine Polacrilex equivalent to 100 mg of nicotine to a 100-mL glass-stoppered tube. Add 20 mL of 1 M ammonium hydroxide, 5 mL of 10 M sodium hydroxide, and 20 mL of *n*-hexane. Shake for 5 min, and allow the phases to separate. Transfer the upper hexane phase to an evaporating dish, and evaporate on a steam bath.

Standard: Use USP Nicotine Bitartrate Dihydrate RS, and prepare as directed for the *Sample*.

Acceptance criteria: Meets the requirements

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy (for Polacrilex)*: 197K or 197A▲ (CN 1-May-2020)

Standard: Transfer a portion of USP Polacrilex Resin RS, equivalent to the amount of Nicotine Polacrilex used to prepare the *Sample solution* in the *Assay*, to a glass-stoppered tube. Add 10 mL of 1 M ammonium hydroxide, shake for 10 min, and then centrifuge. Decant the ammonia solution from the residue, and wash the residue by shaking it with three 10-mL volumes of water, decanting the water phase after each shaking. Wash with 10 mL of 0.1 N hydrochloric acid, decant the liquid, and dry the residue at 105°.

Sample: Use the residue obtained from the *Sample solution* in the *Assay*. Decant the ammonia solution remaining from the residue, and wash the residue by shaking it with three 10-mL volumes of water, decanting the water phase after

USP Monographs

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Table 8 (Nicotine Transdermal System)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)		
			7 mg/24 h	14 mg/24 h	21 mg/24 h
Nicotinic acid[a]	0.15	1.3	0.5	0.5	0.5
Nicotine related compound E (1*R*,2*S* isomer)	0.25	0.76	0.5	0.5	0.5
Nicotine related compound E (1*S*,2*S* isomer)	0.27	0.76	0.5	0.5	0.5
Nicotine related compound F[b,c]	0.38	—	—	—	—
Nicotine related compound C[d]	0.54	1.0	2.8	2.6	1.8
Nicotine related compound G[b,e]	0.64	—	—	—	—
Nicotine related compound A[b,f]	0.74	—	—	—	—
Nicotine related compound D	0.85	1.6	5.6	3.2	2.6
Nicotine	1.00	—	—	—	—
Nicotine related compound B[g]	1.19	1.9	0.5	0.5	0.5
Any other unspecified impurity	—	1.0	0.6	0.5	0.5
Total impurities	—	—	8.3	5.8	4.4

[a] 3-Pyridine carboxylic acid.
[b] Process impurity; controlled in drug substance and not to be included in total impurities.
[c] (*S*)-3-(Pyrrolidin-2-yl)pyridine; also known as nornicotine.
[d] (*S*)-1-Methyl-5-(pyridin-3-yl)pyrrolidin-2-one; also known as cotinine.
[e] (*S*)-3-(Piperidin-2-yl)pyridine; also known as anabasine.
[f] (*S*)-1,2,3,6-Tetrahydro-2,3′-bipyridine; also known as anatabine.
[g] 3-(1-Methyl-1*H*-pyrrol-2-yl)pyridine; also known as nicotyrine.

each shaking. Wash with 10 mL of 0.1 N hydrochloric acid, decant the liquid, and dry the residue at 105°.

Acceptance criteria: Meets the requirements

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Solution A: Add 25 mL of 1 M acetic acid to 900 mL of water, and then add 6 mL of ammonium hydroxide. Adjust with either 2 M acetic acid or 2 M ammonium hydroxide to a pH of 10.0, and dilute with water to 1000 mL.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. [NOTE—Re-equilibration time may be adjusted, if necessary.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.01	95	5
28	74	26
32	60	40
33	100	0
35	100	0

System suitability solution: 1.5 mg/mL of USP Nicotine Bitartrate Dihydrate RS and 6 µg/mL of USP Nicotine Related Compound G RS in water

Standard solution: 1.8 mg/mL of USP Nicotine Bitartrate Dihydrate RS in water

Sample solution: Nominally 0.6 mg/mL of nicotine prepared as follows. Transfer an amount of Nicotine Polacrilex equivalent to 30 mg of nicotine to a glass-stoppered tube. Add 10.0 mL of 1 M ammonium hydroxide, shake vigorously for 10 min, and then centrifuge. Transfer 5.0 mL of the clear solution to a 25-mL volumetric flask, add 5 mL of 1 M acetic acid, and dilute with water to volume. Retain the residue from centrifugation for use in *Identification B.*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between nicotine and nicotine related compound G, *System suitability solution*

Tailing factor: NMT 2.0 for nicotine, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nicotine ($C_{10}H_{14}N_2$) in the portion of Nicotine Polacrilex taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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r_U = peak response of nicotine from the *Sample solution*
r_S = peak response of nicotine from the *Standard solution*
C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)
C_U = nominal concentration of nicotine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of nicotine, 162.23
M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

Acceptance criteria:
Glycerin free: 95.0%–115.0% on the dried basis
Glycerin containing: 95.0%–115.0% on the anhydrous basis

PERFORMANCE TESTS

• **NICOTINE RELEASE**
Solution A: 9 mg/mL of sodium chloride in water
Sample stock solution: Transfer an amount of Nicotine Polacrilex equivalent to 4 mg of nicotine to a glass-stoppered tube, add 10.0 mL of *Solution A* that has been warmed to 37°, and shake by mechanical means for 10 min. Immediately pass the liquid through a dry filter paper, discarding the first milliliter of the filtrate.
Sample solution: Transfer 1.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelengths: 236, 259, and 282 nm
Blank: 1.0 mL of *Solution A* diluted with 0.1 N hydrochloric acid to 25 mL
Analysis
Samples: *Sample solution* and *Blank*
Calculate the percentage of nicotine ($C_{10}H_{14}N_2$) released:

$$\text{Result} = (A_{259} - 0.5A_{236} - 0.5A_{282}) \times (V/E) \times (F/W) \times (1/P) \times 100$$

A_{259} = absorbance of the *Sample solution*, corrected for the *Blank* absorbance, at a wavelength of 259 nm
A_{236} = absorbance of the *Sample solution*, corrected for the *Blank* absorbance, at a wavelength of 236 nm
A_{282} = absorbance of the *Sample solution*, corrected for the *Blank* absorbance, at a wavelength of 282 nm
V = dilution volume, 250 mL
E = specific absorbance of nicotine at a wavelength of 259 nm, 323 mL g^{-1} cm^{-1}
F = unit conversion factor, 1000 mg/g
W = weight of Nicotine Polacrilex (mg)
P = percentage of nicotine in Nicotine Polacrilex determined in the *Assay*

Acceptance criteria: NLT 70% in 10 min

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1.5 mg/mL of USP Nicotine Bitartrate Dihydrate RS and 6 µg/mL each of USP Nicotine Related Compound A RS, USP Nicotine Related Compound B RS, USP Nicotine Related Compound C RS, USP Nicotine Related Compound D RS, USP Nicotine Related Compound E RS, USP Nicotine Related Compound F RS, and USP Nicotine Related Compound G RS in water. [NOTE—The concentration of each related compound is in terms of the free base.]
Standard solution: 1.8 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water
Sensitivity solution: 0.9 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water from the *Standard solution*
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.5 between nicotine and nicotine related compound G, *System suitability solution*
Tailing factor: NMT 2.0 for nicotine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Nicotine Polacrilex taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of nicotine from the *Standard solution*
C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)
C_U = nominal concentration of nicotine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of nicotine, 162.23
M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nicotine related compound E	0.3	0.3
Nicotine related compound C	0.55	0.3
Nicotine related compound F	0.7	0.3
Nicotine related compound A	0.8	0.3
Nicotine related compound D	0.86	0.3
Nicotine related compound G	0.9	0.3
Nicotine	1.00	—
Nicotine related compound B	1.6	0.3
Any other unspecified impurity	—	0.10
Total impurities	—	0.8

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I* (for glycerin-containing Nicotine Polacrilex)
Sample solution: Transfer about 1.0 g of Nicotine Polacrilex to a 50-mL glass-stoppered test tube, and add 20.0 mL of methanol. Shake for 30 min, and allow to stand for 30 min. Use a 10-mL portion of the methanol layer for the titration.

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Acceptance criteria: NMT 5.0%
- **Loss on Drying** ⟨731⟩ (for glycerin-free Nicotine Polacrilex)

Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers, protected from light.
- **Labeling:** Label to indicate if the article contains glycerin.
- **USP Reference Standards** ⟨11⟩

USP Nicotine Bitartrate Dihydrate RS
USP Nicotine Related Compound A RS
Anatabine;
1,2,3,6-Tetrahydro-2,3′-bipyridine.
$C_{10}H_{12}N_2$ 160.22
USP Nicotine Related Compound B RS
3-(1-Methyl-1*H*-pyrrol-2-yl)pyridine; Also known as Nicotyrine.
$C_{10}H_{10}N_2$ 158.20
USP Nicotine Related Compound C RS
(*S*)-1-Methyl-5-(pyridin-3-yl)pyrrolidin-2-one; Also known as Cotinine.
$C_{10}H_{12}N_2O$ 176.22
USP Nicotine Related Compound D RS
3-(4,5-Dihydro-3*H*-pyrrol-2-yl)pyridine fumarate; Also known as Myosamine.
$C_9H_{10}N_2 \cdot C_4H_4O_4$ 262.26
USP Nicotine Related Compound E RS
(1*RS*,2*S*)-1-Methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide oxalate; Also known as Nicotine *N*-oxide.
$C_{10}H_{14}N_2O \cdot C_2H_2O_4$ 268.27
USP Nicotine Related Compound F RS
3-(Pyrrolidin-2-yl)pyridine; Also known as Nornicotine.
$C_9H_{12}N_2$ 148.20
USP Nicotine Related Compound G RS
(*S*)-3-(Piperidin-2-yl)pyridine; Also known as Anabasine.
$C_{10}H_{14}N_2$ 162.23
USP Polacrilex Resin RS

Nicotine Polacrilex Gum

» Nicotine Polacrilex Gum contains an amount of Nicotine Polacrilex $[(C_4H_6O_2)_X(C_{10}H_{10})_Y](C_{10}H_{14}N_2)$ equivalent to not less than 90 percent and not more than 120 percent of the labeled amount of nicotine ($C_{10}H_{14}N_2$).

USP Reference standards ⟨11⟩—
USP Nicotine Bitartrate Dihydrate RS
USP Polacrilex Resin RS

Identification—
A: Developing solvent—Prepare a mixture of chloroform, acetone, and diethylamine (40:5:5).
Test solution—Cut several pieces of Gum into small pieces with scissors, and weigh. Transfer a portion of the Gum, equivalent to about 4 mg of nicotine, to a centrifuge tube. Add 5 mL of chloroform, sonicate for about 30 minutes to dissolve the nicotine, and centrifuge for about 10 minutes. Cool to about 15°, and add two 3-mL portions of 0.5 N hydrochloric acid with gentle mixing, and release excess pressure if necessary. Mix the contents of the tube by shaking, and centrifuge the mixture for about 10 minutes. Transfer 5 mL of the upper aqueous layer to a separatory funnel, and adjust with 0.5 N sodium hydroxide solution to a pH greater than 10.0. Add 3 mL of chloroform, and shake gently. Use the chloroform layer as the *Test solution.*
Standard solution—Transfer 10 mg of USP Nicotine Bitartrate Dihydrate RS to a separatory funnel, add 10 mL of water, and mix to dissolve. Adjust with 0.5 N sodium hydroxide to a pH greater than 10.0. Add 3 mL of chloroform, with gentle shaking, and use the chloroform layer as the *Standard solution.*
Procedure—Separately apply 10 µL each of the *Test solution* and the *Standard solution* about 1.5 cm from the lower edge of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩). Air-dry, place the plate in a chromatographic tank that has been saturated with *Developing solvent,* and develop the chromatogram until the solvent front has moved about 7 cm. Remove the plate from the chamber, and allow to air-dry. Examine the plate under short-wavelength UV light: the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution* (*presence of nicotine*).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—Use USP Polacrilex Resin RS and a test specimen prepared as follows. Cut a piece of Gum into small pieces with scissors. Place the pieces in a 50-mL centrifuge tube, add about 20 mL of *n*-hexane, and place in an ultrasonic bath for about 30 minutes. Centrifuge at about 2500 rpm for about 5 minutes. Decant the hexane phase, and add 10 mL of 2 N hydrochloric acid. Shake the tube carefully, and open the stopper slightly to relieve any excess pressure. Add 10 mL of alcohol, and shake the tube carefully with the stopper slightly open. Centrifuge again as described above, and decant the liquid, taking care to avoid contamination of the precipitate with the gum material. Add 1 mL to 3 mL of water, mix gently to resuspend the precipitate, and filter. Wash the residue on the filter with water and then with alcohol. Dry the filter and residue at about 105° for 1 hour (*presence of polacrilex*).
Uniformity of dosage units ⟨905⟩: meets the requirements.

Assay—
Acetate buffer—Prepare a mixture containing 13.6 g of sodium acetate and 57.2 mL of glacial acetic acid in 1000 mL of water.
Solvent—Prepare a mixture of water, acetonitrile, 0.25 M sodium 1-decanesulfonate, and *Acetate buffer* (785:150:40:25).
Mobile phase—Prepare a mixture containing water, acetonitrile, *Acetate buffer,* and 0.25 M sodium 1-decanesulfonate (685:200:75:40).
Standard preparation—Dissolve an accurately weighed quantity of USP Nicotine Bitartrate Dihydrate RS in *Solvent* to obtain a *Standard stock solution* having a known concentration of about 1.25 mg per mL. Dilute a volume of this solution quantitatively with *Solvent* to obtain a *Standard preparation* having a known concentration of about 125 µg per mL (40 µg of nicotine per mL).
Assay preparation—Place one piece of Gum, accurately weighed, in a stoppered flask, add about 50 mL of *n*-hexane, and transfer 50.0 mL of *Solvent.* Add a stirring bar, insert the stopper in the flask, and stir vigorously for about 30 minutes or until the test specimen has been dispersed. Remove from the stirring mechanism, and allow to stand for about 30 minutes or until the phases have separated. Remove an aliquot of the lower layer, taking care not to remove a large quantity of the insoluble excipients, and filter, discarding the first few mL of the filtrate. Use the clear filtrate as the *Assay preparation.*

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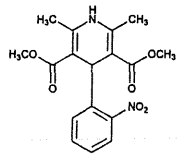
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm stainless steel column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, record the chromatograms, and measure the peak responses as directed for *Procedure:* the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— [NOTE—Perform the following procedure on 10 individual pieces of Gum, and use the average of the calculated values as the assay value. Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of nicotine ($C_{10}H_{14}N_2$) in the Gum taken by the formula:

$$50C(162.23/462.41)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard preparation*, 162.23 and 462.41 are the molecular weights of nicotine and anhydrous nicotine bitartrate, respectively, and r_U and r_S are peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nifedipine



$C_{17}H_{18}N_2O_6$ 346.33

3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester;

Dimethyl 1,4-dihydro-2,6-dimethyl-4-(*o*-nitrophenyl)-3,5-pyridinedicarboxylate [21829-25-4].

DEFINITION

Nifedipine contains NLT 98.0% and NMT 102.0% of nifedipine ($C_{17}H_{18}N_2O_6$), calculated on the dried basis.

[NOTE—Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenylpyridine derivative. Exposure to UV light leads to the formation of a nitrophenylpyridine derivative. Perform the *Assay* and other tests in the dark or under golden fluorescent or other low-actinic light. Use low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)**:** Do not dry samples.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect the *Standard solution* and the *Sample solution* from actinic light. Conduct the *Assay* promptly after preparation of the *Standard solution* and the *Sample solution*.

Mobile phase: Acetonitrile, methanol, and water (25:25:50)

Standard stock solution: 1 mg/mL of USP Nifedipine RS in methanol

Standard solution: 0.1 mg/mL in *Mobile phase* from *Standard stock solution*

Sample solution: 0.1 mg/mL of Nifedipine prepared as follows. Transfer about 25 mg of Nifedipine to a 250-mL flask, dissolve in 25 mL of methanol, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nifedipine ($C_{17}H_{18}N_2O_6$) in the portion of Nifedipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)

C_U = concentration of Nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%, an ignition temperature of 600° being used
- **ORGANIC IMPURITIES**

Protect *Standard stock solution B* and the *Sample solution* from actinic light. Conduct this test promptly after preparation of *Standard stock solution B* and the *Sample solution*.

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution A: 1 mg/mL of USP Nifedipine RS in methanol

Standard solution A: 0.3 mg/mL of USP Nifedipine RS in *Mobile phase* from *Standard stock solution A*

Standard stock solution B: 1 mg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in methanol

Standard solution B: 0.6 µg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in *Mobile phase* from *Standard stock solution B*

Standard stock solution C: 1 mg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS in methanol

Standard solution C: 0.6 µg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS from *Standard stock solution C*, diluted with *Mobile phase*

Standard solution D: *Mobile phase*, *Standard solution B*, and *Standard solution C* (1:1:1)

System suitability solution: *Standard solution A*, *Standard solution B*, and *Standard solution C* (1:1:1)

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System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog; NLT 1.0 between the nifedipine nitrosophenylpyridine analog and nifedipine
Relative standard deviation: NMT 10% for both nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog
Analysis
Samples: *Sample solution* and *Standard solution D*
Calculate the percentage of nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog in the portion of Nifedipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nifedipine nitrophenylpyridine analog or nifedipine nitrosophenylpyridine analog from the *Sample solution*
r_S = peak response of nifedipine nitrophenylpyridine analog or nifedipine nitrosophenylpyridine analog from *Standard solution D*
C_S = concentration of USP Nifedipine Nitrophenylpyridine Analog RS or USP Nifedipine Nitrosophenylpyridine Analog RS in *Standard solution D* (mg/mL)
C_U = concentration of Nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nifedipine nitrophenylpyridine analog[a]	0.8	0.2
Nifedipine nitrosophenylpyridine analog[b]	0.9	0.2
Nifedipine	1.0	—

[a] Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.
[b] Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

• **Limit of Chloride and Sulfate**
Standard solution A: Add 5.0 mL of water to 10 mL of 8.2 µg/mL of sodium chloride (corresponding to 5 µg/mL of chloride).
Standard solution B: Equivalent to 10 µg/mL of sulfate from potassium sulfate in water
Sample solution: To 5.0 g of Nifedipine add 4.0 mL of 6 N acetic acid and 46 mL of water. Bring carefully to a boil on a hot plate. Cool, pass through filter paper that is free of chloride and sulfate, and use the filtrate.
Chloride: Pipet 2.5 mL of the *Sample solution* into a 50-mL color-comparison tube, and add 12.5 mL of water. Into a matched color-comparison tube, pipet 10 mL of *Standard solution A*. To each tube add 0.15 mL of 0.3 M nitric acid and 0.3 mL of silver nitrate TS, and mix.
Acceptance criteria: NMT 0.02%; the opalescence exhibited by the *Sample solution* does not exceed that of *Standard solution A*.
Sulfate: Pipet into each of two 50-mL matched color-comparison tubes 1.5 mL of *Standard solution B*. To each tube add, successively and with continuous shaking, 0.75 mL of alcohol, 0.5 mL of a 6.1% aqueous solution of barium chloride, and 0.25 mL of 6 N acetic acid. Shake for an additional 30 s. Pipet into one tube, designated the standard tube, 15 mL of *Standard solution B*. Pipet into the other tube, designated the sample tube, 3 mL of *Sample solution* and 12 mL of water.
Acceptance criteria: NMT 0.05%; the turbidity exhibited by the *Sample solution* in the sample tube does not exceed that of *Standard solution B* in the standard tube.
• **Perchloric Acid Titration**
Sample solution: Dissolve 4 g of Nifedipine in 160 mL of glacial acetic acid in a 250-mL conical flask with the aid of an ultrasonic bath.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint: Visual
Analysis: Add 3 drops of *p*-naphtholbenzein TS to the *Sample solution*, and titrate with *Titrant* to a green endpoint.
Acceptance criteria: NMT 0.12 mL of 0.1 N perchloric acid is consumed for each g of Nifedipine.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 105° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Nifedipine RS
USP Nifedipine Nitrophenylpyridine Analog RS
Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.
$C_{17}H_{16}N_2O_6$ 344.33
USP Nifedipine Nitrosophenylpyridine Analog RS
Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.
$C_{17}H_{16}N_2O_5$ 328.33

Nifedipine Capsules

DEFINITION
Nifedipine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$).
Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenylpyridine derivative. Exposure to UV light leads to the formation of a nitrophenylpyridine derivative. Perform assays and tests in the dark or under golden fluorescent or other low-actinic light. Use low-actinic glassware.

IDENTIFICATION

Change to read:

• **A.** ▲The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

Change to read:

• **B.** The retention time of the ▲major peak of the▲ (USP 1-May-2019) *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

Change to read:

- **PROCEDURE:** Protect ▲all solutions containing nifedipine▲ (USP 1-May-2019) from actinic light. Conduct the *Assay* promptly after preparation of the *Standard solution* and the *Sample solution.*

Mobile phase: Acetonitrile, methanol, and water (25:25:50)

Standard stock solution: 1 mg/mL of USP Nifedipine RS in methanol

Standard solution: 0.1 mg/mL of USP Nifedipine RS in *Mobile phase* from *Standard stock solution*

▲Sample stock solution: Nominally 1 mg/mL of nifedipine prepared as follows. Transfer the contents of NLT 5 Capsules to a suitable volumetric flask. Dilute with methanol to volume.▲ (USP 1-May-2019)

Sample solution: ▲Nominally 0.1 mg/mL of nifedipine in *Mobile phase* from *Sample stock solution.* Pass through a suitable filter of 0.45-µm pore size.▲ (USP 1-May-2019)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 265 nm. ▲For *Identification A,* use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
Columns
Guard: Packing L1
Analytical: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
▲Run time: NLT 1.5 times the retention time of nifedipine▲ (USP 1-May-2019)
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 4000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nifedipine from the *Sample solution*
r_S = peak response of nifedipine from the *Standard solution*
C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Simulated gastric fluid TS (without pepsin); 900 mL
Apparatus 2: 50 rpm
Time: 20 min
Standard solution: Dissolve a quantity of USP Nifedipine RS in an amount of methanol not exceeding 2% of the final volume, and dilute with *Medium* to obtain a solution of a known suitable concentration.
Sample solution: Pass a portion of solution under test through a suitable filter, and dilute as needed with *Medium,* in comparison with the *Standard solution.* Filters must be checked for absorptive loss of nifedipine.
Instrumental conditions
Mode: UV
Analytical wavelength: 340 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved by using UV absorbances at the specified wavelength.
Tolerances: NLT 80% (*Q*) of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) is dissolved.

Change to read:

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: ▲Meet the requirements▲ (USP 1-May-2019)

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES:** Protect ▲all solutions containing nifedipine▲ (USP 1-May-2019) from actinic light. Conduct this test promptly after preparation of the *Standard solution* and the *Sample solution.*

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*
Standard stock solution A: 1 mg/mL of USP Nifedipine RS in methanol
Standard solution A: 0.3 mg/mL of USP Nifedipine RS from *Standard stock solution A* in *Mobile phase*
Standard stock solution B: 1 mg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in methanol
Standard solution B: 6 µg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in *Mobile phase* from *Standard stock solution B*
Standard stock solution C: 1 mg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS in methanol
Standard solution C: 1.5 µg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS in *Mobile phase* from *Standard stock solution C*
Standard solution D: Mixture of *Standard solution B, Standard solution C,* and *Mobile phase* (1:1:1)
System suitability solution: Mixture of *Standard solution A, Standard solution B,* and *Standard solution C* (1:1:1)
▲▲ (USP 1-May-2019)
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog; NLT 1.0 between nifedipine nitrosophenylpyridine analog and nifedipine
Relative standard deviation: NMT 10% for each nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog
Analysis
Samples: *Sample solution* and *Standard solution D*
Calculate the percentage of nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nifedipine nitrophenylpyridine analog or nifedipine nitrosophenylpyridine analog from the *Sample solution*

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r_S = peak response of nifedipine nitrophenylpyridine analog or nifedipine nitrosophenylpyridine analog from *Standard solution D*
C_S = concentration of the appropriate USP Nifedipine Nitrophenylpyridine Analog RS or USP Nifedipine Nitrosophenylpyridine Analog RS in *Standard solution D* (mg/mL)
C_U = nominal concentration of nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nifedipine ▲nitrophenylpyridine▲ (USP 1-May-2019) analog	0.8	2.0
Nifedipine ▲nitrosophenylpyridine▲ (USP 1-May-2019) analog	0.9	0.5
Nifedipine	1.0	—

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at ▲controlled room temperature.▲ (USP 1-May-2019)
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nifedipine RS
  USP Nifedipine Nitrophenylpyridine Analog RS
  Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.
  $C_{17}H_{16}N_2O_6$ 344.33
  USP Nifedipine Nitrosophenylpyridine Analog RS
  Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.
  $C_{17}H_{16}N_2O_5$ 328.33

Nifedipine Extended-Release Tablets

DEFINITION

Nifedipine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$). [NOTE—Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenylpyridine derivative. Exposure to UV light leads to the formation of a nitrophenylpyridine derivative. Perform assays and tests in the dark or under golden fluorescent or other low-actinic light. Use low-actinic glassware.]

IDENTIFICATION

- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
  Standard stock solution and **Sample stock solution:** Prepare as directed in the *Assay*.
  Standard solution: 0.02 mg/mL of USP Nifedipine RS in *Mobile phase* from the *Standard stock solution*
  Sample solution: Nominally 0.02 mg/mL of nifedipine in *Mobile phase* from the *Sample stock solution*

ASSAY

- **PROCEDURE**
  [NOTE—Conduct the *Assay* promptly after preparation of the *Standard solution* and the *Sample solution*.]
  Mobile phase: Acetonitrile, methanol, and water (25:25:50)
  Standard stock solution: 1 mg/mL of USP Nifedipine RS in methanol
  Standard solution: 0.1 mg/mL of USP Nifedipine RS from the *Standard stock solution* in *Mobile phase*
  Sample stock solution: Dissolve an amount equivalent to 420 mg of nifedipine from powdered Tablets in 130 mL of water in a 250-mL volumetric flask; or transfer the intact Tablets to a 400-mL, high-speed blender cup containing 130 mL of water. Homogenize until a uniform suspension is achieved (about 2 min), and transfer the suspension with the aid of a mixture of acetonitrile and methanol (1:1) to a 250-mL volumetric flask. Dilute with a mixture of acetonitrile and methanol (1:1) to volume, and stir for 30 min. Centrifuge the resulting suspension to obtain a clear supernatant.
  Sample solution: Nominally 0.1 mg/mL of nifedipine prepared as follows. Transfer 3.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and filter. [NOTE—Reserve a portion of this solution for use as the *Sample solution* in the test for *Organic Impurities*.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 265 nm
  Columns
  Guard: 2.1-mm × 3-cm; packing L1
  Analytical: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 25 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 4000 theoretical plates
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩
  Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

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Medium: Water; 50 mL
Apparatus 7: (See *Drug Release* ⟨724⟩.) 15–30 cycles/min. Do not use the reciprocating disk; use a 25-cm Plexiglas rod, the perimeter of the Tablets being affixed to the rod with a water-insoluble glue. The solution containers are 25-mm test tubes, 150–200 mm in length, and the water bath is maintained at 37 ± 0.5°. At the end of each specified test interval, the systems are transferred to the next row of new test tubes containing 50 mL of fresh *Medium*.
Times: 4, 8, 12, 16, 20, and 24 h
Diluent: Methanol and water (1:1)
Standard solution: Transfer 50 mg of USP Nifedipine RS to a 100-mL volumetric flask. Dissolve in 50 mL of methanol, and dilute with water to volume. Quantitatively dilute this solution with *Diluent* to obtain solutions having suitable known concentrations.
Sample solution: Use portions of the solution under test, passed through a suitable filter of 0.4-µm pore size, suitably diluted with methanol, and stepwise if necessary, with *Diluent* to obtain a final mixture consisting of equal parts of methanol and water.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 338 nm
Cell: 0.5 cm
Analysis: Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in the *Sample solution* at each 4-h interval from UV absorbances. [NOTE—For the 4-h time period, determine the absorbance at 456 nm, and use this determination to correct for excipient interference.]
Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved[a] (%)
4	5–17
8	—
12	43–80
16	—
20	—
24	NLT 80

[a] The amount dissolved is expressed in terms of the labeled Tablet strength rather than in terms of the labeled total contents.

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), released at the times specified, conform to *Dissolution* ⟨711⟩,*Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Solution A: Dissolve 330.9 g of dibasic sodium phosphate and 38 g of citric acid in water in a 1-L volumetric flask. Add 10 mL of phosphoric acid, and dilute with water to volume.
Medium: Mix 125.0 mL of *Solution A* and 1 L of 10% sodium lauryl sulfate solution, and dilute to 10 L. Adjust, if necessary, to a pH of 6.8; 900 mL.
Apparatus 2: 50 rpm, with sinkers (see *Dissolution* ⟨711⟩, *Figure 2a*)
Times: 3, 6, and 12 h
Mobile phase: Acetonitrile and water (7:3)
Standard stock solution: 1.11 mg/mL of USP Nifedipine RS in methanol
Standard solution: 0.1 mg/mL of USP Nifedipine RS from the *Standard stock solution* in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 350 nm
Column: 4.0-mm × 125-mm; 3-µm packing L1
Temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
3	10–30
6	40–65
12	NLT 80

The percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), ▲ (RB 1-Aug-2017) dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
For Tablets labeled to contain 30 mg of nifedipine:
Phase 1
Medium: 0.05 M phosphate buffer, pH 7.5; 900 mL
Apparatus 2: 100 rpm
Time: 1 h
Standard solution: 0.034 mg/mL of USP Nifedipine RS in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 238 nm
Cell: 0.5 cm
Analysis: [NOTE—After the run, take the Tablet out of the dissolution vessel, adapt a sinker to it, and transfer the Tablet with the sinker to the dissolution vessel containing the *Medium* for *Phase 2*.] Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in *Phase 1*, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the *Medium* as the blank.
For Tablets labeled to contain 30 mg of nifedipine:
Phase 2
Medium: 0.5% sodium lauryl sulfate in simulated gastric fluid without enzyme, pH 1.2; 900 mL
Apparatus 2: 100 rpm
Times: 1, 4, 8, and 12 h
Standard solution: 0.034 mg/mL of USP Nifedipine RS in *Medium*. [NOTE—If necessary, a volume of methanol,

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not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 238 nm
Analysis: Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in *Phase 2*, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using *Medium* as the blank.
Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved[a] (%)
1	NMT 30
4	30–55
8	NLT 60
12	NLT 80

[a] For each dosage unit, add the amount dissolved in phosphate buffer, pH 7.5 from *Phase 1* to the amount dissolved at each time point in *Phase 2*.

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), ▲ (RB 1-Aug-2017) dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

For Tablets labeled to contain 60 mg of nifedipine:
Phase 1
Medium: 0.05 M phosphate buffer, pH 7.5; 900 mL
Apparatus 2: 100 rpm
Time: 25 min
Standard solution: 0.067 mg/mL of USP Nifedipine RS in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 238 nm
Analysis: [NOTE—After the run, take the Tablet out of the dissolution vessel, adapt a sinker to it, and transfer the Tablet with the sinker to the dissolution vessel containing the *Medium* for *Phase 2*.] Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in *Phase 1*, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the *Medium* as the blank.

For Tablets labeled to contain 60 mg of nifedipine:
Phase 2
Medium: 0.5% sodium lauryl sulfate in simulated gastric fluid without enzyme, pH 1.2; 900 mL
Apparatus 2: 100 rpm
Times: 1, 4, 8, and 12 h
Standard solution: 0.067 mg/mL of USP Nifedipine RS in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 238 nm
Analysis: Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in *Phase 2*, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the *Medium* as the blank.
Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved[a] (%)
1	NMT 30
4	40–70
8	NLT 70
12	NLT 80

[a] For each dosage unit, add the amount dissolved in phosphate buffer, pH 7.5 from *Phase 1* to the amount dissolved at each time point in *Phase 2*.

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), released in vivo and dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.
Medium: 0.5% sodium lauryl sulfate in simulated gastric fluid without enzyme, pH 1.2; 900 mL
Apparatus 2: 100 rpm
Times: 1, 4, and 12 h
Standard solution: 0.067 mg/mL of USP Nifedipine RS for Tablets labeled to contain 60 mg, and 0.034 mg/mL of USP Nifedipine RS for Tablets labeled to contain 30 mg, in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: UV 238 nm
Cell: 1 cm
Analysis: Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the *Medium* as the blank.
Tolerances: See *Table 5* and *Table 6*.

Table 5

For Tablets Labeled to Contain 30 mg of Nifedipine	
Time (h)	Amount Dissolved (%)
1	12–35
4	44–67
12	NLT 80

Table 6

For Tablets Labeled to Contain 60 mg of Nifedipine	
Time (h)	Amount Dissolved (%)
1	10–30
4	40–63
12	NLT 80

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The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), released at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.

Medium: Water; 50 mL

Apparatus 7: (See *Drug Release* ⟨724⟩.) Use a 25-cm Plexiglas rod, the perimeter of the Tablets being affixed to the rod with a water-insoluble glue; 30 dips/min. The solution containers are 25-mm test tubes, 150–200 mm in length, and the water bath is maintained at 37 ± 0.5°.

Times: 4, 12, and 24 h

Diluent A: Methanol and acetonitrile (1:1)

Diluent B: *Diluent A* and water (1:1)

Standard stock solution: ▲0.50 mg/mL of USP Nifedipine RS prepared as follows. Transfer a suitable amount of USP Nifedipine RS to an appropriate volumetric flask. Dissolve in 50% of the flask volume of *Diluent A*. Dilute with water to volume.▲ (ERR 1-Oct-2018)

Standard solutions: 0.01, 0.05, and 0.20 mg/mL solutions, from the *Standard stock solution* in *Diluent B*, that are used at 4-, 12-, and 24-h samplings

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: ▲338 nm▲ (ERR 1-Oct-2018)

Cell: 0.5 cm

Analysis: [NOTE—For the 4-h time period, filter the solution under test, and determine the absorbance at 456 nm. Use this absorbance value to correct for excipient interference at the other time points.] Determine the amount of nifedipine released at each interval on portions of the *Sample solution* passed through a suitable filter of 0.45-µm pore size, suitably diluted, if necessary, with *Diluent A* and water to obtain a final mixture of water, methanol, and acetonitrile (2:1:1), in comparison with the appropriate *Standard solution*, using *Diluent B* as the blank.

Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved (%)
4	NMT 14
12	39–75
24	NLT 75

The cumulative percentages of the labeled amount of nifedipine,▲▲ (RB 1-Aug-2017) dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: Simulated gastric fluid without enzyme containing 0.5% of sodium lauryl sulfate, pH 1.2; 900 mL, deaerated

Apparatus 1: 100 rpm

Times: 1, 4, and 12 h

Standard stock solution: 0.33 mg/mL of USP Nifedipine RS in methanol

Standard solution: Quantitatively dilute the *Standard stock solution* with *Medium* to obtain a solution having a concentration of about 0.033 mg/mL.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 329 nm

Cell: 0.5 cm

Blank: *Medium*

Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved (%)
1	NMT 15
4	20▲▲ (RB 1-Aug-2017)–40
12	NLT 80

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Medium: Simulated gastric fluid without enzyme containing 0.5% sodium lauryl sulfate, pH 1.2; 900 mL

Apparatus 2: 100 rpm, with three-prong sinker

Times: 1, 4, and 12 h

Standard solution: (*L*/900) mg/mL of USP Nifedipine RS in *Medium*, where *L* is the label claim, in mg/Tablet, of nifedipine. A small amount of methanol, not exceeding 6%–7% of the final volume of the first dilution, can be used to solubilize nifedipine.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 238 nm

Cell: 1 mm, flow cell

Blank: *Medium*

Tolerances: See *Table 9*.

Table 9

Time (h)	Amount Dissolved (%)
1	NMT 15
4	25–50
12	NLT 80

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Acid stage medium: Simulated gastric fluid without enzyme containing 3% polysorbate 80, pH 1.2; 250 mL

Apparatus 3: 20 dpm, 20-mesh polypropylene screen on the bottom; 1 min drip time. The Tablet is automatically transferred by the apparatus to the next set of vessels for each time point.

Time: 1 h

Buffer stage medium: 0.01 M sodium phosphate buffer, pH 6.8, containing 3% polysorbate 80 (dissolve 8.3 g of monobasic sodium phosphate and 1 g of sodium hydroxide in 6 L of water, adjust with either diluted sodium hydroxide or phosphoric acid to a pH of 6.8 ± 0.05, and add 180 g of polysorbate 80); 250 mL

Times: 2, 8, 12, and 24 h

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Mobile phase: Acetonitrile, methanol, and water (35:35:30)
Standard stock solution: 1 mg/mL of USP Nifedipine RS in *Buffer stage medium*. An amount of methanol, about 40% of the final volume, can be used to dissolve nifedipine.
Standard solution: (*L*/1000) mg/mL in *Buffer stage medium*, from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 338 nm
Column: 4.6-mm × 25-cm; packing L1
Temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 4000 theoretical plates
Tailing factor: NMT 1.7
Relative standard deviation: NMT 2.0%
Analysis: Calculate the percentage of the labeled amount of nifedipine dissolved at each time point.
At 1 h:

$$D_1 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

At 2 h:

$$D = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$D_2 = D_1 + D$$

At 8 h:

$$D = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$D_8 = D_2 + D$$

At 12 h:

$$D = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$D_{12} = D_8 + D$$

At 24 h:

$$D = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$D_{24} = D_{12} + D$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 250 mL

Tolerances
Acid stage: NMT 5% of the labeled amount of nifedipine is dissolved in 1 h.
Buffer stage: See *Table 10*.

Table 10

Time (h)	Amount Dissolved (%)
1	NMT 5
2	0–10
8	25–60
12	45–85
24	NLT 80

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.
Medium: 0.03 M phosphate/citrate buffer, pH 6.8 with 1% sodium lauryl sulfate (to a solution of 4.1 g/L of dibasic sodium phosphate and 0.475 g/L of citric acid monohydrate in water, add 10 g/L of sodium lauryl sulfate. Adjust if necessary, with phosphoric acid to a pH of 6.8); 900 mL
Apparatus 2: 50 rpm, with a suitable sinker
Times: 3, 6, and 12 h
Standard stock solution: 0.33 mg/mL of USP Nifedipine RS in methanol
Standard solution: Prepare the corresponding USP Nifedipine RS solutions in *Medium* as directed in *Table 11*.

Table 11

Tablet Strength (mg)	Concentration (mg/mL)
30	0.033
60	0.066
90	0.099

Sample solution: Pass a portion of the solution under test at each time point through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 346 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solutions* and *Sample solution*
Calculate the concentration (C_i) of nifedipine ($C_{17}H_{18}N_2O_6$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution* at each time point
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

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$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of nifedipine in the *Sample solution* at the specified time point (*i*) (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (*i*) (mL)

Tolerances: See *Table 12*.

Table 12

Time Point (*i*)	Time (h)	Amount Dissolved (%)	
		Tablets Labeled to Contain 30 mg and 60 mg of Nifedipine	Tablets Labeled to Contain 90 mg of Nifedipine
1	3	15–40	10–35
2	6	43–73	40–65
3	12	NLT 80	NLT 80

The percentages of the labeled amount of Nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 10:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 10*.
Medium: pH 6.8 phosphate buffer with 0.5% sodium lauryl sulfate (transfer 442.1 g of dibasic sodium phosphate and 38 g of citric acid in a 1-L volumetric flask. Add water to dissolve, add 10 mL of phosphoric acid, and dilute with water to volume. Transfer 60 g of sodium lauryl sulfate to a suitable container. Add 150 mL of the phosphate solution above and 11,850 mL of water. Mix well and adjust with phosphoric acid or sodium hydroxide to a pH of 6.8); 900 mL
Apparatus 2: 50 rpm, with sinkers (see *Dissolution* ⟨711⟩, *Figure 2a*)
Times: 2, 8, and 16 h
Mobile phase: Methanol and water (60:40)
Standard solution: 0.06 mg/mL of USP Nifedipine RS prepared as follows. Transfer 12 mg of USP Nifedipine RS into a 200-mL volumetric flask. Add 20 mL of methanol, and dilute with *Medium* to volume. [NOTE—Sonication may be needed to aid dissolution.]
Sample solution: Withdraw a 10-mL aliquot at each time point. Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 380 nm
Column: 4.6-mm × 7.5-mm; 3.5-µm packing L60
Temperature: 45°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of nifedipine ($C_{17}H_{18}N_2O_6$) in the sample withdrawn from the vessel at each time point (*i*):

$$Result_i = (r_U/r_S) \times C_S$$

r_U = peak response of nifedipine in the *Sample solution* at each time point
r_S = peak response of nifedipine in the *Standard solution*
C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at each time point (*i*):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$

$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of nifedipine in the *Sample solution* at the specified time point (*i*) (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (*i*) (mL)

Tolerances: See *Table 13*.

Table 13

Time Point (*i*)	Time (h)	Amount Dissolved (%)	
		Tablets Labeled to Contain 30 and 60 mg of Nifedipine	Tablets Labeled to Contain 90 mg of Nifedipine
1	2	NMT 30	NMT 15
2	8	53–83	35–58
3	16	NLT 80	NLT 75

The percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲ (RB 1-Aug-2017)

▲**Test 11:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 11*.
Medium: 1.25% sodium lauryl sulfate in water (transfer 12.5 g of sodium lauryl sulfate to 1 L of water); 900 mL
Apparatus 2: 100 rpm
Times: 1, 2, 4 and 10 h
Standard solution: 0.033 mg/mL of USP Nifedipine RS for Tablets labeled to contain 30 mg prepared as follows. Transfer an appropriate amount of USP Nifedipine RS into a suitable volumetric flask. Add methanol to 1% volume of the flask, and dilute with *Medium* to volume. [NOTE—Sonication may be needed to aid the dissolution.]
Sample solution: A portion of the solution under test at the time points specified
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV

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Analytical wavelengths: 230–246 nm, from the difference between first derivative values at the wavelengths of maximum and minimum in the wavelength range from 230 to 246 nm
Cell: 1 mm
Blank: *Medium*
Analysis: Use an automatic dissolution system with an appropriate dissolution software. Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved, using portions of the *Sample solution*, in comparison with the *Standard solution*.
Tolerances: See *Table 14*.

Table 14

For Tablets Labeled to Contain 30 mg of Nifedipine	
Time (h)	Amount Dissolved (%)
1	NMT 25
2	15–40
4	35–70
10	NLT 85

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 12: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 12*.
Medium: pH 6.8 phosphate buffer with 1.25% sodium lauryl sulfate (transfer 6 g of *monobasic sodium phosphate* and 112 mL of 0.2 N sodium hydroxide in a 1-L volumetric flask containing 800 mL of water. Mix to dissolve, and dilute with water to volume. Adjust with phosphoric acid or sodium hydroxide to a pH of 6.8. Transfer 12.5 g of sodium lauryl sulfate in 1 L of the phosphate solution); 900 mL
Apparatus 2: 50 rpm
Times: 2, 6, 8 and 16 h
Standard solution: 0.067 mg/mL of USP Nifedipine RS for Tablets labeled to contain 60 mg prepared as follows. Transfer an appropriate amount of USP Nifedipine RS into a suitable volumetric flask. Add methanol to 1% volume of the flask, and dilute with *Medium* to volume. [NOTE—Sonication may be needed to aid the dissolution.]
Sample solution: A portion of the solution under test at the time points specified
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelengths: 230–246 nm, from the difference between first derivative values at the wavelengths of maximum and minimum in the wavelength range from 230 to 246 nm
Cell: 1 mm
Blank: *Medium*
Analysis: Use an automatic dissolution system with an appropriate dissolution software. Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved, using portions of the *Sample solution*, in comparison with the *Standard solution*.
Tolerances: See *Table 15*.

Table 15

For Tablets Labeled to Contain 60 mg of Nifedipine	
Time (h)	Amount Dissolved (%)
2	NMT 20
6	28–53
8	43–68
16	NLT 80

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Oct-2017)
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
[NOTE—Conduct this test promptly after preparation of the *Standard nifedipine solution* and the *Sample solution*.]
Mobile phase: Acetonitrile, methanol, and water (25:25:50)
Quantitative limit stock solution A: 1 mg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in methanol
Quantitative limit solution A: 6 µg/mL of USP Nifedipine Nitrophenylpyridine Analog RS from *Quantitative limit stock solution A* in *Mobile phase*
Quantitative limit stock solution B: 1 mg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS in methanol
Quantitative limit solution B: 1.5 µg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS from *Quantitative limit stock solution B* in *Mobile phase*
Standard nifedipine stock solution: 1 mg/mL of USP Nifedipine RS in methanol
Standard nifedipine solution: 0.3 mg/mL of USP Nifedipine RS from *Standard nifedipine stock solution* in *Mobile phase*
System suitability solution: *Quantitative limit solution A*, *Quantitative limit solution B*, and *Standard nifedipine solution* (1:1:1)
Standard solution: *Mobile phase*, *Quantitative limit solution A*, and *Quantitative limit solution B* (1:1:1)
[NOTE—Each mL of this solution contains about 2 µg of USP Nifedipine Nitrophenylpyridine Analog RS and 0.5 µg of USP Nifedipine Nitrosophenylpyridine Analog RS.]
Sample solution: Use a portion of the *Sample solution* prepared as directed in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Columns
Guard: 2.1-mm × 3-cm; packing L1
Analytical: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between the nitrophenylpyridine analog and nitrosophenylpyridine analog peaks; NLT 1.0 between the nitrosophenylpyridine analog and nifedipine peaks
Relative standard deviation: NMT 10% for each analog

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each analog in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

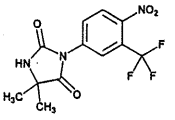
r_U = peak response of each analog from the *Sample solution*
r_S = peak response of each analog from the *Standard solution*
C_S = concentration of the appropriate analog USP Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of nifedipine in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 2.0% of nifedipine nitrophenylpyridine analog and NMT 0.5% of nifedipine nitrosophenylpyridine analog, both relative to the nifedipine content

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** The labeling indicates the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nifedipine RS
  USP Nifedipine Nitrophenylpyridine Analog RS
  Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.
  $C_{17}H_{16}N_2O_6$ 344.33
  USP Nifedipine Nitrosophenylpyridine Analog RS
  Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.
  $C_{17}H_{16}N_2O_5$ 328.33

Nilutamide



$C_{12}H_{10}F_3N_3O_4$ 317.22
2,4-Imidazolidinedione, 5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl]-;
5,5-Dimethyl-3-(α,α,α-trifluoro-4-nitro-*m*-tolyl)hydantoin;
5,5-Dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl]-imidazolidine-2,4-dione [63612-50-0].

DEFINITION

Nilutamide contains NLT 98.0% and NMT 102.0% of nilutamide ($C_{12}H_{10}F_3N_3O_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  [NOTE—Throughout the following procedures, protect the sample, the Reference Standard, and solutions containing them by conducting the procedures under subdued light or using low-actinic glassware.]
  Buffer: 2.0 g/L of monobasic potassium phosphate in water. Adjust with a solution of 1 N sodium hydroxide in water to a pH of 7.5.
  Mobile phase: Acetonitrile and *Buffer* (40:60)
  Diluent: Acetonitrile and water (35:65)
  Standard solution: 0.5 mg/mL of USP Nilutamide RS in *Diluent*
  Sample solution: 0.5 mg/mL of Nilutamide in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 267 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  Run time: NLT 5 times the retention time of nilutamide
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of nilutamide ($C_{12}H_{10}F_3N_3O_4$) in the portion of Nilutamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nilutamide from the *Sample solution*
r_S = peak response of nilutamide from the *Standard solution*
C_S = concentration of USP Nilutamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Nilutamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩
  Analysis: Use a platinum crucible.
  Acceptance criteria: NMT 0.1%
- **ORGANIC IMPURITIES**
  Solution A: 2.0 g/L of monobasic potassium phosphate in water. Adjust with a solution of 1 N sodium hydroxide in water to a pH of 7.5.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	55	45
8	55	45
30	30	70
31	55	45
45	55	45

Diluent: Acetonitrile and water (35:65)

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System suitability solution: 0.04 mg/mL each of USP Nilutamide RS and USP Flutamide Related Compound A RS in *Diluent*
Standard solution: 0.002 mg/mL of USP Nilutamide RS in *Diluent*
Sample solution: 1.0 mg/mL of Nilutamide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between nilutamide and flutamide related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Nilutamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of nilutamide from the *Standard solution*
C_S = concentration of USP Nilutamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Nilutamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
5-Imino nilutamide[a]	0.49	0.10
Flutamide related compound A	0.86	0.10
Nilutamide	1.0	—
3-Oxo nilutamide[b]	2.3	0.10
Nilutamide urea analog[c]	4.6	0.10
Any other individual unspecified impurity	—	0.10
Total impurities	—	0.5

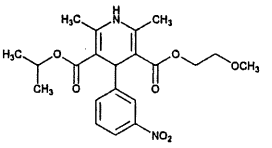
[a] 5-Imino-4,4-dimethyl-1-(4-nitro-3-(trifluoromethyl)phenyl)imidazolidin-2-one.
[b] 5,5-Dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)oxazolidine-2,4-dione.
[c] 1,3-Bis[4-nitro-3-(trifluoromethyl)phenyl]urea.

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Protect from light. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Flutamide Related Compound A RS
4-Nitro-3-(trifluoromethyl)aniline.
$C_7H_5F_3N_2O_2$ 206.12
USP Nilutamide RS

Nimodipine



$C_{21}H_{26}N_2O_7$ 418.44
3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-, 2-methoxyethyl 1-methylethyl ester;
Isopropyl 2-methoxyethyl 1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate [66085-59-4].

DEFINITION
Nimodipine contains NLT 98.0% and NMT 102.0% of nimodipine ($C_{21}H_{26}N_2O_7$), calculated on the dried basis.
[NOTE—Throughout the following procedures, protect samples, the Reference Standards, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, tetrahydrofuran, and water (20:20:60)
Standard solution: 0.3 mg/mL of USP Nimodipine RS prepared as follows. Transfer a quantity of USP Nimodipine RS to a suitable volumetric flask, dissolve in a volume of tetrahydrofuran equivalent to 10% of the total volume, and dilute with *Mobile phase* to volume.
Sample solution: 0.3 mg/mL of Nimodipine prepared as follows. Transfer a quantity of Nimodipine to a suitable volumetric flask, dissolve in a volume of tetrahydrofuran equivalent to 10% of the total volume, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of nimodipine ($C_{21}H_{26}N_2O_7$) in the portion of Nimodipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nimodipine from the *Sample solution*
r_S = peak response of nimodipine from the *Standard solution*
C_S = concentration of USP Nimodipine RS in the *Standard solution* (mg/mL)
C_U = concentration of Nimodipine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution A: 1.6 mg/mL of USP Nimodipine RS prepared as follows. Transfer a quantity of USP Nimodipine RS to a suitable volumetric flask, dissolve in a volume of tetrahydrofuran equivalent to 10% of the total volume, and dilute with *Mobile phase* to volume.

Standard stock solution B: 0.8 mg/mL each of USP Nimodipine RS and USP Nimodipine Related Compound A RS prepared as follows. Transfer quantities of USP Nimodipine RS and USP Nimodipine Related Compound A RS to a suitable volumetric flask, dissolve in a volume of tetrahydrofuran equivalent to 10% of the total volume, and dilute with *Mobile phase* to volume.

Standard solution A: 3.2 µg/mL of USP Nimodipine RS from *Standard stock solution A* in *Mobile phase*

Standard solution B: 1.6 µg/mL each of USP Nimodipine RS and USP Nimodipine Related Compound A RS from *Standard stock solution B* in *Mobile phase*

Sample solution: 1.6 mg/mL of Nimodipine prepared as follows. Dissolve 40 mg of Nimodipine in 2.5 mL of tetrahydrofuran, and dilute with *Mobile phase* to 25 mL.

System suitability

Sample: *Standard solution B*

[NOTE—The relative retention times for nimodipine related compound A and nimodipine are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between nimodipine related compound A and nimodipine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of nimodipine related compound A in the portion of Nimodipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nimodipine related compound A from the *Sample solution*
r_S = peak response of nimodipine related compound A from *Standard solution B*
C_S = concentration of USP Nimodipine Related Compound A RS in *Standard solution B* (µg/mL)
C_U = concentration of Nimodipine in the *Sample solution* (µg/mL)

Calculate the percentage of any other impurity in the portion of Nimodipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of nimodipine from *Standard solution A*
C_S = concentration of USP Nimodipine RS in *Standard solution A* (µg/mL)
C_U = concentration of Nimodipine in the *Sample solution* (µg/mL)

Acceptance criteria

Nimodipine related compound A: NMT 0.1%
Any unspecified impurity: NMT 0.2%
Total impurities: NMT 0.5%

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 50 mg/mL in acetone
Acceptance criteria: –0.10° to +0.10°, at 20°

- **LOSS ON DRYING** ⟨731⟩

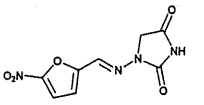
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Nimodipine RS
USP Nimodipine Related Compound A RS
2-Methoxyethyl-1-methylethyl-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate.
$C_{21}H_{24}N_2O_7$ 416.42

Nitrofurantoin



$C_8H_6N_4O_5$ (anhydrous) 238.16

$C_8H_6N_4O_5 \cdot H_2O$ 256.18

2,4-Imidazolidinedione, 1-[[(5-nitro-2-furanyl)methylene] amino]-;
1-[(5-nitrofurfurylidene)amino]hydantoin [67-20-9].
Monohydrate [17140-81-7].

DEFINITION

Nitrofurantoin is anhydrous or contains one molecule of water of hydration. It contains NLT 98.0% and NMT 102.0% of $C_8H_6N_4O_5$, calculated on the anhydrous basis.

[NOTE—Nitrofurantoin and solutions of it are discolored by alkali and by exposure to light and are decomposed upon contact with metals other than stainless steel and aluminum.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Sample: Previously dried at 140° for 30 min
Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

• **PROCEDURE**

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water. Add about 30 mL of 1.0 N sodium hydroxide sufficient to adjust to a pH of 7.0, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (12:88)

Internal standard solution: 1 mg/mL of acetanilide in water

Standard solution: 0.56 mg/mL of USP Nitrofurantoin RS prepared as follows. Dissolve 50 mg of USP Nitrofurantoin RS in 40.0 mL of dimethylformamide, and add 50.0 mL of *Internal standard solution.*

Sample solution: 0.56 mg/mL of Nitrofurantoin prepared as directed for *Standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

[NOTE—Adjust the operating parameters so that the retention time of the nitrofurantoin peak is about 8 min and the peak heights are about half full-scale.]

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Injection size: 5–10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between the acetanilide and nitrofurantoin peaks

Relative standard deviation: NMT 2.0% determined from the ratio of the peak responses

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nitrofurantoin ($C_8H_6N_4O_5$) in the portion of Nitrofurantoin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Sample solution*

R_S = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Standard solution*

C_S = concentration of USP Nitrofurantoin RS in the *Standard solution* (mg/mL)

C_U = concentration of Nitrofurantoin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **LIMIT OF NITROFURFURAL DIACETATE**

Standard solution: 100 µg/mL of USP Nitrofurfural Diacetate RS in dimethylformamide and acetone (1:10)

Sample solution: Dissolve 100 mg of Nitrofurantoin in 1 mL of dimethylformamide, and dilute with acetone to 10.0 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform and methanol (9:1)

Spray reagent: Dissolve 0.75 g of phenylhydrazine hydrochloride in 50 mL of water, and decolorize with activated charcoal. Add 25 mL of hydrochloric acid, and mix with water to produce 200 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Develop in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate, allow to air-dry for 5 min, and heat the plate at 105° for 5 min. While it is still warm, locate the spots by spraying the plate with *Spray reagent.*

Acceptance criteria: Any spot produced by the *Sample solution,* at an R_F value of 0.7, is not greater in size or intensity than that produced by the *Standard solution* at the same R_F value: NMT 1.0% of nitrofurfural diacetate is found.

• **LIMIT OF NITROFURAZONE**

Buffer: Prepare as directed in the *Assay.*

Mobile phase: Tetrahydrofuran and *Buffer* (10:90)

System suitability stock solution: 5.0 µg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in dimethylformamide

System suitability solution: 0.5 µg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in *Mobile phase* from *System suitability stock solution*

Standard stock solution: 5.0 µg/mL of USP Nitrofurazone RS in dimethylformamide

Standard solution: 0.5 µg/mL of USP Nitrofurazone RS in water from *Standard stock solution*

Sample solution: Dissolve 100 mg of Nitrofurantoin in 2.0 mL of dimethylformamide, and add 20.0 mL of water. Mix, and allow to stand for 15 min to allow a precipitate to form. Pass a portion of the solution through a suitable nylon filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

[NOTE—Adjust the operating parameters so that the retention time of the nitrofurazone peak is about 10.5 min and its height is about 0.1 half full-scale.]

Mode: LC

Detector: UV 375 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.6 mL/min

Injection size: 60–100 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between the nitrofurazone and nitrofurantoin peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% determined from the peak height, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The height of any peak appearing in the *Sample solution* at a retention time corresponding to that of the main peak from the *Standard solution* is NMT the height of the main peak from the *Standard solution* (NMT 0.01%).

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method III* ⟨921⟩

Analysis: Dry a sample at 140° for 30 min.

Acceptance criteria: For the anhydrous form, it loses NMT 1.0% of its weight; for the hydrous form, it loses 6.5%–7.5% of its weight.

• **SPECIFIC SURFACE AREA** ⟨846⟩ (where it is labeled as being in the form of macrocrystals)

Sample: Outgas a portion of the powder to be placed under test at 150° for 10 min at ambient pressure with nitrogen.

Acceptance criteria: 0.045–0.20 m^2/g

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

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• **LABELING:** Label it to indicate whether it is anhydrous or hydrous. Nitrofurantoin in the form of macrocrystals is so labeled. The labeling states the specific surface area and which method, specified under *Specific Surface Area* ⟨846⟩, is used.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Nitrofurantoin RS
USP Nitrofurazone RS
USP Nitrofurfural Diacetate RS
$C_9H_9NO_7$ 243.17

Nitrofurantoin Capsules

DEFINITION
Nitrofurantoin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$).

IDENTIFICATION
• **A. INFRARED ABSORPTION**
Sample: Add 10 mL of 6 N acetic acid to a quantity of the contents of Capsules equivalent to 100 mg of nitrofurantoin. Boil the solution for a few min, and filter while hot. Cool to room temperature, collect the precipitate of nitrofurantoin, and dry at 105° for 1 h.
Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the precipitate so obtained exhibits maxima only at the same wavelength as that of a similar solution of USP Nitrofurantoin RS.

• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water. Add a volume of 1.0 N sodium hydroxide (about 30 mL) sufficient to adjust to a pH of 7.0, and dilute with water to 1 L.
Mobile phase: Acetonitrile and *Solution A* (3:22)
Internal standard solution: 1 mg/mL of acetanilide in water
Standard solution: Dissolve 50 mg of USP Nitrofurantoin RS in 40.0 mL of dimethylformamide, and add 50.0 mL of *Internal standard solution*.
Sample solution: Transfer, as completely as possible, the contents of 20 Capsules to a 500-mL flask. Place the emptied Capsules in a beaker, add 25 mL of dimethylformamide, and agitate for 1 min. Decant into the flask containing the Capsule contents. Rinse the emptied Capsules with another two 25-mL portions of dimethylformamide, and decant into the flask. Add sufficient dimethylformamide to bring the volume to about 250 mL. Insert the stopper in the flask, and shake by mechanical means for 15 min. Dilute with dimethylformamide to volume, and mix. If necessary, the sample may be homogenized using a disperser. Pass through a medium-porosity, sintered-glass filter into a suitable flask. Transfer an aliquot, equivalent to 50 mg of nitrofurantoin, to a flask. Add an accurately measured volume of dimethylformamide to bring the volume in the flask to 40.0 mL. To the flask add 50.0 mL of *Internal standard solution*, mix, and cool to room temperature. Pass a portion of the solution through a nylon filter of 0.45-µm pore size, discarding the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Injection volume: 5–10 µL
System suitability
Sample: *Standard solution*
[NOTE—Adjust the operating parameters so that the retention time of the nitrofurantoin peak is about 8 min, and the peak heights are about half full-scale.]
Suitability requirements
Resolution: NLT 3.0 between acetanilide and nitrofurantoin
Relative standard deviation: NMT 2.0%, determined from peak response ratios of replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) in the portion of the powder included in the sample aliquot:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio from the *Sample solution*
R_S = peak response ratio from the *Standard solution*
C_S = concentration of USP Nitrofurantoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1 (where it is labeled as containing nitrofurantoin macrocrystals)
Medium: pH 7.2 (± 0.05) phosphate buffer; 900 mL
Apparatus 1: 100 rpm
Times: 1, 3, and 8 h
Standard solution: USP Nitrofurantoin RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Blank: *Medium*
Instrumental conditions
Mode: UV
Analytical wavelength: 375 nm
Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved
1	20%–60%
3	NLT 45%
8	NLT 60%

The percentage of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the 1-h point conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2* and the percentages dissolved at the 3- and 8-h points conform to the criteria for the final test time in *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Acid medium: 0.01 N hydrochloric acid for 1 h; 900 mL
pH 7.5 buffer medium: Prepare a pH 7.5 buffer concentrate by dissolving 62.2 g of potassium hydroxide

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and 129.3 g of monobasic potassium phosphate in water, dilute with water to 1 L, and mix. After 1 h, change the *Acid medium* to *pH 7.5 buffer medium* by adding 50 mL of pH 7.5 buffer concentrate, and run for an additional 6 h.

Apparatus 2: 100 rpm, with sinkers made of Teflon-coated steel wire prepared by forming a coil approximately 22 mm long from a 13-cm length of 20-gauge wire (see *Figure 1*)

Times: 1, 3, and 7 h

Acid-stage standard solution: 0.025 mg/mL of USP Nitrofurantoin RS in *Acid medium*

Buffer-stage standard solution: 0.075 mg/mL of USP Nitrofurantoin RS in *pH 7.5 buffer medium*

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Analysis: Calculate the percentages of the labeled amount (Q_i) of nitrofurantoin ($C_8H_6N_4O_5$) dissolved from UV absorbances at the isosbestic wavelength at about 375 nm on filtered portions of each solution under test, suitably diluted, if necessary, with *Acid medium* or *pH 7.5 buffer medium* when appropriate, in comparison with the appropriate *Standard solution*.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (Individual)	Amount Dissolved (Mean)
1	2%–16%	5%–13%
3	27%–69%	39%–56%
7	NLT 68%	NLT 81%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to *Table 3*.

Table 3

Level	Number Tested	Criteria
L_1	12	The mean percentage of dissolved label claim lies within the range for the means at each interval and is NLT the stated amount at the final test time. All individual values lie within the ranges for the individuals at each interval and are NLT the stated amount at the final test time.

Table 3 *(continued)*

Level	Number Tested	Criteria
L_2	12	The mean percentage of dissolved label claim lies within the range for the means at each interval and is NLT the stated amount at the final test time. NMT 2 of the 24 individual values lie outside the stated ranges for individuals at each interval, and NMT 2 of 24 are less than the stated amount at the final test time.

Test 3 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Acid medium, pH 7.5 buffer medium, Apparatus 2, Times, Acid-stage standard solution, Buffer-stage standard solution, and **Analysis:** Proceed as directed in *Test 2*.

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (Individual)	Amount Dissolved (Mean)
1	2%–16%	5%–13%
3	50%–80%	55%–75%
7	NLT 85%	NLT 90%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Acid medium: 0.01 N hydrochloric acid for 1 h; 900 mL, deaerated

pH 7.5 buffer medium: Prepare a pH 7.5 buffer concentrate by dissolving 62.2 g of potassium hydroxide and 129.3 g of monobasic potassium phosphate in water, dilute with water to 1 L, and mix. After 1 h change the *Acid medium* to *pH 7.5 buffer medium* by adding 50 mL of pH 7.5 buffer concentrate, and run for an additional 9 h.

Apparatus 2: 100 rpm, with helix sinkers

Times: 1, 3, and 10 h

Standard stock solution: Transfer 25 mg of USP Nitrofurantoin RS to a 10-mL volumetric flask. Add 7.5 mL

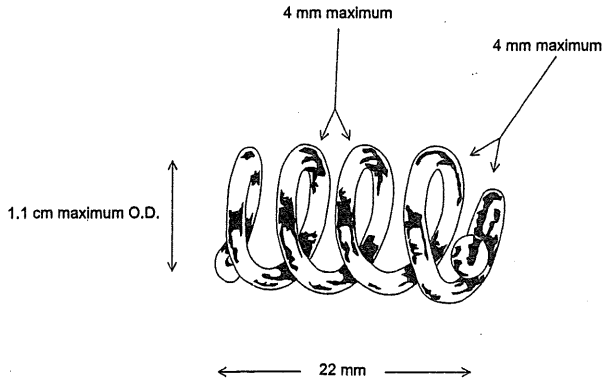


Figure 1. Sinker. (Nitrofurantoin Capsules)

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of dimethylformamide, and sonicate until dissolved. Allow to cool to room temperature, and dilute with dimethylformamide to volume.

Acid-stage standard solution: Dilute 2.0 mL of the *Standard stock solution* with *Acid medium* to 200 mL.

Buffer-stage standard solution: Transfer 3.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *pH 7.5 buffer medium* to volume.

Stock capsule shell blank: Place 10 empty, clean Capsules into a 900-mL volumetric flask, and add 800 mL of *Acid medium*. Gently heat to 37 ± 0.5°, and stir until all the Capsules are dissolved. Allow to cool to room temperature, and dilute with *Acid medium* to volume.

Buffer-stage capsule shell blank: Transfer 100.0 mL of the *Stock capsule shell blank* to a 1000-mL volumetric flask. Add 56 mL of *pH 7.5 buffer medium*, dilute with *Acid medium* to volume, and mix. Filter, using the same filter as for the *Sample solution*.

Sample solution: Pass portions of the solution under test through a 1.2-µm glass/0.45-µm polyethersulfone combination filter, discarding the first few mL.

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Analysis: Calculate the percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved from portions of the *Sample solution* in comparison with the appropriate *Acid-stage standard solution* or *Buffer-stage standard solution*. Correct for the appropriate capsule shell blank absorbance, using a 0.1-cm cell, and the appropriate medium as the blank.

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved
1	NMT 25%
3	25%–50%
10	NLT 80%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Acid medium: 0.01 N hydrochloric acid for 1 h; 900 mL, deaerated

Buffer concentrate: 60 g/L of potassium hydroxide and 129.3 g/L of monobasic potassium phosphate in water

pH 7.5 buffer medium: Prepare by adding 60 mL of *Buffer concentrate* to 890 mL of *Acid medium*.

Apparatus 2: 100 rpm, with Teflon-coated sinkers and paddles

Times: 1, 3, and 7 h

Standard stock solution: 2.48 mg/mL of USP Nitrofurantoin RS in acetonitrile. Sonicate using 50% of the final volume of acetonitrile to dissolve. Use an amber volumetric flask.

Acid-stage standard solution: 24.8 µg/mL of USP Nitrofurantoin RS in *Acid medium* from *Standard stock solution*. Use an amber volumetric flask.

Buffer-stage standard solution: 74.4 µg/mL of USP Nitrofurantoin RS in *pH 7.5 buffer medium* from *Standard stock solution*. Use an amber volumetric flask.

Acid-stage sample solution: After 1 h, collect 10 mL of the solution under test, and pass through a 0.45-µm PVDF filter, discarding the first 5 mL of the filtrate.

Buffer-stage sample solution: After removing 10 mL of *Acid medium*, add 60 mL of *pH 7.5 buffer medium*. The pH of the resulting medium should be about 7.5. Continue the dissolution for another 2 h and 6 h. Collect 10 mL at each time point, and pass through a 0.45-µm PVDF filter, discarding the first 5 mL of the filtrate.

Acid-stage blank: Use *Acid medium*.

Buffer-stage blank: Use *pH 7.5 buffer medium*.

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Cell: 0.5 cm for acid-stage and 0.1 cm for buffer-stage

Analysis

Samples: *Acid-stage standard solution*, *Buffer-stage standard solution*, *Acid-stage sample solution*, *Buffer-stage sample solution*, *Acid-stage blank*, and *Buffer-stage blank*

Calculate the concentration (C_i) of nitrofurantoin ($C_8H_6N_4O_5$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V_1 \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V_2) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V_3) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of nitrofurantoin in the portion of sample withdrawn at the specified time point (mg/mL)
V_1 = volume of medium, 900 mL
L = label claim (mg/Capsule)
V_2 = volume of medium, 950 mL
V_S = volume of the *Sample solution* withdrawn at each time point, 10 mL
V_3 = volume of medium, 940 mL

Tolerances: See *Table 6*.

Table 6

Time Point (i)	Time (h)	Amount Dissolved (Individual)	Amount Dissolved (Mean)
1	1	NMT 12%	NMT 12%
2	3	NLT 80%	80%–100%
3	7	NLT 85%	NLT 90%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to *Table 7*.

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Table 7

Level	Number Tested	Criteria
L_1	12	The mean percentage of dissolved label claim lies within the range for the means at each interval and is NLT the stated amount at the final test time. All individual values lie within the ranges for the individuals at each interval and are NLT the stated amount at the final test time.
L_2	12	If the requirements of level L_1 are not met, test another twelve (12) Capsules. The requirements are met if the mean percentage of dissolved label claim of all 24 Capsules tested lies within the range for the means at each interval and is NLT the stated amount at the final test time. NMT 2 of the 24 individual values of Capsules lie outside the stated range for individuals at each interval, and NMT 2 of 24 Capsules are less than the stated amount at the final test time.

Test 6 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Acid medium: 0.01 N hydrochloric acid; 900 mL

pH 7.5 buffer concentrate: Prepare a pH 7.5 buffer concentrate by dissolving 62.2 g of potassium hydroxide and 129.3 g of monobasic potassium phosphate in water and dilute with water to 1 L.

pH 7.5 buffer medium: 900 mL of *Acid medium* and 50 mL of *pH 7.5 buffer concentrate*

Apparatus 2: 100 rpm, with sinkers made of Teflon-coated steel wire prepared by forming a coil approximately 22 mm long from a 13-cm length of 20-gauge wire (see *Figure 1* in *Dissolution Test 2*)

Times

Acid stage: 1 h

Buffer stage: 3, 4, and 7 h

Acid-stage standard stock solution: 0.11 mg/mL of USP Nitrofurantoin RS in *Acid medium* prepared as follows. Weigh a suitable amount of USP Nitrofurantoin RS in a volumetric flask and add about 2.5% of the flask volume of *N,N*-dimethylformamide. Sonicate to dissolve completely. Dilute with *Acid medium* to final volume.

Acid-stage standard solution: 4.4 µg/mL of USP Nitrofurantoin RS in *Acid medium* from *Acid-stage standard stock solution*

Buffer-stage standard stock solution: 0.11 mg/mL of USP Nitrofurantoin RS in *pH 7.5 buffer medium* prepared as follows. Weigh a suitable amount of USP Nitrofurantoin RS in a volumetric flask and add about 2.5% of the flask volume of *N,N*-dimethylformamide. Sonicate to dissolve completely. Dilute with *pH 7.5 buffer medium* to final volume.

Buffer-stage standard solution: 4.4 µg/mL of USP Nitrofurantoin RS in *pH 7.5 buffer medium* from *Buffer-stage standard stock solution*

Acid-stage sample solution: Pass portions of the solution under test through a suitable filter and discard the first few mL. Dilute with *Acid medium*, if necessary.

Buffer-stage sample solution: Pass portions of the solution under test through a suitable filter and discard the first few mL. Dilute with *pH 7.5 buffer medium*, if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Dissolution medium: After 1 h in the *Acid medium*, withdraw 10 mL of the solution under test and add 50 mL of *pH 7.5 buffer concentrate*.

Analysis: After 1 h in *Acid medium*, withdraw 10 mL of solution under test. Add 10 mL of *Acid medium*, previously warmed to 37 ± 0.5°. Add 50 mL of *pH 7.5 buffer concentrate*, previously warmed to 37 ± 0.5° and continue the test for 6 h more. At specified times, withdraw 10 mL of solution under test and replace with 10 mL of *pH 7.5 buffer medium*, previously warmed to 37 ± 0.5°. Calculate the percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved from portions of the *Acid-stage sample solution* or *Buffer-stage sample solution* in comparison with the appropriate *Acid-stage standard solution* or *Buffer-stage standard solution*. Correct for the appropriate capsule shell blank absorbance and the appropriate medium as the blank.

Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved (Individual)	Amount Dissolved (Mean)
1	2%–16%	3%–11%
3	15%–45%	22%–37%
4	45%–95%	65%–85%
7	NLT 80%	NLT 85%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Acid medium: 0.01 N hydrochloric acid, degassed; 900 mL

Buffer concentrate: 62.2 g/L of potassium hydroxide and 129.3 g/L of monobasic potassium phosphate in water

pH 7.5 buffer medium: Prepare by adding 50 mL of *Buffer concentrate* to 900 mL of *Acid medium*. Adjust to pH 7.5 ± 0.05 with 1 N hydrochloric acid or 1 N potassium hydroxide

Apparatus 2: 100 rpm, with Teflon-coated helix sinkers

Times: 1, 3, and 7 h

Standard stock solution: 2.5 mg/mL of USP Nitrofurantoin RS in dimethylformamide. Sonicate to dissolve prior to final dilution.

Acid stage standard solution: 0.025 mg/mL of USP Nitrofurantoin RS in *Acid medium* from *Standard stock solution*. Prepare this solution immediately before use by diluting from the *Standard stock solution*.

Buffer stage standard solution: 0.075 mg/mL of USP Nitrofurantoin RS in *pH 7.5 buffer medium* from *Standard stock solution*.

Acid stage sample solution: After 1 h, collect 10 mL of the solution under test, and pass through a suitable filter of 0.45-µm pore size, transferring the first 5 mL of the filtrate back into the dissolution vessel.

Buffer stage sample solution: After removing 10 mL of *Acid medium*, add 50 mL of *pH 7.5 buffer medium*. Adjust the pH of the resulting medium to 7.5 ± 0.05 with 1 N hydrochloric acid or 1 N potassium hydroxide, if necessary. Continue collecting 10-mL solution under test at each time point, and pass through a suitable filter of 0.45-µm pore size, transferring the first 5 mL of the filtrate back into the dissolution vessel.

Acid stage blank: Use *Acid medium*.

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Buffer stage blank: Use *pH 7.5 buffer medium.*
Instrumental conditions
Mode: UV
Analytical wavelength: 375 nm
Cell: 0.5 cm for acid stage and 0.1 cm for buffer stage
System suitability
Sample: *Acid stage standard solution*
Suitability requirement
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Acid stage standard solution, Buffer stage standard solution, Acid stage sample solution, Buffer stage sample solution, Acid stage blank,* and *Buffer stage blank*
Calculate the concentration (C_i) of nitrofurantoin ($C_8H_6N_4O_5$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V_1 \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V_2) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V_3) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of nitrofurantoin in the portion of sample withdrawn at the specified time point (mg/mL)
V_1 = volume of medium, 900 mL
L = label claim (mg/Capsule)
V_2 = volume of medium, 945 mL
V_S = volume of the *Sample solution* withdrawn at each time point, 5 mL
V_3 = volume of medium, 940 mL

Tolerances: See *Table 9.*

Table 9

Time point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 23
2	3	55–80
3	7	NLT 85

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to *Dissolution* ⟨711⟩, *Acceptance Table 2.*

▲**Test 8** (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8.*
Acid medium, pH 7.5 buffer medium, Apparatus 2, Times, Acid-stage standard solution, Buffer-stage standard solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Dissolution Test 2.*
Tolerances: See *Table 10.*

Table 10

Time (h)	Amount Dissolved (individual, %)	Amount Dissolved (mean, %)
1	NMT 17	NMT 17
3	27–69	35–59
7	NLT 68	NLT 80

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to *Table 11.*

Table 11

Level	Number Tested	Criteria
L_1	12	The mean percentage of dissolved label claim lies within the range for the means at each interval and is NLT the stated amount at the final test time. All individual values lie within the ranges for the individuals at each interval and are NLT the stated amount at the final test time.
L_2	12	The mean percentage of dissolved label claim lies within the range for the means at each interval and is NLT the stated amount at the final test time. NMT 2 of the 24 individual values lie outside the stated ranges for individuals at each interval, and NMT 2 of 24 are less than the stated amount at the final test time. ▲(RB 1-Jan-2019)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Solution A, Mobile phase, Internal standard solution, Standard solution, Chromatographic system, and **Analysis:** Proceed as directed in the *Assay.*
Sample solution: Transfer the contents of 1 Capsule to a suitable flask, and add a volume of dimethylformamide to obtain a solution having a concentration of about 1.2 mg/mL of nitrofurantoin. Shake the flask for 15 min. If necessary, the sample may be homogenized, using a disperser. In the case of a 50- or 100-mg Capsule, transfer 40.0 mL of this solution to a suitable flask, add 50.0 mL of *Internal standard solution,* mix, and cool to room temperature. Pass a portion of the solution through a nylon filter of 0.45-µm pore size, discarding the first few mL of the filtrate. In the case of a 25-mg Capsule, transfer 20.0 mL of the solution to a suitable flask, and add 25.0 mL of *Internal standard solution* instead of 50.0 mL.
Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES: LIMIT OF NITROFURAZONE**
Solution A: Prepare as directed in the *Assay.*
Mobile phase: Tetrahydrofuran and *Solution A* (1:9)
System suitability stock solution: 5.0 µg/mL each of nitrofurazone and nitrofurantoin in dimethylformamide
System suitability solution: *System suitability stock solution* and *Mobile phase* (1:10)
Standard stock solution: 5.0 µg/mL of USP Nitrofurazone RS in dimethylformamide
Standard solution: Transfer 2.0 mL of the *Standard stock solution* into a glass-stoppered flask, add 20.0 mL of water, and mix.
Sample solution: Transfer a portion of Capsule contents equivalent to 100 mg of nitrofurantoin into a 25-mL glass-stoppered flask. Add 2.0 mL of dimethylformamide, and shake for 5 min. Add 20.0 mL of water, mix, and allow

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to stand for 15 min. Pass a portion of the mixture through a nylon filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 375 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.6 mL/min
Injection volume: 60–100 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Adjust the operating parameters so that the nitrofurazone peak in the chromatogram of the *Standard solution* has a retention time of about 10.5 min and a height of about 0.1 full-scale.]
Suitability requirements
Resolution: NLT 4.0 between the nitrofurazone and nitrofurantoin peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The height of any peak from the *Sample solution* at a retention time corresponding to that of the main peak from the *Standard solution* is NMT the height of the main peak from the *Standard solution*. NMT 0.01% of nitrofurazone is found.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Capsules that contain the macrocrystalline form of nitrofurantoin are so labeled. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Nitrofurantoin RS
USP Nitrofurazone RS

Nitrofurantoin Oral Suspension

DEFINITION

Nitrofurantoin Oral Suspension is a suspension of Nitrofurantoin in a suitable aqueous vehicle. It contains, in each 100 mL, NLT 460 mg and NMT 540 mg of nitrofurantoin ($C_8H_6N_4O_5$).

IDENTIFICATION

- **A. INFRARED ABSORPTION**
Sample solution: 10 mL of Oral Suspension in 15 mL of acetone
Analysis: Warm the *Sample solution* to 50°, with stirring, to coagulate the excipients. Filter, evaporate the acetone with the aid of a warm air blast nearly to dryness, add 10 mL of acetic acid, heat to boiling, and filter while hot. Cool the filtrate to room temperature. Filter the precipitated nitrofurantoin, and dry at 105° for 1 h.
Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the precipitate obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Nitrofurantoin RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water. Add about 30 mL of 1.0 N sodium hydroxide sufficient to adjust to a pH of 7.0, and dilute with water to 1 L.
Mobile phase: Acetonitrile and *Buffer* (12:88)
Internal standard solution: 0.065 mg/mL of acetanilide in *Mobile phase*
Standard stock solution: 0.25 mg/mL of USP Nitrofurantoin RS prepared as follows. Transfer the required amount in suitable volumetric flask, and dissolve in 50% of the final volume of dimethylformamide and 20% of the final volume of water. Cool to room temperature, and dilute with dimethylformamide to volume.
Standard solution: Transfer 4.0 mL of *Standard stock solution* to a glass-stoppered flask, and add 15.0 mL of *Internal standard solution*.
Sample stock solution: Nominally 0.25 mg/mL of nitrofurantoin prepared as follows. Transfer a volume of Oral Suspension to a suitable volumetric flask, add 20% of the final volume of water, and mix. Add 50% of the final volume of dimethylformamide, and shake the flask for 20 min. Cool to room temperature, and dilute with dimethylformamide to volume. Centrifuge a portion of the solution, and use the supernatant to prepare the *Sample solution*.
Sample solution: Transfer 4.0 mL of *Sample stock solution* to a glass-stoppered flask, add 15.0 mL of *Internal standard solution*, and mix. Pass a portion of the solution through a 5-µm pore size polytef filter, discarding the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—Adjust the operating parameters so that the retention time of the nitrofurantoin peak is about 8 min and its peak height is about half-full scale.]
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.2 mL/min
Injection size: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between the acetanilide and nitrofurantoin peaks
Relative standard deviation: NMT 2.0% determined from the ratio of the peak responses
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity per volume, in mg/100 mL, of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) in the Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times C_S \times D$$

R_U = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Sample solution*
R_S = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Standard solution*
C_S = concentration of USP Nitrofurantoin RS in the *Standard solution* (mg/mL)
D = dilution factor, *Sample stock solution* to *Sample solution*, 9500

Acceptance criteria: 460–540 mg/100 mL of $C_8H_6N_4O_5$

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PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩

For Oral Suspension packaged in single-unit containers: Meets the requirements

• **Deliverable Volume** ⟨698⟩**:**

For Oral Suspension packaged in multiple-unit containers: Meets the requirements

IMPURITIES

• **Limit of *N*-(Aminocarbonyl)-*N*-[([5-Nitro-2- Furanyl] Methylene)-Amino]Glycine (NF 250)**

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

Standard solution: 2.5 µg/mL of USP Nitrofurantoin Related Compound A RS in *Mobile phase*

Sample solution: Nominally 0.05 mg/mL of nitrofurantoin in *Mobile phase* from Oral Suspension. Centrifuge a portion of this solution. Pass a portion of the supernatant through a polytef filter having a 5-µm pore size, discarding the first few mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

[Note—Adjust the operating parameters so that the NF 250 peak has a retention time of between 3 and 6 min and its height is about 0.1 full scale.]

Mode: LC

Detector: UV 375 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection size: 30–60 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The height of any peak appearing in the *Sample solution* at a retention time corresponding to that of the main peak from the *Standard solution* is NMT the height of the main peak from the *Standard solution* (NMT 5.0%).

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 4.5–6.5

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards** ⟨11⟩

USP Nitrofurantoin RS

USP Nitrofurantoin Related Compound A RS (*N*-(Aminocarbonyl)-*N*-[([5-nitro-2-furanyl]-methylene)-amino]glycine).

Nitrofurantoin Tablets

DEFINITION

Nitrofurantoin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$).

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197M⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)

Sample: Add 10 mL of 6 N acetic acid to an amount equivalent to 100 mg of nitrofurantoin from powdered Tablets. Boil for a few min, and filter while hot. Cool to room temperature, collect the precipitate of nitrofurantoin, and dry at 105° for 1 h.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the precipitate from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Nitrofurantoin RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water. Add about 30 mL of 1.0 N sodium hydroxide sufficient to adjust to a pH of 7.0, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (12:88)

Internal standard solution: 1 mg/mL of acetanilide in water

Standard solution: 0.56 mg/mL of USP Nitrofurantoin RS prepared as follows. Dissolve 50 mg of USP Nitrofurantoin RS in 40.0 mL of dimethylformamide, and add 50.0 mL of *Internal standard solution*.

Sample solution: Nominally 0.56 mg/mL of nitrofurantoin prepared as follows. Mix an amount equivalent to 50 mg of nitrofurantoin from powdered Tablets (NLT 20) with 40.0 mL of dimethylformamide, and shake mechanically for 15 min. Add 50.0 mL of *Internal standard solution*, mix, and cool to room temperature. Pass a portion of the solution through a nylon filter having a pore size of 0.45-µm, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

[Note—Adjust the operating parameters so that the retention time of the nitrofurantoin peak is about 8 min and the peak height is about half full-scale.]

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Injection size: 5–10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between acetanilide and nitrofurantoin

Relative standard deviation: NMT 2.0% determined from the ratio of the peak responses

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) in the portion of powdered Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Sample solution*

R_S = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Standard solution*

C_S = concentration of USP Nitrofurantoin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nitrofurantoin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: pH 7.2 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 1: 100 rpm

Times: 60 and 120 min

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Standard stock solution: 0.1 mg/mL of USP Nitrofurantoin RS prepared as follows. Dissolve the required amount of USP Nitrofurantoin RS in 5% of the final volume of dimethylformamide. Dilute to final volume with *Medium*.
Standard solution: 10 µg/mL of USP Nitrofurantoin RS in *Medium* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Analysis
Samples: *Standard solution* and *Sample solution*
Analytical wavelength: 375 nm
Blank: *Medium*
Calculate the percentage of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 25% (Q) of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) is dissolved in 60 min, and NLT 85% (Q) of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) is dissolved in 120 min.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

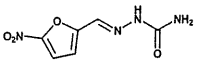
IMPURITIES

• **LIMIT OF NITROFURAZONE**
Buffer: Proceed as directed in the *Assay*.
Mobile phase: Tetrahydrofuran and *Buffer* (10:90)
System suitability stock solution: 5.0 µg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in dimethylformamide
System suitability solution: 0.5 µg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in *Mobile phase* from *System suitability stock solution*
Standard stock solution: 5.0 µg/mL of USP Nitrofurazone RS in dimethylformamide.
Standard solution: Transfer 2.0 mL of the *Standard stock solution* into a glass-stoppered flask, and add 20.0 mL of water.
Sample solution: Transfer an equivalent to 100 mg of nitrofurantoin from powdered Tablets into a 25-mL, glass-stoppered flask. Add 2.0 mL of dimethylformamide, and shake for 5 min. Add 20.0 mL of water, mix, and allow to stand for 15 min. Pass a portion of the mixture through a nylon filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—Adjust the operating parameters so that the nitrofurazone peak has a retention time of about 10.5 min and its height is about 0.1 full-scale.]
Mode: LC
Detector: UV 375 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.6 mL/min
Injection size: 60–100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between the nitrofurantoin and nitrofurazone peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The height of any peak from the *Sample solution* at a retention time corresponding to that of the main peak from the *Standard solution* is NMT the height of the main peak from the *Standard solution* (NMT 0.01%).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Nitrofurantoin RS
USP Nitrofurazone RS

Nitrofurazone



$C_6H_6N_4O_4$ 198.14
Hydrazinecarboxamide, 2-[(5-nitro-2-furanyl)methylene]-; 5-Nitro-2-furaldehyde semicarbazone [59-87-0].

DEFINITION

Nitrofurazone contains NLT 98.0% and NMT 102.0% of nitrofurazone ($C_6H_6N_4O_4$), calculated on the dried basis.
[NOTE—Avoid at all times exposing solutions of nitrofurazone to direct sunlight, excessive heat, strong fluorescent lighting, and alkaline materials.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Sample solution: 8 µg/mL, prepared as directed in the *Assay*
Acceptance criteria: The ratio A_{306}/A_{375} is NMT 0.25.

ASSAY

• **PROCEDURE**
Standard stock solution: 0.4 mg/mL of USP Nitrofurazone RS prepared as follows. Transfer 100 mg of USP Nitrofurazone RS to a 250-mL volumetric flask. Dissolve in 50 mL of dimethylformamide, and dilute with water to volume.
Standard solution: 8 µg/mL of USP Nitrofurazone RS in water from *Standard stock solution*
Sample stock solution: 0.4 mg/mL of Nitrofurazone prepared as follows. Transfer 100 mg of Nitrofurazone to a 250-mL volumetric flask. Dissolve in 50 mL of dimethylformamide, and dilute with water to volume.
Sample solution: 8 µg/mL of Nitrofurazone in water from *Sample stock solution*
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 375 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of nitrofurazone ($C_6H_6N_4O_4$) in the portion of Nitrofurazone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Nitrofurazone RS in the *Standard solution* (µg/mL)
C_U = concentration of Nitrofurazone in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase: Acetonitrile and water (40:60)
Peak identification solution: 0.1 mg/mL each of USP Nitrofurazone Related Compound A RS and USP Nitrofurfural Diacetate RS in *Mobile phase*
System suitability solution: 0.1 mg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in *Mobile phase*
Standard solution: 5 µg/mL of USP Nitrofurazone RS in *Mobile phase*
Sample solution: 1 mg/mL of Nitrofurazone in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 310 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 10 times the retention time of nitrofurazone
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for nitrofurazone and nitrofurantoin are about 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 2.0 between nitrofurazone and nitrofurantoin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Peak identification solution*, *Standard solution*, and *Sample solution*
Calculate the percentage of each impurity in the portion of Nitrofurazone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of nitrofurazone from the *Standard solution*
C_S = concentration of USP Nitrofurazone RS in the *Standard solution* (mg/mL)
C_U = concentration of Nitrofurazone in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nitrofurazone	1.0	—	—
Nitrofurfural diacetate	3.4	2.2	0.5
Nitrofurazone related compound A	6.0	1.6	0.5
Any unspecified impurity	—	1.0	0.1
Total impurities	—	—	2.0

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 1 g of Nitrofurazone in 100 mL of water. Shake for 15 min, allow the suspension to settle, and filter.
Acceptance criteria: 5.0–7.5

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 1 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Nitrofurantoin RS
USP Nitrofurazone RS
USP Nitrofurazone Related Compound A RS
5-Nitro-2-furfuraldazine.
$C_{10}H_6N_4O_6$ 278.18
USP Nitrofurfural Diacetate RS
(5-Nitrofuran-2-yl)methylene diacetate.
$C_9H_9NO_7$ 243.17

Nitrofurazone Ointment

» Nitrofurazone Ointment is Nitrofurazone in a suitable water-miscible base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nitrofurazone ($C_6H_6N_4O_4$).
[NOTE—Avoid exposure at all times to direct sunlight, excessive heat, strong fluorescent lighting, and alkaline materials.]

Packaging and storage—Preserve in tight, light-resistant containers. Avoid exposure to direct sunlight, strong fluorescent lighting, and excessive heat.
USP Reference standards ⟨11⟩—
USP Nitrofurazone RS
Completeness of solution—One g dissolves in 9 mL of water to form a clear solution.
Identification—Dissolve 400 mg of potassium hydroxide in a mixture of 9.5 mL of alcohol and 0.5 mL of methanol. Immediately before use, dilute with dimethylformamide to 100 mL. To 10 mL of this solution add a quantity of Ointment, equivalent to about 10 µg of nitrofurazone, and mix: a purple solution results.
Assay— [NOTE—Protect from light all solutions that contain nitrofurazone.]

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Triethylamine buffer—Transfer 100 mL of triethylamine to a 1000-mL volumetric flask. Add about 800 mL of water, and cautiously add 80 mL of phosphoric acid. Mix, allow to cool to ambient temperature, dilute with water to volume, mix, and pass through a nylon filter having a 0.5-µm or finer porosity.

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and *Triethylamine buffer* (790:200:10). Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 50 mg of USP Nitrofurazone RS, accurately weighed, to a 50-mL low-actinic volumetric flask, add 10 mL of dimethylformamide, and swirl to dissolve. Dilute with alcohol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL low-actinic volumetric flask, dilute with alcohol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL low-actinic volumetric flask containing 15 mL of alcohol, dilute with water to volume, and mix. This solution contains about 0.01 mg of USP Nitrofurazone RS per mL.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 1 mg of nitrofurazone, to a 100-mL low-actinic volumetric flask. Add 0.2 mL of dimethylformamide and about 25 mL of alcohol, and sonicate for about 35 minutes. Dilute with water to volume, mix, and pass through a nylon filter having a 0.5-µm or finer porosity. Use the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 365-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the nitrofurazone peak is not less than 1500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of nitrofurazone ($C_6H_6N_4O_4$) in the portion of Ointment taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Nitrofurazone RS in the *Standard preparation;* and r_U and r_S are the nitrofurazone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nitrofurazone Topical Solution

» Nitrofurazone Topical Solution contains not less than 95.0 percent and not more than 105.0 percent (w/w) of the labeled amount of $C_6H_6N_4O_4$. [NOTE—Avoid exposure at all times to direct sunlight, excessive heat, and alkaline materials.]

Packaging and storage—Preserve in tight, light-resistant containers. Avoid exposure to direct sunlight and excessive heat.

USP Reference standards ⟨11⟩—
USP Nitrofurazone RS

Identification—Dissolve 400 mg of potassium hydroxide in a mixture of 9.5 mL of alcohol and 0.5 mL of methanol. Immediately before use dilute with dimethylformamide to 100 mL. To 10 mL of this solution add 1 drop of Topical Solution: a purple solution results.

Assay— [NOTE—Protect from light all solutions that contain nitrofurazone.]

Triethylamine buffer, Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Nitrofurazone Ointment.*

Assay preparation—Transfer an accurately measured portion of Topical Solution, equivalent to about 1 mg of nitrofurazone, to a 100-mL low actinic volumetric flask. Add 0.2 mL of dimethylformamide and about 25 mL of warm (between 40° and 50°) alcohol. Dilute with water to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Nitrofurazone Ointment.* Calculate the quantity, in mg, of $C_6H_6N_4O_4$ in the portion of Topical Solution taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Nitrofurazone RS in the *Standard preparation,* and r_U and r_S are the nitrofurazone peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ammonia N 13 Injection

DEFINITION

Change to read:

▲Ammonia N 13 Injection is a sterile aqueous solution of [^{13}N] Ammonia ($^{13}NH_3$). It contains NLT 90% and NMT 110% of the labeled amount of [^{13}N] Ammonia ($^{13}NH_3$) expressed in MBq(mCi)/mL at the time indicated in the labeling. It may contain buffering agents, preservatives, stabilizing agents, or sodium chloride. It does not contain any added carrier.▲ 2S (USP41)

IDENTIFICATION

Change to read:

• **A. RADIONUCLIDIC IDENTITY**

▲(See *Radioactivity* ⟨821⟩, *5. Identification of Radionuclides, 5.1 Half-Life Determination.*) The half-life of ^{13}N is 9.5–10.5 min.▲ 2S (USP41)

Change to read:

• **B. RADIOCHEMICAL IDENTITY**

▲**Procedure:** After completing the *Analysis* in the *Radiochemical Purity* test, spray or stain the TLC strip with iodoplatinate TS. Allow the TLC strip to develop for 10 min. Place the developed TLC strip in an iodine chamber. The spot due to ammonium ion on the strip for the *Standard solution* appears as an orange-brown spot against a light maroon background.

Acceptance criteria: The R_F value of $^{13}NH_3$ in the *Sample solution* is 90%–110% of the R_F value of the ammonium ion as determined in the *Standard solution* in the test for *Radiochemical Purity.*▲ 2S (USP41)

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ASSAY

Change to read:

• **Radioactive Concentration (Strength)**
(See *Radioactivity* ⟨821⟩, *6. Assay of Radionuclides*.)
Analysis: Determine the radioactivity, in MBq (or mCi)/mL, of the Injection, using a suitable calibrated system.
Acceptance criteria: ▲90%–110% at the time indicated on the label.▲ 2S (USP41)

PURITY

Change to read:

• **Radionuclidic Purity**
▲(See *Radioactivity* ⟨821⟩, *5. Identification of Radionuclides, 5.2 Gamma-Ray Spectrometry*.)
[Note—This may be a periodic quality indicator test. The Injection may be distributed and dispensed prior to completion of this test.]
Analysis: Determine the purity of ammonia N 13 in the portion of Injection taken for the *Radionuclidic Impurities* test:

$$\text{Result} = [1 - (C_I/C_T)] \times 100$$

C_I = sum of the concentrations of all longer-lived radionuclides, decay corrected to the expiration time from the *Radionuclidic Impurities* test (Bq/mL) or (µCi/mL)
C_T = sum of the concentrations of all long-lived radionuclides and ammonia N 13, all decay corrected to the expiration time from the *Radionuclidic Impurities* test (Bq/mL) or (µCi/mL)

Acceptance criteria: At the time of expiration, NLT 99.5% of radionuclides in the Injection correspond to ^{13}N.▲ 2S (USP41)

Change to read:

• **Radiochemical Purity**
▲**Standard solution:** 100 mg/mL of USP Ammonium Chloride RS in water
Sample solution: A volume of Injection, appropriately diluted if necessary, to a concentration of 5–30 MBq (0.135–0.81 mCi)/mL or to a concentration suitable for the detector to be used
Chromatographic system
Mode: TLC
Detection: Radio-TLC scanner
Adsorbent: Cellulose diethylaminoethyl strips; 1.5-cm × 8-cm
Application volume: 0.5 µL
Developing solvent: Methanol and water (75:25)
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the *Standard solution* and *Sample solution* independently as two adjacent spots on the TLC strip. Allow the spots to dry. Place the TLC strip in the developing chamber with *Developing solvent*. When the solvent front reaches about 1 cm from the top of the strip, remove the strip from the chamber and allow it to dry. Determine the radioactivity distribution by scanning the chromatogram with a suitable radiation detector. After completing the test for *Radiochemical Purity*, use the TLC radiochromatogram for the *Radiochemical Impurities* test. After completing the test for *Radiochemical Purity*, use the TLC strip for the *Radiochemical Identity* test.
Calculate the percentage of the labeled amount of $^{13}NH_3$ in the portion of Injection taken from the chromatogram of the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of $^{13}NH_3$ from the *Sample solution*
r_T = sum of the responses of all peaks including $^{13}NH_3$ from the *Sample solution*

Acceptance criteria: NLT 95% of the total radioactivity is due to ammonia N 13.▲ 2S (USP41)

IMPURITIES

Add the following:

▲• **Radionuclidic Impurities**
[Note—This may be a periodic quality indicator test. The Injection may be distributed or dispensed prior to completion of the test.]
Sample: A suitable volume of Injection, decayed for a suitable length of time to eliminate interference due to ^{13}N emissions
Analysis: Using a suitable gamma-ray spectrometer, count an appropriate aliquot of the sample solution for a period of time sufficient to collect a gamma spectrum. The resultant gamma spectrum should be analyzed for the presence of identifiable photopeaks which are characteristic of radionuclidic impurities.
Determine the concentration of radionuclidic impurities in Bq (or µCi)/mL, decay corrected to the expiration time of the Injection:

$$\text{Result} = (C_I/C_T) \times 100$$

C_I = sum of the concentrations of all longer-lived radionuclides decay, corrected to the expiration time (Bq/mL) or (µCi/mL)
C_T = sum of the concentrations of all long-lived radionuclides and ammonia N 13, all decay corrected to the expiration time (Bq/mL) or (µCi/mL)

Acceptance criteria: Total radionuclidic impurities are NMT 0.5% of the radioactivity of the Injection (see the *Assay*), decay corrected to the time of expiration.▲ 2S (USP41)

Add the following:

▲• **Radiochemical Impurities**
Analysis: Calculate the percentage of radiochemical impurities in the portion of Injection taken from the TLC radiochromatogram of the *Sample solution*, as obtained in the *Radiochemical Purity* test.

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = sum of the responses all peaks excluding the response of $^{13}NH_3$ from the *Sample solution*
r_T = sum of the responses of all peaks including $^{13}NH_3$ from the *Sample solution*

Acceptance criteria: NMT 5%▲ 2S (USP41)

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Delete the following:

▲• **CHEMICAL PURITY**

[NOTE—This article may be synthesized by different methods and processes and, therefore, contains different impurities. The presence of unlabeled ingredients, reagents, and by-products specific to the process must be controlled, and their potential for physiological or pharmacological effects must be considered.]

ALUMINUM (to be determined if Devarda's alloy is used to reduce ^{13}N nitrate/nitrite)

Standard solution: 2 µg/mL of aluminum prepared by dissolving 35.2 mg of aluminum potassium sulfate dodecahydrate in 1 L of water

Blank standard solution Transfer 10 mL of *Standard solution* to a 50-mL volumetric flask. Add 3 drops of methyl orange TS and 2 drops of 6N ammonium hydroxide. Add 0.5N hydrochloric acid dropwise until the solution turns red. Add 1 mL of edetate disodium TS. Add 5 mL of eriochrome cyanine TS and 5 mL of acetate buffer TS. Dilute with water to volume.

Analytical standard solution Transfer 10 mL of *Standard solution* to a 50-mL volumetric flask. Add 3 drops of methyl orange TS and 2 drops of 6N ammonium hydroxide. Add 0.5N hydrochloric acid dropwise until the solution turns red. Add 25 mL of sodium thioglycolate TS. Add 5 mL of eriochrome cyanine TS and 5 mL of acetate buffer TS. Dilute with water to volume.

Sample solution: Use the Injection

Blank sample solution: Transfer 1 mL of Injection to a 50-mL volumetric flask. Add 3 drops of methyl orange TS and 2 drops of 6N ammonium hydroxide. Add 0.5N hydrochloric acid dropwise until the solution turns red. Add 1 mL of edetate disodium TS. Add 5 mL of eriochrome cyanine TS and 5 mL of acetate buffer TS. Dilute with water to volume.

Analytical sample solution: Transfer 1 mL of Injection to a 50-mL volumetric flask. Add 3 drops of methyl orange TS and 2 drops of 6N ammonium hydroxide. Add 0.5N hydrochloric acid dropwise until the solution turns red. Add 25 mL of sodium thioglycolate TS. Add 5 mL of eriochrome cyanine TS and 5 mL of acetate buffer TS. Dilute with water to volume.

Instrumental Conditions:

Mode: Vis

Analytical wavelength: 535 nm

Analysis:

Samples: *Blank Standard solution*, *Analytical standard solution*, *Blank sample solution* and *Analytical sample solution*

Procedure: Measure the absorbance of the *Samples* immediately. Calculate the amount of aluminum in µg/mL in the portion of the Injection taken:

$$\text{Result} = [(A_u - A_{ub})/A_s - A_{sb})] \times C_s \times (V_s/V_u)]$$

A_u = absorbance of *Analytical sample solution*
A_{ub} = absorbance of *Blank sample solution*
A_s = absorbance of *Analytical standard solution*
A_{sb} = absorbance of *Blank standard solution*
C_s = concentration of aluminum in *Standard solution*, µg/mL
V_s = volume of Standard solution in *Analytical standard solution*
V_u = volume of Injection in *Analytical sample solution*

Acceptance criteria: NMT 10 µg/mL ▲2S (USP41)

Add the following:

▲• **LIMIT OF ETHANOL**

[NOTE—Perform this test if ethanol is known to be present in the article. This may be a periodic quality indicator test. The Injection may be distributed and dispensed prior to completion of this test.]

Standard solution: 3.1 mg/mL of ethanol prepared as follows. Transfer 2 mL of USP Alcohol Determination—Alcohol RS to a 10 mL volumetric flask. Dilute with water to volume.

Sample solution: Use the Injection.

Chromatographic system

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused-silica; coated with a 0.5-µm film of phase G16

Temperatures

Injection port: 250°

Detector: 300°

Column: See *Table 1*.

[NOTE—Depending on the syringe wash solvent, an additional ramp at 10°/min to bring the column to 240° with no hold time may be used before returning the column oven to initial conditions.]

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	0.5
40	40	110	1

Carrier gas: Hydrogen

Flow rate: 1.3 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 15:1

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The response for ethanol in the *Sample solution* is NMT the response of ethanol in the *Standard solution* (3.1 mg/mL of ethanol). ▲2S (USP41)

SPECIFIC TESTS

Change to read:

• **pH**

▲**Analysis:** Apply a suitable volume of the Injection on a suitable pH indicator paper, short-range.

Acceptance criteria: 4.5–7.5 ▲2S (USP41)

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements ▲2S (USP41)

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Add the following:

▲• **STERILITY TESTS** ⟨71⟩
[NOTE—The Injection may be distributed or dispensed prior to completion of the test, the latter test being started within 36 h of final manufacture.]
Sample: Use 0.1–0.3 mL of Injection
Acceptance criteria: Meets the requirements ▲2S (USP41)

Add the following:

▲• **APPEARANCE:** Clear, colorless solution, free of foreign particulates ▲2S (USP41)

Delete the following:

▲• **SPECIFIC ACTIVITY:** No carrier added ▲2S (USP41)

Delete the following:

▲• **OTHER REQUIREMENTS:** It meets the requirements under *Injections* ⟨1⟩, except that the Injection may be distributed or dispensed before completion of the test for *Sterility Tests* ⟨71⟩, the latter test being started within 24 h of final manufacture, and except that it is not subject to the recommendation in *Volume in Container*. ▲2S (USP41)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers that are adequately shielded. ▲Store at controlled room temperature. ▲2S (USP41)

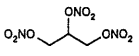
Change to read:

• **LABELING:** ▲The label indicates the time and date of calibration; the concentration of ^{13}N as ammonia expressed in MBq (mCi)/mL, at the time of calibration; the expiration time and date; and the name and quantity of any added preservative or stabilizer. Calculate the correct dosage from date and time of calibration. The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay. The radioactive half-life of ^{13}N is 9.96 min. The label also indicates the following: [**CAUTION**—Radioactive Material. Do not use if cloudy or if it contains visible particulate matter.] ▲2S (USP41)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Alcohol Determination—Alcohol RS ▲2S (USP41)
USP Ammonium Chloride RS
▲▲(CN 1-May-2018)

Diluted Nitroglycerin



$C_3H_5N_3O_9$ 227.09
1,2,3-Propanetriol, trinitrate; Nitroglycerin [55-63-0].

DEFINITION
Diluted Nitroglycerin is a mixture of nitroglycerin ($C_3H_5N_3O_9$) with lactose, dextrose, alcohol, propylene glycol, or other suitable inert excipient to permit safe handling. It contains NLT 90.0% and NMT 110.0% of the labeled amount of $C_3H_5N_3O_9$. It usually contains NMT 10% of nitroglycerin ($C_3H_5N_3O_9$). [**CAUTION**—Taking into consideration the concentration and amount of nitroglycerin ($C_3H_5N_3O_9$) in Diluted Nitroglycerin, exercise appropriate precautions when handling this material. Nitroglycerin is a powerful explosive and can be detonated by percussion or excessive heat. Do not isolate nitroglycerin ($C_3H_5N_3O_9$).]

IDENTIFICATION
• **A.** The R_F value of the principal spot of *Sample solution A* corresponds to that of the *Standard solution*, as obtained in the *Procedure* for *Organic Impurities*.
• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol and water (1:1)
Standard solution: 0.075 mg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in *Mobile phase*
Sample solution: Transfer a portion of Diluted Nitroglycerin, equivalent to 7.5 mg of nitroglycerin, to a 100-mL volumetric flask, and dissolve in 75 mL of *Mobile phase*. If necessary, sonicate for 2 min or until the solid is totally dispersed, then shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1. [NOTE—If necessary a short precolumn that contains packing L1 may be used.]
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.5 for the analyte peak
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_3H_5N_3O_9$ in the portion of Diluted Nitroglycerin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
ORGANIC IMPURITIES
• **Procedure**
Standard solution: 400 µg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in methanol
Sample solution A: Prepare a clear solution containing 400 µg/mL of nitroglycerin from Diluted Nitroglycerin in methanol.

USP Monographs

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Sample solution B: 20 mg/mL of nitroglycerin in methanol from Diluted Nitroglycerin. Centrifuge a portion, if necessary, to obtain a clear liquid phase.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL each of *Sample solution A* and *Sample solution B*; 5, 10, 15, and 20 µL of the *Standard solution*
Developing solvent system: Toluene and ethyl acetate (4:1)
Spray reagent: Diphenylamine in methanol (1 in 100)
Analysis
Samples: *Standard solution*, *Sample solution A*, and *Sample solution B*
Apply the *Samples* to a suitable thin-layer chromatographic plate. Develop the chromatograms in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Spray reagent*, and irradiate the plate with short- and long-wavelength UV light for 15 min.
Acceptance criteria: Any spot from *Sample solution B*, other than the principal spot, is not more intense than the spot from the 20-µL application of the *Standard solution*. Compare the intensities of any secondary spots observed from *Sample solution B* with those of the principal spots from the *Standard solution* (corresponding to 0.5%, 1.0%, 1.5%, and 2.0%, respectively): the sum of the intensities of the secondary spots from *Sample solution B* is NMT 3%. [NOTE—Nitrates of glycerin typically have R_F values of 0.21, 0.37, and 0.61 for mono-, di-, and tri-substituted glycerins, respectively.]

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and prevent exposure to excessive heat. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Diluted Nitroglycerin RS

Nitroglycerin Injection

DEFINITION
Nitroglycerin Injection is a sterile solution prepared from Diluted Nitroglycerin; the solvent may contain Alcohol, Propylene Glycol, and Water for Injection. Nitroglycerin Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$).

IDENTIFICATION
- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Mobile phase: Methanol and water (500:500)
Standard solution: 0.075 mg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in *Mobile phase*
Sample solution: Transfer a measured volume of Injection equivalent to 7.5 mg of nitroglycerin to a 100-mL volumetric flask, and dissolve in and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
[NOTE—If necessary, a short precolumn that contains packing L1 may be used.]
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS
- **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩
Sample: Use isopropyl alcohol as the internal standard.
Acceptance criteria: 90.0%–110.0% of the labeled amount of C_2H_5OH

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.1 USP Endotoxin Unit/µg of nitroglycerin
- **pH** ⟨791⟩
Sample: To 5 mL of the Injection add 5 mL of water and 1 drop of saturated potassium chloride solution.
Acceptance criteria: 3.0–6.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.
- **LABELING:** Where necessary, label it to indicate that it is to be diluted before use.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Diluted Nitroglycerin RS

Nitroglycerin Ointment

DEFINITION
Nitroglycerin Ointment is Diluted Nitroglycerin in a suitable ointment base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$).

IDENTIFICATION
- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

• **PROCEDURE**

Mobile phase: Methanol and water (500:500)

Standard solution: 0.075 mg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in *Mobile phase*

Sample solution: Transfer a quantity of Ointment equivalent to 2.0 mg of nitroglycerin to a glass-stoppered, 50-mL conical flask, and add 25.0 mL of *Mobile phase.* Immerse the flask containing the sample in a water bath maintained at a temperature of 50° for 10 min. Shake intermittently until the sample is dispersed. Remove the flask from the bath, and shake vigorously for 1 min to obtain a coagulated solid. Repeat the heating and shaking steps one more time, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

[NOTE—If necessary, a short precolumn that contains packing L1 may be used.]

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)

C_U = nominal concentration of nitroglycerin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

• **HOMOGENEITY**

Analysis: In the case of single-dose containers, perform the *Assay* on specimens from each of 10 containers. In the case of multiple-dose containers, perform the *Assay* on one sample from the top and one from the bottom of each of five containers.

Acceptance criteria: Each sample contains NLT 90.0% and NMT 110.0% of the mean value.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label multiple-dose containers with a direction to close tightly immediately after each use.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Diluted Nitroglycerin RS

Nitroglycerin Sublingual Tablets

DEFINITION

Nitroglycerin Sublingual Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: Equivalent to 1 mg/mL of nitroglycerin in acetone from USP Diluted Nitroglycerin RS

Sample solution: Equivalent to 1 mg/mL of nitroglycerin from powdered Sublingual Tablets, in acetone. Shake by mechanical means for 30 min, and filter.

Developing solvent system: Toluene, ethyl acetate, and glacial acetic acid (16:4:1)

Analysis: Proceed as directed. Spray with a solution (1 in 100) of diphenylamine in methanol, and irradiate the plate with short- and long-wavelength UV light for 10 min.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and water (50:50)

Standard solution: 0.075 mg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in *Mobile phase*

Sample solution: Nominally equivalent to 0.075 mg/mL of nitroglycerin from powdered Sublingual Tablets (NLT 20 Sublingual Tablets) in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1. [NOTE—If necessary, use a short precolumn that contains packing L1.]

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$) in the portion of Sublingual Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)

C_U = nominal concentration of nitroglycerin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Determined as set forth for Sublingual Tablets

Time: 2 min

Acceptance criteria: Meets the requirements

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• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: 0.075 mg/mL of nitroglycerin in *Mobile phase*, from 1 Sublingual Tablet
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$) in the Sublingual Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

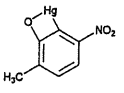
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)
C_U = nominal concentration of nitroglycerin in the *Sample solution* (mg/mL)

Acceptance criteria: The content of each of the 10 Sublingual Tablets is within the range of 75.0%–135.0% of the labeled claim. If the content of NMT 1 Sublingual Tablet is outside the range of 75.0%–135.0% and if the content of none of the Sublingual Tablets is outside the range of 60.0%–150.0%, test 20 additional units. The requirements are met if the content of each of the additional 20 units falls within the range of 75.0%–135.0% of the labeled claim.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, preferably of glass, and store at controlled room temperature. Each container holds NMT 100 Sublingual Tablets.
• **Labeling:** The labeling indicates that the Sublingual Tablets are for sublingual use, and the label directs that the Sublingual Tablets be dispensed in the original, unopened container, labeled with the following statement directed to the patient. "Warning: To prevent loss of potency, keep these tablets in the original container or in a supplemental nitroglycerin container specifically labeled as being suitable for Nitroglycerin Sublingual Tablets. Close tightly immediately after each use."
• **USP Reference Standards** ⟨11⟩
USP Diluted Nitroglycerin RS

Nitromersol



$C_7H_5HgNO_3$ 351.71
7-Oxa-8-mercurabicyclo[4.2.0]octa-1,3,5-triene, 5-methyl-2-nitro-;
5-Methyl-2-nitro-7-oxa-8-mercurabicyclo[4.2.0]octa-1,3,5-triene [133-58-4].

DEFINITION

Nitromersol, dried at 105° for 2 h, contains NLT 98.0% and NMT 100.5% of nitromersol ($C_7H_5HgNO_3$).

IDENTIFICATION

• **A.**
Sample solution: 1 mg/mL in 1 N sodium hydroxide
Analysis: The *Sample solution* possesses a reddish orange color. Add 3 N hydrochloric acid.
Acceptance criteria: The reddish orange color disappears, and a yellowish, flocculent precipitate forms.
• **B.**
Sample solution: Dissolve 250 mg of Nitromersol in 2.5 mL of 1 N sodium hydroxide, and dilute with water to 20 mL.
Analysis 1: Add 3 mL of 3 N hydrochloric acid to the *Sample solution*.
Acceptance criteria 1: A yellowish precipitate is formed.
Analysis 2: Filter the solution obtained after *Analysis 1*. The filtrate is nearly colorless or slightly yellow. Retain the filtrate for the test for *Mercury Ions*. Dissolve the precipitate in 20 mL of water to which 2.5 mL of 1 N sodium hydroxide has been added. Add 0.5 g of sodium hydrosulfite, and heat to boiling.
Acceptance criteria 2: A heavy deposit of metallic mercury is formed.

ASSAY

• **Procedure**
Sample: 200 mg of Nitromersol, previously ground to a fine powder and dried
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N ammonium thiocyanate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 500-mL Kjeldahl flask. Add 15 mL of sulfuric acid, and digest cautiously with occasional swirling over a flame until the solution becomes a clear, light yellowish brown. Cool, and add, dropwise, enough 30% hydrogen peroxide to decolorize the solution. Digest for 2–3 min, adding more hydrogen peroxide if necessary, to produce a colorless solution. Cool, dilute with water to 100 mL, and add potassium permanganate TS until a permanent pink color persists on heating. Then add hydrogen peroxide TS, dropwise, until the color is completely discharged. Cool, and add 5 mL of nitric acid that has been diluted with 10 mL of water. Add 5 mL of ferric ammonium sulfate TS, and titrate with *Titrant*. Each mL of 0.1 N ammonium thiocyanate is equivalent to 17.59 mg of nitromersol ($C_7H_5HgNO_3$).
Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Mercury Ions**
Sample solution: The filtrate obtained in *Identification* test *B, Analysis 2*
Analysis: Add an equal volume of hydrogen sulfide TS to the *Sample solution*.
Acceptance criteria: No darkening in color is produced, although a small amount of a flocculent, light yellow precipitate may form.
• **Alkali-Insoluble Substances**
Sample: 1.0 g of Nitromersol
Analysis: Add 7 mL of 1 N sodium hydroxide to the *Sample*, then dilute with water to 20 mL. The resulting solution, upon standing in a glass-stoppered vessel in the dark for 24 h, shows NMT a slight amount of insoluble material. Collect the insoluble residue, if any, in a tared filter crucible, wash the residue with warm water, and dry at 105° for 1 h.
Acceptance criteria: 0.1%; the weight of the insoluble material is NMT 1 mg.
• **Uncombined Nitrocresol**
Sample: 500 mg of Nitromersol

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Analysis: Shake the *Sample* with 50 mL of benzene, filter, evaporate the filtrate in a tared dish to dryness, and dry the residue at 80° for 2 h.
Acceptance criteria: 1%; the weight of the residue is NMT 5 mg.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Nitromersol Topical Solution

DEFINITION
Nitromersol Topical Solution yields NLT 180.0 mg and NMT 220.0 mg of nitromersol ($C_7H_5HgNO_3$) in each 100 mL.

Nitromersol	2 g
Sodium Hydroxide	0.4 g
Sodium Carbonate, Monohydrate	4.25 g
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Sodium Hydroxide* and the *Sodium Carbonate, Monohydrate* in 50 mL of *Purified Water*. Add the *Nitromersol*, and stir until dissolved. Gradually add *Purified Water* to make 1000 mL.
[NOTE—Prepare dilutions of Nitromersol Topical Solution as needed, because they tend to precipitate upon standing.]

IDENTIFICATION
- **A.**
Sample: 100 mL
Analysis: Add 3 mL of 3 N hydrochloric acid to the *Sample*.
Acceptance criteria: A yellowish precipitate is formed. Filter, and retain both the filtrate and the precipitate.
- **B.**
Sample: The precipitate obtained from *Identification* test *A*
Analysis: Add the *Sample* to 20 mL of water and 2.5 mL of 1 N sodium hydroxide. Add 500 mg of sodium hydrosulfite, and heat to boiling.
Acceptance criteria: A heavy deposit of metallic mercury is formed.

ASSAY
- **PROCEDURE**
Sample: 50.0 mL of Topical Solution
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N ammonium thiocyanate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 500-mL Kjeldahl flask, add a few glass beads, and evaporate to 5 mL. Add 15 mL of sulfuric acid, digest cautiously with occasional swirling over a flame until the solution becomes a clear, light yellowish brown. Cool, and add, dropwise, enough 30% hydrogen peroxide to decolorize the solution. Digest for 2–3 min, adding more hydrogen peroxide, if necessary, to produce a colorless solution. Cool, dilute with water to 100 mL, and add potassium permanganate TS until a permanent pink color persists on heating. Then add hydrogen peroxide TS, dropwise, until the color is completely discharged. Cool, and add 5 mL of nitric acid that has been diluted with 10 mL of water. Add 5 mL of ferric ammonium sulfate TS, and titrate with *Titrant*. Each mL of *Titrant* is equivalent to 17.59 mg of nitromersol ($C_7H_5HgNO_3$).
Acceptance criteria: 180.0–220.0 mg of nitromersol ($C_7H_5HgNO_3$) in each 100 mL

SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨841⟩: 1.005–1.010
- **MERCURY IONS**
Sample: The filtrate obtained from *Identification* test *A*
Analysis: To the *Sample* add an equal volume of hydrogen sulfide TS.
Acceptance criteria: No darkening in color is produced, although a small amount of a flocculent, light yellow precipitate may be formed.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers.

Nitrous Oxide

N_2O 44.01
Nitrogen oxide (N_2O).
Nitrogen oxide (N_2O) [10024-97-2].

» Nitrous Oxide contains not less than 99.0 percent, by volume, of N_2O.

Packaging and storage—Preserve in cylinders.
[NOTE—The following tests are designed to reflect the quality of Nitrous Oxide in both the vapor and liquid phases that are present in previously unopened cylinders. Reduce the container pressure by means of a regulator. Withdraw the samples for the tests with the least possible release of Nitrous Oxide consistent with proper purging of the sampling apparatus. Measure the gases with a gas volume meter downstream from the detector tubes in order to minimize contamination or change of the specimens. Perform tests in the sequence in which they are listed.]
The various detector tubes called for in the respective tests are listed under *Reagents* in the section *Reagents, Indicators, and Solutions*.

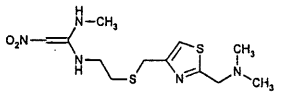
Identification—
A: With the container temperatures the same and maintained between 15° and 25°, concomitantly read the pressure of the Nitrous Oxide container and of a container of nitrous oxide certified standard (see under *Reagents* in the section *Reagents, Indicators, and Solutions*). [NOTE—Do not use the nitrous oxide certified standard if it has been depleted to less than half of its full capacity.] The pressure of the Nitrous Oxide container is within 50 psi of that of the nitrous oxide certified standard.
B: Pass 100 ± 5 mL released from the vapor phase of the contents of the Nitrous Oxide container through a carbon dioxide detector tube at the rate specified for the tube: no color change is observed (*distinction from carbon dioxide*).
C: Collect about 100 mL of the gas under test in a 100-mL tube fitted at the top with a stopcock. Open the stopcock, and quickly add a freshly prepared solution of 500 mg of pyrogallol in 2 mL of water and a freshly prepared solution of 12 g of potassium hydroxide in 8 mL of water. Immediately close the

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stopcock, and mix: the gas is not absorbed, and the solution does not become brown (*distinction from oxygen*).
Water—It meets the requirements of the test for *Water* under *Carbon Dioxide*.
Limit of ammonia—Proceed with Nitrous Oxide as directed in the test for *Carbon monoxide,* except to use an ammonia detector tube: the indicator change corresponds to not more than 0.0025%.
Limit of nitric oxide—Pass 500 ± 50 mL, released from the vapor phase of the contents of the container, through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 1 ppm.
Carbon monoxide—Pass 1000 ± 50 mL, released from the vapor phase of the contents of the container, through a carbon monoxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 0.001%.
Nitrogen dioxide—Arrange a container so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it, and to prevent frost from reaching the inlet of the detector tube. Release into the tubing a flow of liquid sufficient to provide 550 mL of the vaporized sample plus any excess necessary to ensure adequate flushing of air from the system. Pass 550 ± 50 mL of this gas through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 1 ppm.
Halogens—Pass 1000 ± 50 mL, released from the vapor phase of the contents of the container, through a chlorine detector tube at the rate specified for the tube: the indicator change corresponds to not more than 1 ppm.
Carbon dioxide—Pass 1000 ± 50 mL, released from the vapor phase of the contents of the container, through a carbon dioxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 0.03%.
Air—Not more than 1.0% of air is present, determined as directed in the *Assay*.
Assay—Introduce a specimen of Nitrous Oxide taken from the liquid phase, as directed in the test for *Nitrogen dioxide*, into a gas chromatograph by means of a gas-sampling valve. Select the operating conditions of the gas chromatograph such that the peak response resulting from the following procedure corresponds to not less than 70% of the full-scale reading. Preferably, use an apparatus corresponding to the general type in which the column is 6 m in length and 4 mm in inside diameter and is packed with porous polymer beads, which permits complete separation of N_2 and O_2 from N_2O, although the N_2 and O_2 may not be separated from each other. Use industrial grade helium (99.99%) as the carrier gas, with a thermal-conductivity detector, and control the column temperature: the peak response produced by the assay specimen exhibits a retention time corresponding to that produced by an air–helium certified standard (see under *Reagents* in the section *Reagents, Indicators, and Solutions*), and is equivalent to not more than 1.0% of air when compared to the peak response of the air-helium certified standard, indicating not less than 99.0%, by volume, of N_2O.

Nizatidine



$C_{12}H_{21}N_5O_2S_2$ 331.46
1,1-Ethenediamine, *N*-[2-[[[2-[(dimethylamino)methyl]-4-thiazolyl]methyl]thio]ethyl]-*N*′-methyl-2-nitro-.
N-[2-[[[2-[(Dimethylamino)methyl]-4-thiazolyl]methyl]thio]ethyl]- *N*′-methyl-2-nitro-1,1-ethenediamine [76963-41-2].

» Nizatidine contains not less than 98.0 percent and not more than 101.0 percent of $C_{12}H_{21}N_5O_2S_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Nizatidine RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy:* ⟨197K⟩▲ (CN 1-May-2020).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.
Loss on drying ⟨731⟩—Dry about 2 g, accurately weighed, at 100° for 1 hour: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Solution A—Use *Buffer solution* prepared as directed in the *Assay*.
Solution B—Use methanol.
Diluent—Prepare a mixture of *Solution A* and *Solution B* (76:24).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for the *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solutions—Dissolve an accurately weighed quantity of USP Nizatidine RS quantitatively, and stepwise if necessary, in *Diluent*, sonicating if necessary, to obtain a solution having a known concentration of 50 µg per mL (*Standard solution 1*). Quantitatively dilute portions of *Standard solution 1* with *Diluent* to obtain *Standard solution 2* and *Standard solution 3* having known concentrations of 25 µg per mL and 15 µg per mL, respectively.
Test solution—Prepare a solution of Nizatidine in *Diluent* having a concentration of about 5 mg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–3	76	24	isocratic
3–20	76→50	24→50	linear gradient
20–45	50	50	isocratic

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Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
45–50	50→76	50→24	linear gradient
50–70	76	24	isocratic

Make adjustments to the composition of the *Mobile phase*, if necessary, to obtain a retention time of about 12 minutes for the main nizatidine peak (see *System Suitability* under *Chromatography* ⟨621⟩). Chromatograph *Standard solution 1*, and record the peak areas as directed for *Procedure:* the tailing factor is not more than 2.0.

Procedure—Separately inject equal volumes (about 50 µL) of *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than three times the retention time of nizatidine. Record the chromatograms, and measure the areas for all the peaks. The sum of the peak areas, excluding the nizatidine peak area, obtained from the *Test solution* is not more than three times the main peak area obtained from *Standard solution 2;* and no single peak area obtained from the *Test solution* is greater than the main peak area obtained from *Standard solution 3:* not more than 0.3% of any individual impurity is found; and not more than 1.5% of total impurities is found.

Assay—

Buffer solution—Prepare a 0.1 M solution by dissolving 5.9 g of ammonium acetate in 760 mL of water. Add 1 mL of diethylamine, and adjust with acetic acid to a pH of 7.5.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (76:24). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Nizatidine RS in *Mobile phase*, sonicating if necessary, to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of 15 mg of Nizatidine to a 50-mL volumetric flask, dissolve in *Mobile phase*, sonicating if necessary, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure:* the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{12}H_{21}N_5O_2S_2$ in the portion of Nizatidine taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Nizatidine RS in the *Standard preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nizatidine Capsules

» Nizatidine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nizatidine ($C_{12}H_{21}N_5O_2S_2$).

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Nizatidine RS

Identification—

A: Empty the contents of 2 Capsules into a beaker, add 20 mL of methanol, and swirl for approximately 2 minutes. Filter through a filter paper, and evaporate the methanol solution with a current of cool air to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Nizatidine RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.

Procedure—Determine the amount of $C_{12}H_{21}N_5O_2S_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 314 nm using filtered portions of the solution under test, diluted with water if necessary, in comparison with a Standard solution having a known concentration of USP Nizatidine RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{12}H_{21}N_5O_2S_2$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity— [NOTE—Use peak areas where peak responses are indicated.]

Buffer solution and *Mobile phase*—Prepare as directed in the *Assay* under *Nizatidine*.

Standard solution—Dissolve an accurately weighed quantity of USP Nizatidine RS in *Mobile phase* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 40 µg per mL.

Test solution—Remove as completely as possible the contents of not less than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of nizatidine, to a 100-mL volumetric flask, add 50 mL of *Mobile phase*, and sonicate for about 3 minutes. Dilute with *Mobile phase* to volume, mix, and filter.

System suitability solution—Prepare a solution of nizatidine and phenol in *Mobile phase* containing 40 µg of each per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure:* the resolution, *R*, between the nizatidine and phenol peaks is not less than 1.5, the tailing factor for the nizatidine peak is not greater than 1.5, and the relative standard deviation of the nizatidine peak for replicate injections is not more than 2%.

Procedure—Chromatograph about 10 µL of the *Standard solution* and the *Test solution*, and run the chromatograph for twice the elution time of nizatidine. Record the chromatograms, and measure the peak responses. Calculate

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the percentage of each impurity in the portion of Capsules taken by the formula:

$$2(r_i/r_s)$$

in which r_i is the response of each impurity peak in the *Test solution*, and r_s is the response of the nizatidine peak in the *Standard solution:* not more than 0.5% of any individual impurity and not more than 1.5% of total impurities is found.

Assay— [NOTE—Use peak areas where peak responses are indicated.]

Buffer solution and *Mobile phase*—Prepare as directed in the *Assay* under *Nizatidine.*

Internal standard solution—Prepare a solution of phenol in *Mobile phase* having a concentration of 0.1 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Nizatidine RS in *Internal standard solution* to obtain a solution having a known concentration of 0.1 mg per mL.

Assay preparation—Weigh accurately not less than 10 Capsules. Remove as completely as possible the contents of the Capsules, and mix the combined contents. Clean and accurately weigh the Capsule shells, and calculate the net weight of the Capsule contents. Transfer an accurately weighed portion of the mixed Capsule contents equivalent to about 500 mg of nizatidine to a 500-mL volumetric flask, add 200 mL of *Internal standard solution,* and sonicate for a few minutes. Cool, dilute with *Internal standard solution* to volume, and mix. Filter a portion of the solution, transfer 1.0 mL of the filtered solution to a 10-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

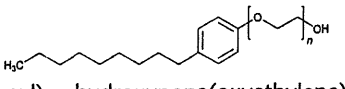
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between nizatidine and the internal standard phenol, is not less than 3; the tailing factor, *T,* for the nizatidine peak is not more than 1.6; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for phenol and 1.0 for nizatidine. Calculate the quantity, in mg, of $C_{12}H_{21}N_5O_2S_2$ in the portion of Capsules taken by the formula:

$$5000C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Nizatidine RS in the *Standard preparation,* and R_U and R_S are the ratios of the peak response of the nizatidine to that of the internal standard for the *Assay preparation* and the *Standard preparation,* respectively.

Nonoxynol 9



α-(*p*-Nonylphenyl)-ω-hydroxynona(oxyethylene) [26027-38-3].

DEFINITION

Nonoxynol 9 is an anhydrous liquid mixture consisting chiefly of monononylphenyl ethers of polyethylene glycols corresponding to the formula:

$$C_9H_{19}C_6H_4(OCH_2CH_2)_nOH$$

in which the average value of n is 9. It contains NLT 90.0% and NMT 110.0% of nonoxynol 9.

IDENTIFICATION

- **A. INFRARED ABSORPTION:** Its IR absorption spectrum, obtained by spreading a capillary film of it between sodium chloride plates, exhibits maxima at 1117 cm^{-1} (strong); at 1512, 1582, and 1610 cm^{-1} (medium, sharp); at 2871, 2928, and 2956 cm^{-1} (strong, unresolved); at 831 cm^{-1} (medium, broad); and at 1250 cm^{-1} (medium, sharp).
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and water (80:20)

System suitability solution: 25 mg/mL each of octoxynol 9 and USP Nonoxynol 9 RS in *Mobile phase*

Standard solution: 25 mg/mL of USP Nonoxynol 9 RS in *Mobile phase*

Sample solution: 25 mg/mL of Nonoxynol 9 in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 25-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

[NOTE—Nonoxynol oligomers elute as a major peak, usually with shoulders and bumps. Include these in the peak response for Nonoxynol 9.]

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the responses for Nonoxynol 9, including any shoulders and bumps.

Calculate the percentage of Nonoxynol 9 in the portion of specimen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nonoxynol 9 from the *Sample solution*

r_S = peak response of nonoxynol 9 from the *Standard solution*

C_S = concentration of USP Nonoxynol 9 RS in the *Standard solution* (mg/mL)

C_U = concentration of Nonoxynol 9 in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **FREE ETHYLENE OXIDE**

Stripped nonoxynol 9: Maintain Nonoxynol 9 at a temperature of 150° with constant stirring in an open vessel until it no longer displays a peak for ethylene oxide when chromatographed as directed below.

USP Monographs

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System suitability solution: 10 µg/mL each of ethylene oxide and acetaldehyde in *Stripped nonoxynol 9*

Standard stock solutions: [NOTE—Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated hood, using great care.] Chill all apparatus and reagents used in the solution of standards in a refrigerator or freezer before use. Fill a chilled pressure bottle with liquid ethylene oxide from a lecture bottle, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Transfer 100 mL of chilled isopropyl alcohol to a 500-mL volumetric flask. Using a chilled graduated cylinder, transfer 25 mL of ethylene oxide to the isopropyl alcohol, and swirl gently to mix. Dilute with additional chilled isopropyl alcohol to volume, replace the stopper, and swirl gently to mix. This stock solution contains 43.6 mg/mL of ethylene oxide.

Pipet 25 mL of 0.5 N alcoholic hydrochloric acid, prepared by mixing 45 mL of hydrochloric acid with 1 L of alcohol, into a 500-mL conical flask containing 40 g of magnesium chloride hexahydrate. Shake the mixture to effect saturation. Pipet 10 mL of the ethylene oxide solution into the flask, and add 20 drops of bromocresol green TS. If the solution is not yellow (acid), add an additional volume of 0.5 N alcoholic hydrochloric acid to give an excess of 10 mL. Record the total volume of 0.5 N alcoholic hydrochloric acid added. Insert the stopper in the flask, and allow to stand for 30 min. Titrate the excess acid with 0.5 N alcoholic potassium hydroxide VS. Perform a blank titration, using 10.0 mL of isopropyl alcohol instead of ethylene oxide solution, adding the same total volume of 0.5 N alcoholic hydrochloric acid, and note the difference in volumes required. Each mL of the difference in volumes of 0.5 N alcoholic potassium hydroxide consumed is equivalent to 22.02 mg of ethylene oxide. Calculate the concentration, in mg/mL, of ethylene oxide in the stock solution. Standardize daily. Store in a refrigerator.

Prepare a 1000-ppm standard by pipeting the calculated volume (2 mL) of cold stock solution that, on the basis of the standardization, contains 88.6 mg of ethylene oxide, into a container and adding 87.0 g of *Stripped nonoxynol 9*. Prepare 10-, 5-, and 0.5-ppm standards by quantitatively diluting the 1000-ppm standard with additional *Stripped nonoxynol 9*.

Standard solutions: Transfer 5 ± 0.01 g of each *Standard stock solution* to suitable serum vials equipped with pressure-tight septum closures designed to relieve any excessive pressure, and seal them.

Sample solution: Transfer 5 ± 0.01 g of Nonoxynol 9 to a serum vial of the same kind as the vials used for the *Standard solutions*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 6.4-m × 2.1-mm nickel; packed with 60- to 80-mesh support S9

Temperatures

Detector: 200°

Injection port: 160°

Column: 100°

Carrier gas: Helium

Flow rate: 30 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution* and *Standard solutions*

Calibration: Place the vial containing the 10-ppm ethylene oxide *Standard solution* in an oven, and heat at 90° for 30 min. Remove the vial from the oven. Using a gas-tight syringe, immediately inject a 100-µL aliquot of the headspace gas into the gas chromatograph. Obtain the area for the ethylene oxide peak (retention time about 8 min). Raise the temperature of the column to 200° after ethylene oxide elutes to volatilize heavy components. Re-equilibrate the column at 100°. Repeat the foregoing steps, using the vials containing the 5- and 0.5-ppm *Standard solution*. Plot area units versus ppm of ethylene oxide for the standards on linear graph paper, and draw the best straight line through the points.

Suitability requirements

Resolution: NLT 1.5 between ethylene oxide and acetaldehyde, *System suitability solution*

Calibration: None of the points used for constructing the straight line *Calibration* curve deviates from the line by more than 10%.

Analysis

Samples: *Standard solutions* and *Sample solution*

Place the vial containing the *Sample solution* in an oven, and heat at 90° for 30 min. Remove the vial from the oven. Immediately inject a 100-µL aliquot of the headspace gas into the gas chromatograph, and obtain the area for the ethylene oxide peak.

Calculate the concentration of ethylene oxide in the sample specimen in ppm:

$$\text{Result} = r_U \times S$$

r_U = peak area from the *Sample solution*

S = slope of the standard curve (ppm/area unit)

Acceptance criteria: NMT 1 ppm

• **LIMIT OF DIOXANE**

Apparatus: Assemble a closed-system vacuum distillation apparatus (see *Figure 1*), using glass vacuum stopcocks (*A*, *B*, and *C*). The concentrator tube (*D*)[1] is made of borosilicate or quartz (not flint) glass, graduated precisely enough to measure the 0.9 mL or more of distillate collected, and marked so that the analyst can dilute accurately to 2.0 mL.

Standard solution: 100 µg/mL of dioxane in water. Use a freshly prepared solution.

Sample solution: Transfer 20.0 g to a 50-mL round-bottom flask (*E*) having a 24/40 ground-glass neck joint. Add 1.0 mL of water. Place a small polytef-covered stirring bar in the flask, insert the stopper, and stir to mix. Immerse the flask in an ice bath, and chill for 1 min. Wrap heating tape around the tube connecting the concentrator tube (*D*) and the round-bottom flask, and apply 10 V to the tape. Apply a light coating of high-vacuum silicone grease to the ground-glass joints, and connect the concentrator tube to the 10/30 joint and the round-bottom flask to the 24/40 joint. Immerse the vacuum trap in a Dewar flask filled with liquid nitrogen, close stopcocks *A* and *B*, open stopcock *C*, and begin evacuating the system with a vacuum pump. Prepare a slurry bath from powdered dry ice and methanol, and raise the bath to the neck of the round-bottom flask. After freezing the contents of the flask for 10 min, and when the vacuum system is operating at a 0.05-mm pressure or lower, open stopcock *A* for 20 s, then close it. Remove the slurry bath, and allow the flask to warm in air for 1 min. Immerse the flask in a water bath maintained at a temperature between 20° and 25°, and after 5 min warm the water bath to between 35° and 40° (sufficient to liquefy most specimens) while stirring slowly but constantly with the magnetic bar. Cool the water in the bath by adding ice, and chill for 2 min. Replace the water bath with the slurry bath, freeze the contents of the round-bottom flask for

[1] A suitable tube is available as Chromaflex concentrator tube, Kontes Glass Co., Vineland, NJ (Catalog No. K42560-0000).

USP Monographs

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10 min, open stopcock *A* for 20 s, and then close it. Remove the slurry bath, and repeat the heating steps as before, this time reaching a final temperature between 45° and 50° or a temperature necessary to melt the specimen completely. If there is any condensation in the tube connecting the round-bottom flask to the concentrator tube, slowly increase the voltage to the heating tape, and heat until condensation disappears.

Stir with the magnetic stirrer throughout the following steps. Very slowly immerse the concentrator tube in a Dewar flask containing liquid nitrogen. [**CAUTION**—When there is liquid distillate in the concentrator tube, immerse the tube in the liquid nitrogen very slowly, or the tube will break.] Water will begin to distill into the concentrator tube. As ice forms in the concentrator tube, raise the Dewar flask to keep the liquid nitrogen level only slightly below the level of ice in the tube. When water begins to freeze in the neck of the 10/30 joint, or when liquid nitrogen reaches the 2.0-mL graduation mark on the concentrator tube, remove the Dewar flask, and allow the ice to melt without heating. After the ice has melted, check the volume of water that has distilled, and repeat the sequence of chilling and thawing until NLT 0.9 mL of water has been collected. Freeze the tube once again for 2 min, and release the vacuum first by opening stopcock *B*, followed by opening stopcock *A*. Remove the concentrator tube from the apparatus, close it with a greased stopper, and allow the ice to melt without heating. Mix the contents of the concentrator tube by swirling, note the volume of distillate, and dilute with water to 2.0 mL, if necessary.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m glass; contains support S10
Temperatures
Detector: 250°
Injection port: 200°
Column: 140°
Carrier gas: Nitrogen or helium
Flow rate: 35 mL/min
Injection volume: 2–4 µL

Install an oxygen scrubber between the carrier gas line and the column. Condition the column for 72 h at 230° with 30–40 mL/min carrier flow. [NOTE—Support S10 is oxygen sensitive. Each time a column is installed, flush with carrier gas for 30–60 min before heating.]

Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The height of the peak of the *Sample solution* is NMT that of the *Standard solution*, corresponding to NMT 10 µg/g.

SPECIFIC TESTS

• **POLYETHYLENE GLYCOL**
Sample: 10 g
Analysis: Transfer the *Sample* to a 250-mL beaker. Add 100 mL of ethyl acetate, and stir on a magnetic stirrer to effect solution. Transfer, with the aid of 100 mL of 5 N sodium chloride, to a pear-shaped, 500-mL separator fitted with a glass stopper. Insert the stopper, and shake vigorously for 1 min. Remove the stopper carefully to release the pressure. Immerse a thermometer in the mixture, and support the separator so that it is partially immersed in a water bath maintained at 50°. Swirl the separator gently while letting the internal temperature rise to between 40° and 45°, then immediately remove the separator from the bath, dry the outside surface, and drain the salt (lower) layer into another pear-shaped, 500-mL separator. In the same manner, extract the ethyl acetate layer a second time with 100 mL of fresh 5 N sodium chloride, combining the two aqueous extracts. Discard the ethyl acetate layer. Wash the combined aqueous layers with 100 mL of ethyl acetate, using the same technique, and drain the salt (lower) layer into a clean pear-shaped, 500-mL separator. Discard the ethyl acetate layer. Extract the aqueous layer with two successive 100-mL portions of chloroform, draining the chloroform (lower) layers through Whatman folded filter paper 2V, and combining them into a 250-mL beaker. Evaporate on a steam bath to dryness, and continue heating until the odor of chloroform is no longer perceptible. Allow the beaker to cool. Add 25 mL of acetone, and dissolve the residue on a magnetic stirrer. Filter through Whatman folded filter paper 2V into a tared 250-mL beaker, rinsing with two 25-mL portions of acetone. Evaporate on a steam bath to dryness. Dry under vacuum at 60° for 1 h. Allow the beaker to cool, and weigh.
Acceptance criteria: NMT 1.0% of polyethylene glycol

• **CLOUD POINT**
Sample: 1.0 g
Analysis: Transfer the *Sample* to a 250-mL beaker, add 99 g of water, and mix to dissolve. Pour 30 mL of the resulting solution into a 70-mL test tube. Support the test tube in a hot water bath, and stir the contents with a thermometer constantly until the solution becomes cloudy, then remove the test tube from the bath immediately so that the temperature rises NMT 2° further, and continue stirring.

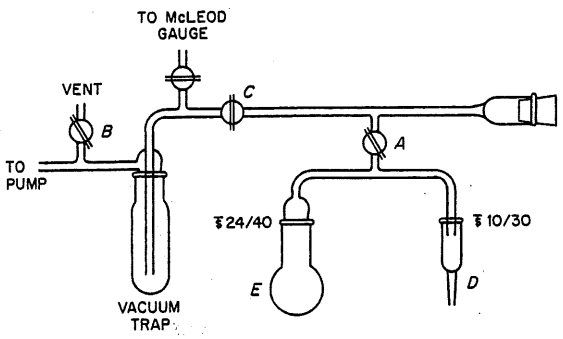


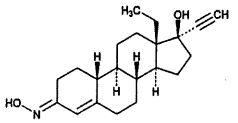
Figure 1. Closed-System Vacuum Distillation Apparatus for Dioxane (Nonoxynol 9)

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The cloud point is the temperature at which the solution becomes sufficiently clear that the entire thermometer bulb is seen plainly.
Acceptance criteria: 52°–56°
- **FATS AND FIXED OILS,** *Acid Value* ⟨401⟩**:** NMT 0.2
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nonoxynol 9 RS

Norelgestromin



$C_{21}H_{29}NO_2$ 327.46
18,19-Dinorpregn-4-en-20-yn-3-one, 13-ethyl-17-hydroxy-, oxime, (17α)-;
13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one oxime [53016-31-2].

DEFINITION
Norelgestromin is a mixture of (*E*)- and (*Z*)-isomers having a ratio of (*E*)- to (*Z*)-isomer between 1.3 and 1.6, and the sum of both isomers is NLT 98.0% and NMT 102.0% of norelgestromin ($C_{21}H_{29}NO_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Cyclohexane and *absolute alcohol* (100:2)
  Diluent: Cyclohexane and *absolute alcohol* (90:10)
  System suitability solution: 1.5 mg/mL of USP Norelgestromin RS and 8 µg/mL of USP Norelgestromin Related Compound A RS in *Diluent*
  Standard solution: 1.5 mg/mL of USP Norelgestromin RS in *Diluent*
  Sample solution: 1.5 mg/mL of Norelgestromin in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L20
  Column temperature: 50°
  Flow rate: 1.2 mL/min
  Injection volume: 25 µL
  Run time: NLT 1.6 times the retention time of (*Z*)-norelgestromin
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for the (*Z*)-isomer of norelgestromin related compound A, (*E*)-norelgestromin, and (*Z*)-norelgestromin are about 0.77, 0.85, and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 1.4 between the (*Z*)-isomer of norelgestromin related compound A and (*E*)-norelgestromin, *System suitability solution*
  Tailing factor: 0.8–1.2 for both (*E*)- and (*Z*)-norelgestromin, *Standard solution*
  Relative standard deviation: NMT 0.73% for both (*E*)- and (*Z*)-norelgestromin, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of norelgestromin ($C_{21}H_{29}NO_2$) in the portion of Norelgestromin taken:

$$\text{Result} = \{[(r_{UE} \times F) + r_{UZ}]/[(r_{SE} \times F) + r_{SZ}]\} \times (C_S/C_U) \times 100$$

r_{UE} = peak response of (*E*)-norelgestromin from the *Sample solution*
F = response factor for (*E*)-norelgestromin, 1.04
r_{UZ} = peak response of (*Z*)-norelgestromin from the *Sample solution*
r_{SE} = peak response of (*E*)-norelgestromin from the *Standard solution*
r_{SZ} = peak response of (*Z*)-norelgestromin from the *Standard solution*
C_S = concentration of USP Norelgestromin RS in the *Standard solution* (mg/mL)
C_U = concentration of Norelgestromin in the *Sample solution* (mg/mL)

Calculate the ratio of (*E*)- to (*Z*)-norelgestromin:

$$\text{Result} = (r_{UE} \times F)/r_{UZ}$$

r_{UE} = peak response of (*E*)-norelgestromin from the *Sample solution*
F = response factor for (*E*)-norelgestromin, 1.04
r_{UZ} = peak response of (*Z*)-norelgestromin from the *Sample solution*

Acceptance criteria
Both isomers: 98.0%–102.0% on the anhydrous basis
Ratio of (*E*)- to (*Z*)-isomer: 1.3–1.6

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
- **ORGANIC IMPURITIES**
  Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Sensitivity solution: 1.5 µg/mL of USP Norelgestromin RS in *Diluent* from the *Standard solution*
  System suitability
  Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*
  Suitability requirements
  Resolution: NLT 1.4 between the (*Z*)-isomer of norelgestromin related compound A and (*E*)-norelgestromin, *System suitability solution*
  Tailing factor: 0.8–1.2 for both (*E*)- and (*Z*)-norelgestromin, *Standard solution*
  Relative standard deviation: NMT 0.73% for both (*E*)- and (*Z*)-norelgestromin, *Standard solution*
  Signal-to-noise ratio: NLT 3 for both (*E*)- and (*Z*)-norelgestromin, *Sensitivity solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of each impurity in the portion of Norelgestromin taken:

$$\text{Result} = \{r_U/[(r_{SE} \times F) + r_{SZ}]\} \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_{SE} = peak response of (*E*)-norelgestromin from the *Standard solution*
F = response factor for (*E*)-norelgestromin, 1.04
r_{SZ} = peak response of (*Z*)-norelgestromin from the *Standard solution*
C_S = concentration of USP Norelgestromin RS in the *Standard solution* (mg/mL)
C_U = concentration of Norelgestromin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard peaks that are less than 0.05% of the total peak areas of (*E*)- and (*Z*)-norelgestromin.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Norgestrel[a]	0.37	0.5
(*E*)- and (*Z*)-Norgestimate[b]	0.43[c], 0.47[d]	0.5[e]
Norelgestromin 5(10)-ene[f,g]	0.68	—
Norelgestromin related compound A[g]	0.73[h], 0.77[i]	—
(*E*)-Norelgestromin	0.85	—
(*Z*)-Norelgestromin	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	1.0

[a] (±)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one.
[b] 18,19-Dinor-17-pregn-4-en-20-yn-3-one, 17-(acetyloxy)-13-ethyl-,oxime, (17α)-(+)-; (+)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one oxime acetate (ester).
[c] (*E*)-Norgestimate.
[d] (*Z*)-Norgestimate.
[e] The combined limits for (*E*)- and (*Z*)-norgestimate are NMT 0.5%.
[f] 13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(10)-en-20-yn-3-one oxime.
[g] This is not a specified impurity and is included in this table for identification only. It is not to be reported or included in the total impurities.
[h] (*E*)-Isomer of norelgestromin related compound A.
[i] (*Z*)-Isomer of norelgestromin related compound A.

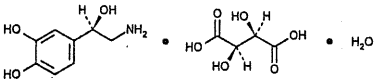
SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 5 mg/mL in alcohol and water (75:25)
  Acceptance criteria: +35° to +41°
- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Norelgestromin RS
  USP Norelgestromin Related Compound A RS
  Mixture of (*E*)- and (*Z*)-isomers.
  13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(6)-en-20-yn-3-one oxime.
  $C_{21}H_{29}NO_2$ 327.46

Norepinephrine Bitartrate



$C_8H_{11}NO_3 \cdot C_4H_6O_6 \cdot H_2O$ 337.28
$C_8H_{11}NO_2 \cdot C_4H_6O_6$ 319.27
1,2-Benzenediol, 4-(2-amino-1-hydroxyethyl)-, (*R*)-,[*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1) (salt), monohydrate;
(–)-α-(Aminomethyl)-3,4-dihydroxybenzyl alcohol tartrate (1:1) (salt), monohydrate [108341-18-0].
Anhydrous [51-40-1].

DEFINITION

Norepinephrine Bitartrate contains NLT 97.0% and NMT 102.0% of $C_8H_{11}NO_3 \cdot C_4H_6O_6$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. PROCEDURE**
  Sample solution: 5 mg/mL in water
  Analysis: Add 1 drop of ferric chloride TS to 2 mL of *Sample solution*.
  Acceptance criteria: An intensely green color develops.
- **C. PROCEDURE**
  Sample solution: 0.01 µg/mL
  Analysis: Add 1.0 mL of 0.10 N iodine to 10 mL of the *Sample solution*. Allow to stand for 5 min, and add 2.0 mL of 0.10 N sodium thiosulfate.
  Acceptance criteria: The solution is colorless, or has at most a slight pink or slight violet color (epinephrine and isoproterenol at the same pH, about 3.5, give a strong red-brown or violet color).

ASSAY

- **PROCEDURE**
  Sample solution: Dissolve 500 mg of Norepinephrine Bitartrate in 20 mL of glacial acetic acid, warming slightly if necessary to effect solution.
  Analysis: Add 2 drops of crystal violet TS to the *Sample solution*, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 31.93 mg of $C_8H_{11}NO_3 \cdot C_4H_6O_6$.
  Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%, from 200 mg

ORGANIC IMPURITIES

- **Procedure: Limit of Arterenone**
  Sample solution: 2 mg/mL in water
  Analysis: Determine the absorptivity of the *Sample solution* (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 310 nm.
  Acceptance criteria: NMT 0.2

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: –10° to –12°
  Sample solution: 50 mg/mL in water
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 4.5%–5.8%

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Norepinephrine Bitartrate RS

Norepinephrine Bitartrate Injection

» Norepinephrine Bitartrate Injection is a sterile solution of Norepinephrine Bitartrate in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of norepinephrine ($C_8H_{11}NO_3$).

Packaging and storage—Preserve in single-dose, light-resistant containers, preferably of Type I glass.
Labeling—Label the Injection in terms of mg of norepinephrine per mL, and, where necessary, label it to indicate that it must be diluted prior to use. The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
USP Reference standards ⟨11⟩—
USP Norepinephrine Bitartrate RS

Color and clarity—
Standard solution—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Visually examine a portion of the Injection *(Test solution)* in a suitable clear glass test tube against a white background: it is not pinkish and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution.*

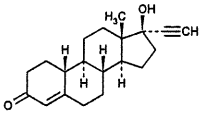
Identification—
A: It responds to *Identification* test *B* under *Norepinephrine Bitartrate.*
B: Dilute the Injection with water to a concentration of 1 mg in 5 mL. To 10 mL of the dilution add 2.0 mL of 0.10 N iodine, allow to stand for 5 minutes, then add 3.0 mL of 0.10 N sodium thiosulfate: the solution is colorless or has at most a slight pink or slight violet color (*epinephrine and isoproterenol at the same pH, about 3.5, give a red-brown or violet color*).
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 83.4 USP Endotoxin Units per mg of norepinephrine.
pH ⟨791⟩: between 3.0 and 4.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Dissolve 1.1 g of sodium 1-heptanesulfonate in 800 mL of water. Add 200 mL of methanol, and adjust with 1 M phosphoric acid to a pH of 3.0 ± 0.1. Pass through a membrane filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Norepinephrine Bitartrate RS in freshly prepared dilute acetic acid (1 in 25), and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.4 mg of norepinephrine bitartrate monohydrate per mL.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of norepinephrine, to a 25-mL volumetric flask, add dilute acetic acid (1 in 25) to volume, and mix.
System suitability preparation—Dissolve a suitable quantity of isoproterenol hydrochloride in the *Standard preparation* to obtain a solution containing, in each mL, 0.4 mg of USP Norepinephrine Bitartrate RS and 0.4 mg of isoproterenol hydrochloride.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed under *Procedure:* the tailing factor for the analyte peak is not more than 2.5, the resolution, *R*, between the norepinephrine and isoproterenol peaks is not less than 4.0, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of norepinephrine ($C_8H_{11}NO_3$) in each mL of the Injection taken by the formula:

$$(169.18/337.29)(25C/V)(r_U/r_S)$$

in which 169.18 and 337.29 are the molecular weights of norepinephrine and norepinephrine bitartrate monohydrate, respectively; *C* is the concentration, in mg per mL, of USP Norepinephrine Bitartrate RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norethindrone



$C_{20}H_{26}O_2$ 298.42
19-Norpregn-4-en-20-yn-3-one, 17-hydroxy-, (17α)-.
17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one [68-22-4].

» Norethindrone contains not less than 97.0 percent and not more than 102.0 percent of $C_{20}H_{26}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Norethindrone RS
Completeness of solution—The solution called for in the test for *Specific rotation* is clear and free from undissolved solid.

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)
Melting range ⟨741⟩: between 202° and 208°.

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Specific rotation ⟨781S⟩: between −30° and −38°.
Test solution: 20 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 3 hours: it loses not more than 0.5% of its weight.
Limit of ethynyl group—Dissolve 200 mg in about 40 mL of tetrahydrofuran. Add 10 mL of silver nitrate solution (1 in 10), and titrate with 0.1 N sodium hydroxide VS, either a glass-calomel or a silver–silver chloride electrode system with potassium nitrate filling solution. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 2.503 mg of ethynyl group (–C≡CH). Not less than 8.18% and not more than 8.43% of ethynyl group is found.

Chromatographic purity—
Test solution—Prepare a solution of Norethindrone in chloroform to contain 10 mg per mL.
Standard solutions—Prepare a solution of USP Norethindrone RS in chloroform to contain 10 mg per mL (*Standard stock solution*). Dilute accurately measured volumes of *Standard stock solution* with chloroform to obtain *Standard solutions A, B,C,* and *D* having known concentrations of 150, 50, 30, and 10 µg per mL, respectively.
Procedure—Separately apply 10 µL of the *Test solution* and 10 µL of each *Standard solution* to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform and methanol (95:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of methanol and sulfuric acid (7:3), then heat the plate at 100° for 5 minutes: the R_F value of the principal spot from the *Test solution* corresponds to that of the principal spot from *Standard solution A*. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solution*:no secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from *Standard solution B* (0.5%), and the sum of the intensities of the secondary spots obtained from the *Test solution* is not more intense than the principal spot obtained from *Standard solution A* (1.5%).
Assay—Dissolve about 100 mg of Norethindrone, accurately weighed, in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a solution containing about 10 µg per mL. Dissolve an accurately weighed quantity of USP Norethindrone RS in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, using alcohol as the blank. Calculate the quantity, in mg, of $C_{20}H_{26}O_2$ in the portion of Norethindrone taken by the formula:

$$10C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Norethindrone RS in the Standard solution, and A_U and A_S are the absorbances of the solution of Norethindrone and the Standard solution, respectively.

Norethindrone Tablets

DEFINITION
Norethindrone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone ($C_{20}H_{26}O_2$).

IDENTIFICATION
• **A. Infrared Absorption**
Analysis: Mix an amount of powdered Tablets equivalent to 50 mg of norethindrone with 15 mL of solvent hexane. Stir the solution occasionally for 15 min. Centrifuge the mixture, then decant and discard the solvent hexane. Extract the residue with two 10-mL portions of solvent hexane, centrifuging and decanting as before, and discard the solvent hexane. Add 25 mL of chloroform to the residue, mix by shaking for 1–2 min, and filter. Evaporate the filtrate to about 3 mL, add a few mL of solvent hexane to induce crystallization, and evaporate to dryness.
Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Norethindrone RS.

ASSAY
• **Procedure**
Solution A: Dissolve 1.0 g of isoniazid in 1000 mL of anhydrous methanol, and add 1.3 mL of hydrochloric acid.
Standard stock solution: 14 µg/mL of USP Norethindrone RS in methanol
Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a suitable container, add 2.0 mL of *Solution A*, mix, seal, and allow to stand for 30 min.
Sample stock solution: Transfer an amount of finely powdered Tablets equivalent to 0.7 mg of norethindrone (powder NLT 20 Tablets) to a 50-mL volumetric flask, and add anhydrous methanol to volume. Mix, and allow to stand for 10 min, with occasional mixing. Filter a portion of the mixture to clarify the solution. Use the filtrate.
Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a suitable container. Add 2.0 mL of *Solution A*, mix, seal, and allow to stand for 30 min.
Sample blank solution: Transfer 10.0 mL of the *Sample stock solution* to a suitable container, add 2.0 mL of methanol, and mix.
Reagent blank solution: Transfer 10.0 mL of methanol to a suitable container, add 2.0 mL of *Solution A*, mix, seal, and allow to stand for 30 min.
Instrumental conditions
Mode: UV
Analytical wavelength: 380 nm
Analysis
Samples: *Standard solution, Sample solution, Sample blank solution,* and *Reagent blank solution*
Concomitantly determine the absorbances of these solutions, using methanol as the reference for the *Sample blank solution,* and using the *Reagent blank solution* as the reference for the *Sample solution* and the *Standard solution.*
Calculate the precentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) in the portion of Tablets taken:

$$\text{Result} = (A_U - A_{UB})/A_S \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_{UB} = absorbance of the *Sample blank solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Norethindrone RS in the *Standard solution* (µg/mL)

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C_U = nominal concentration of norethindrone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.09% Sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 75 rpm

Time: 30 min

Mobile phase: Acetonitrile and water (2:3)

Standard stock solution: 0.07 mg/mL of USP Norethindrone RS in methanol. Sonication may be used to aid dissolution.

Standard solution: (*L*/500) mg/mL of USP Norethindrone RS in *Medium* from the *Standard stock solution*, where *L* is the claim in mg/Tablet of norethindrone

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of norethindrone in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.09% Sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 75 rpm

Time: 45 min

Mobile phase: Acetonitrile and 0.02 M phosphate buffer pH 6.0 (35:65)

Standard stock solution: 0.028 mg/mL of USP Norethindrone RS in methanol

Standard solution: (*L*/500) mg/mL of USP Norethindrone RS in *Medium* from the *Standard stock solution*, where *L* is the claim in mg/Tablet of norethindrone

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size or centrifuge at least 10 mL of the solution under test and use the supernatant.

Use one of the following two chromatographic systems. (See *Chromatography* ⟨621⟩, *System Suitability*.)

Chromatographic system 1

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

Chromatographic system 2

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 10-cm; 3- or 3.5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Use this *System suitability* for either *Chromatographic system 1* or *Chromatographic system 2*.

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of norethindrone in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.5% Sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 75 rpm

Time: 20 min

Mobile phase: Acetonitrile and water (50:50)

Standard stock solution: 0.028 mg/mL of USP Norethindrone RS in methanol. Sonication may be used to aid dissolution.

Standard solution: (*L*/500) mg/mL of USP Norethindrone RS in *Medium* from the *Standard stock solution*, where *L* is the claim in mg/Tablet of norethindrone

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size or centrifuge and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.3 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) dissolved:

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$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of norethindrone in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP Reference Standards** ⟨11⟩
USP Norethindrone RS

Norethindrone and Ethinyl Estradiol Tablets

DEFINITION

Norethindrone and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone ($C_{20}H_{26}O_2$), and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

• **A. Thin-Layer Chromatographic Test**

Standard solution: 1 mg/mL of USP Norethindrone RS and 50 µg/mL of USP Ethinyl Estradiol RS in alcohol

Sample solution: Crush 1 Tablet in 1 mL of alcohol in a 15-mL conical centrifuge tube, and warm to 50° for 10 min with gentle swirling. Cool, and centrifuge to obtain a clear solution.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel and previously activated by heating at 105° for 30 min
Application volume: 20 µL
Developing solvent system: Toluene and ethyl acetate (4:1)
Spray reagent: Sulfuric acid–methanol, prepared by cautiously adding 70 mL of sulfuric acid in small increments to 30 mL of methanol chilled in an ice-bath, and mixing

Analysis
Samples: *Standard solution* and *Sample solution*
Apply both *Standard solution* and *Sample solution* at a line 2.5 cm from the bottom of a thin-layer chromatographic plate. Develop the chromatogram in *Developing solvent system* until the solvent front has moved 10 cm. Remove the plate, and air-dry. Spray the plate with *Spray reagent*.

Acceptance criteria: The spots from the *Sample solution* have the same R_F values as the spots from USP Ethinyl Estradiol RS and from USP Norethindrone RS.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile and water (60:40)
Diluent: Acetonitrile and water (45:55)
Internal standard solution: Transfer 15 mg of valerophenone into a 250-mL volumetric flask, add 125 mL of acetonitrile, and dilute with water to volume.
Ethinyl estradiol standard stock solution: 0.09 mg/mL of USP Ethinyl Estradiol RS in acetonitrile
Norethindrone standard stock solution: 1.25 mg/mL of USP Norethindrone RS in acetonitrile
Standard solution: Transfer 5.0 mL of *Internal standard solution* into a 100-mL volumetric flask. Add volumes of *Ethinyl estradiol standard stock solution* and *Norethindrone standard stock solution* so that the final known concentrations, in mg/mL, of the Reference Standards correspond numerically to one-twentieth of the labeled amounts of the corresponding ingredients in the Tablets. Add ($26 - X$) mL of acetonitrile, X being the total volume of the standard stock solution taken. Dilute with *Diluent* to volume.
Sample stock solution: Transfer 10 Tablets to a 100-mL volumetric flask, add 20 mL of water, and shake by mechanical means until the Tablets are completely disintegrated. Add 10.0 mL of *Internal standard solution* and 60 mL of acetonitrile, and mix. Sonicate for 2 min. Dilute with acetonitrile to volume, and mix. Allow solid particles to settle, or centrifuge if necessary to obtain a slightly turbid solution.
Sample solution: Dilute 5.0 mL of the *Sample stock solution* with *Diluent* to 10.0 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for ethinyl estradiol and norethindrone are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between norethindrone and ethinyl estradiol
Column efficiency: NLT 8000 theoretical plates from the internal standard peak
Relative standard deviation: NMT 2.0% for six replicate injections for norethindrone and ethinyl estradiol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) in each Tablet:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio, at corresponding retention times, of the *Sample solution*
R_S = peak response ratio, at corresponding retention times, of the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding sample in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of norethindrone ($C_{20}H_{26}O_2$), and 90.0%–110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$)

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PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

[NOTE—Exercise care in filtering and handling solutions containing ethinyl estradiol to prevent adsorptive loss of the drug. Centrifugation may be used instead of filtration with nonadsorptive membrane filters. Withdraw dissolution aliquots with glass or polytef pipets or syringes that have been checked for adsorptive loss. Use glass dissolution vessels and polytef-coated or solid polytef paddles.]

Test 1

Medium: 0.09% sodium dodecyl sulfate in 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 75 rpm

Time: 60 min

Buffer: 0.02 M pH 6.0 phosphate buffer

Mobile phase: Acetonitrile and *Buffer* (35:65)

Standard solution: USP Norethindrone RS and USP Ethinyl Estradiol RS in *Medium* with concentrations similar to those expected in the solution under test. [NOTE—A volume of methanol not exceeding 4% of the total final volume of the *Standard solution* may be used in preparing the *Standard solution*.]

Sample solution: A filtered portion of the solution under test.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 5-mm × 8.3-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for norethindrone and ethinyl estradiol are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between norethindrone and ethinyl estradiol

Column efficiency: NLT 7000 theoretical plates for the ethinyl estradiol peak

Tailing factor: NMT 2.0 for either peak

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved by comparison of the corresponding peak responses of the *Standard solution* and the solution under test.

Tolerances: NLT 75% (*Q*) of each of the labeled amounts of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$), respectively, are dissolved in 60 min.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.09% (w/v) sodium dodecyl sulfate in 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 75 rpm

Time: 30 min

Buffer: 0.02 M pH 6.0 phosphate buffer prepared as follows. Dissolve 2.72 g of potassium phosphate, monobasic in 1 L of water. Adjust with 10% potassium hydroxide solution in water to a pH of 6.0.

Mobile phase: Acetonitrile and *Buffer* (42:58)

Standard stock solution 1: 100 µg/mL of USP Norethindrone RS in acetonitrile. Sonicate to dissolve as needed.

Standard stock solution 2: 3.5 µg/mL of USP Ethinyl Estradiol RS in acetonitrile. Sonicate to dissolve as needed.

Standard solution: 0.8 µg/mL of USP Norethindrone RS and 0.07 µg/mL of USP Ethinyl Estradiol RS in *Medium*, from *Standard stock solution 1* and *Standard stock solution 2*.

Sample solutions: Centrifuge a portion of the solution under test at about 3000 rpm for 3 min, and use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 0.8 mL/min

Injection volume: 100 µL

Run time: NLT 2 times the retention time of ethinyl estradiol

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for norethindrone and ethinyl estradiol are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between norethindrone and ethinyl estradiol

Tailing factor: NMT 2.0 for norethindrone and ethinyl estradiol

Relative standard deviation: NMT 3.0% for norethindrone and ethinyl estradiol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of norethindrone or ethinyl estradiol from the *Sample solution*

r_S = peak response of corresponding USP Reference Standard from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)

V = volume of *Medium*, 500 mL

L = label claim of norethindrone or ethinyl estradiol (µg/Tablet)

Tolerances: NLT 80% (*Q*) of each of the labeled amounts of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$), respectively, are dissolved in 30 min.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.09% (w/v) sodium dodecyl sulfate in 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 75 rpm

Time: 30 min

Buffer: 0.02 M pH 6.0 phosphate buffer prepared as follows. Dissolve 2.72 g of potassium phosphate, monobasic in 1 L of water. Adjust with 0.1 N sodium hydroxide solution in water to a pH of 6.0.

Mobile phase: Acetonitrile and *Buffer* (38:62)

Norethindrone standard stock solution: 0.8 mg/mL of USP Norethindrone RS in methanol. Sonicate to dissolve as needed.

Ethinyl estradiol standard stock solution: 0.135 mg/mL of USP Ethinyl Estradiol RS in methanol. Sonicate to dissolve as needed.

Standard solution: USP Norethindrone RS and USP Ethinyl Estradiol RS in *Medium* with concentrations similar to those expected in the solution under test, from

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Norethindrone standard stock solution and *Ethinyl estradiol standard stock solution*. Add intermediate diluting steps as needed.

Sample solution: Pass a portion of the solution under test through a filter of 0.45-µm pore size and use the clear filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: NLT 1.4 times the retention time of ethinyl estradiol

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for norethindrone and ethinyl estradiol are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between norethindrone and ethinyl estradiol
Tailing factor: NMT 2.0 for norethindrone and ethinyl estradiol
Relative standard deviation: NMT 3.0% for norethindrone and ethinyl estradiol

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of norethindrone or ethinyl estradiol from the *Sample solution*
r_S = peak response of corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
V = volume of *Medium*, 500 mL
L = label claim of norethindrone or ethinyl estradiol (µg/Tablet)

Tolerances: NLT 80% (Q) of each of the labeled amounts of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$), respectively, are dissolved in 30 min.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements with respect to norethindrone and to ethinyl estradiol

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethinyl Estradiol RS
USP Norethindrone RS

Norethindrone and Mestranol Tablets

» Norethindrone and Mestranol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norethindrone ($C_{20}H_{26}O_2$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mestranol ($C_{21}H_{26}O_2$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Mestranol RS
USP Norethindrone RS
Identification—Crush 1 Tablet in 1 mL of alcohol in a 15-mL conical centrifuge tube, and centrifuge briefly. Apply 10 µL of this test solution and 10 µL each of solutions containing, respectively, about 1 mg per mL of USP Norethindrone RS in alcohol and about 50 µg per mL of USP Mestranol RS in alcohol at equidistant points along a line about 2.5 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel and previously activated by heating at 105° for 30 minutes. Develop the chromatogram in a mixture of equal volumes of ethyl acetate and cyclohexane in a suitable chamber, previously equilibrated with the solvent mixture, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, air-dry, and observe under short-wavelength UV light: the principal spot from the test solution appears at the same R_F value as the principal spot from USP Norethindrone RS, at about R_F 0.6. Spray the plate with a sulfuric acid and methanol mixture prepared by cautiously adding and mixing sulfuric acid in small increments to 30 mL of chilled anhydrous methanol in a 100-mL volumetric flask. Adjust to room temperature, dilute with sulfuric acid to volume, and mix. Heat the plate at 105° for 10 minutes: the pink spot from the test solution appears at the same R_F value as the pink spot from USP Mestranol RS (about R_F 0.8).
Dissolution ⟨711⟩— [NOTE—Exercise care in filtering solutions containing mestranol to prevent adsorptive loss of the drug. Centrifugation may be used instead of filtration with nonadsorptive membrane filters. Withdraw dissolution aliquots with glass or polytef pipets or syringes that have been checked for adsorptive loss. Use glass dissolution vessels and polytef-coated or solid polytef paddles.]
Medium: 0.09% sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL.
Apparatus 2: 75 rpm.
Time: 60 minutes.
Determine the amounts of norethindrone ($C_{20}H_{26}O_2$) and mestranol ($C_{21}H_{26}O_2$) dissolved, employing the following method.
Mobile phase—Prepare a degassed and filtered mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph replicate injections of a filtered portion of a Standard solution of USP Norethindrone RS and USP Mestranol RS in *Dissolution Medium* having known concentrations similar to those expected in the solution under test, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 3.0%. The minimum number of

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theoretical plates for the mestranol peak is 4000, and the tailing factors for the norethindrone and mestranol peaks do not exceed 1.5.

Procedure—Separately inject equal volumes (about 200 µL) of the Standard solution and a filtered portion of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.4 for norethindrone and 1.0 for mestranol. Calculate the quantities of norethindrone and mestranol dissolved by comparison of the corresponding peak responses obtained from the Standard solution and the test solutions.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{20}H_{26}O_2$ and 75% *(Q)* of the labeled amount of $C_{21}H_{26}O_2$ are dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Content Uniformity* with respect to norethindrone and to mestranol.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer about 80 mg of progesterone into a 100-mL volumetric flask, add 50 mL of acetonitrile, dilute with water to volume, and mix.

Mestranol standard stock solution—Dissolve an accurately weighed quantity of USP Mestranol RS in acetonitrile, and dilute quantitatively and stepwise with acetonitrile to obtain a solution having a known concentration of about 0.05 mg per mL.

Norethindrone standard stock solution—Using an accurately weighed quantity of USP Norethindrone RS, prepare a solution in acetonitrile having a known concentration of about 1 mg per mL.

Standard preparation—Transfer 2.0 mL of *Internal standard solution* into a 100-mL volumetric flask. Add accurately measured volumes of *Mestranol standard stock solution* and *Norethindrone standard stock solution* so that the final known concentrations, in mg per mL, of the Reference Standards correspond numerically to about one-fiftieth of the labeled amounts of the corresponding ingredients in the Tablets. Add 50 mL of water, dilute with acetonitrile to volume, and mix.

Assay preparation—Transfer 10 Tablets to a 250-mL volumetric flask, add 50 mL of water, and shake by mechanical means until the Tablets are completely disintegrated. Add 10.0 mL of *Internal standard solution* and 165 mL of acetonitrile, and mix. Sonicate for about 2 minutes. Dilute with acetonitrile to volume, and mix. Allow solid particles to settle, or centrifuge if necessary, to obtain a slightly turbid solution. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, add 1.0 mL of acetonitrile, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the mestranol peak is not less than 6000 theoretical plates, the resolution, *R*, between the progesterone and mestranol peaks is not less than 5.0, and the relative standard deviation for six replicate injections is not more than 2.0% (both peaks).

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2.5 for mestranol and 1.0 for norethindrone. Calculate the quantities, in mg, of norethindrone ($C_{20}H_{26}O_2$) and mestranol ($C_{21}H_{26}O_2$) in each Tablet taken by the formula:

$$50C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*, and R_U and R_S are the peak response ratios, at corresponding retention times, obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norethindrone Acetate

$C_{22}H_{28}O_3$ 340.46

19-Norpregn-4-en-20-yn-3-one, 17-(acetyloxy)-, (17α).

17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one acetate [51-98-9].

» Norethindrone Acetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{22}H_{28}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Norethindrone Acetate RS

Completeness of solution—The solution prepared for the determination of *Specific rotation* is clear and free from undissolved solids.

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Specific rotation ⟨781S⟩: between –32° and –38°.

Test solution: 20 mg per mL, in dioxane.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Limit of ethynyl group—Proceed as directed in the test for *Ethynyl group* under *Norethindrone*. Not less than 7.13% and not more than 7.57% of ethynyl group is found.

Chromatographic purity—

TEST 1—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Prepare a solution of Norethindrone Acetate in chloroform having a concentration of 10 mg per mL.

Standard stock solution—Prepare a solution of USP Norethindrone Acetate RS in chloroform having a known concentration of 10 mg per mL.

Standard solutions—Dilute accurately measured volumes of the *Standard stock solution* with chloroform to obtain *Standard solutions A, B, C,* and *D* having known concentrations of 150 µg per mL, 50 µg per mL, 30 µg per mL, and 10 µg per mL, respectively.

Application volume: 10 µL, as two 5-µL portions.

Developing solvent system: a mixture of toluene and ethyl acetate (1:1).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩, except to apply the solutions along a line 2.5 cm from the edge of the plate. Spray the plate with a mixture of methanol and sulfuric acid (7:3), and heat at 100° for 5 minutes. The *Test solution* exhibits a principal spot at the same R_F value as the principal

USP Monographs

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spot of *Standard solution A*. Any individual secondary spot is not more intense than the spot in the chromatogram obtained from *Standard solution B:* not more than 0.5% of any individual impurity is found, and the total of impurities found is not more than 1.5%.

TEST 2—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Dissolve accurately weighed quantities of desoxycorticosterone acetate and USP Norethindrone Acetate RS in *Mobile phase* to obtain a solution having concentrations of about 80 µg of each per mL.

Test solution—Transfer about 62.5 mg of Norethindrone Acetate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Diluted test solution—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.83 for desoxycorticosterone acetate and 1.0 for norethindrone acetate; and the resolution, *R*, between desoxycorticosterone acetate and norethindrone acetate is not less than 3.5.

Procedure—Separately inject equal volumes (about 20 µL) of the *Diluted test solution* and the *Test solution* into the chromatograph, record the chromatograms for twice the retention time of norethindrone acetate, and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Norethindrone Acetate taken by the formula:

$$r_i/r_s$$

in which r_i is the peak area for each impurity obtained from the *Test solution;* and r_s is the sum of all the peaks obtained from the *Diluted test solution*. [NOTE—Exclude any peak having a response that is less than 0.025%.] Not more than 0.5% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—Transfer about 100 mg of Norethindrone Acetate, accurately weighed, to a 200-mL volumetric flask, add alcohol to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with alcohol to volume, and mix. Dissolve an accurately weighed quantity of USP Norethindrone Acetate RS in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of $C_{22}H_{28}O_3$ in the portion of Norethindrone Acetate taken by the formula:

$$10C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Norethindrone Acetate RS in the Standard solution, and A_U and A_S are the absorbances of the solution of Norethindrone Acetate and the Standard solution, respectively.

Norethindrone Acetate Tablets

DEFINITION

Norethindrone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$).

IDENTIFICATION

Delete the following:

▲• **A. INFRARED ABSORPTION** ⟨197K⟩

Analysis: Mix an amount of powdered Tablets equivalent to 50 mg of norethindrone with 15 mL of solvent hexane. Stir the solution occasionally for 15 min. Centrifuge the mixture, then decant and discard the solvent hexane. Extract the residue with two 10-mL portions of solvent hexane, centrifuging and decanting as before, and discard the solvent hexane. Add 25 mL of chloroform to the residue, mix by shaking for 1–2 min, and filter. Evaporate the filtrate to about 3 mL, add a few mL of solvent hexane to induce crystallization, and evaporate to dryness.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Norethindrone Acetate RS. ▲ 2S (USP41)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**

▲**Solution A:** Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	60	40
4.0	60	40
40.0	40	60
50.0	10	90
55.0	10	90
56.0	60	40
60.0	60	40

Diluent: Methanol and water (60:40)

System suitability stock solution: 0.1 mg/mL each of USP Norethindrone Acetate RS, USP Norethindrone RS, and USP Norethindrone Related Compound B RS prepared as follows. Transfer the Reference Standards to a suitable

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volumetric flask. Add 50% of the final flask volume of acetonitrile to dissolve, and dilute with *Diluent* to volume.
System suitability solution: 0.05 mg/mL each of USP Norethindrone Acetate RS, USP Norethindrone RS, and USP Norethindrone Related Compound B RS in *Diluent* from *System suitability stock solution*
Standard solution: 0.1 mg/mL of USP Norethindrone Acetate RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of norethindrone acetate in *Diluent* prepared as follows. Transfer a quantity of finely powdered Tablets (NLT 20) equivalent to 20 mg of norethindrone acetate to a 20-mL volumetric flask. Add 15 mL of *Diluent* and sonicate for 30 min. Dilute with *Diluent* to volume. Centrifuge to obtain a clear supernatant. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between norethindrone related compound B and norethindrone, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of norethindrone acetate in the *Sample solution* (mg/mL) ▲2S (USP41)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Dilute hydrochloric acid (1 in 100) containing 0.02% of sodium lauryl sulfate; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Standard solution: A known concentration of USP Norethindrone Acetate RS in *Medium*. [NOTE—The *Standard solution* may be prepared by dissolving the Reference Standard in a volume of methanol, not exceeding 0.5% of the final volume of the solution, and diluting quantitatively with *Medium*.]
Sample solution: Filter portions of the solution under test. Dilute with *Medium* if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 248 nm, measured from a baseline drawn from 350 to 310 nm and extending beyond the maximum peak.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (Q) of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.2 g/L of sodium lauryl sulfate and 10 mL/L of hydrochloric acid in water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Mobile phase: Acetonitrile and water (60:40)
Standard stock solution: 0.275 mg/mL of USP Norethindrone Acetate RS prepared as follows. Transfer an appropriate amount of USP Norethindrone Acetate RS to a suitable volumetric flask and dissolve in 50% of the flask volume of methanol. Sonicate to dissolve as needed. Dilute with *Medium* to volume.
Standard solution: 0.0011 mg/mL of USP Norethindrone Acetate RS from the *Standard stock solution* prepared as follows. Dilute 2 mL of the *Standard stock solution* with *Medium* to 100 mL. Further dilute 5 mL of the resultant solution with *Mobile phase* to 25 mL. Pass a portion through a suitable filter paper.
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute 5 mL of the filtrate with *Mobile phase* to 25 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: 1.5 times the retention time of norethindrone acetate
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times D \times 100$$

r_U = peak response of norethindrone acetate from the *Sample solution*
r_S = peak response of norethindrone acetate from the *Standard solution*

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C_S = concentration of USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*, 5

Tolerances: NLT 80% (Q) of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$) is dissolved.

Change to read:

• **Uniformity of Dosage Units** ⟨905⟩, *Content Uniformity*: Meet the requirements
▲▲ 2S (USP41)

IMPURITIES

Add the following:

▲• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, and System suitability solution: Prepare as directed in the *Assay*.
Standard solution: 0.005 mg/mL of USP Norethindrone Acetate RS in *Diluent*
Sample solution: Nominally 1.0 mg/mL of norethindrone acetate in *Diluent* prepared as follows. Transfer a quantity of finely powdered Tablets (NLT 20) equivalent to 20 mg of norethindrone acetate to a 20-mL volumetric flask. Add 15 mL of *Diluent* and sonicate for 30 min. Dilute with *Diluent* to volume. Centrifuge to obtain a clear supernatant. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm for norethindrone acetate 5(10)-ene isomer; UV 254 nm for all other impurities
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between norethindrone and norethindrone related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of norethindrone acetate 5(10)-ene isomer (detected at UV 210 nm) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of norethindrone acetate 5(10)-ene isomer from the *Sample solution*
r_S = peak response of norethindrone acetate from the *Standard solution*
C_S = concentration of USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of norethindrone acetate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of each specified or unspecified degradation product (detected at UV 254 nm) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified or unspecified degradation product from the *Sample solution*
r_S = peak response of norethindrone acetate from the *Standard solution*
C_S = concentration of USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of norethindrone acetate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6β-Hydroxynorethindrone acetate[a, b]	0.34	0.53	—
Norethindrone related compound B[b]	0.46	1.0	—
Norethindrone	0.51	1.0	0.5
6-Keto norethindrone acetate[c]	0.59	0.73	0.5
6β-Acetylnorethindrone acetate[b, d]	0.67	1.0	—
17-Acetyl norethindrone acetate analog[b, e]	0.82	1.0	—
Norethindrone acetate	1.00	1.0	—
Destrimethylene quingestanol acetate[b, f]	1.63	1.0	—
Desethylene quingestanol acetate[b, g]	1.67	1.0	—
Norethindrone acetate 5-ene isomer[b, h]	1.09	0.57	—
Norethindrone acetate 5(10)-ene isomer[i]	1.15	0.73	0.5
Any individual degradation product	—	1.0	0.5
Total impurities	—	—	2.0

[a] 6β-Hydroxy-3-oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate.
[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities of the drug product.
[c] 3,6-Dioxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate.
[d] 6β-Acetyl-3-oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate.
[e] 3,20-Dioxo-19-nor-17α-pregn-4-en-17-yl acetate.
[f] 3-Ethoxy-19-nor-17βα-pregn-3,5-dien-20-yn-17-yl acetate.
[g] 3-Isopropoxy-19-nor-17βα-pregn-3,5-dien-20-yn-17-yl acetate.
[h] 3-Oxo-19-nor-17α-pregn-5-en-20-yn-17-yl acetate.
[i] 3-Oxo-19-nor-17α-pregn-5(10)-en-20-yn-17-yl acetate. ▲ 2S (USP41)

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ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. ▲Store at controlled room temperature.▲ 2S (USP41)
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  ▲USP Norethindrone RS▲ 2S (USP41)
  USP Norethindrone Acetate RS
  ▲USP Norethindrone Related Compound B RS
  Estr-4-ene-3,17-dione.
  $C_{18}H_{24}O_2$ 272.39▲ 2S (USP41)

Norethindrone Acetate and Ethinyl Estradiol Tablets

DEFINITION

Norethindrone Acetate and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$), and NLT 88.0% and NMT 112.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

A. The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (40:5:55)
Diluent: Acetonitrile and water (1:1)
Standard solution: 90 µg/mL of USP Norethindrone Acetate RS and 1.76 µg/mL of USP Ethinyl Estradiol RS in *Diluent*
Sample solution: Transfer an appropriate number of Tablets, NLT 20, to a volumetric flask, such that the final concentration is 100 µg/mL of norethindrone acetate based on the label claim. Fill the flask with *Diluent* to about 75% of volume, and disintegrate the Tablets by mechanical shaking and sonication. Allow the solution to equilibrate to room temperature, and dilute with *Diluent* to volume. Centrifuge a portion of the solution using glass centrifuge tubes, and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethinyl estradiol and norethindrone acetate are about 0.28 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 10.0 between ethinyl estradiol and norethindrone acetate
Tailing factor: NMT 2.0 for ethinyl estradiol and norethindrone acetate
Relative standard deviation: NMT 2.0% for ethinyl estradiol and norethindrone acetate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$) and ethinyl estradiol ($C_{20}H_{24}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*
r_S = peak response of the corresponding analyte from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$); 88.0%–112.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$)

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

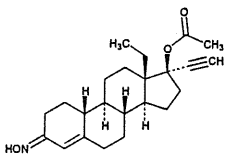
0.025 M acetate buffer solution: Transfer 5.22 g of anhydrous sodium acetate and 2.2 g of glacial acetic acid to a 4-L volumetric flask, add 3.5 L of water, and adjust with 1 N sodium hydroxide to a pH of 5.0 ± 0.2. Dilute with water to volume.
Medium: *0.025 M acetate buffer solution* with 0.15% sodium lauryl sulfate, prepared by weighing 6 g of sodium lauryl sulfate into a 4-L volumetric flask, adding 1.5 L of *0.025 M acetate buffer solution*, mixing, and diluting with *0.025 M acetate buffer solution* to volume; 600 mL
Apparatus 2: 75 rpm
Time: 60 min
Mobile phase: 0.2% phosphoric acid, acetonitrile, and tetrahydrofuran (540:380:80)
Standard solution: USP Norethindrone Acetate RS and USP Ethinyl Estradiol RS dissolved in a minimum amount of acetonitrile, and diluted with *Medium* to obtain a solution having known concentrations equivalent to the expected concentrations of the solution under test
Sample solution: Withdraw a 2-mL aliquot, using a glass pipet or syringe, and centrifuge at about 2000 rpm for about 5 min. Use the supernatant as the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm detector and a fluorescence detector with an excitation wavelength set at 210 nm and an emission wavelength set at 310 nm
Column: 6-mm × 40-mm; 3-µm packing L1
Guard column: 4-mm × 12.5-mm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 200 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 500 theoretical plates for ethinyl estradiol and NLT 1400 theoretical plates for norethindrone acetate
Tailing factor: NMT 2.0 for norethindrone acetate and ethinyl estradiol
Relative standard deviation: NMT 2.5% for norethindrone acetate and ethinyl estradiol

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Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (Q) of the labeled amounts of norethindrone acetate ($C_{22}H_{28}O_3$) and ethinyl estradiol ($C_{20}H_{24}O_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethinyl Estradiol RS
USP Norethindrone Acetate RS

Norgestimate



$C_{23}H_{31}NO_3$ 369.50
18,19-Dinor-17-pregn-4-en-20-yn-3-one, 17-(acetyloxy)-13-ethyl-, oxime, (17α)-(+)-;
(+)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one oxime acetate (ester) [35189-28-7].

DEFINITION
Norgestimate is a mixture of (*E*)- and (*Z*)-isomers having a ratio of (*E*)- to (*Z*)-isomer between 1.27 and 1.78, and it contains NLT 98.0% and NMT 102.0% of norgestimate ($C_{23}H_{31}NO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Use a dispersion prepared by mixing with potassium bromide in a 1-to-100 ratio.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Diluent: Methanol and water (4:1)
Mobile phase: Tetrahydrofuran, acetonitrile, and water (22:18:60)
Standard solution: 0.5 mg/mL of USP Norgestimate RS in *Diluent*
Sensitivity solution: 0.05 µg/mL of USP Norgestimate RS in *Diluent*, from the *Standard solution*
Sample solution: 0.5 mg/mL of Norgestimate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 244 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 25 µL

System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The relative retention times for (*Z*)-norgestimate and (*E*)-norgestimate are about 0.86 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between (*Z*)-norgestimate and (*E*)-norgestimate, *Standard solution*
Tailing factor: NMT 1.5 for (*E*)-norgestimate and for (*Z*)-norgestimate, *Standard solution*
Relative standard deviation: NMT 2.0% for the peak area ratio of (*E*)-norgestimate to (*Z*)-norgestimate, *Standard solution*
Signal-to-noise ratio: NLT 3.0 for (*Z*)-norgestimate, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of norgestimate ($C_{23}H_{31}NO_3$) in the portion of Norgestimate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak areas of (*Z*)-norgestimate and (*E*)-norgestimate from the *Sample solution*
r_S = sum of the peak areas of (*Z*)-norgestimate and (*E*)-norgestimate from the *Standard solution*
C_S = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)
C_U = concentration of Norgestimate in the *Sample solution* (mg/mL)

Calculate the percentages of the (*Z*)- and (*E*)-isomers, U_Z and U_E, respectively, in the portion of Norgestimate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of the appropriate norgestimate isomer from the *Sample solution*
r_S = peak response of the appropriate norgestimate isomer from the *Standard solution*
C_S = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)
C_U = concentration of Norgestimate in the *Sample solution* (mg/mL)
P = fraction of (*E*)- or (*Z*)-norgestimate in USP Norgestimate RS

Calculate the ratio of U_E to U_Z.
Acceptance criteria: 98.0%–102.0% of norgestimate ($C_{23}H_{31}NO_3$) on the dried basis; the ratio of (*E*)- to (*Z*)-isomer is 1.27–1.78.

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
• **ORGANIC IMPURITIES, PROCEDURE 1**
Diluent, Mobile phase, Standard solution, Sensitivity solution, and **Sample solution:** Proceed as directed in the *Assay*.
System suitability solution: 0.5 mg/mL each of USP Norgestimate RS, USP Norgestimate Related Compound A RS, and USP Deacetylnorgestimate RS in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 244 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min

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Injection volume: 25 µL
System suitability
Samples: *Sensitivity solution* and *System suitability solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between (*Z*)-17-deacetylnorgestimate and (*E*)-17-deacetylnorgestimate; NLT 1.5 between (*E*)-17-deacetylnorgestimate and norgestimate related compound A, *System suitability solution*
Tailing factor: NMT 1.5 for (*E*)-norgestimate and for (*Z*)-norgestimate, *System suitability solution*
Relative standard deviation: NMT 2.0% for the peak area ratio of (*E*)-norgestimate to (*Z*)-norgestimate, *System suitability solution*
Signal-to-noise ratio: NLT 3.0 for (*Z*)-norgestimate, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Norgestimate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times P \times 100$$

r_U = peak area for each impurity from the *Sample solution*
r_S = peak area of (*E*)-norgestimate from the *Standard solution*
C_S = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)
C_U = concentration of Norgestimate in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 1*)
P = fraction of (*E*)-norgestimate in USP Norgestimate RS

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
(*Z*)-17-Deacetylnorgestimate[a]	0.50	0.83	0.3
(*E*)-17-Deacetylnorgestimate[a]	0.56	1.13	0.3
Norgestimate related compound A[b]	0.72	0.85	0.3
(*Z*)-Norgestimate	0.86	—	—
(*E*)-Norgestimate	1.0	—	—
Any other impurity	—	1.0	0.1

[a] The combined limits for (*Z*)- and (*E*)-17-deacetylnorgestimate are NMT 0.3%.
[b] Levonorgestrel acetate.

• **ORGANIC IMPURITIES, PROCEDURE 2**
Mobile phase: Cyclohexane and absolute alcohol (50:1)
Standard solution: 1.0 mg/mL of USP Norgestimate RS in *Mobile phase*
Sensitivity solution: 0.5 µg/mL of USP Norgestimate RS in *Mobile phase* from the *Standard solution*
Sample solution: 1.0 mg/mL of Norgestimate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L20
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The retention time for (*E*)-norgestimate is about 18.6 min; see *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between (*Z*)-norgestimate and (*E*)-norgestimate, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, for the peak area ratio of (*Z*)-norgestimate to (*E*)-norgestimate, *Standard solution*
Signal-to-noise ratio: NLT 3.0 for (*E*)-norgestimate, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Norgestimate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times P \times 100$$

r_U = peak area for each impurity from the *Sample solution*
r_S = peak area of (*E*)-norgestimate from the *Standard solution*
C_S = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)
C_U = concentration of Norgestimate in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 2*)
P = fraction of (*E*)-norgestimate in USP Norgestimate RS

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Unidentified impurity	0.74	1.4	0.2
Unidentified impurity	0.78	1.5	0.1
Unidentified impurity	0.91	1.2	0.1
(*E*)-Norgestimate	1.0	—	—
(*Z*)-Norgestimate	1.1	—	—
Total impurities[a]	—	—	1.0

[a] Sum of the impurities found in *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2*.

• **LIMIT OF RESIDUAL SOLVENTS** ⟨467⟩
Internal standard solution: 2 µL/100 mL of isobutyl alcohol in dimethylformamide
Standard solution: 5 µL/100 mL each of acetone, alcohol, chloroform, diisopropyl ether, and methanol in *Internal standard solution*
System suitability solution: 0.05 µL/100 mL each of acetone, alcohol, chloroform, diisopropyl ether, and methanol in *Internal standard solution* from the *Standard solution*

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Sample solution: 40 mg of Norgestimate and 2 mL of *Internal standard solution*; shake well to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica capillary column bonded with a 1-µm layer of phase G16
Temperatures
Injection port: 180°
Detector: 250°
Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
65	—	65	2.5
65	35	100	2
100	30	160	2.5

Carrier gas: Helium
Flow rate: 6 mL/min
Injection mode: Split
Split flow: 16 mL/min
Injection volume: 1 µL
System suitability
Samples: *Internal standard solution*, *Standard solution*, and *System suitability solution*
[NOTE—The retention time of isobutyl alcohol in the *Internal standard solution* is 4–5 min.]
Suitability requirements
There are no interfering peaks due to dimethylformamide.
Relative standard deviation: NMT 3.0%, determined from the peak response ratio of each solvent to the internal standard, *Standard solution*
Signal-to-noise ratio: NLT 2.0 for alcohol, *System suitability solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each solvent in the portion of Norgestimate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/W) \times D \times V \times 100$$

R_U = peak response ratio of the relevant analyte to the internal standard from the *Sample solution*
R_S = peak response ratio of the relevant analyte to the internal standard from the *Standard solution*
C_S = concentration of each solvent in the *Standard solution* (mL/mL)
W = weight of Norgestimate used to prepare the *Sample solution* (mg)
D = density of each solvent (mg/mL)
V = volume of *Internal standard solution* used to prepare the *Sample solution* (mL)

Acceptance criteria
Option 1: NMT 0.5% each of acetone and alcohol is found, NMT 0.05% of diisopropyl ether is found, NMT 0.006% of chloroform is found, and NMT 0.3% of methanol is found.
Option 2: Meets the requirements in *Residual Solvents* ⟨467⟩

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in chloroform
Acceptance criteria: +40° to +46°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Deacetylnorgestimate RS
Mixture of (*E*)- and (*Z*)-17-deacetylnorgestimate.
$C_{21}H_{29}NO_2$ 327.46
USP Norgestimate RS
USP Norgestimate Related Compound A RS
Levonorgestrel acetate.
$C_{23}H_{30}O_3$ 354.48

Norgestimate and Ethinyl Estradiol Tablets

DEFINITION
Norgestimate and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of norgestimate ($C_{23}H_{31}NO_3$) and ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Tetrahydrofuran, methanol, and water (5:2:13)
Internal standard solution: 0.05 mg/mL of dibutyl phthalate in methanol
Standard solution: Dissolve appropriate quantities of USP Ethinyl Estradiol RS and USP Norgestimate RS in a volume of *Internal standard solution* equivalent to 80% of the final volume. Add a volume of water equivalent to 20% of the final volume, and mix to obtain a solution having a known concentration of about 7 µg/mL of ethinyl estradiol and a known concentration of norgestimate similar to that expected in the *Sample solution*. Pass through a suitable filter of 0.45-µm pore size.
Sample solution: Add a number of Tablets, equivalent to 0.35 mg of ethinyl estradiol, to a suitable glass container. Add 10 mL of water, and mix with a vortex mixer until the Tablets are completely disintegrated. Add 40 mL of *Internal standard solution*, and mix with a vortex mixer for at least 23 min. Sonicate the sample for at least 5 min, pass an aliquot through a suitable filter of 0.45-µm pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 2.1 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethinyl estradiol, (*Z*)-norgestimate, (*E*)-norgestimate, and

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dibutyl phthalate are about 0.5, 1.0, 1.2, and 1.5, respectively.]

Suitability requirements

Resolution: NLT 1.5 between (*Z*)-norgestimate and (*E*)-norgestimate

Relative standard deviation: NMT 2.0% for the peak response ratio of ethinyl estradiol, (*Z*)-norgestimate, and (*E*)-norgestimate to dibutyl phthalate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_{UE}/R_{SE}) \times (C_{SE}/C_{UE}) \times 100$$

R_{UE} = ratio of the peak responses of ethinyl estradiol to dibutyl phthalate from the *Sample solution*

R_{SE} = ratio of the peak responses of ethinyl estradiol to dibutyl phthalate from the *Standard solution*

C_{SE} = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)

C_{UE} = nominal concentration of ethinyl estradiol in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of norgestimate ($C_{23}H_{31}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (C_{SN}/C_{UN}) \times [P_A(R_{UA}/R_{SA}) + P_S(R_{US}/R_{SS})] \times 100$$

C_{SN} = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)

C_{UN} = nominal concentration of norgestimate in the *Sample solution* (mg/mL)

P_A = fraction of (*E*)-norgestimate in USP Norgestimate RS

R_{UA} = ratio of the peak responses of (*E*)-norgestimate to dibutyl phthalate from the *Sample solution*

R_{SA} = ratio of the peak responses of (*E*)-norgestimate to dibutyl phthalate from the *Standard solution*

P_S = fraction of (*Z*)-norgestimate in the USP Norgestimate RS

R_{US} = ratio of the peak responses of (*Z*)-norgestimate to dibutyl phthalate from the *Sample solution*

R_{SS} = ratio of the peak responses of (*Z*)-norgestimate to dibutyl phthalate from the *Standard solution*

Calculate the ratio of the content of (*Z*)-norgestimate to ethinyl estradiol in the portion of Tablets taken, for use in the test for *Organic Impurities*:

$$C_Z/C_E = [(C_{SN} \times P_S) \times (R_{US}/R_{SS})]/[C_{SE}(R_{UE}/R_{SE})]$$

The terms are as defined above.

Acceptance criteria: 90.0%–110.0% each of ethinyl estradiol and norgestimate

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩**:** 15 min
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, and Sample solution: Prepare as directed in the *Assay*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: 254 nm

Column: 4.6-mm × 5-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol, (*Z*)-norgestimate, and (*E*)-norgestimate are about 0.5, 1.0, and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.5 between (*Z*)-norgestimate and (*E*)-norgestimate

Relative standard deviation: NMT 2.0% for the (*Z*)-norgestimate and (*E*)-norgestimate peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of any impurity having a relative retention time of about 0.2 or 0.4, relative to the (*Z*)-norgestimate peak, and detected at 254 nm in the portion of Tablets taken:

$$\text{Result} = (r_U/r_Z) \times (C_Z/C_E) \times F \times 100$$

r_U = peak response for each impurity

r_Z = peak response for (*Z*)-norgestimate

C_Z/C_E = ratio of (*Z*)-norgestimate to ethinyl estradiol, as defined in the *Assay*

F = relative response factor of these impurities, 1.54

Acceptance criteria: The sum of the impurities having relative retention times of about 0.2 and 0.4 is NMT 4.0%.

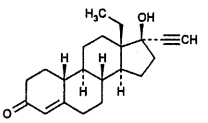
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ethinyl Estradiol RS

USP Norgestimate RS

Norgestrel



$C_{21}H_{28}O_2$ 312.45

18,19-Dinorpregn-4-en-20-yn-3-one, 13-ethyl-17-hydroxy-, (17α)-(±)-.

(±)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one [6533-00-2].

» Norgestrel contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{28}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Norgestrel RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)—If

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differences appear, dissolve portions of both the test specimen and the Reference Standard in ethyl acetate, evaporate the solutions on a steam bath to dryness, and repeat the test.
Melting range, *Class I* ⟨741⟩: between 205° and 212°, but the range between beginning and end of melting does not exceed 4°.
Optical rotation ⟨781A⟩: between −0.1° and +0.1°
Test solution: 50 mg, previously dried, per mL, in chloroform.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.3%.

Chromatographic purity—
Phosphomolybdic acid reagent—Add 10 g of phosphomolybdic acid to 100 mL of alcohol, and stir the mixture for not less than 30 minutes. Filter before use.
Test preparation—Prepare a solution of Norgestrel in chloroform to contain 10.0 mg per mL.
Standard solution and *Standard dilutions*—Prepare a solution of *USP Norgestrel RS* in chloroform to contain 10 mg per mL (*Standard solution*). Prepare a series of dilutions of *Standard solution* in chloroform to contain 0.20, 0.10, 0.05, 0.02, and 0.01 mg per mL (*Standard dilutions*).
Procedure—Apply 10-µL volumes of *Standard solution*, the *Test preparation*, and each of the five *Standard dilutions* at equidistant points along a line 2.5 cm from one edge of a 20- × 20-cm thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously activated by heating at 100° for 15 minutes. Place the plate in a suitable developing chamber that contains and has been equilibrated with a mixture of 96 volumes of chloroform and 4 volumes of alcohol, seal the chamber, and allow the chromatogram to develop until the solvent front has moved 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, then spray uniformly with *Phosphomolybdic acid reagent*, and heat it at 105° for 10 to 15 minutes. The lane of the *Test preparation* exhibits its principal spot at the same R_F as the principal spot of *Standard solution*. If spots other than the principal spot are observed in the lane of the *Test preparation*, estimate the concentration of each by comparison with the *Standard dilutions*. The spots from the 0.20-, 0.10-, 0.05-, 0.02-, and 0.01-mg per mL dilutions are equivalent to 2.0, 1.0, 0.5, 0.2, and 0.1% of impurities, respectively. The requirements of the test are met if the sum of the impurities in the *Test preparation* does not exceed 2.0%.
Limit of ethynyl group—Proceed as directed in the test for *Limit of ethynyl group* under *Norethindrone*. Not less than 7.81% and not more than 8.18% of ethynyl group is found.
Assay—Dissolve about 100 mg of Norgestrel, accurately weighed, in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a solution containing about 10 µg per mL. Dissolve an accurately weighed quantity of *USP Norgestrel RS* in alcohol to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 241 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of $C_{21}H_{28}O_2$ in the portion of Norgestrel taken by the formula:

$$10C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of *USP Norgestrel RS* in the Standard solution, and A_U and A_S are the absorbances of the solution of Norgestrel and the Standard solution, respectively.

Norgestrel Tablets

» Norgestrel Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norgestrel ($C_{21}H_{28}O_2$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Norgestrel RS
Identification—Finely powder 20 Tablets, triturate the powder with 5 mL of chloroform, and allow the solids to settle. Apply 60 µL of the extract and 60 µL of a chloroform solution containing about 300 µg of USP Norgestrel RS per mL at points about 3 cm from one edge of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a developing chamber containing a mixture of chloroform and alcohol (96:4) to a depth of 2 cm, the chamber having been previously equilibrated with the solvent mixture. Remove the plate when the solvent has moved about 15 cm from the line of application, dry at room temperature, spray with a mixture of 80 volumes of sulfuric acid and 20 volumes of alcohol, and heat at 105° for several minutes: the spot from the solution under test exhibits an R_F value identical to that of the spot from the Standard solution, and, when viewed under long-wavelength UV light, exhibits a red fluorescence similar to that from the Standard solution.
Disintegration ⟨701⟩: 15 minutes, the use of disks being omitted.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Isoniazid reagent—Dissolve 0.25 g of isoniazid and 0.3 mL of hydrochloric acid in 500 mL of dehydrated alcohol.
Procedure—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 µg of norgestrel, to a 30-mL separator containing 5 mL of water. Extract with three 5-mL portions of chloroform, shaking for about 1 minute each time, and collecting the chloroform extracts through glass wool, previously moistened with chloroform, into a glass-stoppered test tube. Add 1 mL of dilute hydrochloric acid (1 in 12) to the remaining aqueous phase and extract with a fourth 5-mL portion of chloroform, collecting this chloroform extract as before and combining it with the previous three. To another glass-stoppered test tube transfer 20.0 mL of a solution of USP Norgestrel RS, in chloroform, having a known concentration of about 3.75 µg per mL. Evaporate the contents of both tubes in a water bath with the aid of a current of air to dryness. Add 5.0 mL of *Isoniazid reagent* to each tube, insert the stopper in each tube, and swirl occasionally for 1 hour. Concomitantly determine the absorbances of both solutions in 1-cm cells, at the wavelength of maximum absorbance at about 380 nm, using a suitable spectrophotometer, and using *Isoniazid reagent* as the blank. Calculate the quantity, in µg, of $C_{21}H_{28}O_2$ in the portion of Tablets taken by the formula:

$$20C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Norgestrel RS in the Standard solution, and A_U and A_S are the absorbances of the solutions from the Tablets and the Standard solution, respectively.

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Norgestrel and Ethinyl Estradiol Tablets

» Norgestrel and Ethinyl Estradiol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norgestrel ($C_{21}H_{28}O_2$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Ethinyl Estradiol RS
USP Norgestrel RS

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: 0.0005% (w/v) polysorbate 80; 500 mL.
Apparatus 2: 75 rpm.
Time: 60 minutes.

Determine the amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ dissolved by employing the following method. [NOTE—Do not use plastics during the preparation of solutions.]

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution [NOTE—A volume of alcohol not exceeding 2% of the final volume of the solution may be used to aid in dissolving the USP Reference Standards.] —Dissolve an accurately weighed quantity of USP Norgestrel RS and USP Ethinyl Estradiol RS in *Medium,* and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having known concentrations similar to those expected in the *Test solution.*

Test solution—Use a portion of the solution under test filtered through 0.7-µm borosilicate microfiber filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 247-nm detector (for norgestrel analysis), and a spectrofluorometric detector (for ethinyl estradiol analysis) with an excitation wavelength of about 285 nm and an emission wavelength of 310 nm, and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for ethinyl estradiol and 1.0 for norgestrel; and the relative standard deviation for replicate injections is not more than 3.0% for the ethinyl estradiol and norgestrel peaks.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of norgestrel ($C_{21}H_{28}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved by the formula:

$$(500C)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard solution;* and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution,* respectively.

Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase—Prepare a degassed mixture of water, acetonitrile, and methanol (45:35:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Norgestrel RS and USP Ethinyl Estradiol RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 100 µg of norgestrel per mL and 10 µg of ethinyl estradiol per mL.

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 10 mg of norgestrel, to a 200-mL volumetric flask. Add 100.0 mL of *Mobile phase,* accurately measured, sonicate for 10 minutes to disintegrate the Tablets, and shake by mechanical means for 20 minutes. Centrifuge the clear portion of the solution at about 2000 rpm for 10 minutes, and filter the clear supernatant.

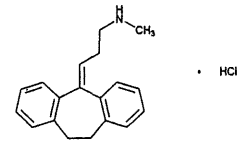
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for ethinyl estradiol and 1.5 for norgestrel; the resolution, *R,* between the two major peaks is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ethinyl estradiol ($C_{20}H_{24}O_2$) and norgestrel ($C_{21}H_{28}O_2$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard preparation;* and r_U and r_S are the peak responses for the relevant analyte obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Nortriptyline Hydrochloride



$C_{19}H_{21}N \cdot HCl$ 299.84
1-Propanamine, 3-(10,11-dihydro-5*H*-dibenzo[*a,d*] cyclohepten-5-ylidene)-*N*-methyl-, hydrochloride;
10,11-Dihydro-*N*-methyl-5*H*-dibenzo[*a,d*]cycloheptene-$\Delta^{5,\gamma}$-propylamine hydrochloride [894-71-3].

DEFINITION
Nortriptyline Hydrochloride contains NLT 97.0% and NMT 101.5% of nortriptyline hydrochloride ($C_{19}H_{21}N \cdot HCl$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements of tests *A* or *B*

ASSAY

- **PROCEDURE**

Solution A: Phosphoric acid and water (1:10)
Buffer: Dissolve 1.4 g of dibasic sodium phosphate in 1 L of water, and adjust with *Solution A* to a pH of 7.7.
Mobile phase: Methanol and *Buffer* (70:30)
Standard solution: 0.2 mg/mL of USP Nortriptyline Hydrochloride RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Nortriptyline Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 1.3 times the retention time of nortriptyline
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nortriptyline hydrochloride ($C_{19}H_{21}N \cdot HCl$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Nortriptyline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Nortriptyline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A: 400 mg/mL of tetrabutylammonium hydroxide in water
Solution B: Phosphoric acid and water (1:7)
Buffer: Dissolve 0.7 g of potassium dihydrogen phosphate in 900 mL water. Add 3.25 mL of *Solution A*, and adjust with *Solution B* to a pH of 7.5. Dilute with water to 1 L.
Mobile phase: Methanol and *Buffer* (75:25). [NOTE—The *Mobile phase* ratio can be adjusted to 70:30 to meet the system suitability requirements.]
System suitability solution: 2.0 µg/mL each of USP Amitriptyline Related Compound B RS, USP Cyclobenzaprine Related Compound B RS, and USP Nortriptyline Hydrochloride RS in *Mobile phase*
Standard solution: 2.0 µg/mL of USP Nortriptyline Hydrochloride RS in *Mobile phase*
Sample solution: 2.0 mg/mL of Nortriptyline Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 35°. [NOTE—The *Column temperature* can be adjusted to 45° to meet the system suitability requirements.]
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 3 times the retention time of nortriptyline
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.4 between the amitriptyline related compound B and cyclobenzaprine related compound B peaks; NLT 2.0 between the cyclobenzaprine related compound B and nortriptyline peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Nortriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of nortriptyline from the *Standard solution*
C_S = concentration of USP Nortriptyline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Nortriptyline Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05% of the area of the principal peak from the *Sample solution*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amitriptyline related compound A[a]	0.5	1.0	0.05
Amitriptyline related compound B	0.8	0.58	0.15
Cyclobenzaprine related compound B	0.9	1.0	0.10
Nortriptyline	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.2

[a] Dibenzosuberone.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 0.5%

USP Monographs

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Amitriptyline Related Compound B RS
5-[3-(Dimethylamino)propyl]-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-ol.
$C_{20}H_{25}NO$ 295.42

USP Cyclobenzaprine Related Compound B RS
3-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*-methyl-1-propanamine hydrochloride.
$C_{19}H_{19}N \cdot HCl$ 297.81

USP Nortriptyline Hydrochloride RS

Nortriptyline Hydrochloride Capsules

» Nortriptyline Hydrochloride Capsules contain nortriptyline hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nortriptyline ($C_{19}H_{21}N$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Nortriptyline Hydrochloride RS

Identification—

A: Transfer the contents of Capsules, equivalent to about 50 mg of nortriptyline hydrochloride, to a suitable flask. Add 15 mL of chloroform, insert the stopper in the flask, and shake for 15 minutes. Transfer the mixture to a suitable centrifuge tube, and centrifuge at about 2900 rpm for about 5 minutes. Pass through a suitable filter paper containing a small amount of anhydrous sodium sulfate. Evaporate the filtrate to dryness, and dissolve the residue in 0.5 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima only at the same wavelengths as that of a Standard solution prepared by dissolving 50 mg of USP Nortriptyline Hydrochloride RS in 0.5 mL of chloroform.

B: A filtered solution in water of the contents of Capsules, equivalent to nortriptyline hydrochloride solution (1 in 20), responds to tests *A* or *B Identification Tests—General* ⟨191⟩, *Chloride.*

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{19}H_{21}N$ dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{19}H_{21}N$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Phosphate buffer—Dissolve 1.63 g of monobasic potassium phosphate in 1 L of water, and adjust with 1 N potassium hydroxide to a pH of 6.7.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and *Phosphate buffer* (40:43:17). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Nortriptyline Hydrochloride RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.38 mg per mL.

Assay preparation—Weigh, empty, and combine the contents of not less than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 76 mg of nortriptyline hydrochloride, to a 200-mL volumetric flask, and dissolve in about 150 mL of methanol. Shake by mechanical means for 15 minutes, dilute with methanol to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 239-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency is not less than 500 theoretical plates, the tailing factor is not more than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of nortriptyline ($C_{19}H_{21}N$) in the portion of Capsules taken by the formula:

$$(263.38/299.84)(200C)(r_U/r_S)$$

in which 263.38 and 299.84 are the molecular weights of nortriptyline and nortriptyline hydrochloride, respectively; C is the concentration, in mg per mL, of USP Nortriptyline Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nortriptyline Hydrochloride Oral Solution

» Nortriptyline Hydrochloride Oral Solution contains nortriptyline hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nortriptyline ($C_{19}H_{21}N$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Nortriptyline Hydrochloride RS

Identification—

A: Transfer a measured volume of Oral Solution, equivalent to about 50 mg of nortriptyline hydrochloride, to a suitable separator, and render the solution distinctly alkaline (to a pH of 11 or above as indicated by pH indicator paper) by the dropwise addition of 1 N sodium hydroxide. Extract with 15 mL of chloroform, and filter the chloroform extract through about 2 g of anhydrous sodium sulfate that has been previously washed with chloroform. Evaporate the chloroform extract with the aid of heat and a current of air to dryness, and dissolve the residue in 0.5 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima only at the same wavelengths as that of a Standard solution obtained by dissolving 50 mg of USP Nortriptyline Hydrochloride RS in 25 mL of water and proceeding as directed for the test specimen.

USP Monographs

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B: It responds to the tests *A* or *B Identification Tests—General* ⟨191⟩ *Chloride*.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH ⟨791⟩: between 2.5 and 4.0.

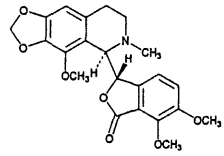
Alcohol Determination, *Method II* ⟨611⟩: between 3.0% and 5.0% of C_2H_5OH.

Assay—Transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of nortriptyline, to a 125-mL separator. Add 20 mL of water, mix, and render the solution distinctly alkaline (to a pH of 11 or above as indicated by pH indicator paper) by the dropwise addition of sodium hydroxide solution (1 in 2). Extract the nortriptyline with four 25-mL portions of chloroform, filtering each extract into a 250-mL beaker through about 12 g of anhydrous sodium sulfate previously washed with 25 mL of chloroform. Rinse the sodium sulfate with four 5-mL portions of chloroform, and collect the rinsings in the beaker. Evaporate the combined chloroform solution with the aid of heat and a current of air to about 10 mL. Transfer the contents of the beaker with the aid of chloroform to a 200-mL volumetric flask. Evaporate the chloroform with the aid of air alone to dryness. [**Caution**—Do not use heat.] Dissolve the residue in 1.7 mL of hydrochloric acid, dilute with water to volume, and mix. Transfer 10.0 mL of the solution to a 50-mL volumetric flask, dilute with water to volume, and mix to obtain the *Assay preparation.* Concomitantly determine the absorbances of the *Assay preparation* and a Standard solution of USP Nortriptyline Hydrochloride RS in water having a known concentration of about 11.4 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{19}H_{21}N$ in the portion of Oral Solution taken by the formula:

$$(263.38/299.84)(C)(A_U/A_S)$$

in which 263.38 and 299.84 are the molecular weights of nortriptyline and nortriptyline hydrochloride, respectively; C is the concentration, in µg per mL, of USP Nortriptyline Hydrochloride RS in the Standard solution; and A_U and A_S are the absorbances of the *Assay preparation* and the Standard solution, respectively.

Noscapine



$C_{22}H_{23}NO_7$ 413.42

1(3*H*)-Isobenzofuranone, 6,7-dimethoxy-3-(5,6,7,8-tetra hydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl), [*S*-(*R**,*S**)]-;
Narcotine [128-62-1].

DEFINITION
Noscapine contains NLT 99.0% and NMT 100.5% of noscapine ($C_{22}H_{23}NO_7$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197K.▲ (CN 1-May-2020) Do not dry specimens.

Change to read:

- **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Solution: 60 µg/mL
  Medium: Methanol
  Acceptance criteria: Meets the requirements
- **C.**
  Analysis: Place about 100 mg in a small porcelain dish, add a few drops of sulfuric acid, and stir.
  Acceptance criteria: A greenish yellow solution is produced, and on warming it becomes red and then turns violet.

ASSAY
- **Procedure**
  Sample solution: Dissolve about 1.5 g of Noscapine in 25 mL of glacial acetic acid.
  Analysis: Add 25 mL of dioxane and 5 drops of crystal violet TS to the *Sample solution*, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.34 mg of noscapine ($C_{22}H_{23}NO_7$).
  Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Chloride and Sulfate,** *Chloride* ⟨221⟩: A 700-mg portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.02%).
- **Limit of Morphine**
  Sample solution: Dissolve 100 mg in 10 mL of 0.1 N hydrochloric acid.
  Analysis: To 1.0 mL of the *Sample solution* add 5.0 mL of diluted ferricyanide reagent (prepared by dissolving 0.50 g of potassium ferricyanide in 50 mL of water, adding 0.50 mL of ferric chloride TS, and diluting 5.0 mL of the resulting solution to 25.0 mL).
  Acceptance criteria: No blue or dark green color develops within 1 min.
- **Ordinary Impurities** ⟨466⟩
  Standard solution: Chloroform
  Test solution: Chloroform
  Eluant: Ethyl acetate and ether (80:20)
  Visualization: 17; then examine the plate immediately.
  Acceptance criteria: The sum of the intensities of all secondary spots of the *Test solution* corresponds to NMT 1.0%.

SPECIFIC TESTS
- **Melting Range or Temperature** ⟨741⟩: 174°–176°
- **Optical Rotation,** *Specific Rotation* ⟨781S⟩
  Sample solution: 20 mg/mL in 0.1 N hydrochloric acid
  Acceptance criteria: +42° to +48°
- **Water Determination,** *Method I* ⟨921⟩: NMT 1.0%

USP Monographs

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Noscapine RS

Novobiocin Sodium

$C_{31}H_{35}N_2NaO_{11}$ 634.61
Benzamide, *N*-[7-[[3-*O*-(aminocarbonyl)-6-deoxy-5-*C*-methyl-4-*O*-methyl-β-L-*lyxo*-hexopyranosyl]oxy]-4-hydroxy-8-methyl-2-oxo-2*H*-1-benzopyran-3-yl]-4-hydroxy-3-(3-methyl-2-butenyl), monosodium salt.
Novobiocin, monosodium salt [1476-53-5].

» Novobiocin Sodium has a potency equivalent to not less than 850 µg of novobiocin ($C_{31}H_{36}N_2O_{11}$) per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Novobiocin RS

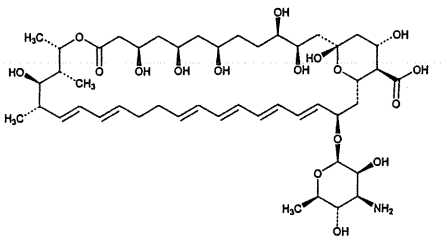
Identification—
A: Prepare a test solution by dissolving a quantity of it in methanol to obtain a concentration of about 1 mg of novobiocin per mL. Similarly prepare a Standard solution, using USP Novobiocin RS. Separately apply 1-µL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Place the plate in a chromatographic chamber equilibrated with a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (75:25:1), and develop the chromatogram. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, and allow to dry. Locate the spots on the plate by examination under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
B: The residue obtained by igniting it responds to the tests for *Sodium* ⟨191⟩.
Specific rotation ⟨781S⟩: between −50° and −58°.
Test solution: 50 mg per mL, in a mixture of methanol and hydrochloric acid (100:1).
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 6.5 and 8.5, in a solution containing 25 mg per mL.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.
Residue on ignition ⟨281⟩: between 10.5% and 12.0%, the charred residue being moistened with 2 mL of sulfuric acid and an ignition temperature of 550 ± 50° being used.
Assay—Dissolve a suitable quantity of Novobiocin Sodium, accurately weighed, in an accurately measured volume of *Buffer B.3* sufficient to obtain a stock solution of convenient concentration. Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock solution diluted quantitatively and stepwise with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Novobiocin Sodium Intramammary Infusion

» Novobiocin Sodium Intramammary Infusion is a suspension of Novobiocin Sodium in a suitable vegetable oil vehicle. It contains suitable preservative and suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of novobiocin ($C_{31}H_{36}N_2O_{11}$).

Packaging and storage—Preserve in disposable syringes that are well-closed containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Novobiocin RS
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay—Proceed as directed for novobiocin under *Antibiotics—Microbial Assays* ⟨81⟩, expelling the contents of a syringe of Intramammary Infusion into a high-speed blender jar containing 1.0 mL of polysorbate 80 and 499.0 mL of *Buffer B.3*, and blend for 3 to 5 minutes. Allow to stand for 10 minutes, and dilute quantitatively and stepwise with *Buffer B.6* to obtain a *Test Dilution* having a concentration of novobiocin assumed to be equal to the median dose level of the Standard.

Nystatin



$C_{47}H_{75}NO_{17}$ 926.09
Nystatin A;
14,39-Dioxabicyclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid, 33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo, (1*S*,3*R*,4*R*,7*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-;
(1*S*,3*R*,4*R*,7*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-[(3-Amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid [1400-61-9].

DEFINITION
Nystatin is a substance or a mixture of two or more substances produced by the growth of *Streptomyces noursei* Brown et al. (Fam. Streptomycetaceae). It has a potency of NLT 4400 USP Nystatin Units/mg or, where intended for use in the extemporaneous preparation of oral suspensions, NLT 5000 USP Nystatin Units/mg.

USP Monographs

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample stock solution: Transfer a suitable quantity of Nystatin to a suitable glass-stoppered volumetric flask, add methanol and glacial acetic acid, using 25% and 5% of the final volumes respectively, mix to dissolve, dilute with methanol to volume, and mix.
Sample solution: 10 µg/mL of Nystatin from the *Sample stock solution* in methanol
Acceptance criteria: $A_{230}/A_{279(sh)}$, 0.90–1.25

ASSAY

• **PROCEDURE**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Analysis: Proceed as directed in the chapter.
Acceptance criteria: NLT 4400 USP Nystatin Units/mg; where intended for use in the extemporaneous preparation of oral suspensions, NLT 5000 USP Nystatin Units/mg

SPECIFIC TESTS

• **SUSPENDABILITY** (where packaged for use in the extemporaneous preparation of oral suspensions)
Analysis
1. Transfer about 200 mg, accurately weighed, to a 250-mL beaker containing 200.0 mL of water, and disperse by stirring gently with a stirring rod. Allow to stand for 2 min, and observe the suspension.
2. If there is any sediment, assay the undisturbed suspension as directed for Nystatin in *Antibiotics—Microbial Assays* ⟨81⟩, using a suitable aliquot blended in a high-speed blender for 3–5 min with a sufficient volume of dimethylformamide to obtain a solution having a concentration of 400 USP Nystatin Units/mL. Dilute this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median level of the standard.

Acceptance criteria
1. The material is in suspension, and little or no sediment is present on the bottom of the beaker.
2. If there is any sediment, the undisturbed suspension contains NLT 90.0% of the expected number of USP Nystatin Units, based on the potency obtained in the *Assay*.

• **CRYSTALLINITY** ⟨695⟩ (where packaged for use in the extemporaneous preparation of oral suspensions)**:** Meets the requirements
• **pH** ⟨791⟩
Sample suspension: 3% aqueous
Acceptance criteria: 6.0–8.0
• **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 5.0%
• **COMPOSITION**
Solution A: Acetonitrile and 0.05 M ammonium acetate (29:71)
Solution B: Acetonitrile and 0.05 M ammonium acetate (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	100	0
35	0	100
40	0	100
45	100	0
50	100	0

System suitability solution: Dissolve 20 mg of Nystatin in 25 mL of methanol, and dilute with water to 50 mL. To 10.0 mL of the resulting solution add 2.0 mL of dilute hydrochloric acid, and allow to stand at room temperature for 1 h.
Standard solution: 0.4 mg/mL of USP Nystatin RS in dimethyl sulfoxide. Protect this solution from light, store refrigerated, and use within 24 h.
Sample solution: 0.4 mg/mL of Nystatin in dimethyl sulfoxide. Protect this solution from light, store refrigerated, and use within 24 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 304 nm
Column: 4.6-mm × 15-cm; base-deactivated, end-capped 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The nystatin A1 peak elutes at 14 min. Identify this peak using the *Standard solution*.]
Suitability requirements
Resolution: NLT 3.5 between the two major peaks, *System suitability solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = area of each individual peak
r_T = total area of all peaks except those eluting in less than 2 min

Acceptance criteria: NLT 85.0% of nystatin A1; NMT 4.0% of any other individual component. Disregard any peaks eluting in less than 2 min.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** Where packaged for use in the extemporaneous preparation of oral suspensions, the label so states.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Nystatin RS

Nystatin Cream

DEFINITION

Nystatin Cream contains NLT 90.0% and NMT 130.0% of the labeled amount of USP Nystatin Units.

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ASSAY
• **PROCEDURE**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: 400 USP Nystatin Units/mL prepared as follows. Blend a suitable portion of Cream in a high-speed blender for 3–5 min with a suitable volume of dimethylformamide.
Analysis: Proceed as directed in the chapter. Dilute an aliquot of the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median level of the standard.
Acceptance criteria: 90.0%–130.0% of the labeled amount of USP Nystatin Units

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes, or in other tight containers, and avoid exposure to excessive heat.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Nystatin RS

Nystatin Lotion

» Nystatin Lotion contains not less than 90.0 percent and not more than 140.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight containers, at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Nystatin RS
pH ⟨791⟩: between 5.5 and 7.5.
Assay—Proceed with Lotion as directed in the *Assay* under *Nystatin Cream.*

Nystatin Lozenges

» Nystatin Lozenges contain not less than 90.0 percent and not more than 125.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Nystatin RS
Disintegration ⟨701⟩: 90 minutes, determined as set forth under *Uncoated Tablets.*
pH ⟨791⟩: between 5.0 and 7.5, in a solution prepared by dissolving 1 Lozenge in 100 mL of water at 37° and allowing the solution to cool to room temperature.
Assay—Proceed as directed for Nystatin under *Antibiotics—Microbial Assays* ⟨81⟩, blending not less than 5 Lozenges for 18 to 20 minutes in a high-speed blender jar containing 100.0 mL of water. Add 400.0 mL of dimethylformamide, and blend for an additional 10 minutes. Dilute an accurately measured volume of this solution quantitatively with a mixture of dimethylformamide and water (4:1) to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median dose level of the Standard. [NOTE—The *Test Dilution* of the specimen and the test dilutions of the Standard contain the same amount of dimethylformamide (about 4%).]

Nystatin Ointment

» Nystatin Ointment contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Nystatin RS
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay—Proceed with Ointment as directed in the *Assay* under *Nystatin Cream.*

Nystatin Topical Powder

» Nystatin Topical Powder is a dry powder composed of Nystatin and Talc. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Nystatin RS
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.
Assay—Proceed with Topical Powder as directed in the *Assay* under *Nystatin Cream.*

Nystatin Vaginal Suppositories

» Nystatin Vaginal Suppositories contain not less than 90.0 percent and not more than 130.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight, light-resistant containers, at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Nystatin RS
Water Determination, *Method I* ⟨921⟩: not more than 1.5%.
Assay—Proceed with Vaginal Suppositories as directed in the *Assay* under *Nystatin Tablets.*

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Nystatin Oral Suspension

» Nystatin Oral Suspension contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of USP Nystatin Units. It contains suitable dispersants, flavors, preservatives, and suspending agents.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Nystatin RS

Uniformity of dosage units ⟨905⟩—
FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Procedure for content uniformity— [NOTE—Use low-actinic glassware.] Transfer the well-shaken contents of 1 container of Oral Suspension to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with methanol to obtain a test solution containing about 25 USP Nystatin Units per mL. Similarly, prepare a Standard solution of USP Nystatin RS in methanol having a known concentration of about 25 USP Nystatin Units per mL. Concomitantly determine the absorbances of the test solution and the Standard solution at the wavelength of maximum absorbance at about 304 nm with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in USP Nystatin Units, in the container taken by the formula:

$$(CL/D)(A_U/A_S)$$

in which C is the concentration, in USP Nystatin Units per mL, of the Standard solution; L is the labeled quantity, in USP Nystatin Units, in the container; D is the concentration, in USP Nystatin Units, in the test solution, on the basis of the labeled quantity in the container and the extent of dilution; and A_U and A_S are the absorbances of the test solution and the Standard solution, respectively.

Deliverable volume ⟨698⟩: meets the requirements.

pH ⟨791⟩: between 4.5 and 6.0; or if it contains glycerin, between 5.3 and 7.5.

Assay—Proceed as directed for Nystatin under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, for 3 to 5 minutes in a high-speed blender with a sufficient accurately measured volume of dimethylformamide to obtain a solution of convenient concentration. Dilute an accurately measured portion of this solution quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Nystatin for Oral Suspension

DEFINITION
Nystatin for Oral Suspension is a dry mixture of Nystatin with one or more suitable colors, diluents, suspending agents, flavors, and preservatives. It contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amount of USP Nystatin Units.

ASSAY
- **PROCEDURE**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: 400 USP Nystatin Units/mL prepared as follows. Constitute Nystatin for Oral Suspension as directed in the labeling. Blend a suitable aliquot of the suspension, freshly mixed and free from air bubbles, for 3–5 min in a high-speed blender with a sufficient volume of dimethylformamide to obtain a solution of suitable concentration. Dilute a portion of the resulting solution with dimethylformamide.
Analysis: Proceed as directed in the chapter. Dilute an aliquot of the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median level of the standard.
Acceptance criteria: 90.0%–140.0% of the labeled amount of USP Nystatin Units

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For powder packaged in single-unit containers
Acceptance criteria: Meets the requirements
- **DELIVERABLE VOLUME** ⟨698⟩

For powder packaged in multiple-unit containers
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **pH** ⟨791⟩

Sample suspension: Constitute as directed in the labeling.
Acceptance criteria: 4.9–5.5
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 7.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nystatin RS

Nystatin Tablets

DEFINITION
Nystatin Tablets contain NLT 90.0% and NMT 130.0% of the labeled amount of USP Nystatin Units.

ASSAY
- **PROCEDURE**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: 400 USP Nystatin Units/mL prepared as follows. Blend NLT 5 Tablets for 3–5 min in a high-speed blender with a sufficient volume of dimethylformamide to obtain a solution of suitable concentration. Dilute a portion of this solution with dimethylformamide.
Analysis: Proceed as directed in the chapter. Dilute an aliquot of the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median level of the standard.
Acceptance criteria: 90.0%–130.0% of the labeled amount of USP Nystatin Units

PERFORMANCE TESTS
- **DISINTEGRATION** ⟨701⟩

Time: If plain-coated, 120 min
Acceptance criteria: Meet the requirements

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩

Sample: 100 mg of powdered Tablets
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

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Acceptance criteria: If plain-coated, NMT 5.0%; if film-coated, NMT 8.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Tablets to indicate that they are intended for oral use only (as distinguished from Vaginal Tablets).
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nystatin RS

Nystatin Vaginal Inserts

DEFINITION
Nystatin Vaginal Inserts are composed of Nystatin with suitable binders, diluents, and lubricants. Vaginal Inserts contain NLT 90.0% and NMT 140.0% of the labeled amount of USP Nystatin Units.

ASSAY
- **NYSTATIN**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample stock solution: 400 USP Nystatin Units/mL in dimethylformamide prepared as follows. Blend NLT 5 Vaginal Inserts for 3–5 min in a high-speed blender with a sufficient volume of dimethylformamide to obtain a solution of suitable concentration. Dilute a portion of this solution with dimethylformamide.
  Test dilution: Dilute a volume of the *Sample stock solution* with *Buffer B.6* to obtain a nystatin concentration assumed to be equal to the median dose level of the standard.
  Acceptance criteria: 90.0%–140.0% of the labeled amount of USP Nystatin Units

PERFORMANCE TESTS
- **DISINTEGRATION** ⟨701⟩
  Time: 60 min
  Analysis: Use the *Procedure* for *Uncoated Tablets* in the chapter.
  Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩**:** Dry 100 mg of powdered Vaginal Inserts in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers and, where so specified in the labeling, in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Nystatin RS

Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Cream

» Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Cream contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of nystatin, neomycin, and gramicidin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Gramicidin RS
USP Neomycin Sulfate RS
USP Nystatin RS
USP Triamcinolone Acetonide RS
Identification—Place 2 g of Cream in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.
Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable, accurately weighed portion of Cream in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.
Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Cream*.
Assay for gramicidin—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Cream dissolved in 50 mL of hexanes in a separator, and extracted with four 20-mL portions of 80% alcohol. Combine the extracts in a suitable volumetric flask, dilute with alcohol to volume, and mix. Dilute an accurately measured volume of the solution so obtained quantitatively and stepwise with alcohol to obtain a *Test Dilution* having a concentration of gramicidin assumed to be equal to the median dose level of the Standard.
Assay for triamcinolone acetonide—Proceed with Cream as directed in the *Assay* under *Triamcinolone Acetonide Cream*.

Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Ointment

» Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Ointment contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of nystatin, neomycin, and gramicidin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Gramicidin RS
USP Neomycin Sulfate RS

USP Monographs

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USP Nystatin RS
USP Triamcinolone Acetonide RS
Identification—Place 2 g of Ointment in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable, accurately weighed portion of Ointment in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.
Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*
Assay for gramicidin—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Ointment dissolved in 50 mL of hexanes in a separator, and extracted with four 20-mL portions of 80% alcohol. Combine the extracts in a suitable volumetric flask, dilute with alcohol to volume, and mix. Dilute an accurately measured volume of the solution so obtained quantitatively and stepwise with alcohol to obtain a *Test Dilution* having a concentration of gramicidin assumed to be equal to the median dose level of the Standard.
Assay for triamcinolone acetonide—Proceed with Ointment as directed in the *Assay* under *Triamcinolone Acetonide Cream*, except to read "Ointment" in place of "Cream" throughout.

Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Cream

» Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Cream contains not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of nystatin, neomycin, and thiostrepton, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Nystatin RS
USP Neomycin Sulfate RS
USP Thiostrepton RS
USP Triamcinolone Acetonide RS
Identification—Place 2 g of Cream in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.
Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable, accurately weighed portion of Cream in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.
Assay for neomycin—Proceed as directed for the turbidimetric assay for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, placing an accurately weighed portion of Cream, equivalent to about 2.5 mg of neomycin, in a 250-mL conical flask, and treating it as follows. Add 50 mL of 0.01 N hydrochloric acid, and shake to disperse the Cream. Transfer the mixture to a 100-mL centrifuge tube. Wash the flask with 40 mL of hexanes, with shaking, and transfer the washing to the centrifuge tube. Stopper the centrifuge tube, shake, and centrifuge for 5 minutes. Draw off the lower aqueous layer, and transfer it to a 250-mL volumetric flask. Repeat the extraction of the hexanes layer remaining in the centrifuge tube with two 50-mL portions of 0.01 N hydrochloric acid, combining the aqueous extracts in the 250-mL volumetric flask. Dilute the contents of the volumetric flask with 0.01 N hydrochloric acid to volume, and mix. Dilute this solution quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose of the Standard.
Assay for thiostrepton—Proceed as directed for thiostrepton under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable, accurately weighed portion of Cream in a high-speed blender with a sufficient, accurately measured volume of dimethyl sulfoxide to give a convenient concentration, and filter. Dilute an accurately measured volume of the filtrate so obtained quantitatively with dimethyl sulfoxide to obtain a *Test Dilution* having a concentration of thiostrepton assumed to be equal to the median dose level of the Standard.
Assay for triamcinolone acetonide—Proceed with Cream as directed in the *Assay* under *Triamcinolone Acetonide Cream.*

Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Ointment

» Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Ointment contains not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of nystatin,

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neomycin, and thiostrepton, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Nystatin RS
USP Neomycin Sulfate RS
USP Thiostrepton RS
USP Triamcinolone Acetonide RS
Identification—Place 2 g of Ointment in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream,* beginning with "Apply 10 µL of this solution": the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.
Assay for nystatin— [NOTE—Protect solutions that contain nystatin from ambient light.]

Ammonium acetate buffer—Dissolve 10.8±1.0 g of ammonium acetate in 2500 mL of water. Adjust with acetic acid to a pH of 6.50±0.05.

Mobile phase—Mix 2500 mL of *Ammonium acetate buffer,* 1000 mL of acetonitrile, and 500 mL of methanol. Pass through a 0.45-µm nylon filter.

BHT solution—Weigh about 1.0 g of butylated hydroxytoluene, and transfer to a 1000-mL volumetric flask. Dilute with methanol to volume, and mix.

Standard preparation—In duplicate, dissolve an accurately weighed quantity of USP Nystatin RS in *BHT solution* to obtain a solution having a known concentration of about 5400 USP Nystatin Units per mL. Store in low-actinic glassware.

System suitability solution—Weigh about 50 mg of USP Nystatin RS, and transfer to a 50-mL low-actinic volumetric flask. Add 0.5 mL of 0.01 N sodium hydroxide, and allow to sit for 1 minute. Add 5 mL of *Ammonium acetate buffer.* Add about 25 mL of methanol, and sonicate to dissolve. Dilute with methanol to volume, and store in low-actinic glassware.

Assay preparation—Thoroughly mix the Ointment prior to sampling. In duplicate, accurately weigh about 1.0 g of Ointment having a known density into a low-actinic sample bottle. Add 20.0 mL of *BHT solution,* and insert a polytef-coated magnetic stir bar having dimensions of about 12.7 × 7.9 mm. Clamp the bottles onto a suitable mixer mill,[1] and mix for a minimum of 5 minutes at about 30 Hz. Centrifuge at about 1350 × *g* for 5 minutes, or until the supernatant is clear. Transfer the supernatant to low-actinic glassware.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 304-nm detector and a 3.9-mm × 15-cm column that contains 4-µm packing L1. The column temperature is maintained at 40°, and the flow rate is about 2.0 mL per minute. [NOTE—Solutions containing nystatin should be stored at 8° until they can be injected into the chromatograph.] Chromatograph the *Standard preparation* and the *System suitability solution,* and record the peak areas as directed for *Procedure:* using the *System suitability solution,* the relative retention times for the nystatin A1 and nystatin A2 peaks are about 1.0 and 1.4, respectively; the column efficiency, using the nystatin A1 peak, is not less than 1200 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%. [NOTE—After the conclusion of the run, rinse the column with a mixture of acetonitrile and water (85:15) until the baseline is stable, and store in this solution. At the beginning of the next run, rinse with *Mobile phase* until the baseline is stable.]

Procedure—Separately inject equal volumes (about 15 µL) of the duplicate *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for nystatin A1 and nystatin A2. Calculate the quantity, in USP Nystatin Units, of nystatin in the portion of Ointment taken by the formula:

$$20(C_S/W_U)(r_u/r_s)$$

in which C_S is the concentration of USP Nystatin RS, in USP Nystatin Units per mL, of the *Standard preparation;* W_U is the weight, in g, of Ointment taken to prepare the *Assay preparation;* and r_u and r_s are the average peak areas of the sum of nystatin A1 and nystatin A2 obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for neomycin—Proceed as directed for the turbidimetric assay for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, placing an accurately weighed portion of Ointment, equivalent to about 2.5 mg of neomycin, in a 250-mL conical flask, and treating it as follows. Add 50 mL of hexanes, and shake to disperse the Ointment. Transfer the mixture to a 250-mL separator. Wash the flask with 50 mL of 0.01 N hydrochloric acid, with shaking, and transfer the washing to a separator. Stopper the separator, shake, and allow the layers to separate. Draw off the lower aqueous layer, collecting it in a 250-mL volumetric flask. Repeat the extraction of the hexanes layer remaining in the separator with two or more 50-mL portions of 0.01 N hydrochloric acid, combining the aqueous extracts in the 250-mL volumetric flask. Dilute the contents of the volumetric flask with 0.01 N hydrochloric acid to volume, and mix. Dilute this solution quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose of the Standard.

Assay for thiostrepton—Proceed as directed for thiostrepton under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable, accurately weighed portion of Ointment in a high-speed blender with a sufficient, accurately measured volume of dimethyl sulfoxide to give a convenient concentration, and filter. Quantitatively dilute an accurately measured volume of the filtrate so obtained with dimethyl sulfoxide to obtain a *Test Dilution* having a concentration of thiostrepton assumed to be equal to the median dose of the Standard.

Assay for triamcinolone acetonide—Proceed with Ointment as directed in the *Assay* under *Triamcinolone Acetonide Cream,* but read "Ointment" in place of "Cream" throughout.

[1] A suitable mixer mill can be obtained from Retsch Inc., 74 Walker Lane, Newtown, PA 18940 (www.retsch-us.com; 267-757-0351), product number MM 301.

Nystatin and Triamcinolone Acetonide Cream

» Nystatin and Triamcinolone Acetonide Cream contains not less than 90.0 percent and not more than 140.0 percent of the labeled amount of USP Nystatin Units and not less than 90.0 percent and

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not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

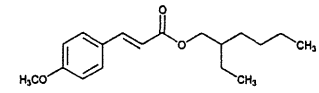
Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Nystatin RS
USP Triamcinolone Acetonide RS
Identification—Place 2 g of Cream in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.
Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable, accurately weighed portion of Cream in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.
Assay for triamcinolone acetonide—Proceed with Cream as directed in the *Assay* under *Triamcinolone Acetonide Cream*.

Nystatin and Triamcinolone Acetonide Ointment

» Nystatin and Triamcinolone Acetonide Ointment contains not less than 90.0 percent and not more than 140.0 percent of the labeled amount of USP Nystatin Units and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Nystatin RS
USP Triamcinolone Acetonide RS
Identification—Place 2 g of Ointment in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable, accurately weighed portion of Ointment in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.
Assay for triamcinolone acetonide—Proceed with Ointment as directed in the *Assay* under *Triamcinolone Acetonide Cream*, except to read "Ointment" in place of "Cream" throughout.

Octinoxate



$C_{18}H_{26}O_3$ 290.40
2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester; 2-Ethylhexyl *p*-methoxycinnamate [5466-77-3].

DEFINITION
Octinoxate contains NLT 95.0% and NMT 105.0% of octinoxate ($C_{18}H_{26}O_3$), calculated on the as-is basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A OR 197F**▲ (CN 1-May-2020)
- **B.** The retention time of the octinoxate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

System suitability stock solution: 5% (v/v) USP Benzyl Benzoate RS in acetone
System suitability solution: 50 mg/mL of USP Octinoxate RS in *System suitability stock solution*
Standard solution: 50 mg/mL of USP Octinoxate RS in acetone
Sample solution: Transfer 5 mL of Octinoxate to a 100-mL volumetric flask, and dilute with acetone to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*..)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 25-m; 0.25-µm thickness of phase G1
Carrier gas: Helium
Temperatures
Injection port: 250°
Detector: 300°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	22	300	10

Flow rate: 2 mL/min
Injection volume: 1 µL

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Injection type: Split ratio, 85:1
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for benzyl benzoate and octinoxate are about 0.68 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 20 between benzyl benzoate and octinoxate, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of octinoxate ($C_{18}H_{26}O_3$) in the portion of Octinoxate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of octinoxate from the *Sample solution*
r_S = peak response of octinoxate from the *Standard solution*
C_S = concentration of USP Octinoxate RS in the *Standard solution* (mg/mL)
C_U = concentration of Octinoxate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the as-is basis

IMPURITIES

• **ORGANIC IMPURITIES**
System suitability stock solution, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Transfer 5 mL of Octinoxate to a 100-mL volumetric flask, and dilute with acetone to volume.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Octinoxate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria
Any individual impurity: NMT 0.5%
Total impurities: NMT 2.0%

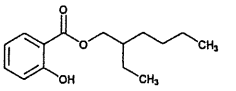
SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩**:** 1.005–1.013
• **REFRACTIVE INDEX** ⟨831⟩**:** 1.542–1.548 at 20°
• **ACIDITY**
Sample: 5 mL of Octinoxate
Analysis: Add 50 mL of alcohol to the *Sample*, and mix. Add 4 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide.
Acceptance criteria: NMT 0.8 mL of 0.1 N sodium hydroxide is required.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cool place.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzyl Benzoate RS
Benzoic acid, phenylmethyl ester.
$C_{14}H_{12}O_2$ 212.24
USP Octinoxate RS

Octisalate



$C_{15}H_{22}O_3$ 250.33
Benzoic acid, 2-hydroxy-, 2-ethylhexyl ester;
2-Ethylhexyl salicylate [118-60-5].

DEFINITION

Octisalate contains NLT 95.0% and NMT 105.0% of octisalate ($C_{15}H_{22}O_3$), calculated on the as-is basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197F▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Standard solution: 20 mg/mL of USP Octisalate RS in *tert*-butyl methyl ether
Sample solution: 20 mg/mL of Octisalate in *tert*-butyl methyl ether
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*..)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 25-m; coated with a 0.1-µm film of phase G1
Temperatures
Injector: 240°
Detector: 260°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	8	240	10

Carrier gas: Helium
Flow rate: 6 mL/min
Injection volume: 1 µL
Injection type: Split; split ratio 50:1. [NOTE—Split ratio can be modified to optimize the performance.]
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between octisalate and any other peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of octisalate ($C_{15}H_{22}O_3$) in the portion of Octisalate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

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C_S = concentration of USP Octisalate RS in the *Standard solution* (mg/mL)
C_U = concentration of Octisalate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the as-is basis

IMPURITIES

• **ORGANIC IMPURITIES**

Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Octisalate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩**:** 1.011–1.016

• **REFRACTIVE INDEX** ⟨831⟩**:** 1.500–1.503 at 20°

• **ACIDITY**

Sample solution: 5.0 mL of measured Octisalate

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide

Endpoint detection: Visual

Analysis: Transfer 50 mL of alcohol to a suitable container, add 1 mL of phenol red TS, and add sufficient *Titrant* to obtain a persistent pink color. Transfer 50 mL of this solution to a suitable container, add the *Sample solution*, mix, and titrate with *Titrant*.

Acceptance criteria: NMT 0.2 mL of 0.1 N sodium hydroxide per milliliter of Octisalate is required for neutralization.

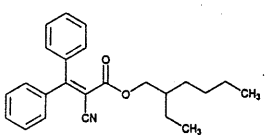
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Octisalate RS

Octocrylene



$C_{24}H_{27}NO_2$ 361.48

2-Propenoic acid, 2-cyano-3,3-diphenyl-, 2-ethylhexyl ester; 2-Ethylhexyl 2-cyano-3,3-diphenylacrylate [6197-30-4].

DEFINITION

Octocrylene contains NLT 95.0% and NMT 105.0% of octocrylene ($C_{24}H_{27}NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Sample solution: 25 µg/mL in methanol

Analytical wavelength: 303 nm

Acceptance criteria: NMT 3.0%, calculated on the as-is basis

• **B.** The retention time of major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Standard solution: 21 mg/mL of USP Octocrylene RS in acetone

Sample solution: 21 mg/mL of Octocrylene in acetone

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*..)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 60-m; coated with a 0.25-µm film of phase G1

Temperatures

Injection port: 300°

Detector: 300°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	4	280	10

Carrier gas: Helium

Flow rate: 6 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 30:1

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between octocrylene and any other adjacent peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of octocrylene ($C_{24}H_{27}NO_2$) in the portion of Octocrylene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Octocrylene RS in the *Standard solution* (mg/mL)
C_U = concentration of Octocrylene in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

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Calculate the percentage of each impurity in the portion of Octocrylene taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria
Individual impurities: NMT 0.5%
Total impurities: NMT 2.0%

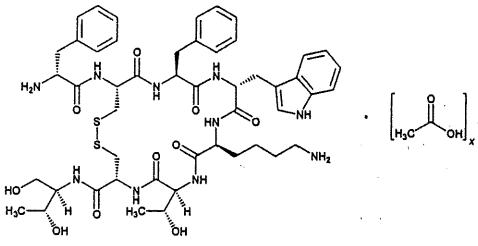
SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨841⟩: 1.045–1.055
- **REFRACTIVE INDEX** ⟨831⟩: 1.561–1.571 at 20°

Change to read:

- **ACIDITY**
Sample: 6 g of Octocrylene
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide
Endpoint detection: Visual
Analysis: To 60 mL of alcohol add 1 mL of phenolphthalein TS and sufficient *Titrant* to obtain a persistent pink color. Transfer 60 mL of this solution to a suitable container, add the *Sample*, mix, and titrate with *Titrant* until a persistent pink color is obtained.
Acceptance criteria: NMT 0.18 mL of ▲*Titrant*/g▲ (ERR 1-Dec-2018) is required

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Octocrylene RS

Octreotide Acetate



$C_{49}H_{66}N_{10}O_{10}S_2 \cdot xC_2H_4O_2$ 1019 (as free base)

L-Cysteinamide, D-phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-*N*-[2-hydroxy-1-(hydroxymethyl)propyl]-, cyclic (2→7)-disulfide, [*R*-(*R**,*R**)]-, acetate (salt);
D-Phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl) propyl]-L-cysteinamide cyclic (2→7)-disulfide acetate (salt);
D-Phenylalanyl-L-hemicystyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-hemicystyl-L-threoninol cyclic (2→7)-disulfide acetate (salt) [79517-01-4].

DEFINITION
Octreotide Acetate is a long-acting synthetic octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. It contains NLT 95.0% and NMT 105.0% of octreotide ($C_{49}H_{66}N_{10}O_{10}S_2$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION
- **A. HPLC**
Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Identification sample solution: Mix an equal volume of the *Standard solution* and the *Sample solution*.
Analysis
Samples: *Identification sample solution, Standard solution,* and *Sample solution*
Examine the chromatograms of the *Identification sample solution, Standard solution,* and *Sample solution*.
Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak of the *Identification sample solution* elutes as a single peak.
- **B. AMINO ACID ANALYSIS**
[NOTE—The following method is given for informational purposes; any validated amino acid analysis method can be used.]
Diluent: 0.1 M hydrochloric acid
Standard amino acid mixture: 2500 nmol/mL each of *Lys, His, NH_3, Arg, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe,* and 1250 nmol/mL of *Cys-Cys* in *Diluent*
Standard tryptophan solution: 0.2042 mg/mL of tryptophan in *Diluent*. This solution contains 1000 nmol/mL of *Trp*.
Standard threonin-ol solution: 0.1052 mg/mL of *Thr-ol* in *Diluent*. This solution contains 1000 nmol/mL *Thr-ol*.
[NOTE—1000 nmol/mL = 1 mM]
Standard solution: *Diluent, Standard amino acid mixture, Standard tryptophan solution,* and *Standard threonin-ol solution* (76:4:10:10). The concentration is 100.0 nmol/mL for each amino acid.
Sample solution A: Weigh 1.00–1.50 mg of Octreotide Acetate into a 5-mL ampule. Add 1000 µL of *Diluent* and 1.2 mL of 30% (w/w) hydrochloric acid, cool the ampule in dry ice, evacuate, and seal by melting. Heat the ampule for 16 h at 115°. Allow to cool and transfer the contents of the ampule quantitatively into a 25-mL round-bottomed flask, rinse with *Diluent*, and evaporate to dryness on a rotary evaporator. Dissolve the residue in 10.00 mL of *Diluent*.
Sample solution B: Weigh 1.00–1.50 mg of Octreotide Acetate into a 5-mL ampule. Add 1000 µL of *Diluent* and 1.2 mL of 30% (w/w) hydrochloric acid with 1% (v/v) thioglycolic acid, cool the ampule in dry ice, evacuate, and seal by melting. Heat the ampule for 16 h in a heating block at 115°. Allow to cool and transfer the contents of the ampule quantitatively into a 25-mL round-bottomed flask, rinse with *Diluent*, and evaporate to dryness on a rotary evaporator. Dissolve the residue in 10.00 mL of *Diluent*.
Analysis
Samples: *Standard solution, Sample solution A,* and *Sample solution B*
Standardize the instrument with the *Standard solution*. Inject a suitable volume of *Standard solution, Sample solution A,* and *Sample solution B*. Evaluate the peak areas of each amino acid found in relation to the peak areas of the respective amino acids in the *Standard solution*, express the content of each amino acid in nmoles.
Calculate *A*, the average number of nmoles of the amino acids found to be stable under hydrolysis conditions (three stable amino acids—2 *Phe* and 1 *Lys*) taken:

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$$A = N_T/3$$

N_T = total nmoles of the stable amino acids

Calculate the ratio of the amino acids taken:

$$\text{Result} = N_E/A$$

N_E = nmoles of each amino acid

[NOTE—For *Trp* use only data obtained with *Sample solution B*. For *Cys* use only data obtained with *Sample solution A*.]

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria
Thr	0.7–1.1
Lys	0.9–1.3
Phe	1.8–2.2
Trp	0.4–1.1
Cys	1.0–2.2
Thr-ol	0.6–1.3

ASSAY

• **PROCEDURE**

Solution A: 0.02% (v/v) of trifluoroacetic acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
25	65	35
30	10	90
35	10	90
40	90	10
45	90	10

System suitability solution: 0.5 mg/mL of USP Octreotide Acetate RS and 0.2 mg/mL of USP Octreotide Non-Cyclic System Suitability Marker RS in *Solution A*

Standard solution: 0.5 mg/mL of USP Octreotide Acetate RS in *Solution A*

Sample solution: 0.5 mg/mL of Octreotide Acetate in *Solution A*. [NOTE—Place Octreotide Acetate in a desiccator containing saturated sodium chloride solution for at least 30 min before weighing. Determine the water content by suitable analysis.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 4-µm packing L87

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The retention times for octreotide and non-cyclic octreotide in the *System suitability solution* are about 16.5 and 18.5 min, respectively.]

Suitability requirements

Resolution: NLT 2.0 between octreotide and non-cyclic octreotide, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of octreotide ($C_{49}H_{66}N_{10}O_{10}S_2$) in the portion of Octreotide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [100/(100 - W - Ac)] \times 100$$

r_U = peak response of octreotide from the *Sample solution*

r_S = peak response of octreotide from the *Standard solution*

C_S = concentration of USP Octreotide Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Octreotide Acetate in the *Sample solution* (mg/mL)

W = water content in the Octreotide Acetate sample (%)

Ac = acetic acid content in the Octreotide Acetate sample (%)

Acceptance criteria: 95.0%–105.0% on the anhydrous, acetic acid-free basis

OTHER COMPONENTS

• **ACETIC ACID IN PEPTIDES** ⟨503⟩**:** 5.0%–12.8%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **OCTREOTIDE ACETATE RELATED COMPOUNDS**

[NOTE—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity taken, disregarding any peak with a retention time of less than 5 min and any peak with an area less than 0.1% of the main peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the peak responses from the *Sample solution*, excluding those of the solvent peaks

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetyl-Lys^5-octreotide[a]	1.4	0.5
Acetyl-Phe^1-octreotide[b]	1.5	0.5
Unspecified impurity	—	0.5

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Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	2.0

[a] D-Phenylalanyl-L-hemicystyl-L-phenylalanyl-D-tryptophyl-(*N*-acetyl)-L-lysyl-L-threonyl-L-hemicystyl-L-threoninol cyclic (2→7)-disulfide.
[b] (*N*-Acetyl)-D-Phenylalanyl-L-hemicystyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-hemicystyl-L-threoninol cyclic (2→7)-disulfide.

PROCESS-RELATED IMPURITIES

- **TRIFLUOROACETIC ACID (TFA) IN PEPTIDES** ⟨503.1⟩: NMT 0.25%.
  [NOTE—Perform this test if trifluoroacetic acid is used in the manufacturing process.]

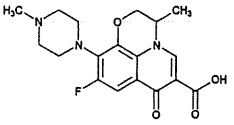
SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 10.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 466 USP Endotoxin Units/mg of octreotide acetate
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ **AND TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 100 cfu/g. The total yeast and mold count is NMT 100 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in air-tight containers. Store at a temperature of 2°–8°, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Endotoxin RS
  USP Octreotide Acetate RS
  USP Octreotide Non-Cyclic System Suitability Marker RS

Ofloxacin



$C_{18}H_{20}FN_3O_4$ 361.37

7*H*-Pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-, (±)-;
(±)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-l-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid [82419-36-1].

DEFINITION

Ofloxacin contains NLT 98.5% and NMT 101.5% of ofloxacin ($C_{18}H_{20}FN_3O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Medium: 0.1 N hydrochloric acid
Sample solution: 6.7 µg/mL in *Medium*
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Sample: 100 mg of Ofloxacin
Analysis: Dissolve the *Sample* in 275 mL of acetic anhydride. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass–silver chloride electrode system (see *Titrimetry* ⟨541⟩). Use the first of the two inflection points. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 36.138 mg of $C_{18}H_{20}FN_3O_4$.
Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ARSENIC**, *Method II* ⟨211⟩: NMT 1 µg/g
- **ORGANIC IMPURITIES**

Diluent: Acetonitrile and water (1:6)
Buffer: 3.1 g/L of ammonium acetate and 5.4 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.2.
Mobile phase: Acetonitrile and *Buffer* (240:1300)
System suitability solution: 0.4 µg/mL each of USP Ofloxacin RS and USP Ofloxacin Related Compound A RS in *Diluent*
Standard solution: 0.4 µg/mL of USP Ofloxacin RS in *Diluent*
Sample solution: 0.2 mg/mL of Ofloxacin in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 294 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 45°
Flow rate: 0.5 mL/min
Injection volume: 10 µL
Run time: 2.5 times the retention time of the ofloxacin peak
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between ofloxacin and ofloxacin related compound A
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Ofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response of ofloxacin from the *Standard solution*
C_S = concentration of USP Ofloxacin RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurities: NMT 0.3%. Disregard any peak less than 0.02%.
Total impurities: NMT 0.5%

- **LIMIT OF METHANOL AND ETHANOL**

Internal standard solution: 5.6 nL/mL of *n*-propyl alcohol in sodium hydroxide solution (1 in 100)
Standard solution: 10.0 µg/mL each of methanol and dehydrated alcohol in *Internal standard solution*. Transfer 2 mL of this solution to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 90° for 2 min, and shake for 6 min.

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Sample solution: Transfer 40 mg of Ofloxacin to a vial fitted with a septum and crimp cap, add 2 mL of *Internal standard solution*, and seal the vial. Heat the sealed vial at 90° for 2 min, and shake for 6 min.
Blank: Transfer 2 mL of *Internal standard solution* to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 90° for 2 min, and shake for 6 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused silica column, coated with a 3.0-µm film of stationary phase G43, and a fused silica precolumn
Temperatures
Injector: 170°
Detector: 250°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	—	35	3
35	20	90	—
90	40	200	2

Condition the column with the helium flowing at 200° for 2 h or until a stable baseline is obtained.
Carrier gas: Helium
Flow rate: 7 mL/min
Injection volume: 1-mL headspace. Use a heated, gas-tight syringe to make injections of the headspace into the chromatograph.
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for methanol, ethanol, and *n*-propyl alcohol are 0.5, 0.6, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between methanol and ethanol
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of methanol and ethanol in the portion of Ofloxacin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methanol or ethanol to the internal standard, corrected for the blank, from the *Sample solution*
R_S = peak response ratio of methanol or ethanol to the internal standard, corrected for the blank, from the *Standard solution*
C_S = concentration of methanol or ethanol in the *Standard solution* (µg/mL)
C_U = concentration of Ofloxacin in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.005% of methanol and NMT 0.05% of ethanol

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in chloroform
Acceptance criteria: +1° to −1°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ofloxacin RS
USP Ofloxacin Related Compound A RS
9-Fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.
$C_{17}H_{18}FN_3O_4$ 347.34

Ofloxacin Ophthalmic Solution

DEFINITION
Ofloxacin Ophthalmic Solution is a sterile aqueous solution of Ofloxacin. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHY**
Standard stock solution: 3.0 mg/mL of USP Ofloxacin RS in a mixture of chloroform and methanol (1:1)
Standard solution: 0.3 mg/mL of USP Ofloxacin RS from *Standard stock solution* prepared as follows. Transfer 5.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 5 mL of water, and dilute with a mixture of chloroform and methanol (1:1) to volume.
Sample solution: 0.3 mg/mL of ofloxacin from a portion of Ophthalmic Solution in a mixture of chloroform and methanol (1:1)
Application volume: 2 µL
Developing solvent system: Chloroform, methanol, and a solution (1 in 30) of ammonium hydroxide (150:75:15). Saturate a paper-lined chromatographic chamber with this mixture.
• **B.** The retention time of the ofloxacin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, 0.24% sodium dodecyl sulfate, and glacial acetic acid (400:580:20)
0.05 N hydrochloric acid: Add 4.0 mL of hydrochloric acid to 500 mL of water, dilute with water to 1000 mL, and mix.
System suitability solution: 0.1 mg/mL of USP Ofloxacin RS and 2.4 mg/mL of propylparaben in acetonitrile
Standard solution: 0.06 mg/mL of USP Ofloxacin RS in *0.05 N hydrochloric acid*
Sample solution: 0.06 mg/mL of ofloxacin from Ophthalmic Solution in *0.05 N hydrochloric acid*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 294 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

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Suitability requirements
Resolution: NLT 2 between the propylparaben and ofloxacin peaks, *System suitability solution*
Tailing factor: NMT 3, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of ofloxacin from the *Sample solution*
r_S = peak area of ofloxacin from the *Standard solution*
C_S = concentration of USP Ofloxacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed for *Membrane Filtration* in the test for *Sterility of the Product to Be Examined.*
- **PH** ⟨791⟩**:** 6.0–6.8

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ofloxacin RS

Ofloxacin Tablets

DEFINITION
Ofloxacin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION
- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
Solution A: Dissolve 2.72 mg/mL of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 3.3 ± 0.1.
Mobile phase: Acetonitrile and *Solution A* (3:22)
Diluent 1: Methanol and glacial acetic acid (3:1)
Diluent 2: Acetonitrile and water (1:9)
Standard stock solution: 1 mg/mL of USP Ofloxacin RS in *Diluent 1*
Standard solution: 20 µg/mL of USP Ofloxacin RS from the *Standard stock solution* in *Diluent 2*
Sample solution: Transfer an equivalent of 100 mg of ofloxacin from powdered Tablets to a 100-mL volumetric flask. Add 70 mL of *Diluent 1*, and sonicate for 20 min. Dilute with *Diluent 1* to volume. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, and collect the filtrate. Dilute 2.0 mL of the filtrate with *Diluent 2* to 100 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 294 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{18}H_{20}FN_3O_4$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ofloxacin RS in the *Standard* solution (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard stock solution: 0.44 mg/mL of USP Ofloxacin RS in methanol
Standard solution: 8.8 µg/mL of USP Ofloxacin RS from the *Standard stock solution* diluted with *Medium*
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Dilute a portion of the filtrate with *Medium* to obtain a final theoretical concentration of 8.8 µg/mL, assuming complete dissolution of the label claim.
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 294 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{18}H_{20}FN_3O_4$ dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (100/L)$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of ofloxacin in the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim of a Tablet (mg)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{18}H_{20}FN_3O_4$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
ORGANIC IMPURITIES
- **Procedure 1**
Phosphate buffer: 2.72 mg/mL of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 3.3 ± 0.1.

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Solution A: Acetonitrile and *Phosphate buffer* (3:22)
Solution B: Acetonitrile and *Phosphate buffer* (3:2)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	100	0
25	40	60
26	100	0
40	100	0

Standard solution: 4 µg/mL of USP Ofloxacin RS in methanol
Sample solution: Transfer an equivalent of 100 mg of ofloxacin from powdered Tablets to a 100-mL volumetric flask. Add 70 mL of methanol, and sonicate for 20 min. Dilute with methanol to volume. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, discarding the first 5 mL. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 294 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of ofloxacin from the *Standard solution*
C_S = concentration of USP Ofloxacin RS in the *Standard* solution (mg/mL)
C_U = nominal concentration of ofloxacin in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity A[a]	0.5	1	0.3
Impurity B[b]	3.6	0.22	0.3
Any other impurity	—	1	0.2
All impurities	—	—	1.0

[a] (2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid).
[b] (9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid).

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ofloxacin RS

Hydrophilic Ointment

DEFINITION
Prepare Hydrophilic Ointment as follows.

Methylparaben	0.25 g
Propylparaben	0.15 g
Sodium Lauryl Sulfate	10 g
Propylene Glycol	120 g
Stearyl Alcohol	250 g
White Petrolatum	250 g
Purified Water	370 g
To make about	1000 g

Melt the *Stearyl Alcohol* and the *White Petrolatum* on a steam bath, and warm to about 75°. Add the other ingredients, previously dissolved in *Purified Water* and warmed to 75°, and stir the mixture until it congeals.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight containers.

White Ointment

DEFINITION
Prepare White Ointment as follows.

White Wax	50 g
White Petrolatum	950 g
To make	1000 g

Melt the *White Wax* in a suitable dish on a water bath, add the *White Petrolatum*, warm until liquefied, then discontinue the heating, and stir the mixture until it begins to congeal.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in well-closed containers.

Yellow Ointment

DEFINITION
Prepare Yellow Ointment as follows.

Yellow Wax	50 g
Petrolatum	950 g
To make	1000 g

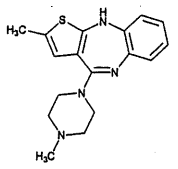
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Melt the *Yellow Wax* in a suitable dish on a steam bath, add the *Petrolatum*, warm until liquefied, then discontinue the heating, and stir the mixture until it begins to congeal.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in well-closed containers.

Olanzapine



$C_{17}H_{20}N_4S$ 312.43

10*H*-Thieno[2,3-*b*][1,5]benzodiazepine, 2-methyl-4-(4-methyl-1-piperazinyl)-;
2-Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine [132539-06-1].

DEFINITION

Olanzapine contains NLT 98.0% and NMT 102.0% of olanzapine ($C_{17}H_{20}N_4S$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 6.9 g of monobasic sodium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 2.5, and dissolve 12 g of sodium dodecyl sulfate in the resulting solution.
Mobile phase: Acetonitrile and *Buffer* (47:53)
System suitability solution: 0.1 mg/mL of USP Olanzapine RS and 0.01 mg/mL of USP Olanzapine Related Compound A RS in *Mobile phase*
Standard solution: 0.1 mg/mL of USP Olanzapine RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Olanzapine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for olanzapine related compound A and olanzapine are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between olanzapine related compound A and olanzapine
Tailing factor: 0.8–1.5 for the olanzapine peak
Relative standard deviation: NMT 1.0% for the olanzapine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of olanzapine ($C_{17}H_{20}N_4S$) in the portion of Olanzapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
C_U = concentration of Olanzapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer: Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water. Add 5 mL of phosphoric acid, and adjust with a sodium hydroxide solution to a pH of 2.5.
Solution A: Acetonitrile and *Buffer* (48:52)
Solution B: Acetonitrile and *Buffer* (70:30)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
20	0	100
25	0	100
27	100	0
35	100	0

Edetate disodium solution: 37 mg/L of edetate disodium in *Buffer*
Diluent: Acetonitrile and *Edetate disodium solution* (40:60)
System suitability solution: 20 µg/mL of USP Olanzapine RS and 2 µg/mL each of USP Olanzapine Related Compound A RS and USP Olanzapine Related Compound B RS in *Diluent*
Standard solution: 2 µg/mL of USP Olanzapine RS in *Diluent*
Sample solution: 0.4 mg/mL of Olanzapine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Temperatures
Column: 35°
Sample: 5°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—Identify the peaks using the *Relative Retention Time* values given in *Table 2*.]
Suitability requirements
Resolution: NLT 3.0 between olanzapine related compound A and olanzapine
Tailing factor: NMT 1.5 for the olanzapine peak

USP Monographs

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Relative standard deviation: NMT 2.0% from four replicate injections for the olanzapine peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Olanzapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of olanzapine from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

C_U = concentration of Olanzapine in the *Sample solution* (mg/mL)

F = relative response factor for each impurity from *Table 2*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olanzapine related compound B[a]	0.3	2.3	0.10
Olanzapine related compound A[b]	0.8	2.3	0.10
Olanzapine	1.0	—	—
Chloromethyl olanzapinium chloride[c] (if present)	1.1	1.0	0.15
Any individual, unspecified impurity	—	—	0.10
Total impurities	—	—	0.4

[a] 2-Methyl-10*H*-thieno-[2,3-*b*][1,5]benzodiazepin-4[5*H*]-one.
[b] 5-Methyl-2-((2-nitrophenyl)amino)-3-thiophenecarbonitrile.
[c] 1-Chloromethyl-1-methyl-4-(2-methyl-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepin-4-yl)piperazin-1-ium chloride.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩

[NOTE—A suitable solvent system for water determination in ketones and aldehydes (e.g., Hydranal composite 5K-working medium K or Aquastar composite 5K-solvent KC or equivalent) is recommended.]

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Olanzapine RS

USP Olanzapine Related Compound A RS
5-Methyl-2-((2-nitrophenyl)amino)-3-thiophenecarbonitrile.
$C_{12}H_9N_3O_2S$ 259.28

USP Olanzapine Related Compound B RS
2-Methyl-10*H*-thieno-[2,3-*b*][1,5]benzodiazepin-4[5*H*]-one.
$C_{12}H_{10}N_2OS$ 230.29

Olanzapine Tablets

DEFINITION

Olanzapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$).

IDENTIFICATION

Change to read:

- **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Standard: Dissolve 10 mg of USP Olanzapine RS in 10 mL of chloroform. Evaporate to dryness on a water bath maintained at 55°. Use about 2 mg of the residue to prepare a potassium bromide pellet.

Sample: Crush NLT 5 Tablets, and transfer the powder equivalent to 30 mg of olanzapine to a suitable container. Add 30 mL of chloroform, and sonicate for 15 min to dissolve. Pass through a suitable filter, and evaporate the filtrate to dryness on a water bath maintained at 55°. Use about 2 mg of the residue to prepare a potassium bromide pellet.

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

[NOTE—A few drops of acetonitrile, not to exceed 5% of the final volume, may be added to the *Standard solution* and *Sample solution* before final dilution to reduce foaming.]

Buffer 1: 6.9 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Buffer 2: 12 g/L of sodium dodecyl sulfate in *Buffer 1*

Mobile phase: Acetonitrile and *Buffer 2* (1:1)

System suitability solution: 0.1 mg/mL of USP Olanzapine RS and 0.01 mg/mL of USP Olanzapine Related Compound A RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Olanzapine RS in *Mobile phase*

Sample solution: Transfer a known quantity of Tablets (NLT 5), equivalent to NLT 25 mg of olanzapine, to a suitable volumetric flask. Dilute with *Mobile phase* to volume, mix, and sonicate for 10 min. Centrifuge a portion of this solution, and dilute the clear supernatant with *Mobile phase* to obtain a solution containing about 0.1 mg/mL of olanzapine. [NOTE—Agitation of the flask may be necessary before sonication to prevent Tablets from adhering to the flask, making disintegration and dissolution difficult.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for olanzapine related compound A and olanzapine are 0.89 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between olanzapine and olanzapine related compound A, *System suitability solution*

Tailing factor: NMT 1.8, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: 10 g/L of ammonium acetate in a mixture of methanol and water (2:3). Adjust with hydrochloric acid to a pH of 4.0.
Standard solution: (*L*/1000) mg/mL of USP Olanzapine RS in *Medium*, where *L* is the label claim in mg/Tablet. Transfer 5.0 mL of this solution to a tube, and add 2.0 mL of *Mobile phase*.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Transfer 5.0 mL of the filtrate to a tube, and add 2.0 mL of *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 20 min
Mobile phase: 10 g/L of ammonium acetate in a mixture of methanol and water (2:3). Adjust with hydrochloric acid to a pH of 4.0. Pass through a suitable filter of 0.45-µm pore size.
Standard stock solution: 0.28 mg/mL of USP Olanzapine RS prepared as follows. Transfer a suitable amount of USP Olanzapine RS to a suitable volumetric flask. Add about 8% of the final flask volume of acetonitrile. Sonicate to dissolve the Reference Standard. Dilute with *Medium* to volume.
Standard solution: (*L*/900) mg/mL of USP Olanzapine RS in *Medium* from *Standard stock solution*, where *L* is the label claim in mg/Tablet. Transfer 5.0 mL of this solution to a tube, and add 2.0 mL of *Mobile phase*.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Transfer 5.0 mL of the filtrate to a tube, and add 2.0 mL of *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L11
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

[Note—A few drops of acetonitrile, not to exceed 5% of the final volume, may be added to the *Standard solution* and *Sample solution* before final dilution to reduce foaming.]
Buffer 1: 3.3 mL/L of phosphoric acid. Adjust with 50% sodium hydroxide to a pH of 2.5.
Buffer 2: 8.7 g/L of sodium dodecyl sulfate in *Buffer 1*
Buffer 3: 18.6 mg/L of edetate disodium (EDTA) in *Buffer 2*
Solution A: Acetonitrile and *Buffer 2* (12:13)
Solution B: Acetonitrile and *Buffer 2* (7:3)
Diluent: Acetonitrile and *Buffer 3* (2:3)
System suitability solution: 20 µg/mL of USP Olanzapine RS and 2 µg/mL each of USP Olanzapine Related Compound B RS and USP Olanzapine Related Compound C RS in *Diluent*
Standard solution: 0.002 mg/mL of USP Olanzapine RS in *Diluent*
Sensitivity solution: 0.4 µg/mL of USP Olanzapine RS in *Diluent* from the *Standard solution*
Sample solution: Nominally 0.375–0.500 mg/mL of olanzapine from a suitable number of Tablets prepared as follows. Transfer a known quantity of Tablets to a suitable volumetric flask, and dilute with *Diluent* to volume. Centrifuge a portion of this solution, and use the

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supernatant. [NOTE—Immediate agitation of the flask may be necessary to prevent Tablets from adhering to the flask, making dissolution and disintegration difficult. [**CAUTION**—Do not sonicate.] The *Sample solution* is stable for 12 h at room temperature and 48 h if refrigerated.]

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
20	0	100
25	0	100
27	100	0
35	100	0

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 3.0 between olanzapine and olanzapine related compound C, *System suitability solution*
Tailing factor: NMT 1.5 for the olanzapine peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)
F = relative response factor for each impurity listed in *Table 2*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olanzapine lactam[a]	0.26	1.0	0.50
Olanzapine related compound B	0.30	2.3	0.50
Olanzapine thiolactam[b]	0.34	1.0	0.50
Olanzapine related compound C	0.83	0.76	0.50
Olanzapine	1.0	—	—
Any individual unspecified degradation product	—	1.0	0.20
Total impurities	—	—	1.5

[a] (*Z*)-4-(4-Methylpiperazin-1-yl)-3-(2-oxopropylidene)-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one.
[b] (*Z*)-1-{4-(4-Methylpiperazin-1-yl)-2-thioxo-1*H*-benzo[*b*][1,4]diazepin-3(2*H*)-ylidene}propan-2-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Olanzapine RS
  USP Olanzapine Related Compound A RS
  5-Methyl-2-((2-nitrophenyl)amino)-3-thiophenecarbonitrile.
  $C_{12}H_9N_3O_2S$ 259.28
  USP Olanzapine Related Compound B RS
  2-Methyl-10*H*-thieno-[2,3-*b*][1,5]benzodiazepin-4[5*H*]-one.
  $C_{12}H_{10}N_2OS$ 230.29
  USP Olanzapine Related Compound C RS
  2-Methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine 4′-*N*-oxide.
  $C_{17}H_{20}N_4OS$ 328.43

Olanzapine and Fluoxetine Capsules

DEFINITION

Olanzapine and Fluoxetine Capsules contain an amount of Olanzapine and Fluoxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% each of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) and fluoxetine ($C_{17}H_{18}F_3NO$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectra of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 37 mg/L of disodium ethylenediaminetetraacetate in water. Add 3.3 mL of phosphoric acid, and adjust with 50% sodium hydroxide to a pH of 2.5. Dissolve 8.7 g of sodium dodecyl sulfate in the resulting solution.

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Mobile phase: Acetonitrile and *Buffer* (1:1)
Standard solution: 0.12 mg/mL of USP Olanzapine RS and 0.45 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 0.06–0.18 mg/mL of olanzapine and 0.25–0.5 mg/mL of fluoxetine in *Mobile phase* from a counted number of Capsules prepared as follows. Place the Capsules (including shells) into a suitable volumetric flask and fill to about half volume with *Mobile phase*. Mix for NLT 30 min. If disintegration is incomplete, sonicate for NMT 5 min. Dilute with *Mobile phase* to volume, mix, and filter or centrifuge.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ (USP 1-Aug-2019)
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 10 µL
Run time: 2.5 times the retention time of olanzapine
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for olanzapine and fluoxetine are 1.0 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 2.0 between olanzapine and fluoxetine
Relative standard deviation: NMT 2.0% for the olanzapine and fluoxetine peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of olanzapine from the *Sample solution*
r_S = peak response of olanzapine from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of fluoxetine from the *Sample solution*
r_S = peak response of fluoxetine from the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluoxetine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Acceptance criteria: 90.0%–110.0% ▲each of olanzapine and fluoxetine▲ (USP 1-Aug-2019)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated. [NOTE—Helium sparging recommended.]
Apparatus 2: 50 rpm, with 3-prong sinkers
Time: 30 min for both olanzapine and fluoxetine
Standard solution: USP Olanzapine RS and USP Fluoxetine Hydrochloride RS in *Medium* to obtain a final concentration of (L/1000) mg/mL each, where L is label claim, in mg/Capsule.
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Buffer, Mobile phase, Chromatographic system, System suitability, and Analysis: Proceed as directed in the *Assay*.
Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of olanzapine from the *Sample solution*
r_S = peak response of olanzapine from the *Standard solution*
C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)
L = label claim for olanzapine (mg/Capsule)
V = volume of *Medium*, 900 mL

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

r_U = peak response of fluoxetine from the *Sample solution*
r_S = peak response of fluoxetine from the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim for fluoxetine (mg/Capsule)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of olanzapine ($C_{17}H_{20}N_4S$) and fluoxetine ($C_{17}H_{18}F_3NO$) are dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
System suitability solution: 0.1 mg/mL of USP Olanzapine RS, 0.11 mg/mL of USP Fluoxetine Hydrochloride RS, and 0.002 mg/mL each of α[(2-methylamino)ethyl] benzyl alcohol, trifluoro-*p*-cresol, USP Fluoxetine Related Compound B RS, and USP Olanzapine Related Compound B RS in *Mobile phase*
▲**Sensitivity solution:** 3 µg/mL of USP Olanzapine RS, 3 µg/mL of USP Fluoxetine Hydrochloride RS, and 0.06 µg/mL each of α[(2-methylamino)ethyl] benzyl alcohol, trifluoro-*p*-cresol, USP Fluoxetine Related Compound B RS, and USP Olanzapine Related Compound B RS in *Mobile phase*▲ (USP 1-Aug-2019)

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Standard solution: 2 µg/mL of USP Olanzapine RS and 8 µg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*

Sample solution: Empty the Capsules, and combine the contents in a suitable container. The contents of the Capsules may be powdered in a mortar, if necessary. Transfer an amount of the sample to a suitable volumetric flask to obtain nominally 0.2 mg/mL of olanzapine and 0.27–1.7 mg/mL of fluoxetine, and fill to about 70% volume with *Mobile phase*. Mix for about 5 min. Dilute with *Mobile phase* to volume, mix, and filter or centrifuge.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Temperatures

Autosampler: 5°

Column: 35°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

Run time: 1.5 times the retention time of fluoxetine

System suitability

Samples: *System suitability solution*, ▲*Sensitivity solution*,▲ (USP 1-Aug-2019) and *Standard solution*

Suitability requirements

[NOTE—Identify the peaks using *Table 1*.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
α[(2-Methylamino)ethyl] benzyl alcohol[a]	0.22	—	0.25
Olanzapine related compound B[b]	0.24	1.73	0.20
Trifluoro-*p*-cresol[a]	0.30	—	0.25
Fluoxetine related compound B[a]	0.31	—	0.25
Olanzapine	0.63	—	—
Fluoxetine	1.0	—	—
Any individual fluoxetine related degradation product	—	—	0.25
Any individual olanzapine related degradation product[c]	—	1.0	0.20
Total impurities (fluoxetine related)[d]	—	—	0.40
Total impurities (olanzapine related)[e]	—	—	1.5

[a] Fluoxetine related degradation product.

[b] Olanzapine related degradation product.

[c] Any other degradation product with a relative retention time <0.63 except fluoxetine related degradation products, and any degradation product with a relative retention time >1.0.

[d] Sum of all specified fluoxetine related degradation products and any other fluoxetine related degradation product with relative retention times ▲between▲ (USP 1-Aug-2019) 0.63 and 1.0.

[e] Sum of all specified olanzapine degradation products, any other degradation product with a relative retention time <0.63 except fluoxetine related degradation products, and any degradation product with relative retention time >1.0.

Resolution: NLT 1.9 between α[(2-methylamino)ethyl] benzyl alcohol and olanzapine related compound B, *System suitability solution*

Tailing factor: NMT 1.8 for olanzapine and fluoxetine, *System suitability solution* and *Standard solution*

▲**Relative standard deviation:** NMT 2.0% for the olanzapine and fluoxetine peaks, *Standard solution*

Signal-to-noise ratio: NLT 3 for the α[(2-methylamino) ethyl] benzyl alcohol peak, *Sensitivity solution*▲ (USP 1-Aug-2019)

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Peaks eluting before a relative retention time of 0.63 and after a relative retention time of 1.0, excluding any peak with relative retention times of 0.22, 0.30, and 0.31, are olanzapine related degradation products.]

Calculate the percentage of each olanzapine related degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of olanzapine from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

[NOTE—Peaks eluting at relative retention times of 0.22, 0.30, and 0.31, and any peaks between a relative retention time of 0.63 and 1.0, are fluoxetine related degradation products.]

Calculate the percentage of each fluoxetine related degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of fluoxetine from the *Standard solution*

C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fluoxetine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of fluoxetine, 309.33

M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Acceptance criteria: See *Table 1*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Fluoxetine Hydrochloride RS

▲▲ (USP 1-Aug-2019)

USP Fluoxetine Related Compound B RS

N-Methyl-3-phenylpropylamine.

$C_{10}H_{15}N$ ▲149.24▲ (USP 1-Aug-2019)

USP Olanzapine RS

▲▲ (USP 1-Aug-2019)

USP Olanzapine Related Compound B RS

2-Methyl-10*H*-thieno-[2,3-*b*][1,5]benzodiazepin-4[5*H*]-one.

$C_{12}H_{10}N_2OS$ 230.29

USP Monographs

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Olanzapine Orally Disintegrating Tablets

DEFINITION

Olanzapine Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Standard: Add 25 mg of USP Olanzapine RS to a suitable container containing 10 mL of water. Centrifuge for 5 min, and discard the supernatant. Dry the olanzapine under vacuum for 1 h at 60°.

Sample: Place 1 Tablet in 10 mL of water in a centrifuge tube. Centrifuge for 5 min. Wash the precipitate with 5 mL of water, and centrifuge for 5 min. Discard the water, and repeat the process a third time. Dry the olanzapine under vacuum for 1 h at 60°.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Acetonitrile and water (20:80). Add 2 mL of perchloric acid to each L of the mixture.

Solution B: Acetonitrile and water (60:40). Add 2 mL of perchloric acid to each L of the mixture.

Diluent: Acetonitrile and water (35:65)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	87	13
10	87	13
20	0	100
26	0	100
26.1	87	13
36	87	13

System suitability solution: 0.1 mg/mL of USP Olanzapine RS and 10 µg/mL of USP Olanzapine Related Compound C RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Olanzapine RS in *Diluent*

Sample stock solution: 0.5–0.6 mg/mL of olanzapine from NLT 10 Tablets, prepared as follows. Transfer the Tablets to a suitable volumetric flask. Add *Diluent* to fill about 80% of the flask volume. Shake mechanically for 10 min, and dilute with *Diluent* to volume.

Sample solution: Nominally, 0.1–0.12 mg/mL of olanzapine from the *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the olanzapine and olanzapine related compound C peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the olanzapine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of olanzapine from the *Sample solution*

r_S = peak response of olanzapine from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION ⟨701⟩**

Test 1: NMT 10 s for each of 6 units

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Disintegration Test 2*

Acceptance criteria: NMT 30 s

- **DISSOLUTION ⟨711⟩**

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 10 min

Standard solution: (*L*/900) mg/mL of USP Olanzapine RS in *Medium*

Sample solution: A portion of the solution under test may be either centrifuged or filtered through a suitable membrane filter of 0.45-µm pore size, discarding the first few mL. The filtrate or centrifugate may be suitably diluted.

Determine the amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved by using either the *Spectrometric procedure* or *Chromatographic procedure* described below:

Spectrometric procedure

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 344 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Chromatographic procedure

Buffer: 6.8 g/L of sodium acetate trihydrate in water. Adjust with glacial acetic acid to a pH of 5.0.

Mobile phase: Acetonitrile and *Buffer* (25:75)

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of olanzapine from the *Sample solution*
r_S = peak response of olanzapine from the *Standard solution*
C_S = concentration of USP Olanzapine RS (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: Acetonitrile and water (20:80). Add 2 mL of perchloric acid to each L of the mixture.
Solution B: Acetonitrile and water (60:40). Add 2 mL of perchloric acid to each L of the mixture.
Diluent: Acetonitrile and water (35:65)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	93	7
13	93	7
28	0	100
33	0	100
33.1	93	7
44	93	7

System suitability solution: 0.1 mg/mL of USP Olanzapine RS and 7 µg/mL of USP Olanzapine Related Compound C RS in *Diluent*
Standard solution: 1.5 µg/mL of USP Olanzapine RS in *Solution A*
Sensitivity solution: 0.15 µg/mL of USP Olanzapine RS from the *Standard solution* in *Diluent*
Sample solution: Nominally, 0.3 mg/mL of olanzapine from NLT 10 Tablets, prepared as follows. Transfer the Tablets to a suitable volumetric flask. Add *Diluent* to fill about 80% of the flask volume. Shake mechanically for 25 min, and dilute with *Diluent* to volume. Dilute a portion of this solution with water to volume. Store the solution at 4°. Use within 7 h.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Sample temperature: 4°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between the olanzapine and olanzapine related compound C peaks, *System suitability solution*
Tailing factor: NMT 2.0 for the olanzapine peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual specified impurity and any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual specified impurity from the *Sample solution*
r_S = peak response of olanzapine from the *Standard solution*
C_S = concentration of olanzapine in the *Standard solution* (mg/mL)
C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. [NOTE—Disregard any peak less than 0.05%.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olanzapine lactam[a]	0.48	1.9	0.50
Olanzapine thiolactam[b]	0.75	1.0	0.50
Olanzapine	1.0	—	—
Olanzapine related compound C	1.2	0.79	0.50
Olanzapine related compound B[c]	2.1	1.9	0.50
Any individual unspecified degradation product	—	1.0	0.20
Total impurities	—	—	1.5

[a] (*Z*)-4-(4-Methylpiperazin-1-yl)-3-(2-oxopropylidene)-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one.
[b] (*Z*)-1-{4-(4-Methylpiperazin-1-yl)-2-thioxo-1*H*-benzo[*b*][1,4]diazepin-3(2*H*)-ylidene}propan-2-one.
[c] 2-Methyl-5,10-dihydro-4*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepin-4-one.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **LABELING:** When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1*

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is not used. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Olanzapine RS
USP Olanzapine Related Compound C RS
2-Methyl-4-(4-methylpiperazin-1-yl)-10*H*-benzo[*b*]thieno[2,3-*e*][1,4]diazepine 4′-*N*-oxide.
$C_{17}H_{20}N_4OS$ 328.43

Oleovitamin A and D

DEFINITION
Oleovitamin A and D is a solution of vitamin A and vitamin D in fish liver oil or in an edible vegetable oil. The vitamin D is present as ergocalciferol or cholecalciferol obtained by the activation of ergosterol or 7-dehydrocholesterol, or from natural sources. Oleovitamin A and D contains NLT 90.0% of the labeled amounts of vitamins A and D.

ASSAY
• **A. VITAMIN A ASSAY** ⟨571⟩
Analysis: The acceptance criteria may be met by following any one of the specified procedures described in the *Chemical Methods* or *Chromatographic Methods* in the chapter. The method and procedure used should be stated in the labeling.
Acceptance criteria: NLT 90.0% of the labeled amount
• **B. VITAMIN D ASSAY** ⟨581⟩
Analysis: The acceptance criteria may be met by following any one of the specified procedures described in the *Chromatographic Methods* in the chapter. The procedure used should be stated in the labeling.
Acceptance criteria: NLT 90.0% of the labeled amount

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and air, preferably under an atmosphere of an inert gas. Store in a dry place.
• **LABELING:** Label it to indicate the content of vitamin A in terms of retinol/g. The vitamin A content may also be expressed in USP Vitamin A Units/g. Label it to show whether it contains ergocalciferol, cholecalciferol, or vitamin D from a natural source. Label it to indicate the vitamin D content, in µg, of ergocalciferol or cholecalciferol/g. Its vitamin D content may be expressed also in USP Vitamin D Units/g.

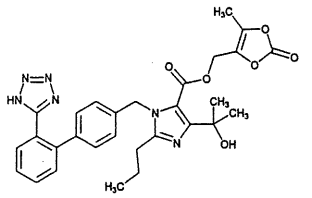
Oleovitamin A and D Capsules

DEFINITION
Oleovitamin A and D Capsules contain NLT 90.0% of the labeled amounts of vitamins A and D. The oil in Oleovitamin A and D Capsules is a solution of vitamin A and vitamin D in fish liver oil or in an edible vegetable oil. The vitamin D is present as ergocalciferol or cholecalciferol obtained by the activation of ergosterol or 7-dehydrocholesterol or from natural sources.

ASSAY
• **VITAMIN A ASSAY** ⟨571⟩
Analysis: Use the appropriate *Chemical Method* or *Chromatographic Method* as described in the chapter.
Acceptance criteria: NLT 90.0% of the labeled amount
• **VITAMIN D ASSAY** ⟨581⟩
Analysis: Use the appropriate *Chromatographic Method* as described in the chapter.
Acceptance criteria: NLT 90.0% of the labeled amount

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a dry place.
• **LABELING:** Label the Capsules to indicate the content, in mg, of vitamin A in each Capsule. The vitamin A content in each Capsule may be expressed also in USP Vitamin A Units. Label the Capsules to show whether they contain ergocalciferol, cholecalciferol, or vitamin D from a natural source. Label the Capsules to indicate also the vitamin D content, in µg, in each Capsule. The vitamin D content may be expressed also in USP Vitamin D Units in each Capsule.

Olmesartan Medoxomil



$C_{29}H_{30}N_6O_6$ 558.59
1*H*-Imidazole-5-carboxylic acid, 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2′-(1*H*-tetrazol-5-yl) [1,1′-biphenyl]-4-yl]methyl]-, (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester; (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylate [144689-63-4].

DEFINITION
Olmesartan Medoxomil contains NLT 98.5% and NMT 101.5% of $C_{29}H_{30}N_6O_6$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The ratio of the retention time of the major peak to that of the internal standard of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
[NOTE—The *Standard solution* and *Sample solution* are stable for 24 h at 5°.]
Diluted phosphoric acid: 0.2% phosphoric acid
Buffer: 0.015 M monobasic potassium phosphate. Adjust the solution with *Diluted phosphoric acid* (w/v) to a pH of 3.4.
Mobile phase: Acetonitrile and *Buffer* (17:33)
Diluent 1: Acetonitrile and water (4:1)
Diluent 2: Acetonitrile and water (2:3)
Internal standard solution: 0.5 mg/mL of 4-hydroxybenzoic acid isobutyl ester in *Diluent 2*. [NOTE—This solution is stable for 1 month at room temperature.]
Standard stock solution: 1 mg/mL of USP Olmesartan Medoxomil RS in *Diluent 1*

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Standard solution: 0.05 mg/mL of USP Olmesartan Medoxomil RS from the *Standard stock solution* and 0.025 mg/mL of *p*-hydroxybenzoic acid isobutyl ester from the *Internal standard solution* in *Diluent 2*
Sample stock solution: 1 mg/mL of Olmesartan Medoxomil in *Diluent 1*
Sample solution: 0.05 mg/mL of Olmesartan Medoxomil from the *Sample stock solution* and 0.025 mg/mL of *p*-hydroxybenzoic acid isobutyl ester from the *Internal standard solution* in *Diluent 2*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4 between olmesartan medoxomil and *p*-hydroxybenzoic acid isobutyl ester
Relative standard deviation: NMT 0.5% for the peak ratio of olmesartan medoxomil and the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of olmesartan medoxomil in the portion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the peak areas of olmesartan medoxomil and *p*-hydroxybenzoic acid isobutyl ester from the *Sample solution*
R_S = ratio of the peak areas of olmesartan medoxomil and *p*-hydroxybenzoic acid isobutyl ester from the *Standard solution*
C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)
C_U = concentration of Olmesartan Medoxomil in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the anhydrous and solvent-free basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%. [NOTE—The ignition temperature range is 450° to 550°.]

ORGANIC IMPURITIES

• **Procedure**
Buffer: Prepare as directed in the *Assay*.
Solution A: Acetonitrile and *Buffer* (1:4)
Solution B: Acetonitrile and *Buffer* (4:1)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	75	25
35	0	100
45	0	100

System suitability solution: 0.01 mg/mL each of USP Olmesartan Medoxomil RS and USP Olmesartan Medoxomil Related Compound A RS in acetonitrile
Standard solution: 0.01 mg/mL of USP Olmesartan Medoxomil RS in acetonitrile
Sample solution: 1 mg/mL of Olmesartan Medoxomil in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—A guard column of 4.6-mm × 5-cm of packing L7 may be used.]
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Suitability requirements
Sample: *System suitability solution*
Resolution: NLT 5 between olmesartan medoxomil and olmesartan medoxomil related compound A
Relative standard deviation: NMT 2.0% for the olmesartan medoxomil peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Olmesartan Medoxomil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of olmesartan medoxomil from the *Standard solution*
C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)
C_U = concentration of Olmesartan Medoxomil in the *Sample solution* (mg/mL)
F = relative response factor (see the *Impurity Table*)

Acceptance criteria
Individual impurities: See the *Impurity Table*.
Total impurities: NMT 1.3%. [NOTE—Disregard any peak below 0.05%.]

Impurity Table

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olmesartan[a]	0.2	1.0	0.5
Olmesartan medoxomil related compound A[b]	0.7	1.6	0.1
Olmesartan medoxomil	1.0	1.0	—
Olefinic impurity[c]	1.6	1.0	0.6
N-alkyl impurity[d]	3.4	0.7	0.1
Any other individual unidentified impurity	—	1.0	0.1

[a] 1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylic acid.
[b] 1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1*H*-furo[3,4-d]imidazol-6(4*H*)-one.
[c] (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 1-((2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl)-4-(prop-1-en-2-yl)-2-propyl-1*H*-imidazole-5-carboxylate.
[d] (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-((2′-(2-trityl-2*H*-tetrazol-5-yl)biphenyl-4-yl)methyl)-1*H*-imidazole-5-carboxylate.

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SPECIFIC TESTS

• **LIMIT OF ACETONE (IF PRESENT)**

Internal standard solution: 1% solution of 1-butanol in dimethyl sulfoxide. [NOTE—This solution is stable for 1 month at room temperature.]

Standard solution: 0.37 µL/mL of acetone and 2 µL/mL of 1-butanol from the *Internal standard solution* in dimethylsulfoxide. [NOTE—This solution is stable for 8 h at room temperature.]

Sample solution: 25 mg/mL of Olmesartan Medoxomil and 2 µL/mL of 1-butanol from the *Internal standard solution* in dimethylsulfoxide. [NOTE—This solution is stable for 8 h at room temperature.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.53-mm column bonded with a 1-µm film of phase G14

Column temperature: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	5
50	10	180	5

Injection port temperature: 200°

Detector temperature: 200°

Autosampler temperature: 80°

Carrier gas: Helium

Flow rate: 4 mL/min. [NOTE—Adjust the flow rate so that the retention time of acetone is 2.5 min.]

Injection size: 1 mL

Split ratio: 5:1

System suitability

Sample: *Standard solution*. [NOTE—Allow the samples to stand for 30 min in the autosampler at 80°.]

Suitability requirements

Resolution: NLT 60 between the acetone and 1-butanol peaks

Relative standard deviation: NMT 5.0% for the peak area ratio of acetone and 1-butanol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetone in the portion of Olmesartan Medoxomil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetone from the *Sample solution*

r_S = peak response of acetone from the *Standard solution*

C_S = concentration of acetone in the *Standard solution* (mg/mL)

C_U = concentration of Olmesartan Medoxomil in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.6%

• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protect from moisture, and store below 25°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Olmesartan Medoxomil RS

USP Olmesartan Medoxomil Related Compound A RS

1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1*H*-furo[3,4-*d*]imidazol-6(4*H*)-one.

$C_{24}H_{24}N_6O_2$ 428.49

Olmesartan Medoxomil Tablets

DEFINITION

Olmesartan Medoxomil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$).

IDENTIFICATION

• **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 3.1 g/L of formic acid

Solution B: Acetonitrile and *Solution A* (10:90)

Solution C: Acetonitrile and *Solution A* (90:10)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	68.8	31.2
1.5	37.5	62.5
1.6	68.8	31.2
3.0	68.8	31.2

Diluent: Acetonitrile and water (60:40)

Standard solution: 40 µg/mL of USP Olmesartan Medoxomil RS in *Diluent*

Sample stock solution: Prepare solutions of nominal concentrations of olmesartan medoxomil in *Diluent* as follows. To NLT 10 Tablets for 5- and 20-mg Tablet strengths and NLT 5 Tablets for 40-mg Tablet strength in a 200-mL volumetric flask, add *Diluent* to volume. Sonicate with occasional shaking to disintegrate the Tablets completely, centrifuge the suspension, and use the supernatant.

Sample solution: Nominally 40 µg/mL of olmesartan medoxomil in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 249 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 2.1-mm × 5-cm; 1.7-µm packing L1

Column temperature: 35°

Flow rate: 0.6 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of olmesartan medoxomil from the *Sample solution*
r_S = peak response of olmesartan medoxomil from the *Standard solution*
C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of olmesartan medoxomil in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
Test 1
Medium: pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
For Tablets labeled to contain 5 mg: 500 mL
For Tablets labeled to contain 20 and 40 mg: 1000 mL
Apparatus 2: 50 rpm
Time: 30 min
Diluent: Acetonitrile and water (60:40)
Standard stock solution: 2 mg/mL of USP Olmesartan Medoxomil RS in *Diluent*
Standard solution: (*L*/*V*) mg/mL of USP Olmesartan Medoxomil RS in *Medium*, where *L* is the label claim in mg/Tablet and *V* is the volume of the *Medium* in mL from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a glass fiber filter of 1.2-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 258 nm
Cells
For Tablets labeled to contain 5 and 20 mg: 1 cm
For Tablets labeled to contain 40 mg: 0.5 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium* (see *Medium*)
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: pH 7.2 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Standard stock solution: 0.2 mg/mL of USP Olmesartan Medoxomil RS prepared as follows. Transfer an appropriate amount of USP Olmesartan Medoxomil RS into a suitable volumetric flask. Dissolve in 30% of the flask volume of acetonitrile. Dilute with *Medium* to volume and mix.
Standard solution: (*L*/1000) mg/mL of USP Olmesartan Medoxomil RS in *Medium*, from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first few milliliters of the filtrate.
Instrumental conditions
Mode: UV
Analytical wavelength: 257 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) is dissolved.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.05 M hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
Solution A: Acetonitrile and *Buffer* (20:80)
Solution B: Acetonitrile and *Buffer* (80:20)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
4.0	52	48
5.0	75	25
7.0	75	25

Diluent A: Acetonitrile, water, and phosphoric acid (50:50:2)
Diluent B: *Medium* and *Diluent A* (50:50)
Standard stock solution: 0.22 mg/mL of USP Olmesartan Medoxomil RS in *Diluent A*, prepared as follows. Transfer an appropriate amount of USP Olmesartan Medoxomil RS to a suitable volumetric flask. Add *Diluent A* to 60% of the total volume and sonicate to dissolve. Dilute with *Diluent A* to volume and mix well.
Standard solution
For Tablets labeled to contain 5 mg: 2.75 µg/mL of USP Olmesartan Medoxomil RS in *Diluent B* from the *Standard stock solution*
For Tablets labeled to contain 20 mg: 11 µg/mL of USP Olmesartan Medoxomil RS in *Diluent B* from the *Standard stock solution*
For Tablets labeled to contain 40 mg: 22 µg/mL of USP Olmesartan Medoxomil RS in *Diluent B* from the *Standard stock solution*

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Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first few milliliters of the filtrate. Transfer 5 mL of the filtered test solution to a 10-mL volumetric flask and dilute with *Diluent A* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperatures
Autosampler: 5°
Column: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for olmesartan and olmesartan medoxomil are 0.45 and 1.00, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for olmesartan medoxomil
Relative standard deviation: NMT 2.0% for olmesartan medoxomil

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_1) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of olmesartan medoxomil from the *Sample solution*
r_S = peak response of olmesartan medoxomil from the *Standard solution*
C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)

Calculate the concentration (C_2) of olmesartan as olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times (1/F) \times (M_{r2}/M_{r1})$$

r_U = peak response of olmesartan from the *Sample solution*
r_S = peak response of olmesartan medoxomil from the *Standard solution*
C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)
F = relative response factor, 0.88
M_{r2} = molecular weight of olmesartan medoxomil, 558.59
M_{r1} = molecular weight of olmesartan, 446.50

Calculate the percentage of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) dissolved:

$$\text{Result} = [(C_1 + C_2) \times D] \times V \times (1/L) \times 100$$

C_1 = concentration of olmesartan medoxomil in the *Sample solution* (mg/mL)
C_2 = concentration of olmesartan as olmesartan medoxomil in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim for olmesartan medoxomil (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) is dissolved.

▲**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.1 M hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Buffer: Dissolve 2.04 g of monobasic potassium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Diluent: Acetonitrile and water (60:40)
Standard stock solution: 1.1 mg/mL of USP Olmesartan Medoxomil RS in *Diluent*
Standard solution
[NOTE—Preserve immediately at 2°–8° after preparation.]
For Tablets labeled to contain 5 mg: 5.5 µg/mL of USP Olmesartan Medoxomil RS in *Medium* from the *Standard stock solution*
For Tablets labeled to contain 20 mg: 22 µg/mL of USP Olmesartan Medoxomil RS in *Medium* from the *Standard stock solution*
For Tablets labeled to contain 40 mg: 44 µg/mL of USP Olmesartan Medoxomil RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first 1 mL of the filtrate. [NOTE—Preserve immediately at 2°–8° after preparation.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.0-mm × 12.5-cm; 5-µm packing L1
Temperatures
Autosampler: 8°
Column: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for olmesartan and olmesartan medoxomil are 0.24 and 1.00, respectively.]
Suitability requirements
Tailing factor: 0.8–1.5 for olmesartan medoxomil
Relative standard deviation: NMT 2.0% for the sum of the peak responses of olmesartan and olmesartan medoxomil

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = sum of the peak responses of olmesartan and olmesartan medoxomil from the *Sample solution*
r_S = sum of the peak responses of olmesartan and olmesartan medoxomil from the *Standard solution*
C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of olmesartan medoxomil (mg/Tablet)

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Tolerances: NLT 80% (*Q*) of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) is dissolved.▲ (RB 19-Mar-2019)

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer: 0.015 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.5.
Solution A: Acetonitrile and *Buffer* (20:80)
Solution B: Acetonitrile and *Buffer* (79:21)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	75	25
35	0	100
45	0	100

Diluent: Acetonitrile and water (90:10)
System suitability solution: 0.01 mg/mL each of USP Olmesartan Medoxomil RS and USP Olmesartan Medoxomil Related Compound A RS in *Diluent*
Standard solution: 0.01 mg/mL of USP Olmesartan Medoxomil RS in *Diluent*
Sensitivity solution: 0.002 mg/mL of USP Olmesartan Medoxomil RS in *Diluent* from the *Standard solution*
Sample solution: Nominally 1 mg/mL of olmesartan medoxomil in *Diluent* prepared as follows. Dissolve a suitable number of Tablets in *Diluent*. Sonicate and/or shake occasionally to disintegrate the Tablets completely. Centrifuge and pass the supernatant through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 5 between olmesartan medoxomil and olmesartan medoxomil related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0% for both peaks, *System suitability solution*
Signal-to-noise ratio: NLT 30, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of olmesartan medoxomil from the *Standard solution*
C_S = concentration of in the *Standard solution* (mg/mL)
C_U = nominal concentration of olmesartan medoxomil in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*. Disregard peaks below 0.1%.

Table 4

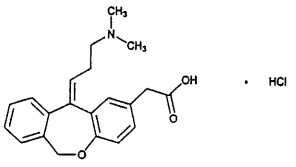
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olmesartan[a]	0.2	1.0	2.5
Olmesartan medoxomil related compound A[b]	0.7	1.6	—
Olmesartan medoxomil	1.0	—	—
Olmesartan dimer[c]	1.2	0.8	0.5
Olefinic impurity[d]	1.5	1.0	0.6
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	4.1

[a] 1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylic acid.
[b] This is a process-related impurity that is controlled in the drug substance.
[c] 1-({2′-(1*H*-Tetrazol-5-yl)-[1,1′-biphenyl]-4-yl}methyl)-4-(2-{[1-({2′-(1*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl}methyl)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbonyl]oxy}propan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylic acid.
[d] (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 1-((2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl)-4-(prop-1-en-2-yl)-2-propyl-1*H*-imidazole-5-carboxylate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Olmesartan Medoxomil RS
  USP Olmesartan Medoxomil Related Compound A RS
  1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1*H*-furo[3,4-*d*]imidazol-6(4*H*)-one.
  $C_{24}H_{24}N_6O_2$ 428.49

Olopatadine Hydrochloride



$C_{21}H_{23}NO_3 \cdot HCl$ 373.87
Dibenz[*b*,*e*]oxepin-2-acetic acid, 11-[3-(dimethylamino) propylidene]-6,11-dihydro-, hydrochloride, (*Z*)-;
11-[(*Z*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b*,*e*]oxepin-2-acetic acid, hydrochloride [140462-76-6].

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DEFINITION

Olopatadine Hydrochloride contains NLT 98.0% and NMT 102.0% of olopatadine hydrochloride ($C_{21}H_{23}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements

ASSAY

- **PROCEDURE**

Protect all solutions containing olopatadine hydrochloride from light.

Buffer: Dissolve 13.6 g of monobasic potassium phosphate in 1 L of water, add 1 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (28:72)

Standard solution: 0.1 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Olopatadine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of olopatadine hydrochloride ($C_{21}H_{23}NO_3 \cdot HCl$) in the portion of Olopatadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Olopatadine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Olopatadine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Protect all solutions containing olopatadine hydrochloride from light.

Mobile phase: Prepare as directed in the *Assay*.

System suitability solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS and 0.02 mg/mL of USP Olopatadine Related Compound B RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Olopatadine Hydrochloride in *Mobile phase*

Blank solution: *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 30 µL

Run time: At least 2.5 times the retention time of the major peak

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for olopatadine and olopatadine related compound B are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between olopatadine and olopatadine related compound B

Relative standard deviation: NMT 2.0%, olopatadine peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Olopatadine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria

Individual impurities: See *Table 1*. Disregard any peaks corresponding to those of the *Blank solution*, and any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
α-Hydroxy olopatadine[a]	0.4	1.0	0.2
Olopatadine *E*-isomer[b]	0.7	1.3	0.1
Olopatadine	1.0	—	—
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.25

[a] (*Z*)-2-(11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b*,*e*]oxepin-2-yl)-2-hydroxyacetic acid.

[b] 11-[(*E*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b*,*e*]oxepin-2-acetic acid.

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample: 10 mg/mL of Olopatadine Hydrochloride in water

Acceptance criteria: 2.0–4.0

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Olopatadine Hydrochloride RS

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USP Olopatadine Related Compound B RS
(*Z*)-3-(2-(Carboxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)-*N,N*-dimethylpropan-1-amine oxide.
$C_{21}H_{23}NO_4$ 353.41

Olopatadine Hydrochloride Ophthalmic Solution

DEFINITION
Olopatadine Hydrochloride Ophthalmic Solution is a sterile aqueous solution of Olopatadine Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of olopatadine ($C_{21}H_{23}NO_3$). It may contain suitable antimicrobial agents.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum in the range of 270–370 nm of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Protect all solutions containing olopatadine hydrochloride from light.
Buffer: Dissolve 13.6 g of monobasic potassium phosphate in 1 L of water, add 1 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (28:72)
Standard solution: 0.1 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*
Sample solution: Nominally equivalent to 0.1 mg/mL of olopatadine in *Mobile phase*, from Ophthalmic Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm or diode array. [NOTE—Use a diode array detector to perform the *Identification B* test.]
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olopatadine ($C_{21}H_{23}NO_3$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Olopatadine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of olopatadine, 337.41
M_{r2} = molecular weight of olopatadine hydrochloride, 373.87

Acceptance criteria: 90.0%–110.0%

IMPURITIES
- **LIMIT OF EARLY ELUTING IMPURITIES**

Protect all solutions containing olopatadine hydrochloride from light.
Mobile phase: Prepare as directed in the *Assay*.
System suitability solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS and 0.02 mg/mL of USP Olopatadine Related Compound B RS in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*
Sample solution: Equivalent to 0.2 mg/mL of olopatadine in *Mobile phase*, from Ophthalmic Solution
Blank solution: *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 30 µL
Run time: At least 1.6 times the retention time of the major peak
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between olopatadine and olopatadine related compound B, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of olopatadine from the *Standard solution*
C_S = concentration of USP Olopatadine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of olopatadine, 337.41
M_{r2} = molecular weight of olopatadine hydrochloride, 373.87
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peaks corresponding to those of the *Blank solution* and any peaks with a relative retention time, measured with respect to olopatadine, greater than 1.5. Disregard any peak less than 0.1%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olopatadine *E*-isomer[a]	0.7	1.3	0.5
Olopatadine	1.0	—	—
Olopatadine related compound B	1.2	1.0	2

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olopatadine carbaldehyde[b]	1.3	4.5	0.5
Any individual unspecified impurity	—	1.0	0.5

[a] 11-[(*E*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepin-2-acetic acid.
[b] (*Z*)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepine-2-carbaldehyde.

• **LIMIT OF LATE ELUTING IMPURITIES**
Protect all solutions containing olopatadine hydrochloride from light.
Buffer: Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile and *Buffer* (1:1)
System suitability solution: 0.02 mg/mL of USP Olopatadine Hydrochloride RS and 0.01 mg/mL of USP Olopatadine Related Compound C RS in *Mobile phase*
Standard solution: 0.01 mg/mL of USP Olopatadine Related Compound C RS in *Mobile phase*
Sample solution: Use the *Sample solution* from the test for *Limit of Early Eluting Impurities*.
Blank solution: *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 30 µL
Run time: At least 3 times the retention time of the olopatadine related compound C peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for olopatadine and olopatadine related compound C are 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 7.0 between olopatadine and olopatadine related compound C, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of olopatadine related compound C from the *Standard solution*
C_S = concentration of USP Olopatadine Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peaks corresponding to those of the *Blank solution* and any peaks with a relative retention time, measured with respect to olopatadine related compound C, less than 0.7. Disregard any peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Olopatadine	0.3	—
Olopatadine related compound C	1.0	1
Any individual unspecified impurity	—	0.5
Total impurities[a]	—	3

[a] Total impurities are the sum of olopatadine related compound B, olopatadine related compound C, olopatadine *E*-isomer, olopatadine carbaldehyde, and all unspecified impurities found in the tests for *Limit of Early Eluting Impurities* and *Limit of Late Eluting Impurities*.

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **pH** ⟨791⟩: 5.0–8.0
• **OSMOLALITY AND OSMOLARITY** ⟨785⟩: 260–340 mOsmol/kg

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 4° and 25°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Olopatadine Hydrochloride RS
USP Olopatadine Related Compound B RS
(*Z*)-3-(2-(Carboxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)-*N,N*-dimethylpropan-1-amine oxide.
$C_{21}H_{23}NO_4$ 353.41
USP Olopatadine Related Compound C RS
11-Oxo-6,11-dihydrodibenzo[*b,e*]oxepin-2-yl acetic acid.
$C_{16}H_{12}O_4$ 268.26

Omega-3-Acid Ethyl Esters

DEFINITION
Omega-3-Acid Ethyl Esters is a mixture of ethyl esters, principally the ethyl esters of eicosapentaenoic acid (EPAee) (C20:5 n–3, EE) and docosahexaenoic acid (DHAee) (C22:6 n–3, EE). It may also contain ethyl esters of alpha-linolenic acid (C18:3 n–3, EE), moroctic acid (C18:4 n–3, EE), eicosatetraenoic acid (C20:4 n–3, EE), heneicosapentaenoic acid (C21:5 n–3, EE), and docosapentaenoic acid (C22:5 n–3, EE). Tocopherol may be added as an antioxidant.

IDENTIFICATION
• **A.** The retention times of the principal peaks in *Test solution 4* correspond to those of eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester in *Standard solution 1b* and *Standard solution 1a*, as obtained in the *Assay*.
• **B.** It meets the acceptance criteria in *Table 1* of the *Assay*.

ASSAY
• **CONTENT OF EPAEE, DHAEE, AND TOTAL OMEGA-3-ACID ETHYL ESTERS**
(See *Fats and Fixed Oils* ⟨401⟩, *Procedures, Omega-3 Fatty Acids Determination and Profile*.)
Test solution 3, Test solution 4, Standard solution 1a, Standard solution 1b, System suitability solution 1, Chromatographic system, and System suitability: Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Procedures, Omega-3 Fatty Acids Determination and Profile*.
Analysis
Samples: *Test solution 3, Test solution 4, Standard solution 1a*, and *Standard solution 1b*

USP Monographs

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Calculate the content of EPAee and DHAee in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from *Test solution 3*
R_S = peak area ratio of the EPAee peak to the internal standard peak from *Standard solution 1b* or DHAee peak to the internal standard peak from *Standard solution 1a*
C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS in *Standard solution 1b* or USP Docosahexaenoic Acid Ethyl Ester RS in *Standard solution 1a* (mg/mL)
C_U = concentration of Omega-3-Acid Ethyl Esters in *Test solution 3* (g/mL)

Calculate the content of total omega-3-acid ethyl esters in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = r_{FAn-3ee}\,[(EPAee + DHAee)/(r_{EPAee} + r_{DHAee})] + EPAee + DHAee$$

$r_{FAn-3ee}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n–3, EE), moroctic acid ethyl ester (C18:4 n–3, EE), eicosatetraenoic acid ethyl ester (C20:4 n–3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n–3, EE), and docosapentaenoic acid ethyl ester (C22:5 n–3, EE) in *Test solution 4*
EPAee = content of EPAee (mg/g)
DHAee = content of DHAee (mg/g)
r_{EPAee} = peak area of EPAee in *Test solution 4*
r_{DHAee} = peak area of DHAee in *Test solution 4*

Acceptance criteria: It conforms to the acceptance criteria in *Table 1*. Articles labeled as Omega-3-Acid Ethyl Esters type A meet *Acceptance Criteria II*.

Table 1

		Acceptance Criteria I		Acceptance Criteria II (for articles labeled as Omega-3-Acid Ethyl Esters type A)	
Name	Relative Retention Time	NLT	NMT	NLT	NMT
C18:3 n–3, EE[a]	0.585	—	—	—	—
C18:4 n–3, EE[b]	0.608	—	—	—	—
C20:4 n–3, EE[c]	0.777	—	—	—	—
C20:5 n–3, EE (EPAee)[d]	0.796	430 mg/g	495 mg/g	365 mg/g	435 mg/g
C21:5 n–3, EE[e]	0.889	—	—	—	—
C22:5 n–3, EE[f]	0.977	—	—	—	—
C22:6 n–3, EE (DHAee)[g]	1.000	347 mg/g	403 mg/g	290 mg/g	360 mg/g
EPAee + DHAee	—	800 mg/g	880 mg/g	700 mg/g	749 mg/g
Total omega-3-acid ethyl esters	—	90% (w/w)	—	78% (w/w)	—

[a] Alpha-linolenic acid ethyl ester.
[b] Moroctic acid ethyl ester.
[c] Eicosatetraenoic acid ethyl ester.
[d] Eicosapentaenoic acid ethyl ester.
[e] Heneicosapentaenoic acid ethyl ester.
[f] Docosapentaenoic acid ethyl ester (clupanodonic acid ethyl ester).
[g] Docosahexaenoic acid ethyl ester.

IMPURITIES

- **Fats and Fixed Oils** ⟨401⟩: NMT 0.1 ppm each of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg)
- **Cholesterol**

Internal standard stock solution: 3 mg/mL of 5α-cholestane in *n*-heptane. [Note—Prepare fresh before use.]
Internal standard solution: 0.3 mg/mL of 5α-cholestane in *n*-heptane. [Note—Prepare fresh before use.]
Standard stock solution: 3.0 mg/mL of cholesterol in *n*-heptane. [Note—This solution is stable for 6 months stored in a freezer.] Transfer 1.0 mL of this solution to a 10.0-mL volumetric flask. Dilute with *n*-heptane to volume. [Note—Prepare this solution fresh daily.]
Standard solution: Transfer 1.0 mL each of the *Standard stock solution* and the *Internal standard solution* to a 15-mL centrifuge tube. Prepare as directed in the *Sample solution* beginning with "Evaporate to dryness".
Alpha tocopherol stock solution: 1.5–2.0 mg/mL of USP Alpha Tocopherol RS in *n*-heptane. [Note—This solution is stable for 12 months stored in a freezer.]
System suitability solution: Mix 1.0 mL of the *Standard stock solution*, 1.0 mL of the *Internal standard stock solution*, and 2.0 mL of the *Alpha tocopherol stock solution* in a 50-mL volumetric flask. Evaporate to dryness with the aid of heat, and dilute with ethyl acetate to volume. Dilute 1.0 mL of this solution with ethyl acetate to 10.0 mL. [Note—This solution is stable for 6 months stored in a freezer.]
Sample solution: Transfer 100 mg of Omega-3-Acid Ethyl Esters to a 15-mL centrifuge tube. Add 1.0 mL of the *Internal standard solution*. Evaporate to dryness at about 50° with a gentle stream of nitrogen. Add 0.5 mL of 50% potassium hydroxide and 3 mL of alcohol, fill the tube with nitrogen, and cap. Heat the sample at 100° for 60 min, using a heating block. Cool for about 10 min. Add 6 mL of water to the tube, and shake for 1 min. Extract the solution four times with 2.5-mL portions of ethyl ether, using a vortex mixer or suitable shaker for 1 min for each extraction. Transfer and combine the extracts into a large centrifuge tube, and wash with 5 mL of water, mixing completely with gentle inversion. Remove the water phase, and add 5 mL of 0.5 M potassium hydroxide to the ether phase, mixing carefully to avoid an emulsion. Remove the potassium hydroxide, and add another 5 mL of water, mixing carefully. Transfer the ether phase to a small centrifuge tube. [Note—If an emulsion has occurred, a small amount of sodium chloride may be added to obtain a

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separation of the phases.] Evaporate the ether phase to dryness under a stream of nitrogen with careful heating. Dissolve the sample in 600 µL of ethyl acetate, and mix well. Transfer 200 µL of this solution to a sample vial, and dilute with ethyl acetate to about 2 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 30-m capillary; coated with a G27 phase of 0.25-µm thickness
Temperatures
Injection port: 320°
Detector: 300°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	1
170	4	320	1.5

Carrier gas: Helium
Flow rate: 1.3 mL/min
Injection volume: 1 µL
Injection type: Splitless injection system
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.2 between alpha tocopherol and cholesterol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the content of total cholesterol in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U)$$

R_U = peak area ratio of the cholesterol peak to the internal standard from the *Sample solution*
R_S = peak area ratio of the cholesterol peak to the internal standard from the *Standard solution*
W_S = weight of cholesterol in the *Standard solution* (mg)
W_U = weight of Omega-3-Acid Ethyl Esters in the *Sample solution* (g)

Acceptance criteria: NMT 3.0 mg/g

• **OLIGOMERS**
Mobile phase: Tetrahydrofuran
System suitability solution: Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin[1] in *Mobile phase*, with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively.
Sample solution 1: 5.0 mg/mL of Omega-3-Acid Ethyl Esters in tetrahydrofuran
Sample solution 2: [NOTE—Use *Sample solution 2* where the results of this test using *Sample solution 1* exceed the *Acceptance criteria* due to the presence of monoglycerides.] Weigh 50 mg of Omega-3-Acid Ethyl Esters into a quartz tube, add 1.5 mL of a 20-g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytef-lined cap, mix, and heat on a water bath for 7 min. Allow to cool. Add 2.0 mL of boron trichloride–methanol solution, cover with nitrogen, cap tightly, mix, and heat on a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and shake vigorously for NLT 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and shake thoroughly for NLT 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer with 1 mL of isooctane. Wash the combined isooctane extracts with two quantities, each of 1 mL of water. Carefully evaporate the solvent under a stream of nitrogen, then add 10.0 mL of tetrahydrofuran to the residue. Add a small amount of anhydrous sodium sulfate, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractometer
Columns: Three concatenated, 7.8-mm × 30-cm; 7-µm packing L21, with pore sizes in the range of 5–50 nm, arranged with decreasing pore size from the injector to the detector to fulfill the system suitability requirements
Flow rate: 0.8 mL/min
Injection volume: 40 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin
Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin
Analysis
Samples: *Sample solution 1* and *Sample solution 2*
Measure the areas of the major peaks.
Calculate the percentage of oligomers in the portion of Omega-3-Acid Ethyl Esters taken to prepare *Sample solution 1*:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = sum of the areas of the peaks with a retention time less than that of the ethyl esters peaks
r_T = sum of the areas of all peaks

Calculate the percentage of oligomers in the portion of Omega-3-Acid Ethyl Esters taken to prepare *Sample solution 2*:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = sum of the areas of all peaks with a retention time less than that of the methyl esters peaks
r_T = sum of the areas of all peaks

Acceptance criteria: NMT 1.0% of oligomers

• **LIMIT OF DIOXINS, FURANS, AND POLYCHLORINATED BIPHENYLS (PCBs):** Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by method No. 1613 revision B of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by method No. 1668 revision A of the Environmental Protection Agency.
Acceptance criteria: The sum of PCDDs and PCDFs is NMT 1 pg/g of WHO toxic equivalents. The sum of PCBs (polychlorinated biphenyls, IUPAC congeners PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180) is NMT 0.5 ppm.

• **LIMIT OF TOTAL UNIDENTIFIED FATTY ACID ETHYL ESTERS** [NOTE—This test is not required for articles labeled as Omega-3-Acid Ethyl Esters type A.]

[1] Suitable grades of monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin may be obtained from Nu-Chek Prep.

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From the chromatogram obtained with *Test solution 4* in the *Assay* for *Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*, determine the peak area of the largest single unidentified peak with a relative retention time different from those in *Table 3*.

Table 3

Identified Ethyl Ester	Relative Retention Time
Phytanic acid	0.416
C16:3 n–4	0.431
C16:4 n–1	0.468
C18:3 n–6	0.557
C18:3 n–4	0.574
C18:3 n–3	0.585
C18:4 n–3	0.608
C18:4 n–1	0.618
Furan acid 5	0.691
C19:5	0.710
C20:3 n–6	0.720
C20:4 n–6	0.736
Furan acid 7	0.744
C20:4 n–3	0.777
Furan acid 8	0.783
EPA	0.796
Furan acid 9	0.867
C21:5 n–3	0.889
C22:4	0.917
Furan acid 10	0.922
C22:5 n–6	0.939
Furan acid 11	0.963
C22:5 n–3	0.977
DHA	1.000

Calculate the content of unidentified fatty acid ethyl esters in area percentage:

$$\text{Result} = 100 - (100 \times \Sigma\, Aiee/r_T)$$

Aiee = peak area of each identified ethyl ester in *Table 3*
r_T = sum of the areas of all peaks except solvents and BHT

Acceptance criteria: The area of the largest single unidentified peak is NMT 0.5% of the total area. The total area of unidentified peaks as calculated above is NMT 2%.

- **LIMIT OF NON-OMEGA-3-ACID ETHYL ESTERS**

[NOTE—This test is only required for articles labeled as Omega-3-Acid Ethyl Esters type A.]

From the chromatogram obtained with *Test solution 4* in the *Assay* for *Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*, calculate the amounts of C18:1 n–9 ethyl ester and C20:4 n–6 ethyl ester in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (Aiee/r_T) \times 100$$

Aiee = peak area of C18:1 n–9 ethyl ester or C20:4 n–6 ethyl ester
r_T = sum of the areas of all peaks except solvents and BHT

Acceptance criteria
C18:1 n–9 ethyl ester: NMT 6.0%
C20:4 n–6 ethyl ester: NMT 4.0%

SPECIFIC TESTS

- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Acid Value*: NMT 2.0
- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Anisidine Value*: NMT 15
- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Peroxide Value*: NMT 10.0
- **ABSORBANCE**

Sample solution: Transfer 300 mg, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute immediately with isooctane to volume. Pipet 2.0 mL into a 50-mL volumetric flask, and dilute with isooctane to volume.
Acceptance criteria: NMT 0.55, determined at 233 nm, with isooctane being used as the blank

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers under a nitrogen atmosphere. Store at controlled room temperature.
- **LABELING:** The label states the content of DHA ethyl ester and EPA ethyl ester in mg/g, the sum of the EPA and DHA ethyl esters contents in mg/g, and the content of the total omega-3-acid ethyl esters in weight percentage (w/w). It also states the name of any added antioxidant. Articles which meet *Acceptance Criteria II* of the *Assay* and the *Limit of Non-Omega-3-Acid Ethyl Esters* are labeled as Omega-3-Acid Ethyl Esters type A.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Alpha Tocopherol RS

Omega-3-Acid Ethyl Esters Capsules

DEFINITION

Omega-3-Acid Ethyl Esters Capsules contain Omega-3-Acid Ethyl Esters, with NLT 95.0% and NMT 105.0% of the labeled sum of eicosapentaenoic acid ethyl ester (EPAee) and docosahexaenoic acid ethyl ester (DHAee) and NLT 95% of the labeled amount of total omega-3-acid ethyl esters, as the sum of alpha-linolenic acid ethyl ester (C18:3 n–3, EE), moroctic acid ethyl ester (C18:4 n–3, EE), eicosatetraenoic acid ethyl ester (C20:4 n–3, EE), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n–3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n–3, EE), docosapentaenoic acid ethyl ester (C22:5 n–3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n–3, EE). Tocopherol may be added as an antioxidant.

IDENTIFICATION

- **A.** The retention times of the peaks for eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay* for *Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*.
- **B.** It complies with the *Acceptance criteria* in the test for *Concentration of Omega-3-Acid Ethyl Esters* in *Specific Tests*.

ASSAY

- **CONTENT OF EPAEE, DHAEE, AND TOTAL OMEGA-3-ACID ETHYL ESTERS**

[NOTE—Carry out the procedure as rapidly as possible, avoiding exposure to actinic light, oxidizing agents, oxidation catalysts (e.g., copper and iron), and air.]

USP Monographs

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Antioxidant solution: 50 mg/L of butylated hydroxytoluene in isooctane

Retention time identification solution: Prepare a mixture containing suitable concentrations of alpha-linolenic acid ethyl ester (C18:3 n–3, EE), moroctic acid ethyl ester (C18:4 n–3, EE), eicosatetraenoic acid ethyl ester (C20:4 n –3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n–3, EE), and docosapentaenoic acid ethyl ester (C22:5 n–3, EE) in *Antioxidant solution*.[1]

Internal standard solution: 7.0 mg/mL of USP Methyl Tricosanoate RS in *Antioxidant solution*

System suitability solution: 5.5 mg/mL of docosahexaenoic acid methyl ester and 0.5 mg/mL of tetracos-15-enoic acid methyl ester in *Antioxidant solution*

Standard solution: Dissolve 60.0 mg of USP Docosahexaenoic Acid Ethyl Ester RS and 90.0 mg of USP Eicosapentaenoic Acid Ethyl Ester RS in 10.0 mL of *Internal standard solution*.

Sample solution: Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp blade, carefully open the Capsules, without loss of shell material, and transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of diethyl ether. Discard the washings, and allow the empty Capsules to air-dry over a period of NMT 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average fill weight per Capsule (W_{AF}). Transfer an amount of the combined Capsule contents equivalent to 225 mg of the labeled amount of total omega-3-acid ethyl esters to a suitable flask, and dissolve with 10.0 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 25–50-m fused silica capillary; coated with a 0.25-µm film of phase G16

Temperatures

Injection port: 250°

Detector: 270°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	2
170	3.5	255	9

Carrier gas: Hydrogen or helium

Linear velocity: Adjust to obtain a retention time for docosahexaenoic acid ethyl ester of 26 ± 3 min.

Injection volume: 1 µL

Injection type: Split; split ratio, 1:220

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between the docosahexaenoic acid methyl ester and tetracos-15-enoic acid methyl ester peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for the ratios of the peak responses of DHAee and EPAee relative to the internal standard, *Standard solution*

Analysis

Samples: *Retention time identification solution*, *Standard solution*, and *Sample solution*

Identify the retention times of the relevant fatty acid ethyl esters by comparing the peaks from the *Sample solution* with those from the *Retention time identification solution*.

Calculate the content, in mg/g, of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Sample solution*

R_S = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Standard solution*

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosahexaenoic Acid Ethyl Ester RS in the *Standard solution* (mg/mL)

C_U = Capsule fill content of the *Sample solution* (g/mL)

Calculate the percentage of the labeled sum of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (EPAee + DHAee) \times W_{AF} \times (100/L)$$

EPAee = content of EPAee in the portion of Capsules taken (mg/g)

DHAee = content of DHAee in the portion of Capsules taken (mg/g)

W_{AF} = average fill weight of the Capsules taken (g)

L = sum of the labeled content of EPAee and DHAee (mg/Capsule)

Calculate the percentage of the labeled amount of total omega-3-acid ethyl esters in the portion of Capsules taken:

$$\text{Result} = \{r_{FAn-3ee} \times [(EPAee + DHAee)/(r_{EPAee} + r_{DHAee})] + EPAee + DHAee\} \times W_{AF} \times (100/L)$$

$r_{FAn-3ee}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n–3, EE), moroctic acid ethyl ester (C18:4 n–3, EE), eicosatetraenoic acid ethyl ester (C20:4 n–3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n–3, EE), and docosapentaenoic acid ethyl ester (C22:5 n–3, EE) from the *Sample solution*

EPAee = content of EPAee (mg/g)

DHAee = content of DHAee (mg/g)

r_{EPAee} = peak area of EPAee from the *Sample solution*

r_{DHAee} = peak area of DHAee from the *Sample solution*

W_{AF} = average fill weight of the Capsules taken (g)

L = label claim of total omega-3-acids ethyl esters (mg/Capsule)

Acceptance criteria: 95.0%–105.0% of the labeled sum of EPAee and DHAee and NLT 95.0% of the labeled amount of total omega-3-acid ethyl esters per Capsule

PERFORMANCE TESTS

- **Uniformity of Dosage Units** ⟨905⟩, *Weight Variation*: Meet the requirements
- **Disintegration** ⟨701⟩

Medium, tier 1: Water

Medium, tier 2: Simulated gastric fluid TS

Time: 30 min

[1] The relevant fatty acid ethyl esters are available from Nu-Chek Prep, Inc. (www.nu-chekprep.com); Cayman Chemical (www.caymanchem.com); and Carbosynth (www.carbosynth.com).

www.webofpharma.com

Analysis: Perform the test with water as *Medium, tier 1*. Repeat the test with simulated gastric fluid TS as *Medium, tier 2*, if the disintegration time is more than 30 min in *Medium, tier 1*.
Acceptance criteria: Meet the requirements

IMPURITIES

• **OLIGOMERS**
Mobile phase: Tetrahydrofuran
System suitability solution: Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin in *Mobile phase*, with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively. [NOTE—Suitable grades of monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin may be obtained from Nu-Chek Prep.]
Sample solution 1: 5.0 mg/mL of the Capsule contents in tetrahydrofuran
Sample solution 2: [NOTE—Use *Sample solution 2* where the results of this test using *Sample solution 1* exceed the *Acceptance criteria* due to the presence of monoglycerides.] Weigh 50 mg of the Capsule contents into a quartz tube, add 1.5 mL of a 20-g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytef-lined cap, mix, and heat on a water bath for 7 min. Allow to cool. Add 2.0 mL of boron trichloride–methanol solution, cover with nitrogen, cap tightly, mix, and heat on a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and shake vigorously for NLT 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and shake thoroughly for NLT 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer with 1 mL of isooctane. Wash the combined isooctane extracts with 2 quantities, each of 1 mL of water. Carefully evaporate the solvent under a stream of nitrogen, then add 10.0 mL of tetrahydrofuran to the residue. Add a small amount of anhydrous sodium sulfate, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractometer
Columns: Three concatenated, 7.8-mm × 30-cm; 7-µm packing L21, with pore sizes in the range of 5–50 nm, arranged with decreasing pore size from the injector to the detector to fulfill the system suitability requirements
Flow rate: 0.8 mL/min
Injection volume: 40 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin
Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin
Analysis
Samples: *Sample solution 1* and *Sample solution 2*
Measure the areas of the major peaks.
Calculate the percentage of oligomers in the portion of omega-3-acid ethyl esters taken to prepare *Sample solution 1*:

$$\text{Result} = (r_I / r_T) \times 100$$

r_I = sum of the peak areas with retention times less than that of the ethyl esters peak from *Sample solution 1*
r_T = sum of the areas of all peaks from *Sample solution 1*

Calculate the percentage of oligomers in the portion of the Capsules contents taken to prepare *Sample solution 2*:

$$\text{Result} = (r_I / r_T) \times 100$$

r_I = sum of the peak areas with retention times less than that of the methyl esters peak from *Sample solution 2*
r_T = sum of the areas of all peaks from *Sample solution 2*

Acceptance criteria: NMT 2% of oligomers

SPECIFIC TESTS

• **CONCENTRATION OF OMEGA-3-ACID ETHYL ESTERS**
Antioxidant solution, Retention time identification solution, Internal standard solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in the *Assay* for *Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*.
Calculate the concentration, in mg/g, of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (R_U / R_S) \times (C_S / C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Sample solution*
R_S = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Standard solution*
C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosahexaenoic Acid Ethyl Ester RS in the *Standard solution* (mg/mL)
C_U = Capsule fill content of the *Sample solution* (g/mL)

Calculate the concentration, in mg/g, of total omega-3-acids ethyl esters in the portion of Capsules taken:

$$\text{Result} = r_{FAn-3ee} \times [(EPAee + DHAee)/(r_{EPAee} + r_{DHAee})] + EPAee + DHAee$$

$r_{FAn-3ee}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n–3, EE), moroctic acid ethyl ester (C18:4 n–3, EE), eicosatetraenoic acid ethyl ester (C20:4 n–3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n–3, EE), and docosapentaenoic acid ethyl ester (C22:5 n–3, EE) from the *Sample solution*
EPAee = content of EPAee (mg/g)
DHAee = content of DHAee (mg/g)
r_{EPAee} = peak area of EPAee from the *Sample solution*
r_{DHAee} = peak area of DHAee from the *Sample solution*

Acceptance criteria: It meets the requirements in *Table 2*. Capsules labeled as containing Omega-3-Acid Ethyl Esters type A meet *Acceptance Criteria II*.

Table 2

Name	Acceptance Criteria I		Acceptance Criteria II (for Capsules labeled as containing Omega-3-Acid Ethyl Esters type A)	
	NLT	NMT	NLT	NMT
EPAee	430 mg/g	495 mg/g	365 mg/g	435 mg/g
DHAee	347 mg/g	403 mg/g	290 mg/g	360 mg/g
EPAee + DHAee	800 mg/g	880 mg/g	700 mg/g	749 mg/g

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Table 2 *(continued)*

Name	Acceptance Criteria I		Acceptance Criteria II (for Capsules labeled as containing Omega-3-Acid Ethyl Esters type A)	
	NLT	NMT	NLT	NMT
Total omega-3-acid ethyl esters[a]	90% (w/w)	—	78% (w/w)	—

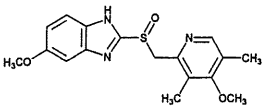
[a] Sum of alpha-linolenic acid ethyl ester (C18:3 n–3, EE), moroctic acid ethyl ester (C18:4 n–3, EE), eicosatetraenoic acid ethyl ester (C20:4 n–3, EE), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n–3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n–3, EE), docosapentaenoic acid ethyl ester (C22:5 n–3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n–3, EE).

- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Acid Value*
  Sample solution: Dissolve about 5.0 g of the oil, accurately weighed, in 100 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 M potassium hydroxide) contained in a flask.
  Acceptance criteria: NMT 2.0
- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Anisidine Value*: NMT 25
- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Peroxide Value*: NMT 10 mEq/kg
- **ABSORBANCE**
  Sample solution: Transfer 300 mg, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute immediately with isooctane to volume. Pipet 2.0 mL into a 50-mL volumetric flask, and dilute with isooctane to volume.
  Acceptance criteria: NMT 0.60, determined at 233 nm in a 1-cm cell, with isooctane being used as the blank
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩: NMT 10^3 cfu/g for the total aerobic microbial count, and NMT 10^2 cfu/g for the total combined yeasts and molds count.
- **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meet the requirements for absence of *Escherichia coli* in 1 g and for absence of *Salmonella* species in 10 g

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature. Do not freeze. Protect from light.
- **LABELING:** The label states the amount of docosahexaenoic acid (DHA) ethyl ester and eicosapentaenoic acid (EPA) ethyl ester, and the minimum amount of total content of omega-3-acid ethyl esters in mg/Capsule. Capsules which meet *Acceptance Criteria II* of the test for *Concentration of Omega-3-Acid Ethyl Esters* are labeled as containing Omega-3-Acid Ethyl Esters type A. It also states the name and content of any added antioxidant.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Docosahexaenoic Acid Ethyl Ester RS
  All *cis*-4,7,10,13,16,19-docosahexaenoic ethyl ester.
  $C_{24}H_{36}O_2$ 356.55
  USP Eicosapentaenoic Acid Ethyl Ester RS
  All *cis*-5,8,11,14,17-eicosapentaenoic ethyl ester.
  $C_{22}H_{34}O_2$ 330.51
  USP Methyl Tricosanoate RS
  Tricosanoic acid methyl ester.
  $C_{24}H_{48}O_2$ 368.64

Omeprazole



$C_{17}H_{19}N_3O_3S$ 345.42
1*H*-Benzimidazole, 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-;
5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl]benzimidazole [73590-58-6].

DEFINITION

Omeprazole contains NLT 98.0% and NMT 102.0% of omeprazole ($C_{17}H_{19}N_3O_3S$), calculated on the dried basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**
  Buffer: Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1000 mL of water. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.
  Mobile phase: Acetonitrile and *Buffer* (1:3)
  Diluent: Acetonitrile and 0.01 M sodium borate (1:3)
  Standard solution: 0.2 mg/mL of USP Omeprazole RS in *Diluent*
  System suitability solution: 0.1 mg/mL of USP Omeprazole RS in *Diluent* from the *Standard solution*
  Sample solution: 0.2 mg/mL of Omeprazole in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L7
  Flow rate: 0.8 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *System suitability solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of omeprazole ($C_{17}H_{19}N_3O_3S$) in the portion of Omeprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Omeprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

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IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A: Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1000 mL of water. Dilute 250 mL of this solution with water to 1000 mL. Adjust with phosphoric acid to a pH of 7.0.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to the original conditions, and re-equilibrate the system for about 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
12	75	25
22	50	50
45	50	50
45.1	75	25

Diluent: *Solution A* and *Solution B* (3:1)

System suitability solution: 0.6 mg/mL of USP Omeprazole RS, 0.6 µg/mL each of USP Omeprazole Related Compound A RS, USP Omeprazole Related Compound E RS, USP Omeprazole Related Compound I RS, and 5-methoxy-1*H*-benzimidazol-2-thiol in *Diluent*. [NOTE—This solution is stable for 14 h when stored at 4°.]

Standard solution: 6.0 µg/mL of USP Omeprazole RS in *Diluent*. [NOTE—This solution is stable for 14 h when stored at 4°.]

Sample solution: 0.6 mg/mL of Omeprazole in *Diluent*. [NOTE—Inject within 1 h of preparation.]

Sensitivity solution: 0.3 µg/mL of USP Omeprazole RS from the *Standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 264 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 0.8 mL/min

Injection volume: 40 µL

Autosampler temperature: 4°

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between omeprazole and omeprazole related compound A, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Omeprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of omeprazole from the *Standard solution*

C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Omeprazole in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Methoxy-1*H*-benzimidazol-2-thiol	0.41	1.0	0.15
Omeprazole *N*-oxide (omeprazole related compound E)	0.53	1.34	0.15
Omeprazole sulfone *N*-oxide (omeprazole related compound I)	0.58	1.48	0.15
Desmethoxy omeprazole[a]	0.97	1.0	0.15
Omeprazole	1.0	—	—
Omeprazole sulfone (omeprazole related compound A)	1.07	1.0	0.15
Omeprazole 4-chloro analog[b]	1.16	1.0	0.15
Ufiprazole[c]	1.25	1.0	0.15
Omeprazole thioxopyrido analogs[d]	1.55 and 1.64	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 2-[(*RS*)-[(3,5-Dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole.

[b] 2-[(*RS*)-[4-Chloro-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole.

[c] 5-Methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylthio)-1*H*-benzimidazole.

[d] Omeprazole related compounds F and G (1,3-dimethyl-8-methoxy-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-a]benzimidazol-2(12*H*)-one and 1,3-dimethyl-9-methoxy-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-a]benzimidazol-2(12*H*)-one). These impurities are controlled in the test for *Limit of Omeprazole Related Compounds F and G*.

SPECIFIC TESTS

- **COMPLETENESS OF SOLUTION** ⟨641⟩

Sample solution: 20 mg/mL of Omeprazole in methylene chloride

Acceptance criteria: Meets the requirements

- **LIMIT OF OMEPRAZOLE RELATED COMPOUNDS F AND G**

Sample solution: 20 mg/mL of Omeprazole in methylene chloride

Instrumental conditions

Mode: Vis

Analytical wavelength: 440 nm

Cell: 1 cm

Blank: Methylene chloride

Acceptance criteria: The absorbance is NMT 0.10, corresponding to NMT 350 ppm of the sum of omeprazole related compounds F and G.

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cold place, protected from moisture.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Omeprazole RS

USP Omeprazole Related Compound A RS
Omeprazole sulfone;
5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfonyl]-1*H*-benzimidazole.
$C_{17}H_{19}N_3O_4S$ 361.42

USP Omeprazole Related Compound E RS
Omeprazole *N*-oxide;
4-Methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl) sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide.
$C_{17}H_{19}N_3O_4S$ 361.42

USP Omeprazole Related Compound I RS
Omeprazole sulfone *N*-oxide;
4-Methoxy-2-[[(5-methoxy-1*H*-benzimidazol-2-yl) sulfonyl]methyl]-3,5-dimethylpyridine 1-oxide.
$C_{17}H_{19}N_3O_5S$ 377.41

Omeprazole Delayed-Release Capsules

DEFINITION

Omeprazole Delayed-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$).

IDENTIFICATION

- **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 6.0 g of glycine in 1500 mL of water, adjust with 50% sodium hydroxide solution to a pH of 9.0, and dilute with water to 2000 mL.

Solution B: Acetonitrile and methanol (85:15)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	88	12
20	40	60
21	88	12
25	88	12

Diluent: Dissolve 7.6 g of sodium borate decahydrate in about 800 mL of water. Add 1.0 g of edetate disodium, and adjust with 50% sodium hydroxide solution to a pH of 11.0 ± 0.1. Transfer the solution to a 2000-mL volumetric flask, add 400 mL of dehydrated alcohol, and dilute with water to volume.

Standard solution: 0.2 mg/mL of USP Omeprazole RS in *Diluent*, using sonication as necessary

Sample solution: Weigh and mix the contents of NLT 20 Capsules. Transfer an accurately weighed portion of the Capsule content, equivalent to 20 mg of omeprazole, to a 100-mL volumetric flask, add about 50 mL of *Diluent*, and sonicate for 15 min. Cool, dilute with *Diluent* to volume, mix, and pass through a membrane filter of 0.45-µm or finer pore size. [NOTE—Bubbles may form just before bringing the solution to volume. Add a few drops of dehydrated alcohol to dissipate the bubbles if they persist for more than a few minutes.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 305 nm
Column: 4.6-mm × 15-cm; 5-µm base-deactivated packing L7
Flow rate: 1.2 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 20,000 theoretical plates
Tailing factor: 0.8–2
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of omeprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1
Acid resistance stage
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 100 rpm
Time: 2 h
Buffer C, Mobile phase, Chromatographic system, and System suitability: Proceed as directed for *Buffer stage*.
Standard solution: Transfer 50 mg of USP Omeprazole RS to a 250-mL volumetric flask, dissolve in 50 mL of alcohol, and dilute with 0.01 M sodium borate solution to volume. Transfer 10.0 mL of this solution into a 100-mL volumetric flask, add 20 mL of alcohol, dilute with 0.01 M sodium borate solution to volume, and mix.
Sample solution: After 2 h, filter the *Medium* containing the pellets through a sieve with an aperture of NMT 0.2 mm. Collect the pellets on the sieve, and rinse them with water. Using approximately 60 mL of 0.01 M sodium borate solution, carefully transfer the pellets quantitatively to a 100-mL volumetric flask. Sonicate for about 20 min until the pellets are broken up. Add 20 mL of alcohol to the flask, dilute with 0.01 M sodium borate solution to volume, and mix. Dilute an appropriate amount of this solution with 0.01 M sodium borate solution to obtain a solution containing 0.02 mg/mL. At level L_1, test 6 units. Test 6 additional units at level L_2, and at level L_3, test an additional 12 units. Continue testing through the three levels unless the results conform at either L_1 or L_2.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved in *Medium*, in mg:

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$$\text{Result} = T - C_S \times D \times (r_U/r_S)$$

T = labeled quantity of omeprazole in the capsule (mg)
C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)
D = dilution factor used in preparing the *Sample solution*
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

Tolerances
Level L_1: No individual value exceeds 15% of the omeprazole dissolved.
Level L_2: The average of 12 units is NMT 20% of omeprazole dissolved, and no individual unit is greater than 35% of omeprazole dissolved.
Level L_3: The average of 24 units is NMT 20% of omeprazole dissolved, NMT 2 units are greater than 35% of omeprazole dissolved, and no individual unit is greater than 45% of omeprazole dissolved.

Buffer stage
Medium: pH 6.8 phosphate buffer, 900 mL
Proceed as directed in *Acid resistance stage* with a new set of Capsules from the same batch. After 2 h, add 400 mL of 0.235 M dibasic sodium phosphate to the 500 mL of 0.1 N hydrochloric acid medium in the vessel. Adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05.
Apparatus 2: 100 rpm
At the end of 30 min, determine the amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved in the pH 6.8 phosphate buffer by using the following method.
Buffer A (0.235 M dibasic phosphate buffer, pH 10.4): 33.36 g/L of anhydrous dibasic sodium phosphate, adjusted with 2 N sodium hydroxide to a pH of 10.4 ± 0.1
Buffer B (phosphate buffer, pH 6.8): 0.1 N hydrochloric acid and *Buffer A* (5:4), adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 6.8 ± 0.05.
Buffer C (phosphate buffer, pH 7.6): 0.718 g/L of monobasic sodium phosphate and 4.49 g/L of dibasic sodium phosphate, adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 7.6 ± 0.1. Dilute 250 mL of this solution with water to 1000 mL.
Mobile phase: Transfer 340 mL of acetonitrile to a 1000-mL volumetric flask, dilute with *Buffer C* to volume, and pass through a membrane filter of 0.5-µm or finer pore size.
Standard solution A (for Capsules labeled to contain 10 mg): Prepare a solution containing 2 mg/mL of USP Omeprazole RS in alcohol. Dilute with *Buffer B* to obtain a solution containing 0.01 mg/mL. Immediately add 2 mL of 0.25 M sodium hydroxide to 10 mL of this solution, and mix. [NOTE—Do not allow the solution to stand before adding the sodium hydroxide solution.]
Standard solution B (for Capsules labeled to contain 20 or 40 mg): Proceed as directed for *Standard solution A*, except to obtain a solution containing 0.02 mg/mL before mixing with 2 mL of 0.25 M sodium hydroxide.
Sample solution A (for Capsules containing 10 or 20 mg): Immediately transfer 5.0 mL of the solution under test to a test tube containing 1.0 mL of 0.25 M sodium hydroxide. Mix well, and pass through a membrane filter of 1.2-µm or finer pore size. Protect from light.
Sample solution B (for Capsules labeled 40 mg): Immediately transfer 5.0 mL of the solution under test to a test tube containing 2.0 mL of 0.25 M sodium hydroxide and 5 mL of *Buffer B*. Mix well, and pass through a membrane filter of 1.2-µm or finer pore size. Protect from light.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 12.5-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution A* or *B*, as appropriate
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak response from the appropriate *Sample solution*
r_S = peak response from the appropriate *Standard solution*
C_S = concentration of the appropriate *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
D = dilution factor used in preparing the appropriate *Sample solution*

Tolerances
For Capsules labeled to contain 10 and 20 mg: NLT 75% (Q) is dissolved.
For Capsules labeled to contain 40 mg: NLT 70% (Q) is dissolved.
The percentages of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved at the time specified conform to *Acceptance Table 1* in *Dissolution* ⟨711⟩.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Acid resistance stage
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 2 h
Sample solution: After 2 h, remove each sample from the basket, and quantitatively transfer into separate volumetric flasks to obtain a solution having a final concentration of about 0.2 mg/mL. Proceed as directed for the *Sample solution* in the *Assay*, starting with "Add about 50 mL of *Diluent*".
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved in *Medium*, in mg:

$$\text{Result} = T - C_S \times D \times (r_U/r_S)$$

T = labeled quantity of omeprazole in the capsule (mg)
C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)
D = dilution factor used in preparing the *Sample solution*
r_U = peak response from the *Sample solution*

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r_S = peak response from the *Standard solution*

Tolerances: See *Table 2*.

Table 2

Level	Criteria
L_1	The average of the 6 units is NMT 10% of omeprazole dissolved.
L_2	The average of the 12 units is NMT 10% of omeprazole dissolved.
L_3	The average of the 24 units is NMT 10% of omeprazole dissolved.

Buffer stage

Medium: 0.05 M pH 6.8 phosphate buffer; 900 mL (see *Reagents, Indicators, and Solutions*)

Apparatus 1: 100 rpm

Time: 45 min

Analysis: Proceed as directed for *Acid resistance stage* with a new set of Capsules from the same batch. After 2 h, replace the acid *Medium* with the buffer *Medium*, and continue the test for 45 more min. Determine the amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 305 nm on portions of the solutions under test passed through a nylon filter of 0.2-µm pore size, in comparison with a *Standard solution* having a known concentration of USP Omeprazole RS in the same *Medium*.

Tolerances: NLT 75% (*Q*) is dissolved.

The percentage of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved at the time specified conforms to *Acceptance Table 1* in *Dissolution* ⟨711⟩.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 1.0 µg/mL of USP Omeprazole RS in *Diluent*

Peak identification solution: 0.2 mg/mL of USP Omeprazole RS, 1.0 µg/mL of USP Omeprazole Related Compound F and G Mixture RS, and 1.0 µg/mL of 5-methoxy-1*H*-benzimidazole-2-thiol in *Diluent*. Sonicate the solution for 15 min, and then heat at 55° for 30 min. [NOTE—The heating step facilitates conversion of omeprazole related compounds F and G into a product with the relative retention time of 0.33. The remaining unconverted omeprazole related compounds F and G may elute as a very broad peak at the relative retention time of about 0.5.]

Analysis

Samples: *Standard solution, Peak identification solution,* and *Sample solution*

Chromatograph the *Peak identification solution*, and identify the components on the basis of their relative retention times, given in the *Table 3*.

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for omeprazole from the *Standard solution*

C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of omeprazole in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Omeprazole related compounds F and G[a]	0.33	1.6	0.5
5-Methoxy-1*H*-benzimidazole-2-thiol	0.64	3.1	0.5
Any other individual impurity	—	1.0	0.5
Total impurities	—	—	2.0

[a] These impurities undergo transformation in the solution to form a conversion product, which elutes at the relative retention time of 0.33.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 15° and 30°.

• **LABELING:** When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.

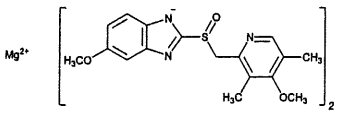
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Omeprazole RS

USP Omeprazole Related Compound F and G Mixture RS

1,3-Dimethyl-8-methoxy-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-*a*]benzimidazol-2(12*H*)-one and 1,3-dimethyl-9-methoxy-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-*a*]benzimidazol-2(12*H*)-one.

Omeprazole Magnesium



$C_{34}H_{36}MgN_6O_6S_2$ 713.12

(*RS*)-5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole, magnesium salt (2:1).

5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]sulfinyl]benzimidazole, (*RS*), magnesium salt (2:1) [95382-33-5].

» Omeprazole Magnesium contains not less than 97.5 percent and not more than 102.0 percent of $C_{34}H_{36}MgN_6O_6S_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, protected from light. Store at room temperature.

USP Reference standards ⟨11⟩—

USP Omeprazole RS

USP Omeprazole Magnesium RS

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USP Omeprazole Related Compound A RS
Omeprazole sulfone, 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole.
$C_{17}H_{19}N_3O_4S$ 361.42
[CAS-88546-55-8].

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The *Test solution,* prepared and tested as directed in the test for *Content of magnesium,* exhibits a significant absorption at the magnesium emission line at 285.2 nm.

Color of solution—Prepare a solution of Omeprazole Magnesium in methanol having a concentration of 20 mg per mL, and filter. Determine the absorbance of this solution at 440 nm, in 1-cm cells, using methanol as the blank: the absorbance is no greater than 0.1.

Water Determination, *Method I* ⟨921⟩**:** between 7% and 10%.

Specific rotation ⟨781S⟩**:** between +0.5° and −0.5°, measured at 20°.

Test solution: 10 mg per mL, in methanol.

Chromatographic purity—

Phosphate buffer pH 7.6—Prepare as directed in the *Assay.*

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer pH 7.6* and acetonitrile (72.5: 27.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—To improve the resolution, the composition may be changed to 75:25, if necessary.]

Test solution—Dissolve about 4 mg of Omeprazole Magnesium in 25 mL of the *Mobile phase.* [NOTE—Prepare this solution fresh.]

System suitability solution—Dissolve about 1 mg of USP Omeprazole RS and 1 mg of USP Omeprazole Related Compound A RS in about 25 mL of *Mobile phase.* [NOTE—Omeprazole related compound A is omeprazole sulfone.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 12.5-cm or 4.6-mm × 15-cm column that contains 5-µm packing L7. (Alternatively, a 3.9-mm × 15-cm column that contains 4-µm packing L1 may be used.) The flow rate is about 0.8 to 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times for omeprazole related compound A and omeprazole are about 0.8 and 1.0, respectively; and the resolution, *R,* between omeprazole related compound A and omeprazole is not less than 3.

Procedure—Inject a volume (about 50 µL) of the *Test solution* into the chromatograph, record the chromatogram for at least 4.5 times the retention time of omeprazole, and measure the peak responses. Identify the impurities based on the relative retention times listed in *Table 1.*

Table 1

Name	Relative Retention Time	Limit (%)
Omeprazole *N*-oxide[1]	0.45	0.1
Omeprazole sulfone[2] (Omeprazole related compound A)	0.8	0.1
Omeprazole	1.0	—

[1] 4-Methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide
[2] 5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole

Calculate the percentage of any individual impurity in the portion of Omeprazole Magnesium taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response of any individual impurity; and r_s is the sum of the responses of all the peaks: in addition to not exceeding the limits in *Table 1,* not more than 0.1% of any other individual impurity is found; and not more than 0.5% of total impurities is found.

Content of magnesium—

Lanthanum solution—Transfer 58.7 g of lanthanum oxide to a 1000-mL volumetric flask, wet the substance with some water, and dissolve by cautious addition of 250 mL of hydrochloric acid in 20- to 30-mL portions, cooling between additions. Add water while stirring, cool to room temperature, and dilute with water to volume. [NOTE—Store the solution in a plastic bottle.]

Magnesium standard stock solution—Quantitatively dilute a suitable amount of a commercially prepared atomic absorption standard solution for magnesium with water to obtain a solution containing 1000 µg of magnesium per mL. [NOTE—Store the solution in a plastic bottle.]

Magnesium intermediate standard solution—Transfer 10.0 mL of *Magnesium standard stock solution* to a 500-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, and dilute with water to volume. Transfer 20.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. This solution contains 2 µg of magnesium per mL.

Standard solutions—Transfer 5.0, 10.0, 15.0, 20.0, and 25.0 mL of *Magnesium intermediate standard solution* to separate 100-mL volumetric flasks. To each flask, add 4.0 mL of *Lanthanum solution,* and dilute with water to volume. These *Standard solutions* contain 0.1, 0.2, 0.3, 0.4, and 0.5 µg of magnesium per mL, respectively. [NOTE—Concentrations of the *Standard solutions* and the *Test solution* may be modified to fit the linear or working range of the instrument. When using instruments with a linear calibration graph, the number of the *Standard solutions* can be reduced.]

Blank solution—Transfer 4.0 mL of *Lanthanum solution* to a 100-mL volumetric flask, and dilute with water to volume.

Test solution—Transfer about 250 mg of Omeprazole Magnesium, accurately weighed, to a 100-mL volumetric flask, add 20 mL of 1 N hydrochloric acid, swirl until dissolved, and dilute with water to volume. Allow to stand for 30 minutes. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of the solution so obtained to another 100-mL volumetric flask, add 4.0 mL of *Lanthanum solution,* and dilute with water to volume.

Procedure—Concomitantly determine the absorbances of the *Standard solutions,* the *Blank solution,* and the *Test solution* at the magnesium emission line at 285.2 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) using an air–acetylene flame. Determine the concentration, *C,* in µg per mL, of magnesium in the *Test solution* using the calibration graph. Calculate the content of

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magnesium, in percentage, in the portion of Omeprazole Magnesium taken by the formula:

$$100(0.001CD/W)[100/(100 - L)]$$

in which the multiplier 0.001 is for conversion of µg per mL to mg per mL; C is as defined above; D is the dilution factor for the *Test solution;* W is the quantity of Omeprazole Magnesium, in mg, taken to prepare the *Test solution;* and L is the content of water, in percentage, as determined in the test for *Water.* The magnesium content, calculated on the anhydrous basis, is between 3.30% and 3.55%.

Assay—

Phosphate buffer pH 7.6—Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, dilute with water to 1000 mL, and mix. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

Mobile phase—Prepare a mixture of *Phosphate buffer pH 7.6* and acetonitrile (650:350).

Phosphate buffer pH 11—Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.

Standard preparation—Transfer about 10 mg of USP Omeprazole RS, accurately weighed, to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of *Phosphate buffer pH 11,* and dilute with water to volume. This solution contains about 0.05 mg of omeprazole per mL.

Assay preparation—Transfer about 10 mg of Omeprazole Magnesium, accurately weighed, to a 200-mL volumetric flask, dissolve in about 10 mL of methanol, add 10 mL of *Phosphate buffer pH 11,* and dilute with water to volume. This solution contains about 0.05 mg of omeprazole magnesium per mL.

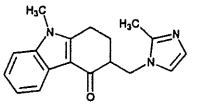
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 12.5-cm or 4.6-mm × 15-cm column that contains 5-µm packing L7. (Alternatively, a 3.9-mm × 15-cm column that contains 4-µm packing L1 may be used.) The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{34}H_{36}MgN_6O_6S_2$ in the portion of Omeprazole Magnesium taken by the formula:

$$100[(713.12/(2 \times 345.42)](C_S/C_U)(r_U/r_S)$$

in which 713.12 and 345.42 are the molecular weights of omeprazole magnesium and omeprazole, respectively; C_S is the concentration, in mg per mL, of omeprazole in the *Standard preparation;* C_U is the concentration, in mg per mL, of omeprazole magnesium in the *Assay preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ondansetron



$C_{18}H_{19}N_3O$ 293.36

4*H*-Carbazol-4-one, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]- (±)-.

(±)-2,3-Dihydro-9-methyl-3-[(2-methylimidazol-1-yl)methyl] carbazol-4(1*H*)-one [99614-02-5].

» Ondansetron contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{19}N_3O$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers at room temperature.

USP Reference standards ⟨11⟩—

USP Ondansetron RS

USP Ondansetron Related Compound C RS
1,2,3,9-Tetrahydro-9-methyl-4*H*-carbazol-4-one.

USP Ondansetron Related Compound D RS
1,2,3,9-Tetrahydro-9-methyl-3-methylene-4*H*-carbazol-4-one.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy:* ⟨197K⟩▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Water Determination, *Method Ia* ⟨921⟩ **:** not more than 3.0%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Chloride ⟨221⟩—To 1 g of the substance under test, add 30 to 40 mL of water, and warm gently, if necessary, until no more dissolves. Mix well, and pass through a filter paper that gives a negative test for chloride. Add 1 mL of nitric acid and 1 mL of silver nitrate TS. Dilute with water to 50 mL. Mix well, and allow to stand for 5 minutes protected from direct sunlight: any turbidity formed is not greater than that produced in a similarly treated control solution containing 0.3 mL of 0.020 N hydrochloric acid (0.02%).

Limit of ondansetron related compound D—

Phosphate buffer—Dissolve about 2.72 g of monobasic potassium phosphate in 900 mL of water. Adjust with 1 N sodium hydroxide or 0.5 N sodium hydroxide to a pH of 5.4, dilute to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an amount of USP Ondansetron Related Compound D RS in *Mobile phase,* and dilute stepwise with *Mobile phase,* to obtain a solution having a known concentration of about 0.4 µg per mL.

Resolution solution—Prepare a solution of USP Ondansetron Related Compound D RS and USP Ondansetron Related Compound C RS in *Mobile phase* having a known concentration of about 0.6 µg per mL and 1.0 µg per mL, respectively.

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Test solution—Transfer about 50 mg of Ondansetron, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 328-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The column temperature is maintained at 30°. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for ondansetron related compound C and 1.0 for ondansetron related compound D; and the resolution, *R,* between ondansetron related compound C and ondansetron related compound D is not less than 1.5. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 8000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of ondansetron related compound D in the ondansetron taken by the formula:

$$10(C/W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Ondansetron Related Compound D RS in the *Standard solution; W* is the weight, in mg, of ondansetron taken to prepare the *Test solution;* and r_U and r_S are the peak responses of ondansetron related compound D obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.10% is found.

Related compounds—

Phosphate buffer, Mobile phase, Resolution solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay.*

Test solution—Use the *Assay preparation* prepared as directed in the *Assay.*

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Ondansetron taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak area for each impurity; and r_s is the sum of the areas of all the peaks: not more than 0.1% of any individual impurity is found; and not more than 0.5% of total impurities is found, including ondansetron related compound D.

[NOTE—Disregard the peak corresponding to ondansetron related compound D at a relative retention time of about 0.4.]

Assay—

Phosphate buffer—Dissolve about 2.72 g of monobasic potassium phosphate in 900 mL of water. Adjust with 1 N sodium hydroxide or 0.5 N sodium hydroxide to a pH of 5.4, dilute to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (52:48). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Prepare a solution of USP Ondansetron RS and USP Ondansetron Related Compound A RS in *Mobile phase* having a known concentration of about 0.09 mg per mL and 0.05 mg per mL, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Ondansetron RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.090 mg per mL.

Assay preparation—Transfer about 45 mg of Ondansetron, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

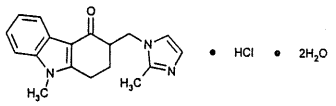
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 216-nm detector and a 4.6-mm 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.1 for ondansetron related compound A and 1.0 for ondansetron; and the resolution, *R,* between ondansetron related compound A and ondansetron is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the ondansetron peaks. Calculate the quantity, in mg, of $C_{18}H_{19}N_3O$ in the portion of Ondansetron taken by the formula:

$$500C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ondansetron RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ondansetron Hydrochloride



$C_{18}H_{19}N_3O \cdot HCl \cdot 2H_2O$ 365.85

4*H*-Carbazol-4-one, 1,2,3,9-tetrahydro-9-methyl-3-(2-methyl-1*H*-imidazol-1-yl)methyl-, monohydrochloride, (±)-, dihydrate.

(±)-2,3-Dihydro-9-methyl-3-(2-methylimidazol-1-yl) methylcarbazol-4(1*H*)-one monohydrochloride dihydrate [103639-04-9].

» Ondansetron Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{19}N_3O \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—

USP Ondansetron Hydrochloride RS

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USP Ondansetron Related Compound A RS
3-[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4*H*-carbazol-4-one hydrochloride.
USP Ondansetron Resolution Mixture RS
Ondansetron hydrochloride having approximately 0.4% w/w of both ondansetron related compound A and 6,6′-methylene bis-[(1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)-methyl]-4*H*-carbazol-4-one)].
USP Ondansetron Related Compound C RS
1,2,3,9-Tetrahydro-9-methyl-4*H*-carbazol-4-one.
USP Ondansetron Related Compound D RS
1,2,3,9-Tetrahydro-9-methyl-3-methylene-4*H*-carbazol-4-one.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: Dissolve 20 mg in 2 mL of water, add 1 mL of 2 M nitric acid, and filter: the filtrate responds to the test for *Chloride* ⟨191⟩.

Water Determination, *Method Ia* ⟨921⟩**:** between 9.0% and 10.5%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Limit of ondansetron related compound D—

Mobile phase—Prepare a filtered and degassed mixture of 0.02 M monobasic potassium phosphate (previously adjusted with 1 M sodium hydroxide to a pH of 5.4) and acetonitrile (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Ondansetron Related Compound D RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.4 µg per mL.

System suitability solution—Dissolve suitable quantities of USP Ondansetron Related Compound D RS and USP Ondansetron Related Compound C RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 0.6 µg per mL and 1 µg per mL, respectively.

Test solution—Transfer about 50 mg of Ondansetron Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 328-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for ondansetron related compound C and 1.0 for ondansetron related compound D; and the resolution, *R,* between ondansetron related compound C and ondansetron related compound D is not less than 1.5. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 400 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of ondansetron related compound D in the portion of Ondansetron Hydrochloride taken by the formula:

$$10{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ondansetron Related Compound D RS in the *Standard solution; W* is the weight, in mg, of Ondansetron Hydrochloride taken to prepare the *Test solution;* and r_U and r_S are the peak areas obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.10% is found.

Chromatographic purity—

METHOD I—

Resolution solution—Dissolve a quantity of USP Ondansetron Resolution Mixture RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of 12.5 mg per mL.

Standard solutions—Dissolve an accurately weighed quantity of USP Ondansetron Hydrochloride RS in methanol, and mix to obtain a solution having a known concentration of about 0.25 mg per mL. Quantitatively dilute this solution with methanol to obtain *Standard solutions,* designated below by letter, having the following compositions:

Standard solution	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimen)
A	(1 in 5)	50	0.4
B	(1 in 10)	25	0.2
C	(1 in 20)	12.5	0.1

Test solution—Dissolve an accurately weighed quantity of Ondansetron Hydrochloride in methanol to obtain a solution containing 12.5 mg per mL.

Procedure—Separately apply 20 µL of the *Test solution,* 20 µL of each *Standard solution,* and 20 µL of the *Resolution solution* to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform, ethyl acetate, methanol, and ammonium hydroxide (90:50:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: complete resolution of the three components of the *Resolution solution* spot is found. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions:* any secondary spot from the chromatogram of the *Test solution* having an R_F value corresponding to that of the uppermost secondary spot of the *Resolution solution* is not larger or more intense than the principal spot obtained from *Standard solution A* (0.4%); and no other secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from *Standard solution B* (0.2%).

METHOD II—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Proceed as directed for *Standard preparation* in the *Assay.*

Test solution—Use the *Assay preparation.*

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in

USP Monographs

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the portion of Ondansetron Hydrochloride taken by the formula:

$$50{,}000(C/W)(1/F)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of USP Ondansetron Hydrochloride RS in the *Standard solution;* W is the weight, in mg, of Ondansetron Hydrochloride taken to prepare the *Test solution;* F is the relative response factor of the impurities as described in the accompanying table; r_i is the peak area for each impurity in the *Test solution;* and r_s is the peak area of ondansetron obtained from the *Standard solution:* it meets the requirements given in the accompanying table.

Compound Name	Relative Retention Time	Relative Response Factor	Limit (%)
Ondansetron related compound C	about 0.32	1.2	0.2
Ondansetron related compound D*	about 0.34	—	0.1
Imidazole	about 0.49	0.3	0.2
2-methylimidazole	about 0.54	0.4	0.2
Ondansetron	1.0	—	—
Ondansetron related compound A	about 1.10	0.8	0.2
Unknown	—	1.0	0.1
Total	—	—	0.5

* Quantified in the test for *Limit of ondansetron related compound D.*

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.02 M monobasic sodium phosphate (previously adjusted with 1 M sodium hydroxide to a pH of 5.4) and acetonitrile (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ondansetron Hydrochloride RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 90 µg per mL.

System suitability solution—Dissolve suitable quantities of USP Ondansetron Hydrochloride RS and USP Ondansetron Related Compound A RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 90 µg per mL and 20 µg per mL, respectively.

Assay preparation—Transfer about 45 mg of Ondansetron Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 216-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for ondansetron and 1.1 for ondansetron related compound A; and the resolution, *R,* between ondansetron related compound A and ondansetron is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{19}N_3O \cdot HCl$ in the portion of Ondansetron Hydrochloride taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ondansetron Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ondansetron Injection

» Ondansetron Injection is a sterile solution of Ondansetron Hydrochloride in Water for Injection or of Ondansetron in Water for Injection prepared with the aid of Hydrochloric Acid. It may contain suitable buffers and/or tonicity adjusting agents. It contains an amount of Ondansetron Hydrochloride equivalent to not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, at a temperature between 2° and 30°, protected from light.

USP Reference standards ⟨11⟩—
USP Ondansetron Hydrochloride RS
USP Ondansetron Related Compound A RS
3-[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4*H*-carbazol-4-one hydrochloride.
USP Ondansetron Related Compound C RS
1,2,3,9-Tetrahydro-9-methyl-4*H*-carbazol-4-one.
USP Ondansetron Related Compound D RS
1,2,3,9-Tetrahydro-9-methyl-3-methylene-4*H*-carbazol-4-one.

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 9.9 USP Endotoxin Units per mg of ondansetron hydrochloride.

pH ⟨791⟩: between 3.3 and 4.0.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Limit of ondansetron related compound D—

Mobile phase, Standard solution, System suitability solution, and *Chromatographic system*—Proceed as directed in the test for *Limit of ondansetron related compound D* under *Ondansetron Hydrochloride.*

Test solution—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of ondansetron, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of

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ondansetron related compound D in the volume of Injection taken by the formula:

$$(2.5/V)(C_S/C_A)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken; C_S is the concentration, in µg per mL, of ondansetron related compound D in the *Standard preparation;* C_A is the concentration, in mg per mL, of ondansetron in the Injection, as determined in the *Assay;* and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation,* respectively: not more than 0.12% is found.

Chromatographic purity—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay.*

System suitability solution—Use the *System suitability solution* prepared as directed in the test for *Limit of ondansetron related compound D* under *Ondansetron Hydrochloride.*

Test solution—Use the *Assay preparation.*

Procedure—Inject about 20 µL of the *System suitability solution,* record the chromatogram, and identify the peaks due to ondansetron related compound C and ondansetron related compound D based on their approximate relative retention times of 0.35 and 0.37, respectively. Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. [NOTE—Ignore the peak due to ondansetron related compound D.] Calculate the percentage of each impurity in the volume of Injection taken by the formula:

$$100(r_i/r_S)$$

in which r_i is the peak response for each impurity; and r_S is the sum of the responses of all of the peaks: not more than 0.2% of any individual impurity is found, and the total of all impurities, including the percentage of ondansetron related compound D determined in the test for *Limit of ondansetron related compound D,* is not more than 0.5%.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.02 M monobasic potassium phosphate (previously adjusted with 1 M sodium hydroxide to a pH of 5.4), and acetonitrile (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ondansetron Hydrochloride RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

System suitability solution—Dissolve suitable quantities of USP Ondansetron Hydrochloride RS and USP Ondansetron Related Compound A RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.1 mg per mL and 50 µg per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 2 mg of ondansetron, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 216-nm detector and a 4.6-mm × 20-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for ondansetron and 1.1 for ondansetron related compound A; and the resolution, R, between ondansetron related compound A and ondansetron is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ondansetron ($C_{18}H_{19}N_3O$) in each mL of the Injection taken by the formula:

$$(293.36/329.83)(25C/V)(r_U/r_S)$$

in which 293.36 and 329.83 are the molecular weights of ondansetron and anhydrous ondansetron hydrochloride, respectively; C is the concentration, in mg per mL, on the anhydrous basis, of USP Ondansetron Hydrochloride RS in the *Standard preparation;* V is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ondansetron Oral Solution

» Ondansetron Oral Solution is a solution of Ondansetron Hydrochloride in a suitable vehicle. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Ondansetron Hydrochloride RS
USP Ondansetron Related Compound A RS
3-[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4*H*-carbazol-4-one hydrochloride.
USP Ondansetron Related Compound C RS
1,2,3,9-Tetrahydro-9-methyl-4*H*-carbazol-4-one.
USP Ondansetron Related Compound D RS
1,2,3,9-Tetrahydro-9-methyl-3-methylene-4*H*-carbazol-4-one.

Identification—

A: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution—Dilute a portion of Oral Solution with a mixture of methanol and water (50:50) to obtain a solution containing about 0.2 mg of ondansetron per mL.
Standard solution: 0.25 mg per mL in methanol.
Developing solvent system: chloroform, ethyl acetate, methanol, and ammonium hydroxide (90:50:40:1).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Escherichia coli.* The total aerobic microbial count does not exceed 100 cfu per g, the *Enterobacteriaceae* count does not exceed 10 cfu per g, and the total combined molds and yeasts count does not exceed 50 cfu per g.

Deliverable volume ⟨698⟩**:** meets the requirements.

pH ⟨791⟩**:** between 3.3 and 4.0.

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Limit of ondansetron related compound D—

Mobile phase—Proceed as directed in the test for *Limit of ondansetron related compound D* under *Ondansetron Hydrochloride.*

System suitability solution—Dissolve suitable quantities of USP Ondansetron Related Compound D RS and USP Ondansetron Related Compound C RS in *Mobile phase;* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.5 µg per mL and 2 µg per mL, respectively.

Standard solution—Dissolve an accurately weighed quantity of USP Ondansetron Related Compound D RS in *Mobile phase;* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.5 µg per mL.

Test solution—Quantitatively dilute, if necessary, an accurately measured volume of Oral Solution with *Mobile phase* to obtain a solution containing about 0.8 mg of ondansetron per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 328-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between ondansetron related compound D and ondansetron related compound C is not less than 2.0; the tailing factor for ondansetron related compound D is not more than 2.0; and the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of ondansetron related compound D in the volume of Oral Solution taken by the formula:

$$100D(C_S/C_A)(r_U/r_S)$$

in which D is the dilution factor for the Oral Solution in the *Test solution;* C_S is the concentration, in µg per mL, of USP Ondansetron Related Compound D RS in the *Standard solution;* C_A is the concentration, in µg per mL, of ondansetron in the Oral Solution, as determined in the *Assay;* and r_U and r_S are the peak responses of ondansetron related compound D obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.1% is found.

Related compounds—

Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Ondansetron Hydrochloride.*

Standard solution—Prepare as directed for the *Standard preparation,* in the *Assay* under *Ondansetron Hydrochloride.*

Test solution—Use the *Assay preparation.*

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each related compound in the volume of Oral Solution taken by the formula:

$$(293.36/329.83)10{,}000(1/F)(1/V)(C_S/C_A)(r_i/r_S)$$

in which 293.36 and 329.83 are the molecular weights of ondansetron and anhydrous ondansetron hydrochloride, respectively; F is the relative response factor for each known and unknown impurity (the values of relative response factors [RRF] and the limits can be obtained from *Table 1*); V is the volume, in mL, of Oral Solution taken; C_S is the concentration, in mg per mL, on the anhydrous basis, of USP Ondansetron Hydrochloride RS in the *Standard solution;* C_A is the concentration, in mg per mL, of ondansetron in the Oral Solution; r_i is the peak response for any related compound obtained from the *Test solution;* and r_S is the peak response for ondansetron obtained from the *Standard solution.*

Table 1

Related Compound	Approx. RRT	RRF	Limit (%)
Ondansetron related compound D*	0.34	—	0.1
Imidazole	0.40	0.46	0.2
2-Methyl imidazole	0.53	0.54	0.2
Des-C-methyl ondansetron hydrochloride	0.62	0.76	0.2
N-Desmethyl ondansetron maleate	0.83	0.73	0.2
Ondansetron related compound A	1.2	0.81	0.2
Unknown		1.0	0.2
Total (including ondansetron related compound D)		—	0.5

* Quantified from Limit of related compound D test

Assay—

Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Ondansetron Hydrochloride.*

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 9 mg of ondansetron, to a 100-mL volumetric flask; dilute with *Mobile phase* to volume; and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 216-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.1 for ondansetron related compound A and 1.0 for ondansetron; and the resolution, *R,* between ondansetron related compound A and ondansetron is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ondansetron ($C_{18}H_{19}N_3O$) in each mL of Oral Solution taken by the formula:

$$(293.36/329.83)100(C/V)(r_U/r_S)$$

in which 293.36 and 329.83 are the molecular weights of ondansetron and anhydrous ondansetron hydrochloride, respectively; C is the concentration, in mg per mL, on the anhydrous basis, of USP Ondansetron Hydrochloride RS in the *Standard preparation;* V is the volume, in mL, of Oral Solution taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Ondansetron Compounded Oral Suspension

DEFINITION

Ondansetron Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$), calculated on the anhydrous basis.

Prepare Ondansetron Compounded Oral Suspension containing 1.0 mg/mL of ondansetron hydrochloride (dihydrate) equivalent to 0.8 mg/mL of ondansetron as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Ondansetron (as ondansetron hydrochloride dihydrate)	80 mg (100 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable glass mortar, and comminute to a fine powder, or add *Ondansetron* hydrochloride powder. Add 50 mL of the *Vehicle* in 5-mL portions, and mix well with each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring the preparation to final volume, and mix well.

ASSAY

- **PROCEDURE**

Mobile phase: 43 mM of monobasic potassium phosphate buffer adjusted with a mixture of 1 N sodium hydroxide and acetonitrile (85:15) to a pH of 5.4

Standard solution: Dissolve USP Ondansetron Hydrochloride RS in *Mobile phase* to obtain a solution with a nominal concentration of 4 µg/mL of ondansetron.

Sample solution: Bring each bottle of Oral Suspension to room temperature. Pipet 500 µL of Oral Suspension from each bottle into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a filter of 0.45-µm pore size, and keep frozen at –70° until assayed.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 216 nm

Columns

Guard: 3.9-mm × 20-cm; 4-µm packing L10

Analytical: 4.6-mm × 25-cm; 5-µm packing L10

Flow rate: 1 mL/min

Injection volume: 80 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for ondansetron is about 30 min.]

Suitability requirements

Relative standard deviation: NMT 1.6% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of ondansetron in the *Standard solution* (µg/mL)

C_U = nominal concentration of ondansetron in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 3.6–4.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 42 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label to indicate that it contains 0.8 mg/mL of ondansetron equivalent to 1 mg/mL of ondansetron hydrochloride (dihydrate).
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ondansetron Hydrochloride RS

Ondansetron Tablets

DEFINITION

Ondansetron Tablets contain Ondansetron Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Transfer a portion of the powder from finely powdered Tablets, equivalent to 100 mg of ondansetron hydrochloride, to a suitable conical flask. Add 50 mL of alcohol, and swirl. Pass the liquid through a PTFE filter of 0.45-µm pore size into a 50-mL beaker. Evaporate the solvent on a rotary evaporator. Dry the precipitate in an air oven for 1 h at 105°. Prepare a suitable dispersion of the residue in potassium bromide, and record the spectra of the *Sample* and the standard specimen in the spectral range 3800–650 cm^{-1}. [NOTE—It is recommended that a solution of USP Ondansetron Hydrochloride RS in alcohol be prepared at a concentration of 2 mg/mL before the evaporation, followed by the drying steps.]

Acceptance criteria: The *Sample* shows strong bands at 1621, 1481, 1281, and 758 cm^{-1}, similar to the potassium bromide dispersion of USP Ondansetron Hydrochloride RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 2.7 g/L of monobasic potassium phosphate. Adjust with 1 N sodium hydroxide to a pH of 5.4.

Mobile phase: Acetonitrile and *Buffer* (1:4)

Diluent: Acetonitrile and *Buffer* (1:1)

Standard solution: 0.05 mg/mL of ondansetron (free base) in *Diluent* from USP Ondansetron Hydrochloride RS

Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to

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50 mg of ondansetron, based on the label claim, to a 100-mL volumetric flask. Add 70 mL of *Diluent*, and sonicate for about 20 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution.

Sample solution: Quantitatively dilute the supernatant with *Diluent* to obtain a solution having a nominal concentration of 0.05 mg/mL of ondansetron, based on the label claim. Pass through a suitable nylon filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 216 nm

Column: 4.6-mm × 25-cm; 5-µm packing L10

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the label claim of ondansetron ($C_{18}H_{19}N_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of ondansetron (free base) in the *Standard solution* (mg/mL)
C_U = nominal concentration of ondansetron in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 500 mL, deaerated

Apparatus 2: 50 rpm

Time: 15 min

Standard solution: USP Ondansetron Hydrochloride RS in *Medium* in a concentration similar to the one expected in the *Sample solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and dilute, if necessary, with *Medium*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 310 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of ondansetron, 293.36
M_{r2} = molecular weight of ondansetron hydrochloride (anhydrous), 329.83
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of $C_{18}H_{19}N_3O$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus 2, Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed for *Test 1*.

Time: 30 min

Tolerances: NLT 80% (*Q*) of the labeled amount of $C_{18}H_{19}N_3O$ is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.01 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Known concentration of USP Ondansetron Hydrochloride RS in *Medium*, close to the expected concentration of the *Sample solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and dilute, if necessary, with *Medium*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 248 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of ondansetron, 293.36
M_{r2} = molecular weight of ondansetron hydrochloride (anhydrous), 329.83
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of $C_{18}H_{19}N_3O$ is dissolved.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: 450 µg/mL of USP Ondansetron Hydrochloride RS in *Medium*

Standard solution: Dilute the *Standard stock solution* quantitatively and stepwise, if necessary, with *Medium* to obtain a final concentration of about (*L*/500) mg/mL, where *L* is the Tablet label claim, in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 249 nm

Cell path: 1 cm for Tablets labeled to contain 4 or 8 mg; 0.2 cm for Tablets labeled to contain 16 or 24 mg

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

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$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of ondansetron, 293.36
M_{r2} = molecular weight of ondansetron hydrochloride (anhydrous), 329.83
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of ondansetron is dissolved.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium, Apparatus 2, Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed for *Test 1*.
Time: 30 min
Tolerances: NLT 70% (*Q*) of the labeled amount of ondansetron is dissolved.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: Water; 500 mL, deaerated
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 3.12 g/L of monobasic sodium phosphate dihydrate. Adjust with 1 N sodium hydroxide to a pH of 5.4.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Standard solution
For Tablets labeled to contain 4 or 24 mg: 0.01 mg/mL of USP Ondansetron Hydrochloride RS in *Medium*
For Tablets labeled to contain 8 mg: 0.02 mg/mL of USP Ondansetron Hydrochloride RS in *Medium*
Sample solution
For Tablets labeled to contain 4 or 8 mg: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
For Tablets labeled to contain 24 mg: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Further dilute 4.0 mL of this solution with *Medium* to 25.0 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 216 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Flow rate: 2.0 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of ondansetron, 293.36
M_{r2} = molecular weight of ondansetron hydrochloride (anhydrous), 329.83
V = volume of *Medium*, 500 mL
D = dilution factor of the *Sample solution*

Tolerances: NLT 75% (*Q*) of the labeled amount of ondansetron is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
System suitability solution: 0.05 and 0.1 mg/mL of USP Ondansetron Related Compound A RS and USP Ondansetron Hydrochloride RS, respectively, in *Mobile phase*
Standard stock solution: Use the *Standard solution* in the *Assay*.
Standard solution: 1.5 µg/mL of ondansetron in *Mobile phase* from the *Standard stock solution*
Sample solution: Weigh and crush NLT 20 Tablets. Transfer a quantity of powder, equivalent to 50 mg of ondansetron, to a 100-mL volumetric flask. Add about 70 mL of *Mobile phase*, and sonicate for about 20 min. Dilute with *Mobile phase* to volume. Centrifuge the solution. Pass a portion of the solution through a suitable nylon filter of 0.45-µm pore size, and use the filtrate.
Chromatographic system: Proceed as directed in the *Assay*.
Run time: At least 45 min for the *Sample solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between ondansetron related compound A and ondansetron, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of ondansetron from the *Standard solution*
C_S = concentration of ondansetron (free base) in the *Standard solution* (mg/mL)
C_U = nominal concentration of ondansetron in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Methyl imidazole[a]	0.22	0.53	0.2
Ondansetron related compound C[b]	0.40	1.2	0.2
Ondansetron related compound D[c]	0.47	1.3	0.1
Ondansetron related compound A[d]	0.87	0.90	0.2

Supplement 1

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desmethylondansetron[a, e]	0.90	0.91	0.2
Ondansetron	1.0	—	—
Any other individual, unspecified degradation product	—	1.0	0.2
Total impurities	—	—	1.0

[a] Not to be included in total impurities.
[b] 1,2,3,9-Tetrahydro-9-methyl-4*H*-carbazol-4-one.
[c] 1,2,3,9-Tetrahydro-9-methyl-3-methylene-4*H*-carbazol-4-one.
[d] 3[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4*H*-carbazol-4-one.
[e] 1,2,3,9-Tetrahydro-9-methyl-3-[1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ondansetron Hydrochloride RS
  USP Ondansetron Related Compound A RS
  3-[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4*H*-carbazol-4-one hydrochloride.

Ondansetron Orally Disintegrating Tablets

DEFINITION

Ondansetron Orally Disintegrating Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  [NOTE—Alternatively, the UV spectra of the major peaks of the *Sample solution* and the *Standard solution*, as obtained in the *Assay*, may be used to meet the *Acceptance criteria*.]
  Diluent: 0.1 N hydrochloric acid
  Standard solution: 8 µg/mL of USP Ondansetron RS in *Diluent*
  Sample solution: Nominally 8 µg/mL of ondansetron from Tablets in *Diluent*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.
  Wavelength range: 200–400 nm
  Cell: 1 cm
  Blank: *Diluent*
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*.

ASSAY

- **PROCEDURE**
  Diluent: 0.01 N hydrochloric acid
  Buffer: 2.72 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide or 0.5 N sodium hydroxide to a pH of 5.4.
  Mobile phase: Acetonitrile and *Buffer* (48:52)
  System suitability solution: 0.02 mg/mL of USP Ondansetron Related Compound A RS and 0.006 mg/mL of USP Ondansetron RS in *Diluent*
  Standard solution: 40 µg/mL of USP Ondansetron RS in *Diluent*
  Sample stock solution: Nominally equivalent to 400 µg/mL of ondansetron prepared as follows. Transfer 10 Tablets to a suitable volumetric flask. Add *Diluent* to fill about 60% of the flask volume. Shake by mechanical means for about 5 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a polypropylene membrane of 0.45-µm pore size, discarding the first 5 mL of the filtrate.
  Sample solution: Nominally 40 µg/mL of ondansetron in *Diluent*, from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 216 nm. If the procedure is used for *Identification B*, use a diode array detector set at 200–400 nm.
  Column: 4.6-mm × 25-cm; packing L10
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for ondansetron and ondansetron related compound A are 1.0 and 1.1, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between ondansetron related compound A and ondansetron, *System suitability solution*
  Tailing factor: NMT 2.0 for the ondansetron peak, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ondansetron RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ondansetron in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩
  Test 1
  Time: NMT 10 s
  Test 2: If the product complies with this test, the labeling indicates that it meets USP *Disintegration Test 2*.
  Time: NMT 60 s
- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid; 500 mL, deaerated
  Apparatus 2: 50 rpm
  Time: 10 min

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Detector: UV 310 nm
Cell
For 4- and 8-mg Tablets: 1 cm
For 16-mg Tablets: 0.5 cm
For 24-mg Tablets: 0.2 cm
Standard solution: (*L*/500) mg/mL of USP Ondansetron RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Ondansetron RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile and *Buffer* (1:4)
System suitability solution: 2 µg/mL each of USP Ondansetron Related Compound D RS, 2-methylimidazole, and USP Ondansetron RS in *Mobile phase*. [NOTE—First dissolve in acetonitrile, and then dilute with *Mobile phase* to volume.]
Standard solution: 2 µg/mL of USP Ondansetron RS in *Mobile phase*
Sensitivity solution: 0.2 µg/mL of USP Ondansetron RS, from the *Standard solution* in *Mobile phase*
Sample solution: Nominally equivalent to 400 µg/mL of ondansetron prepared as follows. Transfer 10 Tablets to a suitable volumetric flask. Add *Mobile phase* to fill about 60% of the flask volume. Shake by mechanical means for about 5 min, and dilute with *Mobile phase* to volume. Centrifuge a portion of this solution at 3000 rpm for 10 min. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 216 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: About 60 min
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between ondansetron and any adjacent peak, *System suitability solution*
Column efficiency: NLT 8000 theoretical plates for ondansetron, *System suitability solution*
Tailing factor: NMT 2.0 for the ondansetron peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 15, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_S = peak area of ondansetron from the *Standard solution*
C_S = concentration of USP Ondansetron RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ondansetron in the *Sample solution* (µg/mL)
F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Methylimidazole	0.16	0.5	0.2
Ondansetron related compound D	0.45	1.2	0.12
Ondansetron	1.0	—	—
Individual unknown impurity	—	1.0	0.2
Total impurities	—	—	0.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.
• **LABELING:** The labeling states the *Disintegration* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ondansetron RS
USP Ondansetron Related Compound A RS
3-[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4*H*-carbazol-4-one hydrochloride.
$C_{16}H_{20}N_2O \cdot HCl$ 292.80
USP Ondansetron Related Compound D RS
1,2,3,9-Tetrahydro-9-methyl-3-methylene-4*H*-carbazol-4-one.
$C_{14}H_{13}NO$ 211.26

Ondansetron Compounded Topical Gel

Change to read:

DEFINITION

Ondansetron Compounded Topical Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$).
Prepare Ondansetron Compounded Topical Gel, 20 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Ondansetron (as Ondansetron Hydrochloride)	200 mg (249.4 mg)
Propylene Glycol	▲3▲ (RB 1-Jun-2018) mL

USP Monographs

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Lipoderm,[a] a sufficient quantity to make	10 mL

[a] PCCA, Houston, TX.

Wet the *Ondansetron Hydrochloride* with the *Propylene Glycol* in a suitable container. Add the *Lipoderm* stepwise and quantitatively. Bring to final volume and mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 2.72 g of monobasic potassium phosphate in 750 mL of water and adjust to a pH of 6.0. Mix with 250 mL of acetonitrile.

Diluent: Methanol and water (50:50)

Standard solution: 0.1 mg/mL of ondansetron (free base) prepared from USP Ondansetron Hydrochloride RS in *Diluent* (approximately equal to 0.1247 mg/mL of ondansetron hydrochloride)

Sample solution: Fill a 1-mL syringe with Topical Gel and weigh. Transfer the sample to a 200-mL volumetric flask, add about 150 mL of *Diluent*, and sonicate until the gel has broken down. Dilute with *Diluent* to final volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.8 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for ondansetron is about 5.3 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$) in the portion of Topical Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ondansetron from the *Sample solution*

r_S = peak response of ondansetron from the *Standard solution*

C_S = concentration of ondansetron in the *Standard solution* (mg/mL)

C_U = nominal concentration of ondansetron in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in a tight, light-resistant calibrated container dispenser. Store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature.
- **LABELING:** Label it to indicate that it is for external use only and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ondansetron Hydrochloride RS

Bland Lubricating Ophthalmic Ointment

DEFINITION

Bland Lubricating Ophthalmic Ointment is a sterile ointment of white petrolatum and mineral oil. It may contain Lanolin, Modified Lanolin, or Lanolin Alcohols. It may also contain a suitable antimicrobial preservative.

SPECIFIC TESTS

- **APPEARANCE**

Analysis: Transfer a portion of it to a suitable test tube, and examine the sample in front of a light source.

Acceptance criteria: The sample appears translucent.

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **ACIDITY OR ALKALINITY**

Sample solution: Transfer 20.0 g of Ophthalmic Ointment to a 400-mL beaker. Add 100 mL of a mixture of neutralized alcohol and water (1 in 2), agitate thoroughly, and gradually heat to boiling. Boil for 10 min.

Analysis: To the *Sample solution* add 1 mL of phenolphthalein TS, and rapidly titrate with vigorous agitation with either 0.1 N sodium hydroxide VS (from a colorless alcohol–water layer to a sharp pink endpoint) or 0.1 N hydrochloric acid VS (from a pink alcohol–water layer to a colorless endpoint).

Acceptance criteria: NMT 0.40 mL of 0.1 N hydrochloric acid VS or 0.1 N sodium hydroxide VS is required to produce the color change.

- **COLOR**

Analysis: Examine the extruded Ophthalmic Ointment for color.

Acceptance criteria: Colorless to light yellow

- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in suitable collapsible ophthalmic ointment tubes.

Opium

» Opium is the air-dried milky exudate obtained by incising the unripe capsules of *Papaver somniferum* L. or its variety *album* De Candolle (Fam. Papaveraceae). It yields not less than 9.5 percent of anhydrous morphine.

Botanic characteristics—More or less rounded, oval, brick-shaped or elongated, somewhat flattened masses usually about 8 cm to 15 cm in diameter and weighing about 300 g to 2 kg each. Externally, it is pale olive-brown or olive-gray, having a coarse surface and being covered with a thin coating consisting of fragments of poppy leaves and, at times, with fruits of a species of *Rumex* adhering from the packing; it is more or less plastic when fresh, becoming hard or tough on storage. Internally, it is reddish brown and coarsely granular.

Assay—

Chromatographic tubes, Citrate buffer, and *Standard preparation*—Prepare as directed in the *Assay* under *Paregoric*.

Assay preparation—Transfer about 2 g of Opium, accurately weighed, to a 250-mL beaker, add 20 mL of dimethyl

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sulfoxide, and heat for 20 minutes on a steam bath, intermittently dispersing the substance with a flat-end stirring rod. Allow to stand for 15 minutes to permit undissolved material to settle, and carefully decant the supernatant into a 100-mL volumetric flask. Add another 20 mL of dimethyl sulfoxide to the residue, rinsing the sides of the beaker with dimethyl sulfoxide. Disperse and heat the substance as before, allow to settle, and decant into the volumetric flask. Repeat the dissolution one or two times, until the opium is dissolved (other than for small leaf fragments, sand-like particles, gelatinous materials, etc.). Rinse the beaker, and transfer the residue to the flask with the aid of water. Dilute with water to about 90 mL, and mix. If necessary, add 1 drop of alcohol to dispel any foam. Cool to room temperature, adjust with water to volume, and mix. Pass the resulting solution through a medium-porosity filter paper, discarding the first 20 mL of the filtrate.

Chromatographic columns—Pack a pledget of glass wool at the base of each of the three tubes, and fill with adsorbent using chromatographic siliceous earth as the base of the adsorbent, and tamping it firmly in place. Prepare the tubes as follows. Pack *Column I* in two layers, the lower layer consisting of 3 g of chromatographic siliceous earth mixed with 2 mL of *Citrate buffer* and the upper layer consisting of 3 g of chromatographic siliceous earth mixed with 2.0 mL of the *Assay preparation* and 0.5 mL of *Citrate buffer.* Dry-rinse the beaker in which the components of the two layers have been mixed with 1 g of chromatographic siliceous earth, and add it also to the top of *Column I.* Pack *Column II* with 3 g of chromatographic siliceous earth mixed with 2 mL of dibasic potassium phosphate solution (1 in 5.75). Pack *Column III* with 3 g of chromatographic siliceous earth mixed with 2 mL of sodium hydroxide solution (1 in 50). Place a small pad of glass wool above each column packing.

Procedure—Proceed as directed in the *Assay* under *Paregoric.* Calculate the percentage of anhydrous morphine in the Opium taken by the formula:

$$0.25(C/W)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of anhydrous morphine in the *Standard preparation; W* is the weight, in g, of Opium taken; and A_U and A_S are the corrected absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Powdered Opium

» Powdered Opium is Opium dried at a temperature not exceeding 70°, and reduced to a very fine powder.

Powdered Opium yields not less than 10.0 percent and not more than 10.5 percent of anhydrous morphine. It may contain any of the diluents, with the exception of starch, permitted for powdered extracts under *Extracts* ⟨1151⟩.

Packaging and storage—Preserve in well-closed containers.

Botanic characteristics—Consists chiefly of yellowish brown to yellow, more or less irregular and granular fragments of latex, varying from 15 to 150 µm in diameter; a few fragments of strongly lignified, thick-walled, 4- to 5-sided or narrowly elongated, epidermal cells of the poppy capsule; very few fragments of tissues of poppy leaves, poppy capsules, and, occasionally, *Rumex* fruits. In addition, there will be the microscopic characteristics of the diluent if any has been used in the preparation of the powder.

Assay—Proceed with Powdered Opium as directed in the *Assay* under *Opium.*

Opium Tincture

» Opium Tincture contains, in each 100 mL, not less than 0.90 g and not more than 1.10 g of anhydrous morphine.

Opium Tincture may be prepared as follows.

Place 100 g of granulated or sliced Opium in a suitable vessel. [NOTE—Do not use Powdered Opium.] Add 500 mL of boiling water, and allow to stand, with frequent stirring, for 24 hours. Transfer the mixture to a percolator, allow it to drain, percolate with water as the menstruum to complete extraction, and evaporate the percolate to a volume of 400 mL. Boil actively for not less than 15 minutes, and allow to stand overnight. Heat the mixture to 80°, add 50 g of paraffin, and heat until the paraffin is melted. Beat the mixture thoroughly, and cool.

Remove the paraffin, and filter the concentrate, washing the paraffin and the filter with sufficient water to make the filtrate measure 750 mL. Add 188 mL of alcohol to the filtrate, mix, and assay a 10-mL portion of the resulting solution as directed in the *Assay.* Dilute the remaining solution with a mixture of 1 volume of alcohol and 4 volumes of water to obtain a Tincture containing 1 g of anhydrous morphine in each 100 mL. Mix.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

Alcohol Determination ⟨611⟩: between 17.0% and 21.0% of C_2H_5OH, determined by the gas-liquid procedure, acetone being used as the internal standard.

Assay—

Chromatographic tubes , Citrate buffer, Standard preparation, and *Chromatographic columns*—Prepare as directed in the *Assay* under *Paregoric.*

Assay preparation—Transfer 10.0 mL of Tincture to a 50-mL volumetric flask containing 10.0 mL of alcohol, add purified water to volume, and mix. Transfer a 2.0-mL aliquot of the solution, equivalent to about 4 mg of Morphine, to a 50-mL beaker, and add 0.5 mL of *Citrate buffer.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Paregoric.* Calculate the weight of anhydrous morphine, in g per 100 mL of the Tincture taken by the formula:

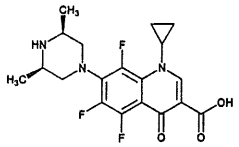
$$0.250W(A_U/A_S)$$

in which W is the weight, in mg, of anhydrous morphine in the 50 mL of *Standard preparation,* and A_U and A_S are the corrected absorbances of the solution from the *Assay preparation* and the *Standard preparation,* respectively.

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Orbifloxacin



$C_{19}H_{20}F_3N_3O_3$ 395.38

1-Cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-5,6,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid [113617-63-3].

» Orbifloxacin contains not less than 98.5 percent and not more than 101.5 percent of $C_{19}H_{20}F_3N_3O_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at room temperature.

USP Reference standards ⟨11⟩—
USP Orbifloxacin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: X-Ray Diffraction ⟨941⟩—The X-ray diffraction pattern conforms to that of USP Orbifloxacin RS, similarly determined.

Microbial enumeration tests ⟨61⟩—The total combined molds and yeasts count does not exceed 100 cfu per g.

pH ⟨791⟩: between 6.5 and 7.8, in a solution containing 10 mg per mL.

Water Determination, *Method Ic* ⟨921⟩: between 1.5% and 2.9%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—

Buffer, Mobile phase, System suitability preparation, Standard preparation, and *Chromatographic system*— Prepare as directed in the *Assay.*

Standard solution—Dilute, quantitatively with *Buffer,* the *Standard preparation* to obtain a solution having a known concentration of about 0.00004 mg per mL.

Test solution—Transfer about 40 mg of Orbifloxacin, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Buffer* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—Inject the *Buffer* as directed for *Procedure* to verify that there are no interfering peaks.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of related compounds in the portion of Orbifloxacin taken by the formula:

$$20{,}000(C_S)(r_i/r_S)(1/F)$$

in which C_S is the concentration, in mg per mL, of orbifloxacin in the *Standard solution;* r_i is the peak area response for each impurity obtained from the *Test solution;* r_S is the peak area response for the orbifloxacin peak obtained from the *Standard solution;* and F is the relative response factor for each impurity, as presented in *Table 1.*

Assay—

Buffer—In a 2-L flask, dissolve about 11.8 g of sodium citrate in 1600 mL of water, and mix. Add 180 mL of acetic acid, and mix. Adjust with 6 N sodium hydroxide to a pH of 3.5, dilute with water to about 2 L, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer,* methanol, and dioxane (86:11:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve in *Buffer* an accurately weighed quantity of USP Orbifloxacin RS to obtain a solution having a known concentration of about 0.2 mg per mL.

Standard preparation—Accurately transfer a quantity of *Standard stock preparation,* and dilute with *Buffer* to obtain a solution having a known concentration of about 0.02 mg per mL.

Table 1 (Orbifloxacin)

Component/Impurity	Approximate Relative Retention Time	Relative Response Factor (*F*)	Limit %
cis, cis-1-Cyclopropyl-5,7-bis(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	0.5	0.36	NMT 0.2
cis-1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-5,6,8-trifluoro-4(1*H*)-quinolinone	0.65	0.27	NMT 0.2
7-[(2-Aminopropyl)amino]-1-cyclopropyl-5,6-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	0.75	0.49	NMT 0.2
Orbifloxacin	1.0	1.00	—
1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	1.4	0.84	NMT 0.2
cis-1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-5-hydroxy-4-oxo-3-quinolinecarboxylic acid	2.7	0.73	NMT 0.2
cis-1-Cyclopropyl-5-(3,5-dimethyl-1-piperazinyl)-6,7,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	3.6	0.11	NMT 0.2
1-Cyclopropyl-5,6,7,8-tetrafluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	6.8	0.16	NMT 0.2
Unknown	—	1.0	—
Total known and unknown	—	—	NMT 0.4

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System suitability preparation—Dissolve about 40 mg of methyl 4-aminobenzoate in 2 mL of methanol, and dilute with *Buffer* to 200 mL. Pipet 10.0 mL of this solution and 10.0 mL of *Standard stock preparation* into a 100-mL volumetric flask. Dilute with *Buffer* to volume, and mix.

Assay preparation—Transfer about 40 mg of Orbifloxacin accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Buffer* to volume, and mix. Dilute with *Buffer* an aliquot of the resulting solution to obtain a solution having a known concentration of about 0.02 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 290-nm detector and 4.6-mm × 3.0-cm column that contains 3-µm packing L1. The flow rate is about 1.0 mL per minute. Prior to injecting the *System suitability preparation,* flush the column with approximately 50 mL of a mixture of acetonitrile and water (9:1). Chromatograph the *System suitability preparation,* and record the peak response as directed for *Procedure:* the relative retention times are about 1.3 for methyl 4-aminobenzoate and 1.0 for orbifloxacin; the resolution, *R,* between methyl 4-aminobenzoate and orbifloxacin is not less than 2; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatographs, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{19}H_{20}F_3N_3O_3$ in the portion of Orbifloxacin taken by the formula:

$$2000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Orbifloxacin RS in the *Standard preparation;* and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Orbifloxacin Tablets

» Orbifloxacin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of orbifloxacin ($C_{19}H_{20}F_3N_3O_3$).

Packaging and storage—Preserve in tight containers, and store between 2° and 30°.

Labeling—Label to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Orbifloxacin RS

Identification—

A: Thin-Layer Chromatographic Identification Test ⟨201⟩.

Absorbent: silica gel.

Diluent: a mixture of chloroform, methanol, and glacial acetic acid (8:1:1).

Test solution—Crush 1 Tablet and transfer into a centrifuge tube. Add *Diluent* quantitatively, and mix to obtain a final concentration of about 0.56 mg per mL of orbifloxacin. Centrifuge the solution.

Standard solution—Prepare a solution of USP Orbifloxacin RS in *Diluent* having a concentration of about 0.56 mg per mL.

Application volume: 5 µL

Developing solvent system: a mixture of chloroform, methanol, water, and ammonium hydroxide (18: 7: 1: 0.02).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 1000 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{19}H_{20}F_3N_3O_3$ dissolved by employing the following procedure.

Standard solution—Prepare a solution of USP Orbifloxacin RS in *Medium* with a final concentration of about $L/100$, where L is the Tablet label claim in mg. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Medium* to volume, and mix.

Test solution—Pass a portion of the solution under test through a suitable 0.8-µm filter, discarding the first 3 mL.

Procedure—Determine the amount of $C_{19}H_{20}F_3N_3O_3$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 291 nm on portions of the *Test solution* in comparison with the *Standard solution* using *Medium* as blank. Calculate the amount of orbifloxacin dissolved by the formula:

$$100{,}000(A_U/A_S)(C_S/L)$$

in which A_U and A_S are the absorbances obtained with the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of orbifloxacin in the *Standard solution;* and L is the Tablet label claim in mg.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{19}H_{20}F_3N_3O_3$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Water Determination, *Method Ic* ⟨921⟩: between 3.5% and 7.0%.

Test preparation—Accurately weigh 5 Tablets, and transfer into a 50-mL centrifuge tube. Add 25 mL of anhydrous methanol, and cap.

Blank: 25 mL of anhydrous methanol in a 50-mL centrifuge tube.

Procedure—Rotate the *Test preparation* and the *Blank* for 16 hours. Centrifuge. Titrate an equal volume of the *Test preparation* and the *Blank* so that the amount of water titrated will be approximately 1000 µg to 1500 µg.

Chromatographic purity—

Buffer, Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay.*

Standard solution—Dilute quantitatively with *Buffer* the *Standard preparation* to obtain a solution having a known concentration of about 0.00004 mg per mL.

Test solution—Transfer 10 Tablets into a volumetric flask. Add *Buffer* to fill the flask about 70%, shake for 2 hours, and sonicate for 5 minutes. Dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a concentration of about 0.22 mg per mL. Pass a portion of the solution through a 0.8-µm filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—Inject the *Buffer* as directed for *Procedure* to verify that there are no interfering peaks.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of related compounds in the portion of Tablets taken by the formula:

$$100(C_S/C_T)(r_i/r_S)(1/F)$$

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in which C_S is the concentration, in mg per mL, of USP Orbifloxacin RS in the *Standard solution;* C_T is the concentration, in mg per mL, of the *Test solution;* r_i is the peak area response for each impurity obtained from the *Test solution;* r_S is the peak area response for the orbifloxacin peak obtained from the *Standard solution;* and F is the relative response factor for each impurity, as presented in *Table 1*.

Assay—

Buffer—In a 2-L flask, dissolve about 11.8 g of sodium citrate in 1600 mL of water, and mix. Add 180 mL of glacial acetic acid, and mix. Adjust with 6 N sodium hydroxide to a pH of 3.5, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer*, methanol, and dioxane (91:6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve an accurately weighed quantity of USP Orbifloxacin RS in *Buffer*, and dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a known concentration of about 0.2 mg per mL.

Standard preparation—Accurately transfer a quantity of *Standard stock preparation* and dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a known concentration of about 0.02 mg per mL.

System suitability preparation—Dissolve about 40 mg of methyl 4-aminobenzoate in 2 mL of methanol, and dilute with *Buffer* to 200 mL. Pipet 10.0 mL of this solution and 10.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask. Dilute with *Buffer* to volume, and mix.

Assay preparation—Transfer 10 Tablets into a volumetric flask. Add *Buffer* to fill the flask about 70%, shake for 2 hours, and sonicate for 5 minutes. Dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a known concentration of about 0.02 mg per mL. Pass a portion of the solution through a 0.8-µm filter.

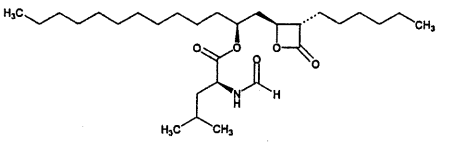
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 290-nm detector and 4.6-mm × 3-cm column that contains 3-µm packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *System suitability preparation,* and record the peak response as directed for *Procedure:* the relative retention times are about 1.3 for methyl 4-aminobenzoate and 1.0 for orbifloxacin; the resolution, *R,* between methyl 4-aminobenzoate and orbifloxacin is not less than 2; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of orbifloxacin ($C_{19}H_{20}F_3N_3O_3$) in the portion of Tablets taken by the formula:

$$C(D_U)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Orbifloxacin RS in the *Standard preparation;* D_U is the dilution factor of the *Assay preparation,* in mL; and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Orlistat



$C_{29}H_{53}NO_5$ 495.73

L-Leucine, *N*-formyl-, 1-[(3-hexyl-4-oxo-2-oxetanyl)methyl] dodecyl ester, [2*S*-[2α(*R**), 3β]]-;
N-Formyl-L-leucine, ester with (3*S*,4*S*)-3-hexyl-4-[(2*S*)-2-hydroxytridecyl]-2-oxetanone [96829-58-2].

DEFINITION

Orlistat contains NLT 98.0% and NMT 101.5% of orlistat ($C_{29}H_{53}NO_5$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **Procedure**

[Note—Avoid the use of plastic flasks for the preparation or containment of any solution in this analysis.]

Mobile phase: Acetonitrile, phosphoric acid, and water (860: 0.05: 140)

Standard solution: 0.5 mg/mL of USP Orlistat RS in *Mobile phase.* Inject immediately after preparation or store at 5°.

Sample solution: 0.5 mg/mL of Orlistat in *Mobile phase.* Inject immediately after preparation or store at 5°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 195

Column: 3.9-mm × 15-cm; 4-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Table 1 (Orbifloxacin Tablets)

Component	Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
cis-1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-5,6,8-trifluoro-4(1*H*)-quinolinone	0.57	0.29	NMT 0.5
Orbifloxacin	1.0	1.00	—
cis-1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-5-hydroxy-4-oxo-3-quinolinecarboxylic acid	2.9	0.71	NMT 0.5
All other related compounds and impurities	—	0.11	NMT 0.5
Total known and unknown	—	—	NMT 1

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Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of orlistat ($C_{29}H_{53}NO_5$) in the portion of Orlistat taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
C_U = concentration of Orlistat in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous, solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **LIMIT OF ORLISTAT RELATED COMPOUND A**
Standard solution: 0.1 mg/mL of USP Orlistat Related Compound A RS in acetone
Sample solution: 50 mg/mL of Orlistat in acetone
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Toluene and ethyl acetate (4:1)
Detection solution: Transfer 2.5 g of phosphomolybdic acid and 1 g of ceric sulfate into a 100-mL volumetric flask, and dissolve in and dilute with methanol to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Remove the plate, and air-dry it thoroughly. Spray the dried plate with *Detection solution*, and place the plate in an oven at 120° for 30 min.
Acceptance criteria: Any secondary spot from the *Sample solution* corresponding to orlistat related compound A is not more intense than the corresponding spot from the *Standard solution* (0.2%).

• **LIMIT OF ORLISTAT RELATED COMPOUND B**
Standard solution: 0.025 mg/mL of USP Orlistat Related Compound B RS in methylene chloride
Sample solution: 50 mg/mL of Orlistat in methylene chloride
Spiked sample solution: 50 mg/mL of Orlistat in *Standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m fused silica, coated with a 0.25-µm G27 stationary phase
Temperatures
Injector: 270°
Detector: 280°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	4	170	—
170	30	300	30

Carrier gas: Helium
Flow rate: 1 mL/min
Split ratio: 20:1
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Standard solution*, *Sample solution*, and *Spiked sample solution*
Calculate the percentage of orlistat related compound B in the portion of Orlistat taken:

$$\text{Result} = \{r_U/[r_{SP} - r_U \times (C_T/C_U)]\} \times (C_S/C_U) \times 100$$

r_U = peak response of orlistat related compound B from the *Sample solution*
r_{SP} = peak response of orlistat related compound B from the *Spiked sample solution*
C_T = concentration of Orlistat in the *Spiked sample solution* (mg/mL)
C_U = concentration of Orlistat in the *Sample solution* (mg/mL)
C_S = concentration of USP Orlistat Related Compound B RS in the *Standard solution* (mg/mL)

Acceptance criteria: NMT 0.05% of orlistat related compound B is found.

• **ORGANIC IMPURITIES**
[NOTE—Avoid the use of plastic flasks for the preparation or containment of any solution in this analysis.]
Mobile phase, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.
System suitability solution: 10 µg/mL of USP Orlistat RS and 0.1 µg/mL of USP Orlistat Related Compound C RS in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Signal-to-noise ratio: NLT 3 for orlistat related compound C
Relative standard deviation: NMT 10.0% for the orlistat peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity other than orlistat related compound A, orlistat related compound B, orlistat related compound D, orlistat open ring amide, and orlistat related compound E in the portion of Orlistat taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

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r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of orlistat from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
C_U = concentration of Orlistat in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Formylleucine[a]	0.10	4.0	0.2
Orlistat related compound C	0.13	33	0.05
Orlistat open ring epimer[b]	0.44	1.0	0.2
Orlistat related compound D[c]	0.90	—	see *Table 3*
Orlistat open ring amide[c, d]	0.90	—	see *Table 3*
Orlistat	1.00	—	—
D-Leucine orlistat[e]	1.18	1.0	0.2
Individual unidentified impurity	—	1.0	0.1
Total impurities[f]	—	—	1.0

[a] *N*-Formyl-L-leucine.
[b] (2*S*,3*R*,5*S*)-5-[(*S*)-2-Formylamino-4-methyl-pentanoyloxy]-2-hexyl-3-hydroxy-hexadecanoic acid.
[c] Coelutes in this LC system, determined using *Limits of Orlistat Related Compound D and Orlistat Open Ring Amide.*
[d] *N*-Formyl-L-leucine (*S*)-1-[(2*S*,3*S*)-2-hydroxy-3-[1-phenyl-*R*-ethylcarbomoyl] nonyl]-dodecyl ester.
[e] *N*-Formyl-D-leucine (*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester or enantiomer.
[f] Sum of results obtained in the tests for *Limit of Orlistat Related Compound A, Limit of Orlistat Related Compound B, Organic Impurities, Limits of Orlistat Related Compound D and Orlistat Open Ring Amide,* and *Limit of Orlistat Related Compound E.*

• **LIMITS OF ORLISTAT RELATED COMPOUND D AND ORLISTAT OPEN RING AMIDE**

Mobile phase: Methanol and water (830:170)
System suitability solution: 4 mg/mL of USP Orlistat RS and 2.4 µg/mL of USP Orlistat Related Compound D RS in acetonitrile, respectively
Standard solution: 5.0 mg/mL of USP Orlistat RS in acetonitrile
Sample solution: 5.0 mg/mL of Orlistat in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: 205 nm
Column: 4.0-mm × 25-cm; 5-µm packing L7
Flow rate: 0.6 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Signal-to-noise ratio: NLT 3 for the orlistat related compound D peak
Relative standard deviation: NMT 10.0% for the orlistat peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of orlistat related compound D and orlistat open ring amide in the portion of Orlistat taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of orlistat related compound D or orlistat open ring amide from the *Sample solution*
r_S = peak response of orlistat from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (µg/mL)
C_U = concentration of Orlistat in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Orlistat related compound D	0.94	1.0	0.2
Orlistat	1.00	—	—
Orlistat open ring amide[a]	1.25	4.3	0.1

[a] *N*-Formyl-L-leucine (*S*)-1-[(2*S*,3*S*)-2-hydroxy-3-[1-phenyl-*R*-ethylcarbomoyl] nonyl]-dodecyl ester.

• **LIMIT OF ORLISTAT RELATED COMPOUND E**

Buffer: 0.4 N borate solution, adjusted to a pH of 10.2
Derivatizing agent: *o*-Phthalaldehyde (OPA) solution. [NOTE—If unable to obtain commercially, the *Derivatizing agent* can be prepared as 1% each of 3-mercaptopropionic acid and *o*-phthalaldehyde in 0.4 M borate buffer solution.]
Solution A: Transfer 4.1 g of sodium acetate trihydrate and 40 mg of ethylenediaminetetraacetic acid (EDTA) into a 1-L volumetric flask. Dissolve in 950 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 7.2. Dilute with water to volume, add 2.5 mL of tetrahydrofuran, and mix. Filter, and degas.
Solution B: Transfer 2.7 g of sodium acetate trihydrate and 40 mg of EDTA into a 1-L volumetric flask. Dissolve in 200 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 7.2. Add 800 mL of acetonitrile, filter, and degas.
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	96.7	3.3
20	60	40
24	0	100
38	0	100
38	96.7	3.3
45	96.7	3.3

Standard solution: Transfer a weighed quantity of about 0.2 mg of USP Orlistat Related Compound E RS into a 20-mL headspace vial. Add 10 mL of 4 N sodium hydroxide, and close the vial. Heat the vial to 100° for 1 h, then allow

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to cool to room temperature. Transfer 2 mL of the resulting solution into a 50-mL volumetric flask, and dilute with water to volume. To 0.5 mL of this solution add 2.0 mL of *Buffer* and 0.5 mL of *Derivatizing agent*.

Sample solution: Proceed as directed for the *Standard solution*, except use 25 mg of Orlistat to replace the 0.2 mg of USP Orlistat Related Compound E RS.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence 340 nm (excitation); 450 nm (emission)
Columns
Guard: 2.1-mm × 2-cm; 5-µm packing L1
Analytical: 2.1-mm × 20-cm; 5-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 6.0% for the orlistat related compound E peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of orlistat related compound E in the portion of Orlistat taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of orlistat related compound E from the *Sample solution*
r_S = peak response of orlistat related compound E from the *Standard solution*
C_S = concentration of USP Orlistat Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Orlistat in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2% of orlistat related compound E is found.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 30 mg/mL in dehydrated alcohol
Acceptance criteria: Between –48.0° and –51.0°, at 20°
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers between 2° and 8°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Orlistat RS
USP Orlistat Related Compound A RS
(3*S*,4*S*)-3-Hexyl-4-[(2*R*)-2-hydroxytridecyl]-2-oxetanone.
$C_{22}H_{42}O_3$ 354.57
USP Orlistat Related Compound B RS
Diisopropyl hydrazine-1,2-dicarboxylate.
$C_8H_{16}N_2O_4$ 204.22
USP Orlistat Related Compound C RS
Triphenylphosphine oxide.
$C_{18}H_{15}OP$ 278.28
USP Orlistat Related Compound D RS
N-Formyl-L-leucine (3*S*,4*R*,6*S*)-3-hexyl-2-oxo-6-undecyltetrahydro-2*H*-pyran-4-yl ester.
$C_{29}H_{53}NO_5$ 495.73
USP Orlistat Related Compound E RS
N-Formyl (*S*)-isoleucine (*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester.
$C_{29}H_{53}NO_5$ 495.73

Orlistat Capsules

DEFINITION

Orlistat Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of orlistat ($C_{29}H_{53}NO_5$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**
Mobile phase: Acetonitrile, phosphoric acid, and water (860: 0.05: 140)
Standard solution: 0.6 mg/mL of USP Orlistat RS in *Mobile phase*
Sample solution: Nominally 0.6 mg/mL of orlistat in *Mobile phase* prepared as follows. Transfer a quantity of orlistat from the contents of NLT 10 Capsules to a suitable volumetric flask. Add 70% of the final volume of *Mobile phase* and sonicate for 1 min. Shake the resulting solution mechanically for 15 min and dilute with *Mobile phase* to volume. Pass a portion of this solution through a filter of 0.45-µm or finer pore size, discarding the first few milliliters of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of orlistat ($C_{29}H_{53}NO_5$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of orlistat from the *Sample solution*
r_S = peak response of orlistat from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of orlistat in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
Medium: 3% sodium lauryl sulfate and 0.5% sodium chloride in water. To each 10 L of media, add 1–2 drops of *n*-octanol, and adjust with phosphoric acid to a pH of 6.0; 900 mL
Apparatus 2: 75 rpm, with coil wire sinker
Time: 45 min
Mobile phase: Acetonitrile and water (86:14)
Standard solution: 0.13 mg/mL of USP Orlistat RS prepared as follows. Transfer a quantity of USP Orlistat RS to a suitable

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volumetric flask. Dissolve in 2% of the final volume of acetonitrile, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.2-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode, Detector, and **Column:** Proceed as directed in the *Assay*.
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of orlistat ($C_{29}H_{53}NO_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of orlistat from the *Sample solution*
r_S = peak response of orlistat from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of orlistat ($C_{29}H_{53}NO_5$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.5 µg/mL of USP Orlistat Related Compound D RS and about 0.6 mg/mL of USP Orlistat RS in *Mobile phase*
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.4 between orlistat and orlistat related compound D, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of orlistat from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of orlistat in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

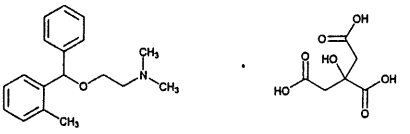
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Orlistat open-ring epimer[a]	0.45	1.0	1.5
Orlistat open ring[b]	0.5	1.0	0.3
Orlistat related compound D	0.9	1.0	1.0
Orlistat	1.0	—	—
Hexyl undecyl pyranone[c]	2.0	1.0	0.2
Henicosenyl leucinate[d]	4.7	2.3	0.3
Any other identified impurity	—	1.0	0.3
Any unidentified impurity	—	1.0	0.2
Total impurities	—	—	3.0

[a] (2*S*,3*R*,5*S*)-5-[(Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.
[b] (2*S*,3*S*,5*S*)-5-[(Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.
[c] (*S*)-3-Hexyl-6-undecyl-5,6-dihydro-2*H*-pyran-2-one.
[d] (*S*,*E*)-Henicos-7-en-10-yl formyl-L-leucinate.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at 25°, excursions permitted between 15° and 30°.
• **USP Reference Standards** ⟨11⟩
USP Orlistat RS
USP Orlistat Related Compound D RS
(3*S*,4*R*,6*S*)-3-Hexyl-2-oxo-6-undecyltetrahydro-2*H*-pyran-4-yl formyl-L-leucinate.
$C_{29}H_{53}NO_5$ 495.73

Orphenadrine Citrate



$C_{18}H_{23}NO \cdot C_6H_8O_7$ 461.50
Ethanamine, *N*,*N*-dimethyl-2-[(2-methylphenyl) phenylmethoxy]-, (±)-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1);
(±)-*N*,*N*-Dimethyl-2-[(*o*-methyl-α-phenylbenzyl)oxy] ethylamine citrate (1:1) [4682-36-4].

DEFINITION

Orphenadrine Citrate contains NLT 98.0% and NMT 101.5% of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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• **C. Identification Tests—General,** *Citrate* ⟨191⟩: Meets the requirements

ASSAY

• **Procedure**

Buffer: 5.8 g/L of monobasic ammonium phosphate in water. Adjust with ammonium hydroxide or phosphoric acid to a pH of 7.9.

Mobile phase: Methanol, acetonitrile, and *Buffer* (45:15:40)

System suitability solution: 0.01 mg/mL each of USP Orphenadrine Related Compound B RS, USP Orphenadrine Related Compound C RS, and USP Methylbenzhydrol RS and 1.0 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Standard solution: 1.0 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sample solution: 1.0 mg/mL of Orphenadrine Citrate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: NLT 2.5 times the retention time of orphenadrine

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between orphenadrine related compound B and C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Orphenadrine Citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

• **Organic Impurities, Procedure 1**

[Note—If methyl orphenadrine is a known manufacturing impurity, *Procedure 1* and the test for *Isomer Content* are recommended. If diphenhydramine and didesmethyl orphenadrine are known manufacturing process impurities, *Procedure 2* is recommended.]

Buffer, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sensitivity solution: 0.5 µg/mL of USP Orphenadrine Citrate RS, from the *Standard solution*, in *Mobile phase*

Sample solution: 1 mg/mL of Orphenadrine Citrate in *Mobile phase*

System suitability

Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between orphenadrine related compound B and C; NLT 3.0 between orphenadrine related compound C and 2-methylbenzhydrol, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of Orphenadrine Citrate from the *Standard solution*

C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citric acid[a]	0.14	—	—
Orphenadrine related compound B	0.25	1.3	0.1
Orphenadrine related compound C	0.39	1.0	0.3
Methylbenzhydrol	0.51	2.4	0.1
Diphenhydramine	0.69	1.0	0.3
Orphenadrine	1.0	—	—
Methyl orphenadrine[b]	1.54	1.9	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities[c]	—	—	0.5

[a] Counter ion peak; not to be reported; not to be included in total impurities.

[b] 2-(Di-*o*-tolylmethoxy)-*N*,*N*-dimethylethan-1-amine.

[c] Excluding orphenadrine related compound E and orphenadrine related compound F from the *Isomer Content* test.

• **Organic Impurities, Procedure 2**

[Note—If didesmethyl orphenadrine is a known manufacturing process impurity, *Procedure 2* is recommended. The labeling indicates that the article complies with *Organic Impurities, Procedure 2*.]

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System suitability solution: 0.3 mg/mL each of USP Orphenadrine Citrate RS, USP Diphenhydramine Citrate RS, USP Methylbenzhydrol RS, USP Orphenadrine Related Compound E RS, and USP Orphenadrine Related Compound F RS in toluene prepared as follows. Dissolve 6 mg each of the USP Reference Standards in 10 mL of water. Add 0.2 mL of ammonium hydroxide and shake with 3 mL of toluene. Separate the organic layer. Repeat the extraction of the aqueous layer two more times with 3 mL of toluene. To the combined organic layer add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.

Sample solution: 25 mg/mL of Orphenadrine Citrate in toluene prepared as follows. Dissolve 500 mg of Orphenadrine Citrate in 50 mL of water. Add 2 mL of ammonium hydroxide and shake with 10 mL of toluene. Separate the organic layer. Repeat the extraction of the aqueous layer two more times with 10 mL of toluene. To the combined organic layers add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 60-m; 1-µm thick coating of phenyl methylpolysiloxane, phase G27
Carrier gas: Helium at 1 mL/min
Temperatures
Injection port: 290°
Detector: 290°
Column: 240°
Injection volume: 2 µL
Injection type: Split ratio, 1:25
Run time: 1.3 times the retention time of orphenadrine

System suitability
Sample: *System suitability solution*
[NOTE—Relative retention times for the peaks are given in *Table 2*.]
Suitability requirements
Resolution: NLT 1.5 between orphenadrine related compound E and orphenadrine; NLT 2.0 between orphenadrine and orphenadrine related compound F

Analysis
Sample: *Sample solution*
Calculate the sum of the percentage of any individual impurity in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%, w/w)
Methylbenzophenone[a]	0.5	0.3
Methylbenzhydrol	0.6	0.3
Diphenhydramine	0.8	0.3
Didesmethyl orphenadrine[b]	0.9	0.3
Orphenadrine related compound E	0.98	0.3
Orphenadrine	1.0	—
Orphenadrine related compound F	1.1	0.3
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] Phenyl(*o*-tolyl)methanone.
[b] 2-[Phenyl(*o*-tolyl)methoxy]ethanamine.

• **ISOMER CONTENT**
If methyl orphenadrine is a known manufacturing impurity, the test for *Isomer Content* is to be performed.
System suitability solution: 0.3 mg/mL each of USP Orphenadrine Citrate RS, USP Orphenadrine Related Compound E RS, and USP Orphenadrine Related Compound F RS in toluene prepared as follows. Dissolve 6 mg each of the USP Reference Standards in 10 mL of water. Add 0.2 mL of ammonium hydroxide and shake with 3 mL of toluene. Separate the organic layer. Repeat the extraction of the aqueous layer two more times with 3 mL of toluene. To the combined organic layer add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.
Sample solution and **Chromatographic system:** Proceed as directed in *Organic Impurities, Procedure 2*.
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times of orphenadrine related compound E, orphenadrine, and orphenadrine related compound F are about 0.98, 1.0, and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between orphenadrine related compound E and orphenadrine; NLT 2.0 between orphenadrine and orphenadrine related compound F
Analysis
Sample: *Sample solution*
Calculate the percentage of orphenadrine related compound E and orphenadrine related compound F in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of orphenadrine related compound E or orphenadrine related compound F from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: NMT 0.3% each of orphenadrine related compound E and orphenadrine related compound F

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

USP Monographs

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• **LABELING:** The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1*, is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diphenhydramine Citrate RS
USP Methylbenzhydrol RS
2-Methylbenzhydrol;
Also known as Phenyl(*o*-tolyl)methanol.
$C_{14}H_{14}O$ 198.26
USP Orphenadrine Citrate RS
USP Orphenadrine Related Compound B RS
N-Ethyl-*N*,*N*-dimethyl-2-[phenyl(*o*-tolyl)methoxy] ethanaminium chloride.
$C_{20}H_{28}ClNO$ 333.90
USP Orphenadrine Related Compound C RS
N-Methyl-2-[phenyl(*o*-tolyl)methoxy]ethanamine hydrochloride.
$C_{17}H_{21}NO \cdot HCl$ 291.82
USP Orphenadrine Related Compound E RS
N,*N*-Dimethyl-2-[phenyl(*m*-tolyl)methoxy]ethanamine.
$C_{18}H_{23}NO$ 269.38
USP Orphenadrine Related Compound F RS
N,*N*-Dimethyl-2-[phenyl(*p*-tolyl)methoxy]ethanamine.
$C_{18}H_{23}NO$ 269.38

Orphenadrine Citrate Injection

DEFINITION
Orphenadrine Citrate Injection is a sterile solution of Orphenadrine Citrate in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains NLT 93.0% and NMT 107.0% of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$).

IDENTIFICATION
• **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Citrate*: Meets the requirements

ASSAY
• **PROCEDURE**

Buffer: 5.8 g/L of monobasic ammonium phosphate in water. Adjust with ammonium hydroxide or phosphoric acid to a pH of 7.9 ± 0.05.

Mobile phase: Methanol, acetonitrile, and *Buffer* (45:15:40)

System suitability solution: 0.01 mg/mL each of USP Orphenadrine Related Compound B RS, USP Orphenadrine Related Compound C RS, USP Methylbenzhydrol RS, and 0.9 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Standard solution: 0.9 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sample solution: Nominally 0.9 mg/mL of orphenadrine citrate from a known volume of the Injection containing NLT 90 mg of orphenadrine citrate in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention time of orphenadrine

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between orphenadrine related compound B and orphenadrine related compound C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

IMPURITIES
• **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.002 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sensitivity solution: 0.001 mg/mL of USP Orphenadrine Citrate RS from the *Standard solution* in *Mobile phase*

System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 3.0 between orphenadrine related compound B and orphenadrine related compound C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of orphenadrine from the *Standard solution*
C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

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Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%, w/w)
Citric acid[a]	0.14	—	—
Orphenadrine related compound B	0.25	1.3	0.2
Orphenadrine related compound C	0.39	1.0	0.2
Methylbenzhydrol	0.51	2.4	0.2
Orphenadrine	1.0	—	—
Methyl orphenadrine[b]	1.54	1.9	0.2
Any individual unspecified degradation product	—	1.0	0.20
Total degradation products	—	—	4.0

[a] Counter ion peak; not to be reported; not to be included in total impurities.
[b] 2-(Di-*o*-tolylmethoxy)-*N*,*N*-dimethylethan-1-amine.

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.0–6.0
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 5.8 USP Endotoxin Units/mg of orphenadrine citrate
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩

USP Methylbenzhydrol RS
2-Methylbenzhydrol;
Also known as phenyl(*o*-tolyl)methanol.
$C_{14}H_{14}O$ 198.26

USP Orphenadrine Citrate RS

USP Orphenadrine Related Compound B RS
N-Ethyl-*N*,*N*-dimethyl [2-2(methylbenzhydryloxy)ethyl] ammonium chloride; also known as *N*-ethyl-*N*,*N*-dimethyl-2-[phenyl(*o*-tolyl)methoxy]ethanaminium chloride.
$C_{20}H_{28}ClNO$ 333.90

USP Orphenadrine Related Compound C RS
N-Methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride; also known as *N*-methyl-2-[phenyl(*o*-tolyl)methoxy]ethanamine hydrochloride.
$C_{17}H_{21}NO \cdot HCl$ 291.82

Orphenadrine Citrate Extended-Release Tablets

DEFINITION
Orphenadrine Citrate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Buffer: 5.8 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.2.
Mobile phase: Acetonitrile and *Buffer* (40:60)
System suitability solution: 0.1 mg/mL of USP Orphenadrine Citrate RS and 0.01 mg/mL each of USP Orphenadrine Related Compound B RS and USP Orphenadrine Related Compound C RS, in *Mobile phase*
Standard solution: 0.1 mg/mL of USP Orphenadrine Citrate RS
Sample stock solution: Nominally 0.5 mg/mL of orphenadrine citrate prepared as follows. Transfer a quantity of powder equivalent to NLT 100 mg of orphenadrine citrate, from finely powdered Tablets (NLT 20), to a suitable volumetric flask. Add 50% of the flask volume of *Mobile phase*. Sonicate for 5 min and shake for 15 min. Dilute with *Mobile phase* to volume. Pass through a suitable filter.
Sample solution: Nominally 0.1 mg/mL of orphenadrine citrate in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 3.9-mm × 30-cm; 10-µm L1 packing
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between orphenadrine and orphenadrine related compound C; NLT 2.0 between orphenadrine citrate and orphenadrine related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of orphenadrine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩

Test 1
Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Times: 1, 2, 6, and 12 h
Buffer: 5.8 g/L of monobasic ammonium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (40:60). Adjust with phosphoric acid to a pH of 3.2 ± 0.1.

USP Monographs

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Standard stock solution: 1 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*. Sonication may be used to promote dissolution.
Standard solution 0.1 mg/mL of USP Orphenadrine Citrate RS in *Medium* from a suitable volume of *Standard stock solution* and *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis:
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved in the portion of the sample withdrawn at each time point (*i*) (mg/mL):

$$C_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{C_3 \times [V - (2 \times V_S)] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{C_4 \times [V - (3 \times V_S)] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of orphenadrine citrate in the portion of the sample withdrawn at time point (*i*) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	10–40
2	2	30–50
3	6	50–80
4	12	NLT 80

The percentages of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 1, 4, and 12 h
Standard solution: 0.02 mg/mL of USP Orphenadrine Citrate RS in *Medium*
Sample solution: Withdraw 10 mL of the solution under test from each vessel at each specified time point. Replace 10 mL of *Medium* in each vessel. Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Transfer 1.0 mL of the filtrate to a 50-mL volumetric flask, and dilute with *Medium* to volume.
Blank: *Medium*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 210 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the sample withdrawn from the vessel at each time point (*i*):

$$C_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts (Q_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of orphenadrine citrate in the portion of the sample withdrawn at time point (*i*) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	10–40
2	4	40–70
3	12	NLT 80

The percentages of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

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Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Solution A: 0.45 M monobasic potassium phosphate prepared as follows. Mix 6.12 g of monobasic potassium phosphate and 12 mL of 10 N sodium hydroxide. Dilute with water to 100 mL.
Acid stage medium: 0.1 N hydrochloric acid; 800 mL
Buffer stage medium: pH 7.5 phosphate buffer (add 100 mL of *Solution A* to the *Acid stage medium* after 1 h); 900 mL
Apparatus 2: 50 rpm
Times: 1 h in *Acid stage medium*; 4 and 10 h in *Buffer stage medium*. The time in the *Buffer stage medium* includes the time in the *Acid stage medium*.
Standard solution: 0.11 mg/mL of USP Orphenadrine Citrate RS in *Acid stage medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Blank: *Acid stage medium*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 232 nm
Cell: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the sample withdrawn from the vessel at each time point (i):

$$C_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts (Q_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V_A \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V_B - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V_B - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of orphenadrine citrate in the portion of the sample withdrawn at time point (i) (mg/mL)
V_A = volume of *Acid stage medium*, 800 mL
L = label claim (mg/Tablet)
V_B = volume of *Buffer stage medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	40–55
2	4	50–70
3	10	NLT 70

The percentages of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of orphenadrine from the *Sample solution*
F = relative response factor for each degradation product (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citric acid[a]	0.4	—	—
Orphenadrine related compound C	0.9	1.5	0.5
Orphenadrine citrate	1	—	—
Orphenadrine related compound B	1.3	1.3	0.5
2-Methylbenzhydrol[b]	2.1	2.1	0.5
2-Methylbenzophenone[c]	4	1.0	0.5
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.5

[a] The peak is due to counter ion and is not to be reported or included in total degradation products.
[b] Also known as Phenyl(o-tolyl)methanol.
[c] Also known as Phenyl(o-tolyl)methanone.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, tight, light-resistant containers, and store at controlled room temperature.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP Reference Standards** ⟨11⟩
USP Orphenadrine Citrate RS
USP Orphenadrine Related Compound B RS
N-Ethyl-*N*,*N*-dimethyl [2-2(methylbenzhydryloxy)ethyl] ammonium chloride;

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Also known as *N*-Ethyl-*N*,*N*-dimethyl-2-[phenyl(*o*-tolyl) methoxy]ethanaminium chloride.
$C_{20}H_{28}ClNO$ 333.90

USP Orphenadrine Related Compound C RS
N-Methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride;
Also known as *N*-Methyl-2-[phenyl(*o*-tolyl)methoxy] ethanamine hydrochloride.
$C_{17}H_{22}ClNO$ 291.82

Orphenadrine Citrate, Aspirin, and Caffeine Tablets

DEFINITION

Orphenadrine Citrate, Aspirin, and Caffeine Tablets contain NLT 90.0% and NMT 110.0% each of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$).

IDENTIFICATION

- **A.** The retention times of the orphenadrine, aspirin, and caffeine peaks in the chromatogram of the *Sample solution* correspond to those of the orphenadrine, aspirin, and caffeine peaks in the chromatogram of the *Standard solution*, as obtained in the *Orphenadrine Citrate* and *Aspirin and Caffeine* tests in the *Assay*.

ASSAY

- **ORPHENADRINE CITRATE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.6. To each L of this solution add 5.8 g of sodium dodecyl sulfate, and dissolve.
Mobile phase: Acetonitrile and *Buffer* (50:50)
Diluent: Methanol and glacial acetic acid (92:8)
Standard solution: 0.1 mg/mL of USP Orphenadrine Citrate RS prepared as follows. Transfer the weighed amount of standard to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. Filter a portion through a suitable filter of 0.45-µm pore size, discarding at least the first 3 mL of the filtrate.
Sample stock solution: Nominally 0.5 mg/mL of orphenadrine citrate from Tablets (NLT 5), prepared as follows. To the volumetric flask containing the Tablets, add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Shake mechanically for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. [NOTE—This solution also contains 7.7 mg/mL of aspirin and 0.6 mg/mL of caffeine.]
Sample solution: Nominally 0.1 mg/mL of orphenadrine citrate by diluting a suitable portion of the *Sample stock solution* with *Diluent*. Filter a portion through a suitable filter of 0.45-µm pore size, discarding at least the first 3 mL of the filtrate. [NOTE—This solution also contains 1.5 mg/mL of aspirin and 0.1 mg/mL of caffeine.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 15 µL
Run time: 1.5 times the retention time of orphenadrine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of orphenadrine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

- **ASPIRIN AND CAFFEINE**

Buffer: Dissolve 0.8 g/L of hexanesulfonic acid sodium salt in water. Adjust with glacial acetic acid to a pH of 3.0.
Mobile phase: Methanol and *Buffer* (40:60)
Diluent: Methanol and glacial acetic acid (92:8)
Standard solution: 1.5 mg/mL of USP Aspirin RS and 0.1 mg/mL of USP Caffeine RS prepared as follows. Transfer the weighed amount of standards to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. Filter a portion through a suitable filter of 0.45-µm pore size, discarding at least the first 3 mL of the filtrate.
Sample stock solution: Nominally 7.7 mg/mL of aspirin from Tablets (NLT 5), prepared as follows. To the volumetric flask containing the Tablets, add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. [NOTE—This solution also contains 0.6 mg/mL of caffeine and 0.5 mg/mL of orphenadrine citrate.]
Sample solution: Nominally 1.5 mg/mL of aspirin by diluting a suitable portion of the *Sample stock solution* with *Diluent*. Filter a portion through a suitable filter of 0.45-µm pore size, discarding at least the first 3 mL of the filtrate. [NOTE—This *Sample solution* also contains 0.1 mg/mL of caffeine and 0.1 mg/mL of orphenadrine citrate.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 15 µL
Run time: 3 times the retention time of aspirin
System suitability
[NOTE—The relative retention time for caffeine and for aspirin is 0.70 and 1.0, respectively.]
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between caffeine and aspirin peaks
Relative standard deviation: NMT 2.0% each for both the caffeine and aspirin peaks

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of caffeine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of aspirin and caffeine

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 45 min for aspirin and caffeine; 60 min for orphenadrine
Separate dissolution baths must be run for the different time points.
Orphenadrine citrate
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.6. To each L of this solution add 5.8 g of sodium dodecyl sulfate, and dissolve.
Mobile phase: Acetonitrile and *Buffer* (50:50)
Standard solution: (*L*/900) mg/mL of USP Orphenadrine Citrate RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 100 µL
Run time: 1.2 times the retention time of orphenadrine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 3.0%
Calculate the percentage (*Q*) of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response for orphenadrine from the *Sample solution*
r_S = peak response for orphenadrine from the *Standard solution*
C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)
L = label claim for orphenadrine (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of orphenadrine is dissolved in 60 min.
Aspirin and caffeine
Buffer: 0.8 g/L of hexanesulfonic acid sodium salt in water
Mobile phase: Methanol and *Buffer* (40:60). Adjust with glacial acetic acid to a pH of 3.0.
Standard stock solution: 1.0 mg/mL of USP Aspirin RS, 0.1 mg/mL of USP Caffeine RS, and 0.1 mg/mL of USP Salicylic Acid RS prepared as follows. Transfer the weighed amount of standards to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume.
Standard solution: Dilute a suitable volume of the *Standard stock solution* with methanol to obtain a final concentration of about 0.25 mg/mL of USP Aspirin RS and 0.025 mg/mL each of USP Caffeine RS and USP Salicylic Acid RS.
Sample solution: At the time specified, withdraw the solution under test and pass through a suitable filter. If necessary, dilute the filtrate with methanol to obtain a final concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 15 µL
Run time: 2 times the retention time of aspirin
System suitability
[NOTE—The relative retention times for caffeine, aspirin, and salicylic acid are 0.70, 1.0, and 1.3, respectively.]
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0% for both the caffeine and aspirin peaks
Calculate the percentage (*Q*) of the labeled amount of aspirin ($C_9H_8O_4$) or caffeine ($C_8H_{10}N_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response for aspirin or caffeine from the *Sample solution*
r_S = peak response for aspirin or caffeine from the *Standard solution*
C_S = concentration of USP Aspirin RS or USP Caffeine RS in the *Standard solution* (mg/mL)
L = label claim for aspirin or caffeine (mg/Tablet)
D = dilution factor, if applicable
V = volume of *Medium*, 900 mL

Calculate the percentage of salicylic acid ($C_7H_6O_3$), relative to the labeled amount of aspirin, dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response for salicylic acid from the *Sample solution*
r_S = peak response for salicylic acid from the *Standard solution*

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C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
L = label claim for aspirin (mg/Tablet)
D = dilution factor
M_{r1} = molecular weight of aspirin, 180.16
M_{r2} = molecular weight of salicylic acid, 138.12
V = volume of *Medium,* 900 mL

The percentage of salicylic acid dissolved should be added to the percentage of aspirin dissolved.

Tolerances: NLT 70% (Q) of the labeled amounts of aspirin and caffeine is dissolved in 45 min.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Limit of 2-Methylbenzhydrol**

Buffer: 6.8 g/L of monobasic potassium phosphate in water

Mobile phase: To 1 L of *Buffer* add 5.8 g of sodium dodecyl sulfate and 1 L of acetonitrile. Adjust with phosphoric acid to a pH of 3.6.

Standard solution: 7.5 µg/mL of USP 2-Methylbenzhydrol RS in methanol

Sample solution: Nominally 1.5 mg/mL of orphenadrine citrate in methanol from a portion of finely powdered Tablets (NLT 20 Tablets). [Note—This *Sample solution* also contains 0.6 mg/mL of caffeine and 0.5 mg/mL of orphenadrine citrate.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 213 nm
Column: 4.6-mm × 150-mm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: 2.5 times the retention time of 2-methyl benzhydrol

System suitability
Sample: *Standard solution*
Suitability requirements:
Tailing factor: NMT 1.5
Relative standard deviation: NMT 5.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 2-methylbenzhydrol in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP 2-Methylbenzhydrol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of orphenadrine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

• **Organic Impurities**

Buffer: 0.8 g/L of sodium 1-hexanesulfonate in water. Adjust with glacial acetic acid to a pH of 3.0.

Mobile phase: Methanol and *Buffer* (40:60)

Diluent: Methanol and glacial acetic acid (92:8)

System suitability solution: 0.6 mg/mL of USP Salicylic Acid RS, 1.5 mg/mL of USP Aspirin RS, and 0.1 mg/mL of USP Caffeine RS prepared as follows. Transfer the weighed amount of standards to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. Filter a portion through a suitable filter of 0.45-µm pore size, discarding at least the first 3 mL of the filtrate.

Standard solution: 0.04 mg/mL of USP Salicylic Acid RS prepared as follows. Transfer the weighed amount of standard to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume, and dilute with methanol to volume. Filter a portion through a suitable filter of 0.45-µm pore size, discarding at least the first 3 mL of the filtrate.

Sample stock solution: Nominally 7.7 mg/mL of aspirin from Tablets (NLT 5), prepared as follows. To the volumetric flask containing the Tablets, add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. [Note—This solution also contains 0.5 mg/mL of orphenadrine citrate and 0.6 mg/mL of caffeine.]

Sample solution: Nominally 1.5 mg/mL of aspirin by diluting a suitable portion of the *Sample stock solution* with *Diluent.* Filter a portion through a suitable filter of 0.45-µm pore size, discarding at least the first 3 mL of the filtrate. [Note—This *Sample solution* also contains 0.1 mg/mL of orphenadrine citrate and 0.1 mg/mL of caffeine.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 25 µL
Run time: 3 times the retention time of the aspirin peak

System suitability
[Note—Refer to *Table 1* for relative retention times.]
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the caffeine and aspirin peaks and NLT 1.5 between the aspirin and salicylic acid peaks, *System suitability solution*
Relative standard deviation: NMT 2.0% each for aspirin and caffeine peaks, *System suitability solution;* NMT 3.0% for salicylic acid, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of salicylic acid in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Calculate the percentage of other organic impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the areas of all other organic impurities from the *Sample solution*
r_T = sum of the areas of all peaks from the *Sample solution*

Acceptance criteria: See *Table 1.*

USP Monographs

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Table 1

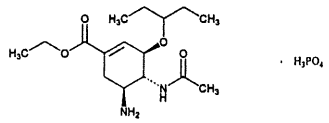
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Caffeine	0.70	—
Aspirin	1.0	—
Salicylic acid	1.3	3.0
Total impurities[a]	—	2.0

[a] Does not include salicylic acid and 2-methylbenzhydrol.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aspirin RS
  USP Caffeine RS
  USP 2-Methylbenzhydrol RS
  2-Methylbenzhydrol.
  $C_{14}H_{14}O$ 198.26
  USP Orphenadrine Citrate RS
  USP Salicylic Acid RS

Oseltamivir Phosphate



$C_{16}H_{28}N_2O_4 \cdot H_3PO_4$ 410.40

[3*R*-(3α,4β,5α)]-Ethyl 4-(acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate (1:1);
Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate, phosphate (1:1) [204255-11-8].

DEFINITION

Oseltamivir Phosphate contains NLT 98.0% and NMT 101.5% of $C_{16}H_{28}N_2O_4 \cdot H_3PO_4$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 6.8 g of potassium dihydrogen phosphate in 980 mL of water. Adjust with 1 M potassium hydroxide solution to a pH of 6.0, and dilute with water to 1 L.
Mobile phase: Methanol, acetonitrile, and *Solution A* (245:135:620)
Diluent: Methanol, acetonitrile, and water (245:135:620)
Standard solution: 1 mg/mL of USP Oseltamivir Phosphate RS in *Diluent*
Sample solution: 1 mg/mL of Oseltamivir Phosphate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 207 nm
Column: 4.6-mm × 25-cm; packing L7
Column temperature: 50°
Flow rate: 1.2 mL/min
Injection size: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{28}N_2O_4 \cdot H_3PO_4$ in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oseltamivir Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous basis

IMPURITIES

ORGANIC IMPURITIES

- **Procedure 1**

Solution A, Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of oseltamivir phosphate from the *Standard solution*
C_S = concentration of USP Oseltamivir Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)
F = relative response factor from *Impurity Table 1*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.7%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oseltamivir acid[a]	0.17	1.4	0.3
Oseltamivir phenol[b]	0.51	2.7	0.1
Oseltamivir phosphate	1.00	1.0	—
Unspecified impurity	—	1.0	0.1

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Impurity Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total unspecified impurity	—	—	0.4

[a] (3*R*,4*R*,5*S*)-4-Acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid.
[b] 4-Acetylamino-3-hydroxybenzoic acid ethyl ester.

• Procedure 2: Oseltamivir Related Compound A
Buffer: 1.54 g/L of ammonium acetate in water
Mobile phase: Acetonitrile, water, and *Buffer* (3:6:1)
Stock solution A: 50 µg/mL of USP Oseltamivir Related Compound A RS, prepared as follows: Dissolve in alcohol, using 5% of final volume, and dilute with water to volume.
Solution A: 1 µg/mL of USP Oseltamivir Related Compound A RS in water from *Stock solution A*
Standard solution: 10 mg/mL of USP Oseltamivir Phosphate RS in *Solution A*
Sample solution: 10 mg/mL of Oseltamivir Phosphate in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors: UV 210 nm and mass spectrometer
Column: 3.0-mm × 5-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 1 µL
Temperature: 40°
Use electrospray (+) ionization, a selected ion monitoring mode with m/z of 356.2 (protonated oseltamivir related compound A). Adjust the dwell time, fragmentation voltage, drying gas temperature, drying gas flow, nebulizer pressure, and capillary voltage as appropriate for an optimal response. [NOTE—A postcolumn flow splitter with a split ratio of about 3:1 is used.]
System suitability
Sample: *Standard solution*
[NOTE—The relative retention time for oseltamivir related compound A versus oseltamivir is about 2.6.]
Suitability requirements
Resolution: The oseltamivir related compound A peak (detected by MD-SIM mode) and the oseltamivir peak (detected by UV) are baseline resolved.
[NOTE—The resolution of the two components minimizes background noise and ion suppression effects for the trace of oseltamivir related compound A by the oseltamivir matrix.]
Relative standard deviation: NMT 15.0%, oseltamivir related compound A peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oseltamivir related compound A in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oseltamivir related compound A from the *Sample solution*
r_S = peak response of oseltamivir related compound A from the *Standard solution*
C_S = concentration of USP Oseltamivir Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of oseltamivir phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.01%

• Procedure 3: Limit Of Tributyl Phosphine Oxide
Blank: Transfer 1.0 mL of suitable derivatizing reagent[1] to a vial. Close the vial, shake, and heat for 20 min at 60°. Centrifuge the pyridinium salt precipitate.
Standard stock solution 1: 21 mg/mL of USP Tributyl Phosphine Oxide RS in pyridine
Standard stock solution 2: 21 mg/mL of USP Oseltamivir Phosphate RS in suitable derivatizing reagent. Close the vial, mix, and heat for 20 min at 60°. Centrifuge the pyridinium salt precipitate.
Standard solution: 21 µg/mL each of USP Tributyl Phosphine Oxide RS and USP Oseltamivir Phosphate RS in pyridine from *Standard stock solution 1* and *Standard stock solution 2*, respectively
Sample solution: Transfer 15 mg of Oseltamivir Phosphate to a vial. Add 1.0 mL of suitable derivatizing reagent. Close the vial, mix, and heat for 20 min to 60°. Centrifuge the pyridinium salt precipitate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m capillary column coated with a 0.25-µm phase G1
Split ratio: 1:50
Split flow: 64 mL/min
Injection size: 1 µL
Temperature
Detector: 260°
Injection port: 260°
Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
180	0	180	2
180	8	250	10

Linear velocity: 27 cm/s
Carrier gas: Helium
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tributyl phosphine oxide and oseltamivir phosphate are about 0.54 and 1.00, respectively.]
Suitability requirements
Relative standard deviation: NMT 10.0% for the tributyl phosphine oxide and osteltamivir phosphate peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tributyl phosphine oxide in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tributyl phosphine oxide from the *Sample solution*
r_S = peak response of tributyl phosphine oxide from the *Standard solution*
C_S = concentration of tributyl phosphine oxide in the *Standard solution* (mg/mL)
C_U = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

[1] Tri-Sil Reagent (product number: 48999 0049001) may be obtained from Pierce: www.piercenet.com.

USP Monographs

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SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: Between –30.7 and –32.6

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oseltamivir Phosphate RS
  USP Oseltamivir Related Compound A RS
  (3*S*,4*R*,5*S*)-Ethyl 4-acetamido-5-amino-2-azido-3-(pentan-3-yloxy)cyclohexanecarboxylate.
  $C_{16}H_{29}N_5O_4$ 355.43
  USP Tributyl Phosphine Oxide RS
  $C_{12}H_{27}OP$ 218.32

Oseltamivir Phosphate Capsules

DEFINITION
Oseltamivir Phosphate Capsules contain Oseltamivir Phosphate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of oseltamivir ($C_{16}H_{28}N_2O_4$).

IDENTIFICATION
- The retention time of the major peaks of the *Sample solution* corresponds to those of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **OSELTAMIVIR**
  Solution A: Dissolve 6.8 g of potassium dihydrogen phosphate in 980 mL of water. Adjust with 1 M potassium hydroxide solution to a pH of 6.0, and dilute with water to 1 L.
  Mobile phase: Methanol, acetonitrile, and *Solution A* (245:135:620)
  Diluent: Methanol, acetonitrile, and 0.01 N phosphoric acid (245:135:620)
  Standard solution: 1 mg/mL of USP Oseltamivir Phosphate RS in *Diluent*
  Sample solution: Weigh the contents of 20 Capsules, and mix. Prepare the equivalent of about 1 mg of oseltamivir phosphate per mL, based on the label claim, by first dispersing a suitable portion of the powder in about 40% of the flask volume of *Diluent* using an ultrasonic bath for about 20 min, and diluting with *Diluent* to volume. Centrifuge an aliquot of this solution, and use the supernatant.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 207 nm
  Column: 4.6-mm × 25-cm; packing L7
  Column temperature: 50°
  Flow rate: 1.2 mL/min
  Injection size: 15 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of the labeled amount of oseltamivir ($C_{16}H_{28}N_2O_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

  r_U = peak response from the *Sample solution*
  r_S = peak response from the *Standard solution*
  C_S = concentration of USP Oseltamivir Phosphate RS in the *Standard solution* (mg/mL)
  C_U = nominal concentration of oseltamivir in the *Sample solution* (mg/mL)
  M_{r1} = molecular weight of oseltamivir, 312.40
  M_{r2} = molecular weight of oseltamivir phosphate, 410.40

  Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 2: 50 rpm
  Time: 20 min
  Detector: UV 240 nm
  Standard solution: Prepare a solution in *Medium* having a known concentration of about 0.11 mg/mL of USP Oseltamivir Phosphate RS. Quantitatively dilute a portion of this solution with *Medium* to obtain a solution having a known concentration similar to the expected concentration in the solution under test.
  Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size.
  Excipients solution: Suspend an amount of the placebo mixture equivalent to the weight of the excipients in one dosage unit and one empty Capsule shell in 900 mL of *Medium.* Heat to 37°, and filter.
  Analysis
  Samples: *Medium, Standard solution, Sample solution,* and *Excipients solution*
  Determine the amount of oseltamivir phosphate ($C_{16}H_{28}N_2O_4 \cdot H_3PO_4$) dissolved by measuring the absorbance at about 240 nm of the *Sample solution* and *Excipients solution* in comparison with the *Standard solution,* using the *Medium* as the blank. Calculate the percentage of oseltamivir phosphate dissolved:

$$\text{Result} = [(A_U - A_E) \times C_S \times V \times 100]/(A_S \times L)$$

  A_U = absorbance of the *Sample solution*
  A_E = absorbance of the *Excipients solution*
  C_S = concentration of USP Oseltamivir Phosphate RS in the *Standard solution*
  V = volume of *Medium,* 900 mL
  A_S = absorbance of the *Standard solution*
  L = label claim for oseltamivir phosphate (mg/Capsule)

  Tolerances: NLT 75% (*Q*) of the labeled amount of oseltamivir phosphate is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A, Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*
  Analysis
  Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of individual impurities in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oseltamivir Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oseltamivir in the *Sample solution* (mg/mL)
F = relative response factor from *Table 1*
M_{r1} = molecular weight of oseltamivir, 312.40
M_{r2} = molecular weight of oseltamivir phosphate, 410.40

Acceptance criteria: See *Table 1*.

Table 1

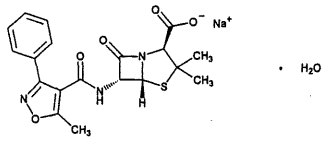
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity A[a]	0.18	1.4	2.0
Impurity B[b]	0.49	2.7	0.3
Oseltamivir phosphate	1.00	1.0	—
Impurity C[c]	1.45	0.9	0.5
Individual unidentified impurity	—	1.0	0.2
Total unidentified impurities	—	1.0	0.5
Total impurities	—	1.0	3.0

[a] (3*R*,4*R*,5*S*)-4-Acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid.
[b] 4-Acetylamino-3-hydroxybenzoic acid ethyl ester.
[c] (3*R*,4*R*,5*S*)-4-Amino-5-acetylamino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oseltamivir Phosphate RS

Oxacillin Sodium



$C_{19}H_{18}N_3NaO_5S \cdot H_2O$ 441.43
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-6-[[(5-methyl-3-phenyl-4-isoxazolyl)-carbonyl] amino]-7-oxo-, monosodium salt, monohydrate, [2*S*-(2α, 5α,6β)]-;
Monosodium (2*S*,5*R*,6*R*)-3,3-dimethyl-6-(5-methyl-3-phenyl-4-isoxazolecarboxamido)-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate monohydrate [7240-38-2].
Anhydrous
$C_{19}H_{18}N_3NaO_5S$ 423.43
[1173-88-2].

DEFINITION

Oxacillin Sodium contains the equivalent of NLT 815 µg/mg and NMT 950 µg/mg of oxacillin ($C_{19}H_{19}N_3O_5S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*: Meets the requirements

ASSAY

- **PROCEDURE**

Protect solutions containing oxacillin from light.
Solution A: 1.18 g/L of sodium 1-hexanesulfonate monohydrate and 0.8 mL/L of ammonium hydroxide in water, adjusted with phosphoric acid to a pH of 2.8–3.2
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	30	70

Return to the original conditions and re-equilibrate the system.
Diluent: Acetonitrile and water (15:85)
System suitability stock solution: 0.1 mg/mL of USP Oxacillin Related Compound C RS in *Diluent*. Sonicate as needed to dissolve.
System suitability solution: 0.01 mg/mL of USP Oxacillin Related Compound C RS from *System suitability stock solution* and 1 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Store this solution at 4°.
Standard solution: 1 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.
Sample solution: 1 mg/mL of Oxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 40°
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for oxacillin related compound C and oxacillin are about 0.96 and 1.0, respectively.]

USP Monographs

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Suitability requirements
Resolution: NLT 1.5 between oxacillin related compound C and oxacillin, *System suitability solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of oxacillin ($C_{19}H_{19}N_3O_5S$) in the portion of Oxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxacillin Sodium in the *Sample solution* (mg/mL)
P = potency of oxacillin in USP Oxacillin Sodium RS (µg/mg)

Acceptance criteria: 815–950 µg/mg

IMPURITIES

• ORGANIC IMPURITIES

Protect solutions containing oxacillin from light.
Solution A: 6 g/L of anhydrous monobasic sodium phosphate, 0.56 g/L of sodium 1-hexanesulfonate monohydrate, and 0.05 g/L of edetate disodium. Adjust with phosphoric acid to a pH of 3.0–3.2.
Solution B: Acetonitrile
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3	90	10
30	50	50
40	15	85
45	15	85

Return to the original conditions and re-equilibrate the system.
Diluent: Acetonitrile and water (15:85)
System suitability stock solution: 0.1 mg/mL of USP Oxacillin Related Compound C RS in *Diluent*. Do not sonicate.
System suitability solution: 0.01 mg/mL of USP Oxacillin Related Compound C RS from *System suitability stock solution* and 1 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Store this solution at 4°.
Standard solution: 0.01 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Do not sonicate. Store this solution at 4°.
Sample solution: 1 mg/mL of Oxacillin Sodium in *Diluent*. Do not sonicate. Store this solution at 4°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 22°
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between oxacillin related compound C and oxacillin, *System suitability solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 2.5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxacillin Sodium in the *Sample solution* (mg/mL)
P = potency of oxacillin in USP Oxacillin Sodium RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound A[a]	0.08	0.22	0.5
Oxacillin penicilloic acid[b, c]	0.66 0.69	0.40	1.5
Imidazothiazole analog[d]	0.68	1.0	0.5
Oxacillin penilloic acid[c, e]	0.83 0.84	0.79	0.5
Thiooxacillin[f]	0.93	1.0	0.5
Oxacillin related compound C[g]	0.97	2.0	0.5
Oxacillin	1.0	—	—
Cloxacillin	1.09	1.0	1.0
Cloxacillin isomers[h]	1.17	1.0	0.5
N-(Penicillan-6-yl) oxacillinamide[i]	1.19	1.0	0.5
N-(Penicillan-6-yl) open ring oxacillinamide[i]	1.31	1.0	0.5
Any individual unspecified impurity	—	1.0	0.2

USP Monographs

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Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	3.0

[a] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[b] (4*S*)-2-[Carboxy(5-methyl-3-phenylisoxazole-4-carboxamido)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[c] The system resolves two isomers. The limit is for the sum of the isomers.
[d] (3*S*,7*R*)-2,2-Dimethyl-5-(5-methyl-3-phenylisoxazol-4-yl)-2,3,7,7a-tetrahydroimidazo[5,1-*b*]thiazole-3,7-dicarboxylic acid.
[e] (4*S*)-5,5-Dimethyl-2-[(5-methyl-3-phenylisoxazole-4-carboxamido)methyl] thiazolidine-4-carboxylic acid.
[f] (2*R*,5*R*,6*R*)-3,3-Dimethyl-6-(5-methyl-3-phenylisoxazole-4-carboxamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbothioic acid.
[g] Isoxazole carboxylic analog; 5-Methyl-3-phenylisoxazole-4-carboxylic acid.
[h] (2*S*,5*R*,6*R*)-6-[3-(Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[i] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-3,3-Dimethyl-6-(5-methyl-3-phenylisoxazole-4-carboxamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[j] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(2*R*,4*S*)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-(5-methyl-3-phenylisoxazole-4-carboxamido)acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 30 mg/mL
  Acceptance criteria: 4.5–7.5
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 3.5%–5.0%
- **STERILITY TESTS** ⟨71⟩: Where the label states that Oxacillin Sodium is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Oxacillin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.2 USP Endotoxin Units/mg of oxacillin.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxacillin Related Compound C RS
  Isoxazole carboxylic analog;
  5-Methyl-3-phenylisoxazole-4-carboxylic acid.
  $C_{11}H_9NO_3$ 203.20
  USP Oxacillin Sodium RS

Oxacillin Injection

DEFINITION
Oxacillin Injection is a sterile isoosmotic solution of Oxacillin Sodium in Water for Injection. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$). It contains dextrose as a tonicity-adjusting agent and one or more suitable buffer substances. It contains no preservatives.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 2.7 g/L of monobasic potassium phosphate
  Mobile phase: Acetonitrile, methanol, and *Solution A* (300:100:700)
  Standard solution: 0.11 mg/mL of USP Oxacillin Sodium RS. Use this solution on the day prepared.
  Sample solution: Nominally 0.1 mg/mL of oxacillin from one container of Injection that has been allowed to thaw. Use this solution on the day prepared.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 225 nm
  Column: 4-mm × 30-cm; packing L11
  Flow rate: 2 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 2000 theoretical plates
  Tailing factor: NMT 1.6
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxacillin in the *Sample solution* (mg/mL)
P = potency of oxacillin in USP Oxacillin Sodium RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH** ⟨791⟩: 6.0–8.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **PYROGEN TEST** ⟨151⟩: It meets the requirements, the test dose being a volume of undiluted Injection providing the equivalent of 20 mg/kg of oxacillin.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
- **LABELING:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use; it describes conditions for proper storage of the resultant solution; and it directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxacillin Sodium RS

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Oxacillin for Injection

DEFINITION

Oxacillin for Injection contains an amount of Oxacillin Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 2.7 g/L of monobasic potassium phosphate

Mobile phase: Acetonitrile, methanol, and *Solution A* (300:100:700)

Standard solution: 0.11 mg/mL of USP Oxacillin Sodium RS. Use on the day prepared.

Sample solution 1 (where it is represented as being in a single-dose container): Nominally 0.1 mg/mL of oxacillin in water, prepared as follows. Constitute Oxacillin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents using a suitable hypodermic needle and syringe, and dilute with water to obtain a solution containing nominally 0.1 mg/mL of oxacillin. Use on the day prepared.

Sample solution 2 (where the label states the quantity of oxacillin in a given volume of constituted solution): Nominally 0.1 mg/mL of oxacillin in water, prepared as follows. Constitute Oxacillin for Injection with a volume of water corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution with water to obtain a solution containing nominally 0.1 mg/mL of oxacillin. Use on the day prepared.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4-mm × 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 1.6

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$) in the portion of Oxacillin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from *Sample solution 1* or *Sample solution 2*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxacillin in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of oxacillin in USP Oxacillin Sodium RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Where the test for *Uniformity of Dosage Units* has been performed, report the average of the determinations as the *Assay* value.

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for *Content Uniformity* for individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate, prepared as directed in the *Assay*.

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.2 USP Endotoxin Units/mg of oxacillin.
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Specific Tests, Completeness and clarity of solutions***:** Meets the requirements
- **WATER DETERMINATION** ⟨921⟩, *Method I***:** NMT 6.0%
- **pH** ⟨791⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 6.0–8.5

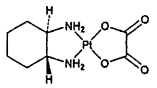
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Oxacillin Sodium RS

Oxaliplatin



$C_8H_{14}N_2O_4Pt$ 397.29

[*SP*-4-2-(1*R*-*trans*)]-(1,2-Cyclohexanediamine-*N*,*N*′)[ethanedioato(2-)-*O*,*O*′]platinum; *cis*-[(1*R*,2*R*)-1,2-Cyclohexanediamine-*N*,*N*′][oxalato(2-)-*O*,*O*′]platinum [61825-94-3].

DEFINITION

Oxaliplatin contains NLT 98.0% and NMT 102.0% of oxaliplatin ($C_8H_{14}N_2O_4Pt$), calculated on the dried basis. [**CAUTION**—Great care should be taken in handling Oxaliplatin, because it is a potentially cytotoxic agent.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation.

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Polypropylene HPLC autosampler vials should be used.]

Buffer: Weigh 2.72 g of monobasic potassium phosphate (anhydrous) and 1.80 g of 1-pentanesulfonic acid sodium salt into a suitable container. Add 2000 mL of water, and mix well to completely dissolve all solids. Transfer 0.5 mL of triethylamine to the buffer solution, and mix thoroughly. Adjust the solution by dropwise addition of phosphoric acid to a pH of 4.30 ± 0.05.

Mobile phase: Methanol and *Buffer* (3:17)

Oxaliplatin standard stock solution: 0.5 mg/mL of USP Oxaliplatin RS in water

Oxaliplatin related compound B standard stock solution: Transfer USP Oxaliplatin Related Compound B RS to a suitable volumetric flask, add 25% of the final volume of methanol, and sonicate for approximately 2 min to disperse the solids. Add approximately 65% of the final volume of 0.001 M nitric acid, and sonicate for an additional 30 min to dissolve the solids. Allow to cool if necessary. Dilute with 0.001 M nitric acid to volume, and mix to obtain a solution having a known concentration of 0.125 mg/mL. [NOTE—USP Oxaliplatin Related Compound B RS is converted to (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*']platinum during preparation of this solution.]

Oxaliplatin related compound C standard stock solution: 0.1 mg/mL of USP Oxaliplatin Related Compound C RS in water

System suitability solution: 2 mg/mL of Oxaliplatin in 0.005 M sodium hydroxide. Allow this solution to stand at room temperature for at least 5 days. Transfer 10 mL of this solution, 10 mL of *Oxaliplatin related compound B standard stock solution*, and 5 mL of *Oxaliplatin related compound C standard stock solution* into a 100-mL volumetric flask, and dilute with water to volume. [NOTE—The preparation of the *System suitability solution* forms diaquodiaminocyclohexaneplatinum dimer.]

Standard solution: 0.1 mg/mL of USP Oxaliplatin RS in water from *Oxaliplatin standard stock solution*

Sample solution: 0.1 mg/mL of Oxaliplatin in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times, measured with respect to oxaliplatin, of oxaliplatin related compound C, oxaliplatin related compound B, and diaquodiaminocyclohexaneplatinum dimer are 0.8, 2.7, and 6, respectively.]

Suitability requirements

Resolution: NLT 2.0 between oxaliplatin and oxaliplatin related compound C, *System suitability solution*

Tailing factor: Between 0.8 and 2.0 for oxaliplatin, *System suitability solution*

Relative standard deviation: NMT 2.0% for oxaliplatin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxaliplatin ($C_8H_{14}N_2O_4Pt$) in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• LIMIT OF SILVER

Sample stock solution: Dissolve 100 mg of Oxaliplatin, weighed, in 50 mL of water to obtain a solution having a concentration of 2 mg/mL.

Sample solution: 1 mg/mL of Oxaliplatin in 0.5 M nitric acid from the *Sample stock solution*

Standard stock solution: Dilute a commercially available silver nitrate atomic absorption standard solution containing 1000 ppm of silver in 0.5 M nitric acid quantitatively, and stepwise if necessary, with 0.5 M nitric acid to obtain a 10-ppb solution.

Standard solution 1: Mix 20 µL of the *Sample stock solution* and 8 µL of the *Standard stock solution*, and dilute with 0.5 M nitric acid to 40 µL.

Standard solution 2: Mix 20 µL of the *Sample stock solution* and 16 µL of the *Standard stock solution*, and dilute with 0.5 M nitric acid to 40 µL.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometer equipped with a silver hollow-cathode lamp and graphite furnace

Analytical wavelength: Silver emission line of 328.1 nm

Blank: 0.5 M nitric acid

Analysis

Samples: *Sample solution*, *Standard solution 1*, and *Standard solution 2*

Plot the absorbances of the *Sample solution*, *Standard solution 1*, and *Standard solution 2* versus their concentrations, in ppb, of silver, and draw the straight line best fitting the three plotted points. The intercept on the *x*-axis of the extended regression line indicates the silver concentration in the *Sample solution*.

Calculate the concentration of silver, in ppm, in the portion of Oxaliplatin taken:

$$\text{Result} = (C/W) \times 100$$

C = absolute value of the intercept, in ppb of silver, on the *x*-axis

W = weight of Oxaliplatin taken for the preparation of the *Sample stock solution* (mg)

Acceptance criteria: NMT 5 ppm

• CONTENT OF PLATINUM

Sample: Ignite an empty porcelain crucible fitted with a lid in a furnace at 800° for 30 min. Cool in a desiccator, and weigh. Add 200 mg of the Oxaliplatin, weighed, to the crucible, and ignite in a furnace by stepwise increments as follows. Introduce into the furnace, and increase the temperature to 200° within 15 min, then to 400° within 15 min, then to 600° within 15 min, then finally to 800° within 15 min. Allow to remain in the furnace at 800° for 30 min. Remove, cool in a desiccator, and reweigh.

Calculate the percentage of platinum in the portion of Oxaliplatin taken:

$$\text{Result} = (W_2/W_1) \times 100$$

W_2 = weight of residue after ignition (mg)

W_1 = weight of oxaliplatin before ignition (mg)

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Acceptance criteria: 48.1%–50.1% of the oxaliplatin taken, on the dried basis

• **ORGANIC IMPURITIES, PROCEDURE 1: LIMIT OF OXALIC ACID**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Buffer: Add 1.36 g of potassium dihydrogen phosphate to 10 mL of 10% tetrabutylammonium hydroxide in water, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 6.0.

Mobile phase: Acetonitrile and *Buffer* (1:4)

Standard stock solution: 0.06 mg/mL of USP Oxaliplatin Related Compound A RS in water

Standard solution: 15 µg/mL of USP Oxaliplatin Related Compound A RS in water from the *Standard stock solution*

System suitability solution: 0.05 mg/mL of sodium nitrate in water. Transfer 2 mL of this solution and 25 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume.

Sensitivity solution: 1.5 µg/mL of USP Oxaliplatin Related Compound A RS in water from the *Standard solution*

Sample solution: 2 mg/mL of Oxaliplatin in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

[NOTE—The elution order is sodium nitrate, followed by oxalic acid.]

Suitability requirements

Resolution: NLT 2.0 between oxalic acid and sodium nitrate, *System suitability solution*

Relative standard deviation: NMT 3.0% for oxalic acid, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxalic acid in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxalic acid from the *Sample solution*

r_S = peak response of oxalic acid from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous oxalic acid, 90.03

M_{r2} = molecular weight of USP Oxaliplatin Related Compound A RS, 126.07

Acceptance criteria: NMT 0.1%

• **ORGANIC IMPURITIES, PROCEDURE 2: LIMIT OF (*SP*-4-2-)-DIAQUA[(1*R*, 2*R*)-CYCLOHEXANE-1,2-DIAMINE-*N*,*N*′]PLATINUM, OXALIPLATIN RELATED COMPOUND C, AND UNSPECIFIED IMPURITIES**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Mobile phase, Oxaliplatin standard stock solution, Oxaliplatin related compound B standard stock solution, Oxaliplatin related compound C standard stock solution, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of oxaliplatin, 0.01 mg/mL of oxaliplatin related compound B, and 0.004 mg/mL of oxaliplatin related compound C in water from *Oxaliplatin standard stock solution*, *Oxaliplatin related compound B standard stock solution*, and *Oxaliplatin related compound C standard stock solution*, respectively

Sample solution: 2 mg/mL of Oxaliplatin in water

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between oxaliplatin and oxaliplatin related compound C, *System suitability solution*

Tailing factor: Between 0.8 and 2.0 for oxaliplatin, *System suitability solution*

Relative standard deviation: NMT 3.0% for oxaliplatin, oxaliplatin related compound B, and oxaliplatin related compound C, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of (*SP*-4-2)-diaqua[(1*R*, 2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of (*SP*-4-2)-diaqua[(1*R*, 2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum from the *Sample solution*

r_S = peak response of (*SP*-4-2)-diaqua[(1*R*, 2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of (*SP*-4-2)-diaqua[(1*R*, 2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum, 345.30

M_{r2} = molecular weight of USP Oxaliplatin Related Compound B RS, 433.28

[NOTE—USP Oxaliplatin Related Compound B RS is converted to (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum in solution preparation.]

Calculate the percentage of oxaliplatin related compound C in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxaliplatin related compound C from the *Sample solution*

r_S = peak response of oxaliplatin related compound C from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound C RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

USP Monographs

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Calculate the percentage of diaquodiaminocyclo hexaneplatinum dimer in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of diaquodiaminocyclo hexaneplatinum dimer from the *Sample solution*
r_S = peak response of oxaliplatin related compound B from the *Standard solution*
C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of (*SP*-4-2)-diaqua[(1*R*, 2*R*)-cyclohexane-1,2-diamine-*N*,*N*']platinum, 345.30
M_{r2} = molecular weight of USP Oxaliplatin Related Compound B RS, 433.28
F = relative response factor for diaquodiami nocyclohexaneplatinum dimer, measured with respect to USP Oxaliplatin Related Compound B RS, 2.5

Calculate the percentage of any other unspecified impurity in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other unspecified impurity from the *Sample solution*
r_S = peak response of oxaliplatin from the *Standard solution*
C_S = concentration of oxaliplatin in the *Standard solution* (mg/mL)
C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxaliplatin related compound C	0.8	—	0.1
Oxaliplatin	1.0	—	—
(*SP*-4-2)-Diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*']platinum	2.7	—	0.1
Diaquodiaminocyclo-hexaneplatinum dimer	6	2.5	0.1
Any individual unspecified impurity	—	—	0.10
Total impurities[a]	—	—	0.30

[a] Total impurities include oxalic acid (from *Procedure 1*) and all impurities from *Procedure 2*.

• **Organic Impurities, Procedure 3: Limit of Oxaliplatin Related Compound D**

[Note—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Mobile phase: Methanol and ethanol (7:3)

Oxaliplatin related compound D standard stock solution: 0.05 mg/mL of USP Oxaliplatin Related Compound D RS in methanol

Oxaliplatin related compound D standard solution: 15 µg/mL of USP Oxaliplatin Related Compound D RS in methanol from *Oxaliplatin related compound D standard stock solution*

Oxaliplatin standard stock solution: 0.75 mg/mL of USP Oxaliplatin RS in methanol

Oxaliplatin standard solution: 37.5 µg/mL of USP Oxaliplatin RS in methanol from *Oxaliplatin standard stock solution*

Oxaliplatin blank solution: Transfer 40 mL of *Oxaliplatin standard stock solution* to a 50-mL volumetric flask, and dilute with methanol to volume.

Standard solutions: Transfer 40 mL of *Oxaliplatin standard stock solution* to separate 50-mL volumetric flasks. Add 1.0, 3.0, and 5.0 mL of *Oxaliplatin related compound D standard solution* to each flask, and dilute with methanol to volume. The concentration of oxaliplatin in these solutions is 0.6 mg/mL. The concentrations of oxaliplatin related compound D in these solutions are 0.3, 0.9, and 1.5 µg/mL, respectively.

System suitability solution: Transfer 5.0 mL of *Oxaliplatin standard solution* and 4.0 mL of *Oxaliplatin related compound D standard stock solution* to a 50-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Transfer 30 mg of Oxaliplatin into a 50-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L70
Column temperature: 40°
Flow rate: 0.3 mL/min
Injection volume: 20 µL
Run time: 30 min

System suitability
Samples: 0.9-µg/mL *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between oxaliplatin and oxaliplatin related compound D, *System suitability solution*
Relative standard deviation: NMT 3.0% for the peak height ratio of oxaliplatin related compound D to the sum of oxaliplatin and oxaliplatin related compound D; 0.9-µg/mL *Standard solution*

Analysis
Samples: *Standard solutions* and *Sample solution*
Subtract the oxaliplatin related compound D peak height obtained in the *Oxaliplatin blank solution* from the oxaliplatin related compound D peak height obtained in the *Standard solutions*. [Note—USP Oxaliplatin RS may contain a small amount of oxaliplatin related compound D.] Plot a calibration curve for the *Standard solutions* with the peak height ratios of oxaliplatin related compound D to the sum of oxaliplatin and oxaliplatin related compound D on the *y*-axis and the concentration ratios of oxaliplatin related compound D, in µg/mL, to the sum of oxaliplatin and oxaliplatin related compound D concentrations, in mg/mL, on the *x*-axis. Read the concentration ratio of oxaliplatin related compound D, in µg/mL, to the sum of oxaliplatin and oxaliplatin related compound D, in mg/mL, in the *Sample solution* from the calibration curve.
Calculate the percentage of oxaliplatin related compound D in the portion of Oxaliplatin taken:

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Result = $R/10$

R = concentration ratio of oxaliplatin related compound D, in µg/mL, to the sum of oxaliplatin and oxaliplatin related compound D, in mg/mL, in the *Sample solution* from the calibration curve

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **ACIDITY**
  Sample solution: Dissolve 100 mg in 50 mL of carbon dioxide-free water, and add 0.5 mL of phenolphthalein TS.
  Acceptance criteria: The solution is colorless, and NMT 0.6 mL of 0.01 M sodium hydroxide is required to change the color to pink.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 1.0 USP Endotoxin Unit/mg of oxaliplatin
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry 1 g at 100°–105° for 2 h.
  Acceptance criteria: NMT 0.5%
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 20 cfu/g, and the total combined molds and yeast count does not exceed 5 cfu/g.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 5 mg/mL in water
  Acceptance criteria: Between +74.5° and +78.0°, measured at 20°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxaliplatin RS
  USP Oxaliplatin Related Compound A RS
  Oxalic acid dihydrate.
  $C_2H_2O_4 \cdot 2H_2O$ 126.07
  USP Oxaliplatin Related Compound B RS
  [*SP*-4-2-(1*R*-*trans*)]-(1,2-Cyclohexanediamine-*N*,*N*′) dinitratoplatinum(II).
  $C_6H_{14}N_4O_6Pt$ 433.28
  USP Oxaliplatin Related Compound C RS
  [1*R*-*trans*-(1,2-Cyclohexanediamine-*N*,*N*′)]-*trans*-dihydroxido-[oxalato(2-)-*O*,*O*′]platinum(IV).
  $C_8H_{16}N_2O_6Pt$ 431.30
  USP Oxaliplatin Related Compound D RS
  cis-[(1*S*,2*S*)-1,2-Cyclohexanediamine-*N*,*N*′][oxalato(2-)-*O*,*O*′]platinum.
  $C_8H_{14}N_2O_4Pt$ 397.29

Oxaliplatin Injection

DEFINITION

Oxaliplatin Injection is a sterile solution of Oxaliplatin in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: 100 µg/mL
  Medium: Water
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  [NOTE—All HPLC autosampler vials should be made of polypropylene.]
  Acidified water: Adjust ▲water▲ (USP 1-Aug-2019) with phosphoric acid to a pH of 3.0.
  Mobile phase: Acetonitrile and *Acidified water* (1:99)
  System suitability solution: 0.1 mg/mL of USP Oxaliplatin RS and 0.1 mg/mL of USP Oxaliplatin System Suitability RS in water. [NOTE—USP Oxaliplatin System Suitability RS is [*SP*-4-2-(1*R*-*trans*)]-(1,2-cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II).]
  Standard solution: 0.1 mg/mL of USP Oxaliplatin RS in water
  Sample solution: Nominally 0.1 mg/mL of oxaliplatin in water from the combined contents of NLT 3 vials of Injection
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1.2 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for [*SP*-4-2-(1*R*-*trans*)]-(1,2-cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II) and oxaliplatin are 0.9 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between [*SP*-4-2-(1*R*-*trans*)]-(1,2-cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II) and oxaliplatin
  Tailing factor: NMT 2.0 for the oxaliplatin peak
  Relative standard deviation: NMT 1.0% for the oxaliplatin peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

- **LIMIT OF OXALIC ACID**
  [NOTE—All HPLC autosampler vials should be made of polypropylene.]

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▲**Mobile phase:** 0.43 g/L of sodium carbonate and 0.04 g/L of sodium bicarbonate in water

Standard stock solution: 0.32 mg/mL of USP Oxaliplatin Related Compound A RS in water. [NOTE—USP Oxaliplatin Related Compound A RS is available as dihydrate oxalic acid.]

Tartaric acid standard stock solution: 0.25 mg/mL of tartaric acid in water

System suitability solution: 23 µg/mL of USP Oxaliplatin Related Compound A RS and 12.5 µg/mL of tartaric acid in water from *Standard stock solution* and *Tartaric acid standard stock solution*.

Standard solution: 16.2 µg/mL of USP Oxaliplatin Related Compound A RS in water from *Standard stock solution*

Sensitivity solution: 1.94 µg/mL USP Oxaliplatin Related Compound A RS in water from *Standard solution*

Sample solution: Nominally equivalent to 2 mg/mL of oxaliplatin in water from Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Conductivity with anion suppression. [NOTE—A recycle mode may be used.]

Column: 4.0-mm × 25-cm; packing L31

Autosampler temperature: 5°

Flow rate: 2.0 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—The relative retention times for tartaric acid and oxalic acid are 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between tartaric acid and oxalic acid, *System suitability solution*

Tailing factor: 0.5–1.5 for the oxalic acid peak, *Standard solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*▲ (USP 1-Aug-2019)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxalic acid in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxalic acid from the *Sample solution*

r_S = peak response of oxalic acid from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous oxalic acid, 90.03

M_{r2} = molecular weight of oxaliplatin related compound A, 126.07

Acceptance criteria: NMT 0.6%

• **LIMIT OF (*SP*-4-2)-DIAQUA[(1*R*,2*R*)-CYCLOHEXANE-1,2-DIAMINE-*N*,*N*′]PLATINUM AND UNSPECIFIED IMPURITIES**

[NOTE—All HPLC autosampler vials should be made of polypropylene.]

Solution A: Dissolve 1.36 g of monobasic potassium phosphate and 0.55 g of sodium heptanesulfonate in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Methanol and *Solution A* (19:81)

Solution C: Methanol and *Solution A* (50.5:49.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
45.0	0	100
45.5	100	0
53.0	100	0

System suitability solution: 2 mg/mL of USP Oxaliplatin RS in 0.005 M sodium hydroxide. Allow this solution to stand at room temperature for at least 5 days. Transfer 5 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume. [NOTE—The preparation of the *System suitability solution* forms (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum and diaquodiaminocyclohexaneplatinum dimer.]

Standard stock solution: Transfer a weighed quantity of USP Oxaliplatin Related Compound B RS into a suitable volumetric flask, add a volume of methanol equivalent to about 25% of the final volume, and sonicate for approximately 2 min to disperse the solids. Add a volume of 0.01 M nitric acid equivalent to about 65% of the final volume, and sonicate for approximately 30 min to dissolve. Allow to cool, if necessary, and dilute with 0.01 M nitric acid to volume to obtain a solution with a concentration of 0.125 mg/mL.

Standard solution: 31.25 µg/mL of USP Oxaliplatin Related Compound B RS in 0.01 M nitric acid from the *Standard stock solution*. [NOTE—USP Oxaliplatin Related Compound B RS is converted to (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum in the *Standard solution* preparation.]

Sample solution: Combined contents of NLT 3 vials of Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 7.5-cm; 3-µm packing L1

Column temperature: 10°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between the peaks of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′] platinum and diaquodiaminocyclohexaneplatinum dimer, *System suitability solution*

Tailing factor: NMT 2.0 for the (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum peak, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

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r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum from the *Standard solution*
C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum, 345.30
M_{r2} = molecular weight of oxaliplatin related compound B, 433.28
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
(*SP*-4-2)-Diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′] platinum	1.0	1.0	0.65
Diaquodiaminocyclohexaneplatinum dimer[a]	1.4	2.5	0.50
Any individual unspecified impurity	—	4.0	0.2
Total impurities	—	—	2.45[b]

[a] (*SP*-4-2)-Di-μ-oxobis[(1*R*,2*R*)-cyclohexane-1,2-diamine-k*N*,k*N*′]diplatinum.
[b] From the tests for *Limit of Oxalic Acid* and *Limit of (SP-4-2)-Diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N′]platinum and Unspecified Impurities*.

SPECIFIC TESTS

Change to read:

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements▲ (USP 1-Aug-2019)
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **pH** ⟨791⟩: 4.0–7.0 using a polymer combination electrode
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature. Do not freeze.
- **LABELING:** Label it to indicate that it is to be diluted with a 5% dextrose solution. Oxaliplatin Injection must not be diluted in sodium chloride solutions or in chloride-containing solutions.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxaliplatin RS
  USP Oxaliplatin Related Compound A RS
  Oxalic acid dihydrate.
  $C_2H_2O_4 \cdot 2H_2O$ 126.07
  USP Oxaliplatin Related Compound B RS
  [*SP*-4-2-(1*R*-*trans*)]-(1,2-Cyclohexanediamine-*N*,*N*′) dinitratoplatinum(II).
  $C_6H_{14}N_4O_6Pt$ 433.28
  USP Oxaliplatin System Suitability RS
  [*SP*-4-2-(1*R*-*trans*)]-(1,2-Cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II).
  $C_6H_{14}Cl_2N_2Pt$ 380.17

Oxaliplatin for Injection

DEFINITION

Oxaliplatin for Injection is a sterile, lyophilized mixture of Oxaliplatin and Lactose Monohydrate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

- **PROCEDURE**
  [NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Use polypropylene HPLC autosampler vials.]
  Acidified water: Adjust water with phosphoric acid to a pH of 3.0.
  Mobile phase: Acetonitrile and *Acidified water* (1:99)
  System suitability solution: 0.1 mg/mL each of USP Oxaliplatin RS and USP Oxaliplatin System Suitability RS in water. [NOTE—USP Oxaliplatin System Suitability RS is [*SP*-4-2-(1*R*-*trans*)]-(1,2-cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II).]
  Standard solution: 0.1 mg/mL of USP Oxaliplatin RS in water
  Sample solution: Nominally equivalent to 0.1 mg/mL of oxaliplatin obtained by constituting a suitable number of vials of Oxaliplatin for Injection with the appropriate amount of water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ 2S (USP41)
  Column: 4.6-mm × 25-cm; 5-μm packing L1
  Column temperature: 40°
  Flow rate: 1.2 mL/min
  Injection volume: 20 μL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for [*SP*-4-2-(1*R*-*trans*)]-(1,2-cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II) and oxaliplatin are about 0.9 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between the peaks of [*SP*-4-2-(1*R*-*trans*)]-(1,2-cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II) and oxaliplatin, *System suitability solution*

USP Monographs

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Tailing factor: NMT 2.0 for the oxaliplatin peak, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$) in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

IMPURITIES

Change to read:

• **Limit of Oxalic Acid**
[Note—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Use polypropylene HPLC autosampler vials.]
Buffer: Add 1.36 g of ▲monobasic potassium phosphate▲ 2S (USP41) to 10 mL of 10% tetrabutylammonium hydroxide in water, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (1:4)
Standard stock solution: 0.06 mg/mL of USP Oxaliplatin Related Compound A RS in water. [Note—USP Oxaliplatin Related Compound A RS is available as oxalic acid dihydrate.]
Standard solution: 15 µg/mL of USP Oxaliplatin Related Compound A RS in water, from the *Standard stock solution*
System suitability stock solution: 0.05 mg/mL of sodium nitrate in water
System suitability solution: 1.0 µg/mL of sodium nitrate and 15 µg/mL of oxaliplatin related compound A in water, from the *System suitability stock solution* and *Standard stock solution*, respectively
Sensitivity solution: ▲1.5 µg/mL of USP Oxaliplatin Related Compound A RS in water, from the *Standard solution*▲ 2S (USP41)
Sample solution: Nominally equivalent to 2.0 mg/mL of oxaliplatin in water from Oxaliplatin for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
[Note—The relative retention times for the sodium nitrate and oxalic acid peaks are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the oxalic acid and sodium nitrate peaks, *System suitability solution*
Relative standard deviation: NMT 3.0% for the oxalic acid peak, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxalic acid in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxalic acid from the *Sample solution*
r_s = peak response of oxalic acid from the *Standard solution*
C_S = concentration of USP Oxaliplatin Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of anhydrous oxalic acid, 90.03
M_{r2} = molecular weight of USP Oxaliplatin Related Compound A RS, 126.07

Acceptance criteria: NMT 0.5%

Change to read:

• **Limit of (*SP*-4-2)-Diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum**
[Note—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Use polypropylene HPLC autosampler vials.]
Buffer: Dissolve 1.36 g of ▲monobasic potassium phosphate▲ 2S (USP41) and 1 g of sodium 1-heptanesufonate in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (1:4)
System suitability solution: 2 mg/mL of USP Oxaliplatin RS in 0.005 M sodium hydroxide. Allow this solution to stand at room temperature for at least 5 days. [Note—Sonicate if necessary.] Transfer 5 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume. [Note—The preparation of the *System suitability solution* forms diaquodiaminocyclohexaneplatinum dimer and (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′] platinum.]
Standard solution: 0.0125 mg/mL of USP Oxaliplatin Related Compound B RS prepared as follows. Transfer USP Oxaliplatin Related Compound B RS to a suitable volumetric flask, add 25% of the final volume of methanol, and sonicate for approximately 30 min to dissolve. Allow to cool, if necessary, and dilute with water to volume. [Note—When preparing the solution, USP Oxaliplatin Related Compound B RS is converted to (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′] platinum.]
Sample solution: Use the *Sample solution* from the test for *Limit of Oxalic Acid*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 20 µL

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System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum and diaquodiaminocyclohexaneplatinum dimer are about 1.0 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 2.0 between (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum and diaquodiaminocyclohexaneplatinum dimer, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum from the *Sample solution*
r_S = peak response of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum from the *Standard solution*
C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum, 345.30
M_{r2} = molecular weight of USP Oxaliplatin Related Compound B RS, 433.28

Acceptance criteria: NMT 0.5%

Change to read:

- **LIMIT OF RELATED COMPOUND C AND UNSPECIFIED IMPURITIES**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Use polypropylene HPLC autosampler vials.]
Mobile phase: Prepare as directed in the *Assay*.
Standard stock solution: 0.1 mg/mL each of USP Oxaliplatin RS and USP Oxaliplatin Related Compound C RS in water
Standard solution: 0.01 mg/mL each of USP Oxaliplatin RS and USP Oxaliplatin Related Compound C RS in water, from the *Standard stock solution*
System suitability stock solution: 0.1 mg/mL of USP Oxaliplatin System Suitability RS in methanol. Sonicate for approximately 10 min to aid the dissolution.
System suitability solution: Transfer 10 mL each of the *Standard stock solution* and the *System suitability stock solution* into a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Use the *Sample solution* from the test for *Limit of Oxalic Acid*.
Chromatographic system: Proceed as directed in the *Assay*, except for the *Injection volume*.
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between [*SP*-4-2-(1*R-trans*)]-(1,2-cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II) and oxaliplatin, *System suitability solution*
Tailing factor: NMT 2.0 for the oxaliplatin peak, *System suitability solution*
Relative standard deviation: NMT 3.0% each for the oxaliplatin and oxaliplatin related compound C peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxaliplatin related compound C in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxaliplatin related compound C from the *Sample solution*
r_S = peak response of oxaliplatin related compound C from the *Standard solution*
C_S = concentration of USP Oxaliplatin Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of oxaliplatin from the *Standard solution*
C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxaliplatin related compound C ▲▲ 2S (USP41)	0.6	0.3
[*SP*-4-2-(1*R-trans*)]-(1,2-Cyclohexanediamine-*N*,*N*′) dichloridoplatinum(II)[a]	0.8	—
Oxaliplatin	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities[b]	—	1.5

▲▲ 2S (USP41)
[a] The relative retention time is included for system suitability purposes only.
[b] Includes oxalic acid, (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*′]platinum, oxaliplatin related compound C, and the total of the individual unspecified impurities.

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.0–7.0 using a polymer combination electrode, determined in a solution constituted as directed in the labeling
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.

USP Monographs

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- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1.0 USP Endotoxin Unit/mg of oxaliplatin
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 4.0%
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

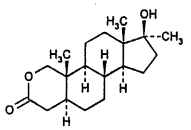
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for Constitution.* Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

▲▲ (CN 1-May-2018)
USP Oxaliplatin RS
▲▲ 2S (USP41)
USP Oxaliplatin Related Compound A RS
Oxalic acid dihydrate.
$C_2H_2O_4 \cdot 2H_2O$ 126.07
USP Oxaliplatin Related Compound B RS
[*SP*-4-2-(1*R-trans*)]-(1,2-Cyclohexanediamine-*N,N'*) dinitratoplatinum(II).
$C_6H_{14}N_4O_6Pt$ 433.28
USP Oxaliplatin Related Compound C RS
[1*R-trans*-(1,2-Cyclohexanediamine-*N,N'*)]-*trans*-dihydroxido-[oxalato(2-)-*O,O'*]platinum(IV).
$C_8H_{16}N_2O_6Pt$ 431.30
USP Oxaliplatin System Suitability RS
[*SP*-4-2-(1*R-trans*)]-(1,2-Cyclohexanediamine-*N,N'*) dichloridoplatinum(II).
$C_6H_{14}Cl_2N_2Pt$ 380.17

Oxandrolone



$C_{19}H_{30}O_3$ 306.44
2-Oxaandrostan-3-one, 17-hydroxy-17-methyl-, (5α,17β)-.
17β-Hydroxy-17-methyl-2-oxa-5α-androstan-3-one [53-39-4].

» Oxandrolone contains not less than 98.0 percent and not more than 102.0 percent of $C_{19}H_{30}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Oxandrolone RS
USP Oxandrolone Related Compound A RS
(7,8-Didehydro-oxandrolone) or (17β-hydroxy-17α-methyl-2-oxa-5α-androst-7-en-3-one).
USP Oxandrolone Related Compound B RS
(4-Oxa-isomer) or (17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one).
USP Oxandrolone Related Compound C RS
Anhydro-oxandrolone or (17,17-dimethyl-18-nor-2-oxa-5α-androst-13-en-3-one).

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Specific rotation ⟨781S⟩: between −18° and −24°.
Test solution: 10 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Related compounds—

Solution A: acetonitrile.
Solution B: water.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Blank solution—Prepare a mixture of *Solution A* and *Solution B* (50:50).
Standard stock solution—Dissolve accurately weighed quantities of USP Oxandrolone Related Compound A RS, USP Oxandrolone Related Compound B RS, USP Oxandrolone Related Compound C RS, and USP Oxandrolone RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having known concentrations of about 4 µg of USP Oxandrolone Related Compound A RS per mL, 120 µg of USP Oxandrolone Related Compound B RS per mL, 4 µg of USP Oxandrolone Related Compound C RS per mL, and 200 µg of USP Oxandrolone RS per mL. [NOTE—Sonicate if necessary to dissolve.]
Standard solution—Dilute 1.0 mL of the *Standard stock solution* with 4.0 mL of acetonitrile and 5.0 mL of water, and mix.
Test solution—Weigh accurately 40 mg of Oxandrolone into a 10-mL volumetric flask, dissolve in 5.0 mL of acetonitrile using an ultrasonic bath, dilute with water to volume, and mix. [NOTE—The *Test solution,* the *Standard solution,* and the *Blank solution* are made up fresh and injected immediately.]
Chromatographic system—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column temperature is maintained at 40°. The flow rate is about 0.7 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	50	50	equilibration
0–30	50→100	50→0	linear gradient
30–32	100→50	0→50	linear gradient
32–40	50	50	re-equilibration

Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between

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oxandrolone related compound A and oxandrolone related compound B is not less than 1.5, and the resolution, *R*, between oxandrolone related compound B and oxandrolone is not less than 2.0; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 5.0%.

Change to read:

Procedure—Separately inject equal volumes (about 50 µL) of the *Blank solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of oxandrolone related compound A in the portion of Oxandrolone taken by the formula:

$$(C/W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of oxandrolone related compound A in the *Standard solution*; *W* is the weight, in mg, of Oxandrolone taken to prepare the *Test solution*; r_U is the peak area of oxandrolone related compound A in the chromatogram of the *Test solution*; and r_S is the peak area obtained for oxandrolone related compound A in the chromatogram of the *Standard solution*.

Calculate the percentage of oxandrolone related compound C, methyltestosterone, Δ1-mestalone, specified unknown impurity 1, and each impurity eluting at a relative retention time greater than or equal to 2.2 (relative to retention time of oxandrolone) by the formula:

$$(1/F)(C/W)(r_U/r_S)$$

in which *F* is the relative response factor (see accompanying table for values); *C* is the concentration, in µg per mL, of oxandrolone related compound C in the *Standard solution*; *W* is the weight, in mg, of Oxandrolone taken to prepare the *Test solution*; r_U is the peak area of oxandrolone related compound C, methyltestosterone, Δ1-mestalone, specified unknown impurity 1, or each impurity eluting at a relative retention time greater than or equal to 2.2 in the chromatogram of the *Test solution*; and r_S is the peak area obtained for oxandrolone related compound C in the chromatogram of the *Standard solution*.

Calculate the percentage of each impurity, except oxandrolone related compound A, oxandrolone related compound C, methyltestosterone, Δ1-mestalone, specified unknown impurity 1, and other impurities eluting at relative retention times greater than or equal to 2.2 by the formula:

$$(1/F)(C/W)(r_U/r_S)$$

in which *F* is the relative response factor for each impurity (see accompanying table for values); *C* is the concentration, in µg per mL, of USP Oxandrolone RS in the *Standard solution*; *W* is the weight, in mg, of Oxandrolone taken to prepare the *Test solution*; r_U is the peak area of each impurity, in the chromatogram of the *Test solution*, other than peak areas of oxandrolone related compound A, oxandrolone related compound C, methyltestosterone, Δ1-mestalone, specified unknown impurity 1, and other impurities eluting at relative retention times greater than or equal to 2.2; and r_S is the peak area obtained for oxandrolone in *Standard solution*. Disregard any peak observed in the chromatogram obtained from the *Blank solution*. Disregard any impurity peak that is less than 0.05%. The impurities meet the requirements specified in the accompanying table.

Compound	Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
Secodicarboxylic acid[1]	0.46	4.1	0.1
7,8-Didehydro-oxandrolone[2] (Oxandrolone related compound A)	0.90	—	0.1
4-Oxa-isomer[3] (Oxandrolone related compound B)	0.94	1.4	0.3
Oxandrolone	1.00	—	—
Oxandrolone open lactone methyl ester[4]	1.09	1.5	0.1
Secoacid anhydride[5]	1.12	2.5	0.1
Methyltestosterone[6]	1.25	0.8[a]	0.1
17-epi-Oxandrolone[7]	1.33	1.0	0.3
Δ1-Mestalone[8]	1.48	1.3[a]	0.1
4-Oxa-isomer (beta epimer)[9]	1.52	1.4	0.3
Specified unknown impurity 1	1.63	0.6[a]	0.1
Oxandrolone-17-acetate[10]	2.14	1.9	0.1
Anhydro-oxandrolone[11] (Oxandrolone related compound C)	3.29	—	0.5
Individual unknown impurity	—	1.0	0.1
Total impurities	—	—	1.0

[1] 17β-Hydroxy-17α-methyl-2-nor-5α-androstan-1,3-dioic acid.
[2] 17β-Hydroxy-17α-methyl-2-oxa-5α-androst-7-en-3-one.
[3] 17β-Hydroxy-17α-methyl-4-oxa-5α-androstan-3-one.
▲[4] Methyl 1,17β-dihydroxy-17α-methyl-1,3-seco-2-nor-5α-androstan-3-oate.▲ (ERR 1-Mar-2019)
[5] 17β-Hydroxy-17α-methyl-2-oxa-5α-androstan-1,3-dione.
[6] 17β-Hydroxy-17α-methyl-5α-androst-4-ene-3-one.
[7] 17α-Hydroxy-17β-methyl-2-oxa-5α-androstan-3-one.
[8] 17β-Hydroxy-17α-methyl-5α-androst-1-ene-3-one.
[9] 17β-Hydroxy-17α-methyl-4-oxa-5β-androstan-3-one.
[a] *F* values relative to oxandrolone related compound C.
[10] 17β-Hydroxy-17α-methyl-2-oxa-5α-androstan-3-one 17-acetate.
[11] 17,17-Dimethyl-18-nor-2-oxa-5α-androst-13-en-3-one.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Oxandrolone RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 3 mg per mL. [NOTE—Sonicate if necessary to dissolve.]

Assay preparation—Transfer to a suitable volumetric flask an accurately weighed quantity of Oxandrolone, and dissolve in and dilute with acetonitrile to volume to obtain a solution having a concentration of about 3 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg,

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of $C_{19}H_{30}O_3$ in the portion of Oxandrolone taken by the formula:

$$VC(r_U/r_S)$$

in which V is the final volume, in mL, of the *Assay preparation;* C is the concentration, in mg per mL, of USP Oxandrolone RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Oxandrolone Tablets

» Oxandrolone Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of oxandrolone ($C_{19}H_{30}O_3$).

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.
USP Reference standards ⟨11⟩—
USP Oxandrolone RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Standard solution: 5 mg per mL in chloroform.
Test solution—Transfer a portion of finely powdered Tablets, equivalent to about 20 mg of oxandrolone, to a 50-mL stoppered centrifuge tube, add 4 mL of chloroform, shake by mechanical means for 10 minutes, centrifuge for about 15 minutes, and filter a portion of the chloroform layer.
Application volume: 10 µL.
Developing solvent system: a mixture of chloroform and methanol (19:1).
Procedure—Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2) and heating on a hot plate or under a lamp until spots appear: the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution.*
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
TEST 1—
Medium: a solution of water and isopropanol (7:3); 500 mL.
Apparatus 2: 100 rpm.
Time: 60 minutes.
Determine the amount of $C_{19}H_{30}O_3$ dissolved by employing the following method.
Internal standard solution—Dissolve accurately weighed quantities of 17α-methyltestosterone, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a concentration of about 0.2 mg per mL (for Tablets with a 2.5-mg label claim) and about 0.8 mg per mL (for Tablets with a 10-mg label claim).
Standard solution—Dissolve an accurately weighed quantity of USP Oxandrolone RS, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a concentration of about 1 mg per mL.
Working standard solution—For Tablets labeled to contain 2.5 mg: combine 100 µL of the *Standard solution,* 400 µL of the *Internal standard solution,* and 1500 µL of acetonitrile. For Tablets labeled to contain 10 mg: combine 100 µL of the *Standard solution,* 100 µL of the *Internal standard solution,* and 1800 µL of acetonitrile.
Test solution—Withdraw 25 mL of the solution under test from the vessel. Pass through a 0.45-µm polytef filter. Transfer 20 mL of the filtrate to a separatory funnel, add 400 µL of the *Internal standard solution,* 40 mL of a 10% potassium chloride solution, and 8 mL of chloroform. In separate separatory funnels, prepare an extraction blank and an internal standard blank in a similar manner using 20 mL of filtered *Medium* in place of the solution under test and excluding the *Internal standard solution* from the extraction blank. Shake each funnel, and allow the layers to separate. Collect the lower chloroform layer. Repeat the extraction procedure one more time. Evaporate the solvents under a stream of nitrogen at 45° until just dry. Reconstitute the dried residue with 2 mL of acetonitrile (for Tablets with a 2.5-mg label claim) or with 8 mL of acetonitrile (for Tablets with a 10-mg label claim), and sonicate for 10 minutes.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m column coated with a 0.5-µm phase G27. The carrier gas is helium, flowing at a rate of about 16.8 mL per minute. The injection port and detector temperatures are maintained at 190° and 320°, respectively. The chromatograph is programmed as follows. The column temperature is initially 180°. Upon injection, the column temperature is increased at a rate of 25° per minute to 280°, and maintained at 280° for 3 minutes. Then the column temperature is increased at a rate of 10° per minute to 320°, and maintained at 320° for 3 minutes. Chromatograph the acetonitrile, the extraction blank, and the internal standard blank, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5. Make two injections of the *Working standard solution,* and record the peak responses. The average oxandrolone/*Internal standard solution* peak area percent comparison is between 98.0% and 102.0%. The resolution, *R,* between the oxandrolone peak and the nearest eluting peak is equal to or greater than 1.5.
Procedure—Separately inject equal volumes (0.5 µL) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{19}H_{30}O_3$ released by the formula:

$$\frac{C_S \times \text{sample ratio} \times V_{UF} \times 500 \times 100}{\text{Standard ratio} \times V_{UI} \times LC}$$

in which C_S is the concentration, in mg per mL, of oxandrolone in the *Standard solution;* sample ratio is the area ratio of oxandrolone to 17α-methyltestosterone in the sample injection for each *Test solution;* V_{UF} is the final volume, in mL, of the sample after reconstitution of the dry residue; 500 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; standard ratio is the mean area ratio of oxandrolone to 17α-methyltestosterone in all injections of the *Standard solution;* V_{UI} is the initial sample volume, in mL, used in the extraction; and LC is the Tablet label claim, in mg.
Tolerances—Not less than 75% *(Q)* of the labeled amount of oxandrolone ($C_{19}H_{30}O_3$) is dissolved in 60 minutes.
TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*
Medium: 1% polysorbate 80 in water; 500 mL, deaerated.
Apparatus 2: 100 rpm.
Time: 120 minutes.
Determine the amount of $C_{19}H_{30}O_3$ dissolved by employing the following method.

USP Monographs

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Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard stock solution—Transfer about 20 mg of USP Oxandrolone RS, accurately weighed, to a 200-mL volumetric flask. Add about 20 mL of acetonitrile, and sonicate to dissolve. Dilute with *Medium* to volume, and mix.
Working standard solution—Quantitatively dilute the *Standard stock solution* with *Medium* to obtain a solution having a final concentration of about 5 µg per mL for Tablets with a label claim of 2.5 mg, or a final concentration of about 20 µg per mL for Tablets with a label claim of 10 mg.
Test solution—Withdraw about 10 mL of the solution under test from the vessel. Centrifuge in a glass tube at 2000 rpm for 10 minutes.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column is maintained at 30°, and the detector is maintained at 50°. The flow rate is about 1.5 mL per minute. Chromatograph the *Working standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; the column efficiency is not less than 4000 theoretical plates; and the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 100 µL) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{19}H_{30}O_3$ released by the formula:

$$\frac{r_U \times C_S \times D \times 500 \times 100}{r_S \times LC}$$

in which r_U and r_S are the peak responses obtained from the *Test solution* and *Working standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Working standard solution; D* is the dilution factor of the *Test solution;* 500 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and *LC* is the Tablet label claim, in mg.
Tolerances—Not less than 65% *(Q)* of the labeled amount of $C_{19}H_{30}O_3$ is dissolved in 120 minutes.

TEST 3—If the product complies with this test, the labeling indicates that it meets *USP Dissolution Test 3.*
Medium: 0.1 N hydrochloric acid containing 0.75% of sodium lauryl sulfate; 500 mL for Tablets labeled to contain 2.5 mg, 900 mL for Tablets labeled to contain 10 mg, deaerated with helium.
Apparatus 2: 75 rpm.
Time: 90 minutes.

Determine the amount of $C_{19}H_{30}O_3$ released by employing the following method.
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard stock solution—Transfer about 20 mg, accurately weighed, of USP Oxandrolone RS to a 100-mL volumetric flask. Dissolve in approximately 5 mL of acetonitrile, and sonicate for 10 minutes. Dilute with *Medium* to volume, and mix.
Working standard solution—Transfer 8.0 mL of the *Standard stock solution* to a 200-mL volumetric flask, dilute with *Medium* to volume, and mix.
Test solution—Pass the solution under test through a suitable filter having a porosity of 0.45 µm.
Chromatographic system—The liquid chromatograph is equipped with a refractive index detector and a 4.6-mm × 3-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. The temperatures of the detector and the column are both maintained at 35°. Chromatograph the *Working standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 200 µL) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of $C_{19}H_{30}O_3$ dissolved by the formula:

$$\frac{r_U \times C_S \times 500 \times 100}{r_S \times LC}$$

in which r_U and r_S are the peak responses for the *Test solution* and the *Working standard solution,* respectively; C_S is the concentration, in mg per mL, of oxandrolone in the *Working standard solution;* 500 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and *LC* is the Tablet label claim, in mg.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{19}H_{30}O_3$ is dissolved in 90 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

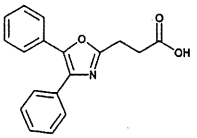
Assay—
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (62:38). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Prepare a mixture of water and acetonitrile (1:1).
Standard preparation—Dissolve an accurately weighed quantity of USP Oxandrolone RS in *Diluent,* and dilute with *Diluent* to obtain a solution having a known concentration of about 0.5 mg per mL.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of oxandrolone, to a 100-mL volumetric flask. Add 50 mL of *Diluent,* sonicate for 30 minutes with frequent shaking, and shake for an additional 15 minutes using a mechanical shaker. Dilute with *Diluent* to volume, and centrifuge. Use the supernatant as the *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column is maintained at 30°, and the detector is maintained at 50°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; the column efficiency is not less than 5000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the oxandrolone peaks. Calculate the quantity, in percent of label claim, of oxandrolone ($C_{19}H_{30}O_3$) in the portion of Tablets taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of oxandrolone in the *Standard preparation;* C_U is the concentration, in mg per mL, of oxandrolone in the *Assay preparation,* based on the label claim; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Oxaprozin



$C_{18}H_{15}NO_3$ 293.32
2-Oxazolepropanoic acid, 4,5-diphenyl-;
4,5-Diphenyl-2-oxazolepropionic acid [21256-18-8].

DEFINITION

Oxaprozin contains NLT 98.0% and NMT 102.0% of oxaprozin ($C_{18}H_{15}NO_3$), calculated on the dried basis.
[NOTE—Because of light sensitivity, protect all oxaprozin samples and *Standard solutions* from light.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020) ▲or ⟨197A⟩▲ (USP 1-Dec-2019)
- **B.** The retention time of the oxaprozin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% phosphoric acid, pH 2.0 ± 0.1
Mobile phase: Acetonitrile and *Solution A* (45:55)
Standard solution: 0.2 mg/mL of USP Oxaprozin RS in acetonitrile
Sample solution: 0.2 mg/mL of Oxaprozin in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 25°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxaprozin ($C_{18}H_{15}NO_3$) in the portion of Oxaprozin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxaprozin from the *Sample solution*
r_S = peak response of oxaprozin from the *Standard solution*
C_S = concentration of USP Oxaprozin RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxaprozin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **ARSENIC** ⟨211⟩, *Procedures, Method II*: NMT 1 ppm

Change to read:

- **ORGANIC IMPURITIES**

Solution A: 0.1% phosphoric acid, ▲pH 2.0 ± 0.1▲ (USP 1-Dec-2019)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
20	70	30
21	70	30
60	0	100
61	70	30
70	70	30

Diluent A: Acetonitrile, methylene chloride, and water (48:1:1)
Diluent B: Acetonitrile and water (1:1)
Standard stock solution: 200 µg/mL of USP Oxaprozin RS in acetonitrile
Standard solution: 5 µg/mL of USP Oxaprozin RS in *Diluent A* from the *Standard stock solution*
▲Sensitivity solution: 0.3 µg/mL of USP Oxaprozin RS in *Diluent A* from the *Standard solution*▲ (USP 1-Dec-2019)
System suitability stock solution: 200 µg/mL of benzil in acetonitrile
System suitability solution: 10 µg/mL each of benzil and USP Oxaprozin RS in *Diluent A* from the *System suitability stock solution* and *Standard stock solution*, respectively
Sample solution A: 1 mg/mL of Oxaprozin prepared as follows. Transfer about 100 mg of Oxaprozin to a 100-mL volumetric flask. Add 2 mL of methylene chloride, 2 mL of water, and 75 mL of acetonitrile. Sonicate after each solvent is added. Dilute with acetonitrile to volume. [NOTE—This is used to monitor all known and unknown impurities, except ▲oxaprozin imidazole analog and oxaprozin amide.▲ (USP 1-Dec-2019)]
Sample solution B: 1 mg/mL of Oxaprozin in *Diluent B*. [NOTE—This is used to monitor only ▲oxaprozin imidazole analog and oxaprozin amide.▲ (USP 1-Dec-2019)]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution*, ▲*Sensitivity solution*,▲ (USP 1-Dec-2019) and *System suitability solution* ▲▲ (USP 1-Dec-2019)
Suitability requirements
Resolution: NLT 3.0 between oxaprozin and benzil, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
▲Signal-to-noise ratio: NLT 10, *Sensitivity solution*▲ (USP 1-Dec-2019)
Analysis
Samples: *Sample solution A* and *Sample solution B*

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Calculate the percentage of ▲oxaprozin imidazole analog and oxaprozin amide▲ (USP 1-Dec-2019) in the portion of Oxaprozin taken:

$$\text{Result} = (r_U/r_T) \times ▲(1/F)▲_{\text{(USP 1-Dec-2019)}} \times 100$$

r_U = peak area of ▲oxaprozin imidazole analog or oxaprozin amide▲ (USP 1-Dec-2019) from *Sample solution B*
r_T = sum of the peak areas from *Sample solution B*
F = relative response factor (see *Table 2*)

Calculate the percentage of any other impurity in the portion of Oxaprozin taken:

$$\text{Result} = (r_U/r_T) \times ▲(1/F)▲_{\text{(USP 1-Dec-2019)}} \times 100$$

r_U = peak area of each individual impurity from *Sample solution A*
r_T = sum of the peak areas from *Sample solution A*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. ▲The reporting threshold is 0.03%.▲ (USP 1-Dec-2019)

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
▲Oxaprozin imidazole analog[a]▲ (USP 1-Dec-2019)	0.14	▲0.87▲ (USP 1-Dec-2019)	0.1
▲Benzoin[b]▲ (USP 1-Dec-2019)	0.42	▲0.83▲ (USP 1-Dec-2019)	0.1
▲Oxaprozin amide[c]▲ (USP 1-Dec-2019)	0.73	▲1.10▲ (USP 1-Dec-2019)	0.1
▲Benzoin succinate[d]▲ (USP 1-Dec-2019)	0.84	▲1.18▲ (USP 1-Dec-2019)	0.1
▲Oxaprozin	1.00	1.00	—▲ (USP 1-Dec-2019)
▲Benzil▲ (USP 1-Dec-2019)	1.08	▲0.78▲ (USP 1-Dec-2019)	0.1
▲Oxaprozin bisoxazole analog[e]▲ (USP 1-Dec-2019)	1.50	▲0.68▲ (USP 1-Dec-2019)	0.1
▲Tetraphenylpyrazine[f]▲ (USP 1-Dec-2019)	1.57	▲0.48▲ (USP 1-Dec-2019)	0.1
▲Individual unspecified impurity	—	1.00	0.06▲ (USP 1-Dec-2019)
Total impurities	—	—	0.5

▲[a] 3-(4,5-Diphenyl-1*H*-imidazol-2-yl)propanoic acid.
[b] 2-Hydroxy-1,2-diphenylethan-1-one.
[c] 3-(4,5-Diphenyloxazol-2-yl)propanamide.
[d] 4-Oxo-4-(2-oxo-1,2-diphenylethoxy)butanoic acid.
[e] 1,2-Bis(4,5-diphenyloxazol-2-yl)ethane.
[f] 2,3,5,6-Tetraphenylpyrazine.▲ (USP 1-Dec-2019)

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Oxaprozin RS

Oxaprozin Tablets

DEFINITION

Oxaprozin Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of oxaprozin ($C_{18}H_{15}NO_3$).
[Note—Because of light sensitivity, protect all oxaprozin samples and standard solutions from light.]

IDENTIFICATION

- **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
  Sample solution: 2 mg/mL of oxaprozin in acetone
  Developing solvent system: Ethyl acetate and glacial acetic acid (99:1)
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**
  Solution A: 0.1% Phosphoric acid. Add phosphoric acid, dropwise, to water to obtain a pH of 2.00 ± 0.10.
  Mobile phase: Acetonitrile and *Solution A* (45:55)
  Standard solution: Dissolve an accurately weighed quantity of USP Oxaprozin RS in acetonitrile to obtain a solution having a concentration of about 12 µg/mL of oxaprozin.
  Sample stock solution: Nominally 0.6 mg/mL of oxaprozin prepared as follows. Transfer a suitable amount of oxaprozin from NLT 20 powdered Tablets to an appropriate volumetric flask. Add water to 10% of the final volume, and sonicate for 10 min. Add 40% of the final volume of acetonitrile, and sonicate for 30 min. Shake by mechanical means for an additional 30 min. Add 30% of the final volume of acetonitrile, and sonicate for 10 min. Dilute with acetonitrile to volume. Pass through a suitable filter. Use the filtrate.
  Sample solution: Nominally equivalent to 12 µg/mL of oxaprozin from the *Sample stock solution* in acetonitrile
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 285 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L7
  Flow rate: 1.0 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of oxaprozin ($C_{18}H_{15}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxaprozin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxaprozin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

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PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: 0.05 M monobasic potassium phosphate buffer, pH 7.4; 1000 mL

Apparatus 2: 75 rpm

Time: 45 min

Detector: UV 286 nm (maximum absorbance)

Standard solution: A known concentration of USP Oxaprozin RS in *Medium*. [Note—A quantity of methanol, not exceeding 5% of the final volume, can be added to help solubilize the USP Reference Standard.]

Sample solution: Filter portions of the solution under test, suitably diluted with *Medium*, if necessary.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxaprozin ($C_{18}H_{15}NO_3$) dissolved by using UV absorption from the *Sample solution* in comparison with the *Standard solution*.

Tolerances: NLT 75% (*Q*) of the labeled amount of oxaprozin ($C_{18}H_{15}NO_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Solution A and **Mobile phase:** Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL of USP Oxaprozin RS in acetonitrile

Sample solution: Nominally 1 mg/mL of oxaprozin prepared as follows. Transfer a suitable amount of oxaprozin from NLT 20 powdered Tablets to an appropriate volumetric flask. Add water to 10% of the final volume, and sonicate for 10 min. Add 40% of the final volume of acetonitrile, and sonicate for 30 min. Shake by mechanical means for an additional 30 min. Add 30% of the final volume of acetonitrile, and sonicate for 10 min. Dilute with acetonitrile to volume. Pass through a suitable filter. Use the filtrate.

Chromatographic system: Proceed as directed in the *Assay* except use a column temperature of 20°.

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of benzoin, benzil, and each individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of benzoin, benzil, or each individual unspecified degradation product from the *Sample solution*

r_S = peak response of oxaprozin from the *Standard solution*

C_S = concentration of USP Oxaprozin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxaprozin in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzoin[a]	0.6	0.12	0.2
Oxaprozin	1.0	—	—
Benzil	1.9	0.62	0.2
Any individual unspecified degradation product	—	1.0	0.2

[a] 2-Hydroxy-1,2-diphenylethan-1-one.

SPECIFIC TESTS

• **Water Determination,** *Method Ia* ⟨921⟩**:** NMT 3.5%

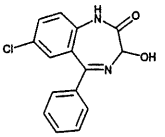
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Oxaprozin RS

Oxazepam



$C_{15}H_{11}ClN_2O_2$ 286.71

2*H*-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-, (±)-;

(±)-7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one [604-75-1].

DEFINITION

Oxazepam contains NLT 98.0% and NMT 102.0% of oxazepam ($C_{15}H_{11}ClN_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **Procedure**

▲Prepare the *System suitability solution*, *Standard solution*, and *Sample solution* immediately before use.

Solution A: Dissolve 3.5 g of dibasic potassium phosphate in 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 10.5, and dilute with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
4	75	25
19	50	50
20	25	75
25	25	75
26	75	25
30	75	25

Diluent: Acetonitrile and water (50:50)
System suitability solution: 1.6 µg/mL each of USP Oxazepam Related Compound A RS and USP Chlordiazepoxide Related Compound A RS, and 0.8 mg/mL of USP Oxazepam RS in *Diluent*
Standard solution: 0.08 mg/mL of USP Oxazepam RS in *Diluent*
Sample solution: 0.08 mg/mL of Oxazepam in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between chlordiazepoxide related compound A and oxazepam related compound A, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxazepam ($C_{15}H_{11}ClN_2O_2$) in the portion of Oxazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxazepam RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxazepam in the *Sample solution* (mg/mL) ▲(USP 1-May-2019)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.3%

Change to read:

• **ORGANIC IMPURITIES**
Prepare the ▲*System suitability solution*, *Standard solution*, and *Sample solution*▲ (USP 1-May-2019) immediately before use.
Solution A, Solution B, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
4	75	25
34	25	75
45	25	75
50	75	25
60	75	25

Standard solution: 1.6 µg/mL of USP Oxazepam RS in *Diluent*
Sample solution: 0.8 mg/mL of Oxazepam in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between chlordiazepoxide related compound A and oxazepam related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oxazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of any impurity from the *Sample solution*
r_S = peak area of oxazepam from the *Standard solution*
C_S = concentration of USP Oxazepam RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxazepam in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. ▲The reporting threshold is 0.05%.▲ (USP 1-May-2019)

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A	0.7	1.0	0.2
Oxazepam related compound A	0.8	0.25	0.2
Oxazepam	1.0	—	—
Oxazepam related compound B[a]	1.2	0.90	0.2
Oxazepam related compound C[b]	1.4	1.0	0.2
Oxazepam related compound D[c]	2.0	1.0	0.2
Any individual unknown impurity	—	—	0.10
Total impurities	—	—	1.0

[a] 7-Chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzodiazepin-3-yl acetate.
[b] 6-Chloro-4-phenylquinazoline-2-carbaldehyde.
[c] 5-Chloro-2-aminobenzophenone.

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SPECIFIC TESTS
• **pH** ⟨791⟩
Sample: A suspension of 1 g of Oxazepam in 50 mL of water
Acceptance criteria: 4.8–7.0
• **Loss on Drying** ⟨731⟩
Analysis: Dry at a pressure not exceeding 5 mm of mercury at 105° for 3 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Chlordiazepoxide Related Compound A RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.
$C_{15}H_{11}ClN_2O_2$ 286.71
USP Oxazepam RS
USP Oxazepam Related Compound A RS
7-Chloro-5-phenyl-4,5-dihydro-1*H*-benzodiazepine-2,3-dione.
$C_{15}H_{11}ClN_2O_2$ 286.71

Oxazepam Capsules

DEFINITION
Oxazepam Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{15}H_{11}ClN_2O_2$.

IDENTIFICATION
• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Diluent: Methanol and water (9:1)
Buffer: 8.5 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 6.5.
Mobile phase: Methanol and *Buffer* (3:2)
Standard solution: 0.1 mg/mL of USP Oxazepam RS in *Diluent*
Sample solution: 0.1 mg/mL of oxazepam in *Diluent*, from the contents of NLT 20 Capsules. [Note—Sonicate for 15 min and shake for 15 min. Pass through a filter of 0.45-µm pore size.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: At least 1.7 times the retention time of oxazepam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{11}ClN_2O_2$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxazepam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 1000 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard stock solution: 0.1 mg/mL of USP Oxazepam RS in methanol
[Note—Prepare NMT 30 min before use.]
Standard solution: 0.01 mg/mL of USP Oxazepam RS in *Medium* from the *Standard stock solution*. [Note—Keep it at about 6° for the *Analysis*. This solution is stable for 72 h if kept refrigerated.]
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Keep it at about 6° for the *Analysis*.
Mobile phase: Methanol, water, and glacial acetic acid (60:40:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm
Column: 4-mm × 15-cm; packing L7
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the oxazepam peak
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 75% (Q) of the labeled amount of $C_{15}H_{11}ClN_2O_2$ is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES
Organic Impurities
• **Procedure**
Diluent, Buffer, and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: 2 µg/mL of USP Oxazepam RS in *Diluent*
Sample solution: 0.2 mg/mL of oxazepam in *Diluent*, from the contents of NLT 20 Capsules. [Note—Sonicate for 15 min and shake for 15 min. Pass through a filter of 0.45-µm pore size.]
Chromatographic system: Proceed as directed in the *Assay*.
Run time: 3.5 times the retention time of oxazepam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of oxazepam from the *Standard solution*
C_S = concentration of USP Oxazepam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of oxazepam in the *Sample solution* (µg/mL)

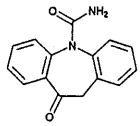
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
[NOTE—Disregard peaks less than 0.05%.]
Total impurities: NMT 0.5%, not including 2-amino 5-chlorobenzophenone

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxazepam	1.0	—
2-Amino 5-chlorobenzophenone	2.7	0.5
Any individual unspecified degradation product	—	0.1

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxazepam RS

Oxcarbazepine



$C_{15}H_{12}N_2O_2$ 252.27
5H-Dibenz[*b*,*f*]azepine-5-carboxamide, 10,11-dihydro-10-oxo-;
10,11-Dihydro-10-oxo-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide [28721-07-5].

DEFINITION
Oxcarbazepine contains NLT 98.0% and NMT 102.0% of oxcarbazepine ($C_{15}H_{12}N_2O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
[NOTE—If the spectrum obtained shows differences, dissolve the substance to be examined in chloroform, and evaporate to dryness. Compare the spectrum of the residue to that of a similarly prepared USP Oxcarbazepine RS.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. For each liter prepared, add 2 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 6.0 ± 0.1.
Mobile phase: Methanol, acetonitrile, and *Buffer* (11:8:31)
Standard solution: 0.1 mg/mL of USP Oxcarbazepine RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Oxcarbazepine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxcarbazepine ($C_{15}H_{12}N_2O_2$) in the portion of Oxcarbazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxcarbazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES, PROCEDURE 1**
[NOTE—If oxcarbazepine related compound A and oxcarbazepine related compound B are known process impurities, *Organic Impurities, Procedure 2* is recommended.]
Mobile phase: Prepare as directed in the *Assay*.
System suitability solution: 0.1 mg/mL each of USP Oxcarbazepine RS and USP Carbamazepine RS in *Mobile phase*
Standard solution: 0.25 µg/mL of USP Oxcarbazepine RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Oxcarbazepine in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except to use a run time 10 times the retention time of oxcarbazepine.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 8.0 between oxcarbazepine and carbamazepine, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oxcarbazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

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r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of oxcarbazepine from the *Standard solution*
C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxcarbazepine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxcarbazepine	1.0	1.0	—
Carbamazepine[a]	1.7	1.9	0.5
Oxcarbazepine related compound E	2.1	1.2	0.05
Methoxycarbamazepine[b]	2.5	1.6	0.05
Carbamazepine related compound B[c]	7.4	1.3	0.05
Methoxydibenzazepine[d]	7.9	1.5	0.05
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	1.0

[a] 5*H*-Dibenz[*b*,*f*]azepine-5-carboxamide.
[b] 10-Methoxy-5*H*-Dibenz[*b*,*f*]azepine-5-carboxamide.
[c] 5*H*-Dibenz[*b*,*f*]azepine.
[d] 10-Methoxy-5*H*-Dibenz[*b*,*f*]azepine.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Buffer A: 0.004 mol/L of monobasic potassium phosphate and 0.063 mol/L of dibasic sodium phosphate
Buffer B: To 1 L of 3.6 g/L edetate disodium in water add 1 L of *Buffer A*.
Diluent: 1.8 g/L of ascorbic acid in water
Solution A: Acetonitrile, tetrahydrofuran, *Buffer B*, and water (1:2:2:15)
Solution B: Acetonitrile, tetrahydrofuran, *Buffer B*, and water (6:1:1:2)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
1	80	20
29	30	70
30	30	70
33	80	20
42	80	20

System suitability solution: 2 µg/mL each of USP Oxcarbazepine Related Compound A RS, USP Oxcarbazepine Related Compound B RS, USP Oxcarbazepine Related Compound D RS, and USP Oxcarbazepine Related Compound E RS in a 1:1 mixture of acetonitrile and *Diluent*
Standard stock solution: 0.1 mg/mL of USP Oxcarbazepine RS in acetonitrile
Standard solution: 2 µg/mL of USP Oxcarbazepine RS in a 1:1 mixture of acetonitrile and *Diluent*
Sample solution: 1.0 mg/mL of Oxcarbazepine in a 1:1 mixture of acetonitrile and *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Column temperature: 50°
Flow rate: 0.8 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between oxcarbazepine related compound A and oxcarbazepine related compound B; NLT 1.2 between oxcarbazepine related compound D and oxcarbazepine related compound E, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oxcarbazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of oxcarbazepine from the *Standard solution*
C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxcarbazepine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. [NOTE—Disregard any peak below 0.03%.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxcarbazepine related compound F[a]	0.76	0.59	0.2
Oxcarbazepine	1.0	—	—
N-Carbamoyl oxcarbazepine[b]	1.1	0.91	0.05
Oxcarbazepine related compound A[c]	1.2	1.1	0.2
Oxcarbazepine related compound B[d]	1.3	1.1	0.1
Dibenzazepinodione[e]	1.7	2.0	0.1
Oxcarbazepine related compound D[f]	2.3	1.7	0.2
Oxcarbazepine related compound E	2.4	3.3	0.05
Any individual unspecified impurity	—	1.0	0.05

USP Monographs

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Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	1.0

[a] 10,11-Dioxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.
[b] *N*-Carbamoyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.
[c] *N*-Formyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.
[d] *N*-Acetyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.
[e] 5*H*-Dibenzo[*b,f*]azepine-10,11-dione.
[f] 10-(10-Oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamido)-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Oxcarbazepine RS
USP Carbamazepine RS
USP Oxcarbazepine Related Compound A RS
N-Formyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.
$C_{16}H_{12}N_2O_3$ 280.28
USP Oxcarbazepine Related Compound B RS
N-Acetyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.
$C_{17}H_{14}N_2O_3$ 294.30
USP Oxcarbazepine Related Compound D RS
10-(10-Oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamido)-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.
$C_{30}H_{22}N_4O_3$ 486.52
USP Oxcarbazepine Related Compound E RS
10(11*H*)-Oxo-5*H*-Dibenz[*b,f*]azepine.
$C_{14}H_{11}NO$ 209.24

Oxcarbazepine Oral Suspension

DEFINITION

Oxcarbazepine Oral Suspension contains NLT 95.0% and NMT 105.0% of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect all solutions from light.
Buffer: Dissolve 1.36 g of sodium acetate trihydrate and 0.6 g of glacial acetic acid in 1 L of water. Adjust with glacial acetic acid to a pH of 4.4.
Solution A: Acetonitrile, tetrahydrofuran, *tert*-butyl methyl ether, and *Buffer* (130:30:9:830)
Solution B: Acetonitrile, tetrahydrofuran, *tert*-butyl methyl ether, and *Buffer* (670:30:9:290)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
2	90	10
10	90	10
25	10	90
26	93	7
35	93	7

Diluent: Dissolve 0.1 g of ascorbic acid and 1 mL of acetonitrile in 1 L of water.
Standard stock solution: 1 mg/mL of USP Oxcarbazepine RS in acetonitrile. Sonicate to aid in dissolution.
Standard solution: 0.25 mg/mL of USP Oxcarbazepine RS from the *Standard stock solution*, prepared as follows. Dilute a suitable volume of the *Standard stock solution* first in *Diluent*, using 70% final volume. Allow the solution to equilibrate to room temperature, and dilute with acetonitrile to volume.
Sample solution: 0.25 mg/mL of oxcarbazepine from a portion of Oral Suspension, prepared as follows. Dissolve first in *Diluent* using 8% of final volume, fill 30% of final volume with acetonitrile. Sonicate for 15 min. Add *Diluent* to fill 36% of final volume. Shake the flask vigorously. Allow the solution to equilibrate to room temperature, and dilute with *Diluent* to volume.
System suitability stock solution: 0.01 mg/mL of USP Oxcarbazepine Related Compound A RS and 0.02 mg/mL of USP Oxcarbazepine Related Compound C RS in acetonitrile
System suitability solution: 0.5 µg/mL of USP Oxcarbazepine Related Compound A RS and 1 µg/mL of USP Oxcarbazepine Related Compound C RS from the *System suitability stock solution*, in *Standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.0-mm × 25-cm; 3-µm packing L1
Column temperature: 50°
Flow rate: 0.6 mL/min
Injection volume: 5 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—Refer to *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.3 between oxcarbazepine related compound C and oxcarbazepine related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxcarbazepine from the *Sample solution*
r_S = peak response of oxcarbazepine from the *Standard solution*

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C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 1% sodium dodecyl sulfate in water; 890 mL
Apparatus 2: 75 rpm
Time: 30 min
Analysis: Shake manually a bottle of Oral Suspension for about 20 s. Using a 10-mL syringe, draw 10.0 mL of the Oral Suspension. Attach a long needle to the syringe. Deliver carefully 10.0 mL of Oral Suspension through the needle to the bottom of the vessel containing preheated *Medium*. Take about 10 mL of the *Medium* from the vessel to clean the syringe, and transfer it back to the vessel. Start the paddle rotation immediately after introduction of each sample.
Mobile phase: Methanol, acetic acid, and water (24:1:75)
Standard solution: 0.7 mg/mL of USP Oxcarbazepine RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size, discarding the first few mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 310 nm
Column: 4.6-mm × 25-cm; 10-µm packing L10
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)
L = label claim (mg in 10 mL)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) is dissolved.

• **Deliverable Volume** ⟨698⟩**:** Meets the requirements

IMPURITIES

• **Organic Impurities**
Protect all solutions from light.
Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.5 mg/mL of USP Carbamazepine RS in acetonitrile. Sonicate to aid in dissolution.
Standard solution: 0.5 µg/mL of USP Carbamazepine RS from the *Standard stock solution* prepared as follows. Dilute a volume of the *Standard stock solution* first in *Diluent*, using 70% of final volume. Cool to room temperature, and dilute with acetonitrile to volume.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.3 between oxcarbazepine related compound C and oxcarbazepine related compound A peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of carbamazepine from the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Acridine carboxylic acid[a]	0.24	11.1	0.1
Carbamazepinedione[b]	0.65	0.68	0.2
Oxcarbazepine	1.0	1.0	—
Oxcarbazepine related compound C[c]	1.33	12.5	0.1
Oxcarbazepine related compound A[d,e]	1.38	—	—
Carbamazepine	1.66	1.0	—
Dibenzazepinodione[f]	1.97	1.1	0.2
Acridine[g]	2.49	11.1	0.1
Dibenzazepinone[h]	2.62	2.9	0.1
Any unspecified individual degradation product	—	1.0	0.1
Total impurities	—	—	0.8

[a] Acridine-9-carboxylic acid.
[b] 10,11-Dioxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide.
[c] Acridin-9(10*H*)-one.
[d] *N*-Formyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide.
[e] For system suitability purposes only.
[f] 5*H*-Dibenzo[*b*,*f*]azepine-10,11-dione.
[g] Acridine.
[h] 10(11*H*)-Oxo-5*H*-dibenz[*b*,*f*]azepine.

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 2.5–3.7
• **Microbial Enumeration Tests** ⟨61⟩ and **Test for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 10^1 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.

USP Monographs

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Carbamazepine RS
  USP Oxcarbazepine RS
  USP Oxcarbazepine Related Compound A RS
  N-Formyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide.
  $C_{16}H_{12}N_2O_3$ 280.28
  USP Oxcarbazepine Related Compound C RS
  Acridin-9(10*H*)-one.
  $C_{13}H_9NO$ 195.22

Oxcarbazepine Tablets

DEFINITION

Oxcarbazepine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Weigh about 840 mg of crushed Tablet powder, and add to a 50-mL volumetric flask. Add 45 mL of chloroform, and shake the flask for 30 min on a mechanical shaker. Add chloroform to volume, centrifuge, and collect the supernatant in a Petri dish. Evaporate the supernatant on a water bath at 60°. Dry the residue, and crush the residue thoroughly with potassium bromide in the ratio of 1:100.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 6.8 g/L of monobasic potassium phosphate in water. Add 2 mL of triethylamine. Adjust with phosphoric acid to a pH of 6.0.
  Diluent: Methanol and water (80:20)
  Mobile phase: Methanol, acetonitrile, and *Buffer* (22:16:62)
  Standard stock solution: 0.5 mg/mL of USP Oxcarbazepine RS in *Diluent*. Sonication may be used to aid in dissolution.
  Standard solution: 0.1 mg/mL of USP Oxcarbazepine RS in *Mobile phase* from the *Standard stock solution*
  Sample stock solution: Nominally equivalent to 1.2 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets, prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to 600 mg of oxcarbazepine, to a 500-mL volumetric flask. Add *Diluent* to fill 50% of the final volume. Sonicate for 15 min with intermittent swirling, cool to room temperature, and dilute with *Diluent* to volume. Pass this solution through a suitable 2-µm glass filter, and discard the first portion of the filtrate.
  Sample solution: 0.1 mg/mL of oxcarbazepine in *Mobile phase* from a portion of the filtrate obtained from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 215 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  Temperatures
  Sample: 5°
  Column: 50°
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium
  For Tablets labeled to contain 150 mg: 0.3% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated
  For Tablets labeled to contain 300 mg: 0.6% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated
  For Tablets labeled to contain 600 mg: 1.0% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated
  Apparatus 2: 60 rpm
  Times: 30 and 60 min
  Standard stock solution: 0.35 mg/mL of USP Oxcarbazepine RS in methanol
  Standard solution: Dilute the *Standard stock solution* with the corresponding *Medium* to obtain a final concentration of 0.0175 mg/mL of USP Oxcarbazepine RS.
  Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45-µm pore size. The volume of the solution under test withdrawn must be replaced by the same volume of corresponding *Medium*. Dilute with the appropriate *Medium* if necessary, according to the Tablet strength, to obtain a final concentration similar to that of the *Standard solution*.
  Instrumental conditions
  Mode: UV-Vis
  Analytical wavelength: 256 nm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 30 min (Q_{30}):

$$Q_{30} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

  Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 60 min (Q_{60}):

$$Q_{60} = [(A_U/A_S) \times (C_S/L) \times D \times V \times 100] + [Q_{30} \times (V_S/V)]$$

A_U = absorbance of the *Sample solution*

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A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
V_S = volume of the solution under test withdrawn (mL)

Tolerances: NLT 70% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium

For Tablets labeled to contain 150 mg: 0.3% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 300 mg: 0.6% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 600 mg: 1.0% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

Apparatus 2: 60 rpm

Times: 30 and 60 min

Standard stock solution: 3.3 mg/mL of USP Oxcarbazepine RS in methanol. [NOTE—This solution is stable for 22 h at 10°.]

Standard solution: Dilute the *Standard stock solution* with the corresponding *Medium*, according to the Tablet strength, to obtain a final concentration of (L/900) mg/mL, where L is the label claim in mg/Tablet.

Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45-µm pore size. The volume of the solution under test withdrawn must be replaced by the same volume of corresponding *Medium*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 304 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 30 min (Q_{30}):

$$Q_{30} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 60 min (Q_{60}):

$$Q_{60} = [(A_U/A_S) \times (C_S/L) \times D \times V \times 100] + [Q_{30} \times (V_S/V)]$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
V_S = volume of the solution under test withdrawn (mL)

Tolerances

For Tablets labeled to contain 150 or 300 mg: NLT 70% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

For Tablets labeled to contain 600 mg: NLT 50% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

[NOTE—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*. If methoxycarbamazepine is a potential degradation product, *Procedure 1* is recommended. If carbamazepinedione or dibenzazepinodione is a potential degradation product, *Procedure 2* is recommended.]

• **ORGANIC IMPURITIES, PROCEDURE 1**

Buffer and **Chromatographic system:** Proceed as directed in the *Assay*.

Diluent: Methanol and water (60:40)

Mobile phase: Methanol, acetonitrile, and *Buffer* (29:21:75)

System suitability solution: 0.5 mg/mL of USP Oxcarbazepine RS and 1.0 µg/mL of USP Carbamazepine RS in *Mobile phase*

Standard solution: 0.5 µg/mL of USP Oxcarbazepine RS in *Mobile phase*

Sample stock solution: Nominally equivalent to 1.2 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets, prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to 600 mg of oxcarbazepine, to a 500-mL volumetric flask. Add *Diluent* to fill 50% of the final volume. Sonicate for 15 min with intermittent swirling, cool to room temperature, and dilute with *Diluent* to volume. Pass this solution through a suitable 2-µm glass filter, and discard the first portion of the filtrate.

Sample solution: 0.5 mg/mL of oxcarbazepine from the *Sample stock solution* in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between oxcarbazepine and carbamazepine, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of oxcarbazepine from the *Standard solution*
C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxcarbazepine	1.0	1.0	—
Carbamazepine	1.6	1.5	0.5
Dibenzazepinone[a]	2.0	1.0	0.05
Methoxycarbamazepine[b]	2.3	1.3	0.05

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any unspecified individual degradation product	—	1.0	0.10
Total impurities	—	—	0.75

[a] 10(11*H*)-Oxo-5*H*-dibenz[*b*,*f*]azepine.
[b] 10-Methoxy-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Buffer A: 4.2 g of tris(hydroxymethyl)amino methane and 0.2 g of edetate disodium in 1 L of water

Buffer B: 18 g of tris(hydroxymethyl)amino methane and 0.9 g of edetate disodium in 1 L of water

Diluent: Acetonitrile and 1.8 g/L of ascorbic acid in water (1:99)

Solution A: Acetonitrile, tetrahydrofuran, and *Buffer A* (5:10:85)

Solution B: Acetonitrile, tetrahydrofuran, and *Buffer B* (70:10:20)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
33.0	30	70
33.1	95	5
45.0	95	5

System suitability stock solution: 1 µg/mL of USP Oxcarbazepine Related Compound C RS and 12 µg/mL of USP Carbamazepine RS in acetonitrile. Sonication may be used to aid in dissolution. [NOTE—The water bath temperature should not exceed 23°.]

System suitability solution: 0.05 µg/mL of USP Oxcarbazepine Related Compound C RS, 0.6 µg/mL of USP Carbamazepine RS, and 0.06 mg/mL of USP Oxcarbazepine RS prepared as follows. Transfer a suitable volume of *System suitability stock solution* to a volumetric flask containing 50% of the final volume of *Diluent*. Allow the solution to reach ambient temperature, and dilute with acetonitrile to volume.

Standard stock solution: 12 µg/mL of USP Carbamazepine RS in acetonitrile. Sonication may be used to aid in dissolution. [NOTE—The water bath temperature should not exceed 23°.]

Standard solution: 0.6 µg/mL of USP Carbamazepine RS from *Standard stock solution* prepared as follows. Transfer a suitable volume of *Standard stock solution* to a flask containing 50% of the final volume of *Diluent* and 20% of the final volume of acetonitrile. Allow the solution to reach ambient temperature, and dilute with acetonitrile to volume.

Sample stock solution: 1.5 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to about 375 mg of oxcarbazepine, to a 250-mL volumetric flask. Add 150 mL of acetonitrile, and sonicate for 15 min. Shake for 15 min, and dilute with acetonitrile to volume. Mix, and allow the suspension to settle for 30 min. Use the supernatant. [NOTE—The water bath temperature should not exceed 23°.]

Sample solution: 0.3 mg/mL of oxcarbazepine from the *Sample stock solution* prepared as follows. Transfer a suitable volume of *Sample stock solution* to a volumetric flask containing 50% of the final volume of *Diluent* and 20% of the final volume of acetonitrile. Allow the solution to warm to ambient temperature, and dilute with acetonitrile to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.0-mm × 25-cm; 5-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 20 µL
Temperatures
Sample: 5°
Column: 35°

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between oxcarbazepine related compound C and carbamazepine, *System suitability solution*
Relative standard deviation: NMT 15%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of carbamazepine from the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

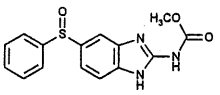
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carbamazepinedione[a]	0.72	0.70	0.2
Oxcarbazepine	1.0	1.0	—
Oxcarbazepine related compound C[b]	1.3	—	—
Carbamazepine	1.4	1.0	0.5
Dibenzazepinodione[c]	1.7	2.8	0.2
Any unspecified individual degradation product	—	1.0	0.1
Total impurities	—	—	1.0

[a] 10,11-Dioxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide.
[b] For system suitability and identification purposes only. Process impurity. Not included in total.
[c] 5*H*-Dibenzo[*b*,*f*]azepine-10,11-dione.

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Carbamazepine RS
  USP Oxcarbazepine RS
  USP Oxcarbazepine Related Compound C RS
  Acridin-9(10*H*)-one.
  $C_{13}H_9NO$ 195.22

Oxfendazole



$C_{15}H_{13}N_3O_3S$ 315.35
Carbamic acid, 5-(phenylsulfinyl)-1*H*-benzimidazol-2-yl-, methyl ester.
Methyl 5-(phenylsulfinyl)-2-benzimidazolecarbamate [53716-50-0].

» Oxfendazole contains not less than 98.0 percent and not more than 100.5 percent of $C_{15}H_{13}N_3O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Fenbendazole RS
USP Oxfendazole RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The appearance of the principal spot in the chromatogram of the *Test solution* corresponds to that in the chromatogram of *Standard solution 1*, as obtained in the test for *Related compounds*.

Loss on drying ⟨731⟩—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 105° for 2 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—
Diluent—Prepare a mixture of ethyl acetate and glacial acetic acid (4:1).
Standard solution 1—Dissolve a quantity of USP Oxfendazole RS in *Diluent* to obtain a solution having a concentration of 0.1 mg per mL.
Standard solution 2—Dissolve a quantity of USP Fenbendazole RS in *Diluent* to obtain a solution having a concentration of 0.05 mg per mL.
Standard solution 3—Prepare a mixture of *Standard solution 1* and *Standard solution 2* (1:2).
Test solution—Dissolve 25 mg of Oxfendazole in *Diluent*, dilute with *Diluent* to 5 mL, and mix.
Procedure—Separately apply 20 µL portions of *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and the *Test solution* to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate and glacial acetic acid (95:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, and allow to air-dry. Examine the plate under short-wavelength UV light: the chromatogram obtained from *Standard solution 3* shows two clearly separated principal spots. In the chromatogram obtained from the *Test solution*, no spot corresponding to fenbendazole is more intense than the spot in the chromatogram obtained from *Standard solution 2* (1%), and no spot other than the principal spot and no spot corresponding to fenbendazole is more intense than the spot in the chromatogram obtained from *Standard solution 1* (2%).
Assay—Dissolve about 300 mg of Oxfendazole, accurately weighed, in 3 mL of anhydrous formic acid. Add 40 mL of acetic anhydride, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 31.54 mg of $C_{15}H_{13}N_3O_3S$.

Oxfendazole Oral Suspension

» Oxfendazole Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of oxfendazole ($C_{15}H_{13}N_3O_3S$).

Packaging and storage—Preserve in tight containers, and protect from excessive heat.
Labeling—Label the Suspension to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Oxfendazole RS
Identification—The relative retention time of the oxfendazole peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
pH ⟨791⟩: between 4.3 and 4.9.

Assay—
Mobile phase—Prepare a solution of sodium acetate in water containing 2.5 mg per mL, and adjust with glacial acetic acid to a pH of 4.75 ± 0.1. Prepare a mixture of this solution and acetonitrile (800:225). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Prepare a solution in *Mobile phase* containing in each mL about 1.2 µg of methylparaben, 12 µg of sulfabenzamide, and 72 µg of USP Oxfendazole RS.
Internal standard solution—Prepare a solution of sulfabenzamide in *Mobile phase* containing about 0.3 mg per mL.
Standard preparation—Prepare a solution of USP Oxfendazole RS in methanol having a known concentration of about 900 µg per mL. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, add 4.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. This solution contains about 180 µg of USP Oxfendazole RS per mL.

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Assay preparation—Transfer an accurately measured volume of the Suspension, previously well-mixed and free from air bubbles, equivalent to about 450 mg of oxfendazole, to a 500-mL volumetric flask. Add about 30 mL of water, and swirl to disperse. Add about 300 mL of methanol, and dissolve with the aid of sonication. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, add 4.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

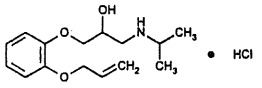
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, a guard column containing packing L2, and a 4.6-mm × 25-cm analytical column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for sulfabenzamide, 0.8 for methylparaben, and 1.0 for oxfendazole; the resolution, *R*, between the methylparaben peak and the oxfendazole peak is not less than 2.0; the column efficiency determined from the oxfendazole peak is not less than 2000 theoretical plates; and the relative standard deviation of replicate injections is not more than 1.5%. [NOTE—The detector sensitivity may be changed between the peaks to keep the responses on scale.]

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Calculate the quantity, in mg, of oxfendazole ($C_{15}H_{13}N_3O_3S$) in each mL of the Suspension taken by the formula:

$$2.5(C/V)(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Oxfendazole RS in the *Standard preparation*; V is the volume, in mL, of Suspension taken to prepare the *Assay preparation*; and R_U and R_S are the ratios of the oxfendazole peak response to the sulfabenzamide peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oxprenolol Hydrochloride



$C_{15}H_{23}NO_3 \cdot HCl$ 301.81

2-Propanol, 1-(*o*-allyloxyphenoxy)-3-isopropylamino-, hydrochloride.

1-(*o*-Allyloxyphenoxy)-3-isopropylamino-2-propanol hydrochloride [6452-73-9].

» Oxprenolol Hydrochloride contains not less than 98.5 percent and not more than 101.0 percent of $C_{15}H_{23}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Oxprenolol Hydrochloride RS

Clarity of solution—Dissolve 1 g in 10 mL of water: solution is clear.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: A solution of it responds to the tests for *Chloride* ⟨191⟩.

pH ⟨791⟩: between 4.0 and 6.0, in a solution (1 in 10).

Loss on drying ⟨731⟩—Dry about 3 g of it in vacuum at 60° for 6 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—

Diluting solvent—Prepare a mixture of chloroform and dehydrated alcohol (1:1).

Standard solution A—Prepare a solution of USP Oxprenolol Hydrochloride RS in *Diluting solvent* containing 20 mg per mL.

Standard solution B—Dilute an accurately measured volume of *Standard solution A* quantitatively, and stepwise if necessary, with *Diluting solvent* to obtain a solution containing 0.08 mg per mL.

Test solution—Transfer 200 mg of it to a 10-mL volumetric flask, dissolve in *Diluting solvent*, dilute with *Diluting solvent* to volume, and mix.

Procedure—Apply separate 5-µL portions of the *Test solution* and the *Standard solutions* to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, previously washed with methanol until the solvent front reaches the top of the plate, dried first in air and then at 100° for 20 minutes, and cooled in a desiccator. Allow the spots to dry. Line a suitable chromatographic chamber with filter paper, saturate the paper with 100 mL of a solvent system consisting of a mixture of ethyl acetate, glacial acetic acid, and water (15:5:5), and allow to stand for about 30 minutes. Place the plate in the chamber, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber and dry at 100° for 15 minutes. Spray the plate uniformly with a detection reagent consisting of a freshly prepared mixture of equal volumes of potassium ferricyanide solution (1 in 100) and ferric chloride solution (1 in 5). Dry the plate in a current of warm air for about 5 minutes or until a spot from *Standard solution B* is visible. Examine the chromatograms in ordinary light: the R_F value of the principal spot from the *Test solution* corresponds to that obtained from *Standard solution A*. No spot other than the principal spot obtained from the *Test solution* exceeds in size or intensity the principal spot obtained from *Standard solution B* (0.4%, corresponding to 0.2% of related compounds, the response factors for which are about double that of oxprenolol hydrochloride).

Assay—Dissolve about 450 mg of Oxprenolol Hydrochloride in 100 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass-calomel electrode system (with a salt bridge of a saturated solution of lithium chloride in glacial acetic acid). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.18 mg of $C_{15}H_{23}NO_3 \cdot HCl$.

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Delete the following:

▲Oxprenolol Hydrochloride Extended-Release Tablets

» Oxprenolol Hydrochloride Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of oxprenolol hydrochloride ($C_{15}H_{23}NO_3 \cdot HCl$).

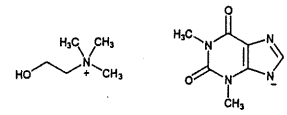
Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards 〈 11 〉 —
USP Oxprenolol Hydrochloride RS
Identification—Tablets respond to the *Identification* test under *Oxprenolol Hydrochloride Tablets.*

Dissolution 〈711〉—
Acid medium: 0.1 N hydrochloric acid; 900 mL.
Dissolution medium: simulated intestinal fluid TS (without enzyme); 900 mL.
Apparatus 1: 100 rpm.
Times: 1 hour in *Acid medium;* 1, 3, and 7 hours in *Dissolution medium.*
Procedure—Determine the amount of $C_{15}H_{23}NO_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorption at about 272 nm on the first solution under test, suitably diluted with *Acid medium,* in comparison with a Standard solution having a known concentration of USP Oxprenolol Hydrochloride RS in the same medium. Promptly transfer the basket containing the Tablet to *Dissolution medium.* After 1, 3, and 7 hours, respectively, remove 9.0 mL of the test solution and determine the amount of $C_{15}H_{23}NO_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorption at about 272 nm on the solution under test, suitably diluted with *Dissolution medium,* in comparison with a Standard solution having a known concentration of USP Oxprenolol Hydrochloride RS in the same medium. [NOTE—Replace the aliquots withdrawn for analysis with fresh portions of *Dissolution medium.*]
Tolerances—The percentages of the labeled amount of $C_{15}H_{23}NO_3 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1, in *Acid medium*	between 15% and 45%
1, in *Dissolution medium*	between 30% and 60%
3, in *Dissolution medium*	between 50% and 80%
7, in *Dissolution medium*	not less than 75%

Uniformity of dosage units 〈905〉: meet the requirements.
Procedure for content uniformity—Proceed as directed for *Procedure for the content uniformity* in the test for *Uniformity of dosage units* under *Oxprenolol Hydrochloride Tablets.*
Assay—Determine the mean value of the $C_{15}H_{23}NO_3 \cdot HCl$ contents of the Tablets as directed for *Uniformity of dosage units* under *Oxprenolol Hydrochloride Tablets.* ▲ (USP 1-May-2020)

Oxtriphylline



$C_{12}H_{21}N_5O_3$ 283.33
Ethanaminium, 2-hydroxy-*N,N,N*-trimethyl-, salt with 3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione;
Choline salt with theophylline (1:1) [4499-40-5].

DEFINITION
Oxtriphylline contains NLT 61.7% and NMT 65.5% of anhydrous theophylline ($C_7H_8N_4O_2$), calculated on the dried basis.

IDENTIFICATION
• **A.**
Sample solution: 50 mg/mL in water
Analysis: To 10 mL of the *Sample solution* add 5 mL of mercuric–potassium iodide TS.
Acceptance criteria: A pale yellow precipitate is formed (presence of choline).
• **B.**
Sample solution: 50 mg/mL in water
Analysis: To 10 mL of the *Sample solution* add 5 drops of 6 N ammonium hydroxide and 5 mL of silver nitrate TS.
Acceptance criteria: A gelatinous precipitate is formed, and it coagulates on heating (presence of theophylline).

ASSAY
• **THEOPHYLLINE**
Sample solution: The solution retained in the test for *Choline Content*
Analysis: Add 35 mL of silver nitrate TS to the *Sample solution,* and swirl gently to promote complete precipitation. Titrate with 0.1 N sodium hydroxide VS to a green endpoint. Each mL of 0.1 N sodium hydroxide is equivalent to 18.02 mg of theophylline ($C_7H_8N_4O_2$).
Acceptance criteria: 61.7%–65.5% on the dried basis

OTHER COMPONENTS
• **CHOLINE CONTENT**
Indicator solution: Dissolve 30 mg of methyl red in 100 mL of methanol, and add 15 mL of methylene blue solution (1 in 1000).
Sample solution: 900 mg of Oxtriphylline in 50 mL of water
Analysis: Add 4 drops of *Indicator solution* to the *Sample solution,* and titrate with 0.1 N sulfuric acid VS to a purple endpoint. Each mL of 0.1 N sulfuric acid is equivalent to 12.12 mg of choline ($C_5H_{15}NO_2$). Retain the final solution for the *Assay* for *Theophylline.*
Acceptance criteria: The content of choline ($C_5H_{15}NO_2$) is between 652 and 693 mg/g of theophylline ($C_7H_8N_4O_2$) found in the *Assay.*

IMPURITIES
• **RESIDUE ON IGNITION** 〈281〉: NMT 0.3%
• **CHLORIDE AND SULFATE,** *Chloride* 〈221〉
Standard: 0.15 mL of 0.020 N hydrochloric acid
Sample: 0.50 g
Acceptance criteria: 0.02%; the *Sample* shows no more chloride than corresponds to the *Standard.*
• **ORDINARY IMPURITIES** 〈466〉
Standard solution: Chloroform, alcohol, and formic acid (88:10:2)
Sample solution: Chloroform, alcohol, and formic acid (88:10:2)
Eluant: Chloroform, alcohol, and formic acid (88:10:2)

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Visualization: 1
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩**:** 185°–189°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 80° for 4 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxtriphylline RS

Oxtriphylline Extended-Release Tablets

DEFINITION
Oxtriphylline Extended-Release Tablets contain an amount of Oxtriphylline equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

IDENTIFICATION
- **A.** The retention time of the theophylline peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHY**
  Standard solution: 10 mg/mL of USP Oxtriphylline RS in methanol
  Sample solution: Nominally 10 mg/mL of oxtriphylline prepared as follows. Transfer a quantity of finely ground Tablets, equivalent to 100 mg of oxtriphylline, to a suitable test tube. Add 10 mL of methanol, shake on a vortex mixer for several min, and filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 10 µL
  Developing solvent system: Chloroform, alcohol, and formic acid (88:10:2)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.
  Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

ASSAY
- **PROCEDURE**
  Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 0.1 N potassium hydroxide to a pH of 5.8 ± 0.1.
  Mobile phase: Methanol and *Buffer* (1:4)
  System suitability solution: 60 µg/mL of USP Oxtriphylline RS and 30 µg/mL of theobromine in water
  Standard solution: 0.1 mg/mL of USP Oxtriphylline RS in water
  Sample stock solution: Place 10 Tablets in a 1000-mL volumetric flask. Add 700 mL of water, and heat on a steam bath, with occasional shaking, until the Tablets have disintegrated. Cool to room temperature, dilute with water to volume, and filter.
  Sample solution: Nominally 0.1 mg/mL of oxtriphylline, equivalent to 0.0636 mg/mL of theophylline, prepared by diluting the *Sample stock solution* with water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 275 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for theobromine and theophylline are about 0.7 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 3.0 between the theobromine and theophylline peaks, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of theophylline from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Oxtriphylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of anhydrous theophylline, 180.17
M_{r2} = molecular weight of oxtriphylline, 283.33

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Test 1 (for products labeled as 400-mg Tablets)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*. Proceed as directed for *Method B* under *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms*, except use *Acceptance Table 2*.
  Buffer: Transfer 27.22 g of monobasic potassium phosphate to a 4-L volumetric flask. Add 1 L of water and 816 mL of 0.2 N sodium hydroxide, and dilute with water to 3800 mL. Adjust with 0.2 N sodium hydroxide or phosphoric acid to a pH of 7.5, and dilute with water to volume.
  Medium: 0.1 N hydrochloric acid for the first hour, then *Buffer*; 900 mL
  Apparatus 2: 50 rpm
  Times: 1, 3, 5, and 7 h
  Standard solution: Prepare a solution having a known concentration of USP Oxtriphylline RS in *Medium*.
  Sample solution: A filtered portion of the solution under test, diluted with *Medium* if necessary
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum at about 248 nm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Tolerances: See *Table 1*.

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Table 1

Time (h)	Amount Dissolved
1	5%–30%
3	50%–70%
5	65%–85%
7	NLT 75%

Test 2 (for products labeled as 600-mg Tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. Proceed as directed for *Method B* under *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms*, except use *Acceptance Table 2*.
Buffer, Medium, Apparatus 2, Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed for *Test 1*.
Times: 1, 3, and 7 h
Tolerances: See *Table 2*.

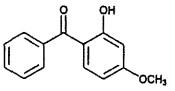
Table 2

Time (h)	Amount Dissolved
1	15%–40%
3	50%–70%
7	NLT 75%

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets to state both the content of oxtriphylline and the content of anhydrous theophylline. The labeling indicates the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxtriphylline RS

Oxybenzone



$C_{14}H_{12}O_3$ 228.24
Methanone, (2-hydroxy-4-methoxyphenyl)phenyl-;
2-Hydroxy-4-methoxybenzophenone [131-57-7].

DEFINITION
Oxybenzone contains NLT 97.0% and NMT 103.0% of oxybenzone ($C_{14}H_{12}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Methanol and water (70:30)
  Standard solution: 0.1 mg/mL of USP Oxybenzone RS in *Mobile phase*. Sonicate if necessary to dissolve.
  Sample solution: 0.1 mg/mL of Oxybenzone in *Mobile phase*. Sonicate if necessary to dissolve.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 289 nm
  Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of oxybenzone ($C_{14}H_{12}O_3$) in the portion of Oxybenzone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

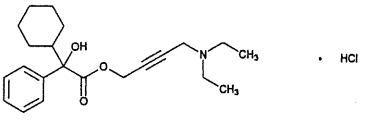
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxybenzone RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxybenzone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

SPECIFIC TESTS
- **CONGEALING TEMPERATURE** ⟨651⟩: NLT 62.0°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample in a vacuum at 40° for 2 h.
  Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxybenzone RS

Oxybutynin Chloride



$C_{22}H_{31}NO_3 \cdot HCl$ 393.95
Benzeneacetic acid, α-cyclohexyl-α-hydroxy-, 4-(diethyl amino)-2-butynyl ester hydrochloride, (±)-;
4-(Diethylamino)-2-butynyl (±)-α-phenylcyclohexaneglycolate hydrochloride [1508-65-2].

DEFINITION
Oxybutynin Chloride contains NLT 97.0% and NMT 102.0% of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: A solution containing 6.67 g/L of monobasic potassium phosphate and 8.55 g/L of dibasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (49:51)
Standard solution: 0.1 mg/mL of USP Oxybutynin Chloride RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Oxybutynin Chloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 7.5-cm; 3-µm or 3.5-µm packing L7
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) in the portion of Oxybutynin Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxybutynin Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

OTHER COMPONENTS

- **CHLORIDE CONTENT**

Sample: 600 mg of Oxybutynin Chloride, previously dried
Analysis: Dissolve the *Sample* in 100 mL of water, and add 5 mL of nitric acid. Titrate (see *Titrimetry* ⟨541⟩) with 0.1 N silver nitrate VS, using a platinum–silver chloride electrode system. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).
Acceptance criteria: 8%–10%

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability stock solution: 100 µg/mL each of USP Oxybutynin Related Compound B RS and USP Oxybutynin Related Compound C RS in *Mobile phase*
Standard stock solution: 1.0 mg/mL of USP Oxybutynin Chloride RS in *Mobile phase*
System suitability solution: Transfer 10.0 mL of the *System suitability stock solution* to a 100-mL volumetric flask, add 10.0 mL of *Standard stock solution*, and dilute with *Mobile phase* to volume.
Standard solution: 7.5 µg/mL of USP Oxybutynin Chloride RS from *Standard stock solution* in *Mobile phase*
Sample solution: 5.0 mg/mL of Oxybutynin Chloride in *Mobile phase*
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 1.1 between oxybutynin related compound B and oxybutynin related compound C
Relative standard deviation: NMT 2.0% for the oxybutynin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for a total time of NLT twice the retention time of the oxybutynin peak.
Calculate the percentage of each impurity in the portion of Oxybutynin Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of oxybutynin from the *Standard solution*
C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxybutynin Chloride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxybutynin related compound A[a]	0.08	1.4	0.5
Diphenyl analog of oxybutynin chloride[b]	0.37	2.7	0.1
Oxybutynin related compound B[c]	0.65	1.3	1.0
Oxybutynin related compound C[d]	0.79	1.0	1.0
Oxybutynin	1.0	—	—
Cyclohexenyl analog of oxybutynin chloride[e]	1.8	0.4	1.0
Ethylpropyl analog of oxybutynin chloride[f]	1.9	1.0	0.1
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] Phenylcyclohexylglycolic acid (cyclohexylmandelic acid, or CHMA).
[b] 4-(Diethylamino)but-2-ynyl 2-hydroxy-2,2-diphenylacetate.
[c] Methyl ester of phenylcyclohexylglycolic acid (methyl ester of cyclohexylmandelic acid, or CHMME).
[d] Methylethyl analog of oxybutynin chloride (4-(ethylmethylamino) but-2-ynyl (±)-2-cyclohexyl-2-hydroxy-2-phenylacetate).
[e] 4-(Diethylamino)but-2-ynyl (±)-2-(cyclohex-3-enyl)-2-cyclohexyl-2-hydroxyacetate.
[f] 4-(Ethylpropylamino)but-2-ynyl (±)-2-cyclohexyl-2-hydroxy-2-phenylacetate.

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SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 3%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Oxybutynin Chloride RS
USP Oxybutynin Related Compound B RS
Methyl ester of phenylcyclohexylglycolic acid, or CHMME (cyclohexyl mandelic acid methyl ester).
USP Oxybutynin Related Compound C RS
Methylethyl analog of oxybutynin chloride, or 4-(ethylmethylamino) but-2-ynyl (±) 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride.

Oxybutynin Chloride Oral Solution

» Oxybutynin Chloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{22}H_{31}NO_3 \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Oxybutynin Chloride RS
Identification—Place a volume of Oral Solution, equivalent to about 50 mg of oxybutynin chloride, in a separator, and extract with 10 mL of chloroform. The extract so obtained responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, methanol being used as the developing solvent, and iodine vapor being used to visualize the spots.

Assay—
pH 4 Phosphate buffer—Place 38 mL of 0.2 M dibasic sodium phosphate in a 100-mL volumetric flask. Dilute with 0.1 M citric acid to volume, and mix. Adjust the pH, if necessary, with either the dibasic sodium phosphate solution or the citric acid solution.
pH 5.6 Phosphate buffer—Place 58 mL of 0.2 M dibasic sodium phosphate in a 100-mL volumetric flask. Dilute with 0.1 M citric acid to volume, and mix. Adjust the pH, if necessary, with either the dibasic sodium phosphate solution or the citric acid solution.
Bromocresol green solution—Transfer 125 mg of bromocresol green to a 25-mL volumetric flask, dissolve in 3.5 mL of 0.05 N sodium hydroxide, dilute with water to volume, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Oxybutynin Chloride RS in 0.05 N sulfuric acid to obtain a solution having a known concentration of about 100 µg per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of oxybutynin chloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Separately transfer 10.0 mL of the *Standard preparation* and the *Assay preparation* to separate 125-mL separators. Add 20 mL of *pH 4 Phosphate buffer* to each separator, and extract each solution with a 25-mL portion of chloroform. [Note—Allow at least 10 minutes for the layers to separate.] Collect the chloroform extracts in respective 125-mL separators, each containing a mixture of 2 mL of *pH 5.6 Phosphate buffer* and 1 mL of *Bromocresol green solution*. Shake the separators, and filter the chloroform extracts through rayon pledgets, collecting the extracts in respective 100-mL volumetric flasks. Repeat the double extractions with 25-mL portions of chloroform. Wash the rayon pledgets with chloroform, collecting the washings in the respective 100-mL volumetric flasks. Dilute both solutions with chloroform to volume, and mix. Concomitantly determine the absorbances of both solutions at the wavelength of maximum absorbance at about 415 nm, with a suitable spectrophotometer, against a blank prepared using 10 mL of 0.05 N sulfuric acid treated in the same manner as the *Standard preparation* and the *Assay preparation*. Calculate the quantity, in mg, of $C_{22}H_{31}NO_3 \cdot HCl$ in each mL of Oral Solution taken by the formula:

$$(0.1C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Oxybutynin Chloride RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Oxybutynin Chloride Tablets

DEFINITION
Oxybutynin Chloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{22}H_{31}NO_3 \cdot HCl$.

IDENTIFICATION
• **Thin-Layer Chromatographic Identification Test** ⟨201⟩
Sample solution: Add a portion of powdered Tablets, equivalent to about 50 mg of oxybutynin chloride, to 10 mL of chloroform. Mix for two minutes, and centrifuge. Use the supernatant layer.
Developing solvent system: Methanol
Visualization: Iodine vapor

ASSAY
• **Procedure**
Solution A: Methanol, water, and triethylamine (800: 3200: 0.9). Adjust with phosphoric acid to a pH of 3.5 ± 0.05.
Mobile phase: Acetonitrile and *Solution A* (1:4)
Standard solution: 0.05 mg/mL of USP Oxybutynin Chloride RS in *Mobile phase*
Sample solution: Transfer an amount of powdered Tablets (from NLT 20 Tablets) nominally equivalent to 50 mg of oxybutynin chloride to a 1000-mL volumetric flask. Add about 400 mL of *Mobile phase*, sonicate for about 10 min, shake by mechanical means for about 45 min, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 203 nm
Column: 4-mm × 30-cm; packing L10
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{31}NO_3 \cdot HCl$ in the portion of Tablets taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Dilute with *Medium* if necessary.
Analysis: Determine the amount of $C_{22}H_{31}NO_3 \cdot HCl$ dissolved using the method set forth in the *Assay*, making any necessary modifications to the concentration of the *Standard solution* to correspond to that of the solution under test and injecting 100 µL of both solutions.
Tolerances: NLT 80% (Q) of the labeled amount of $C_{22}H_{31}NO_3 \cdot HCl$ is dissolved.

Test 2: If the products complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: 5 µg/mL of USP Oxybutynin Chloride RS in *Medium*. This solution is stable for 5 days at ambient conditions.
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter, discarding the first few mL.
Mobile phase: Water, acetonitrile, and phosphoric acid (760:240:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 203 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxybutynin chloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of oxybutynin chloride in the *Standard solution*
L = Tablet label claim (mg)
V = volume of *Medium* (mL), 900

Tolerances: NLT 80% (Q) of the labeled amount of oxybutynin chloride is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxybutynin Chloride RS

Oxybutynin Chloride Extended-Release Tablets

DEFINITION

Oxybutynin Chloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)
Standard: Dissolve 15 mg of USP Oxybutynin Chloride RS in 5 mL of water. Adjust with 0.1 N sodium hydroxide to a pH of between 7 and 8. Extract the solution twice with 10 mL of ether. Combine the extracts, evaporate the ether, and dry under vacuum over silica gel for at least 30 min. Redissolve the dried residue in a small amount of acetone, transfer the solution to an IR salt plate, and evaporate to cast a thin film.
Sample: Add a quantity of finely powdered Tablets, equivalent to about 15 mg of oxybutynin chloride, to 5 mL of water per Tablet. Mix for 1 min. Adjust with 0.1 N sodium hydroxide to a pH between 7 and 8. Extract the solution twice with 10 mL of ether. Combine the extracts, evaporate the ether, and dry under vacuum over silica gel for at least 30 min. Redissolve the dried residue in a small amount of acetone, transfer the solution to an IR salt plate, and evaporate to cast a thin film.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Use water adjusted with phosphoric acid to a pH of 3.5.
Solution A: Methanol and acetonitrile (1:1)
Mobile phase: Acetonitrile, triethylamine, and water (700:3:1300). Adjust with phosphoric acid to a pH of 3.9.
Impurity stock solution: 0.11 mg/mL of USP Oxybutynin Related Compound A RS in acetonitrile
Standard stock solution: 0.37 mg/mL of USP Oxybutynin Chloride RS in acetonitrile
System suitability solution: Transfer 10 mL of the *Standard stock solution* and 1 mL of the *Impurity stock solution* to a 100-mL volumetric flask, and dilute with *Diluent* to volume.
Standard solution: 0.1 mg/mL of USP Oxybutynin Chloride RS in *Diluent* from the *Standard stock solution*
Sample solution
For Tablets that contain 5 mg of oxybutynin chloride: Place 10 Tablets in a 500-mL volumetric flask, add 150 mL of *Solution A*, and stir for at least 4 h or until

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dissolved. Dilute with *Diluent* to volume. Mix thoroughly, centrifuge, and use the clear supernatant.

For Tablets that contain 10 mg or more of oxybutynin chloride: Place 10 Tablets in a 1000-mL volumetric flask, add 300 mL of *Solution A*, and stir for at least 4 h or until dissolved. Dilute with *Diluent* to volume. If necessary, make a further dilution with *Diluent* to obtain a solution having a final concentration equivalent to 0.1 mg/mL of oxybutynin chloride. Mix thoroughly, centrifuge, and use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; packing L11
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for oxybutynin and oxybutynin related compound A are about 1.0 and 1.6, respectively.]
Suitability requirements
Resolution: NLT 1.5 between oxybutynin and oxybutynin related compound A
Tailing factor: Greater than 0.75 and NMT 2.5 for each peak
Relative standard deviation: NMT 3% for each compound for six replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxybutynin chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Test 1
Medium: Simulated gastric fluid without enzyme; 50 mL
Apparatus 7: See *Drug Release* ⟨724⟩, 30 cycles/min; 2–3-cm amplitude, at 37.0 ± 0.5°
Times: 4, 10, and 24 h
Solution A: 4.83 g/L of monobasic sodium phosphate in water. Add 2.3 mL/L of triethylamine, and adjust with phosphoric acid to a pH of 2.2 ± 0.2.
Mobile phase: Acetonitrile and *Solution A* (7:13)
Solution B: To 1 L of water add phosphoric acid dropwise to a pH of 3.5, and mix well.
Standard stock solutions: 250, 300, and 350 µg/mL of USP Oxybutynin Chloride RS in acetonitrile
Standard solutions: Prepare a series of dilutions of the *Standard stock solutions* in *Solution B* having final concentrations similar to those expected in the *Sample solution*.
System suitability solution: Use a medium range *Standard solution* of USP Oxybutynin Chloride RS.
Sample solution: Use portions of the solution under test. If the solution is cloudy, centrifuge at 2000 rpm for 10 min, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 5-cm; packing L11
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Tailing factor: Greater than 0.5 and less than 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solutions* and *Sample solution*
Construct a calibration curve by plotting the peak response versus concentration of the *Standard solutions*. A weighing factor, $1/x$, is applied to the regression line of the calibration curve to enhance the accuracy of the low standard concentrations. Determine the percentage of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved in each interval from a linear regression analysis of the calibration curve.
Tolerances: See *Tables 1* and *2*.

Table 1. For Tablets Labeled to Contain 5 or 10 mg of Oxybutynin Chloride

Time (h)	Amount Dissolved
4	NMT 20%
10	34.5%–59.5%
24	NLT 80%

Table 2. For Tablets Labeled to Contain 15 mg of Oxybutynin Chloride

Time (h)	Amount Dissolved
4	NMT 20%
10	34.5%–59.5%
24	NLT 75%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Acid stage medium: Simulated gastric fluid, without enzymes, pH 1.2 ± 0.05; 250 mL (first row)
Buffer stage medium: Simulated intestinal fluid, without enzymes, pH 6.8 ± 0.1; 250 mL (rows 2–4)
Apparatus 3: 25 dips/min; 20-mesh polypropylene screen on top and bottom; 30 s drip time
Times: 2 h in the *Acid stage medium* (first row); 4, 8, and 16 h (corresponding to 2, 6, and 14 h after changing the medium) in the *Buffer stage medium* (rows 2–4)
Solution A: Transfer 1 mL of triethylamine to 1000 mL of water. Adjust with phosphoric acid to a pH of 3.50 ± 0.05.
Mobile phase: Acetonitrile and *Solution A* (4:1)
Standard stock solution: 0.2 mg/mL of USP Oxybutynin Chloride RS in *Acid stage medium*
Working standard solution: Transfer 5.0 mL of the *Standard stock solution* for Tablets labeled to contain 5 mg,

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transfer 10 mL for Tablets labeled to contain 10 mg, or transfer 15 mL for Tablets labeled to contain 15 mg to a 100-mL volumetric flask. Dilute with *Buffer stage medium* to volume.

Sample solution: Centrifuge a portion of the solution under test at approximately 3000 rpm for 10 min. Use the supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 203 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1.5 mL/min
Injection volume: 25 µL

System suitability
Sample: *Working standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%

Analysis
Samples: *Working standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at each time point (C_{T2}, C_{T4}, C_{T8}, C_{T16}):

$$C_i = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Working standard solution*
C_S = concentration of the *Working standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 250 mL
C_{T2} = percentage dissolved at 2 h, C_2
C_{T4} = percentage dissolved at 4 h, $C_2 + C_4$
C_{T8} = percentage dissolved at 8 h, $C_2 + C_4 + C_8$
C_{T16} = percentage dissolved at 16 h, $C_2 + C_4 + C_8 + C_{16}$

Tolerances: See *Tables 3* and *4*.

Table 3. For Tablets Labeled to Contain 5 or 10 mg of Oxybutynin Chloride

Time (h)	Amount Dissolved
2	0%–10%
4	10%–30%
8	40%–65%
16	NLT 80%

Table 4. For Tablets Labeled to Contain 15 mg of Oxybutynin Chloride

Time (h)	Amount Dissolved
2	0%–10%
4	10%–30%
8	35%–65%
16	NLT 75%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: Simulated gastric fluid without enzyme; 50 mL
Apparatus 7: See *Drug Release* ⟨724⟩. Use acrylic rods. 30 dips/min, 37.0 ± 0.5°, 10 s drip time. Dip time interval: row 1, 1 h; row 2, 3 h; row 3, 6 h; row 4, 5 h; row 5, 9 h.
Times: 4, 10, and 24 h
pH 2.3 phosphate buffer: 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or 2 N potassium hydroxide to a pH of 2.30 ± 0.05.
Standard solution: (L/200) mg/mL of USP Oxybutynin Chloride RS in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable nylon filter of 0.45-µm pore size, discarding the first few mL.
Mobile phase: *pH 2.3 phosphate buffer* and acetonitrile (7:3)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; packing L10
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount, in mg, of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at each time interval:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 50 mL

Calculate the percentage of the labeled amount of oxybutynin dissolved:

$$\text{Result} = \Sigma(\text{amount dissolved at current time interval} + \text{amount dissolved at previous time intervals}) \times 100/L$$

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved
4	NMT 25%
10	40%–65%
24	NLT 75%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.
Acid stage medium: 0.1 N hydrochloric acid; 900 mL
Buffer stage medium: pH 6.0 sodium phosphate buffer with 0.2% of sodium lauryl sulfate; 900 mL

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Apparatus 2: 50 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWHT-2S from www.QLA-LLC.com.]
Times: 2 h in the *Acid stage medium*; 4, 6, and 14 h (corresponding to 2, 4, 12 h after changing the medium) in the *Buffer stage medium*
Standard solution: (*L*/1000) mg/mL of USP Oxybutynin Chloride RS in *Buffer stage medium*, where *L* is the label claim, in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size.
pH 3.5 phosphate buffer: 6.94 g/L of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 3.50 ± 0.05.
Mobile phase: *pH 3.5 phosphate buffer* and acetonitrile (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; packing L7
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) in mg/mL of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) at each time point (*i*):

$$C_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved ($Q_i$) at each time point (*i*):
At $i = 1$

$$Q_1 = (C_1 \times V/L) \times 100$$

At $i = 2$ to n

$$\frac{(C_1 \times 900) + \Sigma_{j=2}^{n-1} C_j V_s + C_n \times [900 - (n-2)V_s]}{L} \times 100$$

i = 1, 2, ..., n
j = 2, 3, ..., n–1
C_i = concentration of oxybutynin chloride in the *Sample solution* at time point *i* (mg/mL)
C_j = concentration of oxybutynin chloride in the *Sample solution* at time point 2 through n–1 (mg/mL)
V_S = sampling volume (mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved
2	NMT 10%
4	10%–40%
6	40%–75%
14	NLT 85%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.
Medium: Acetate buffer pH 4.5, prepared as follows. Transfer 2.99 g of sodium acetate to a 1000-mL volumetric flask, dissolve in 700 mL of water, adjust with glacial acetic acid to a pH of 4.5, and dilute with water to volume; 900 mL.
Apparatus 2: 75 rpm
Times: 2, 8, 12, and 24 h
Standard stock solution: 0.28 mg/mL of USP Oxybutynin Chloride RS in acetonitrile. Use sonication, if necessary.
Standard solution: (*L*/900) mg/mL of USP Oxybutynin Chloride RS in *Medium*, where *L* is the label claim, in mg/Tablet, from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size, discarding the first few mL of the filtrate. Replace the portion of solution withdrawn with an equal volume of *Medium*.
pH 3.5 phosphate buffer: 6.94 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.50 ± 0.05.
Mobile phase: *pH 3.5 phosphate buffer* and acetonitrile (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i), in mg/mL, of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

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$$Result_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$Result_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$Result_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of oxybutynin chloride in the portion of the sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved
2	NMT 10%
8	30%–50%
12	55%–75%
24	NLT 85%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 6*.
Medium: Simulated gastric fluid without enzyme; 50 mL
Apparatus 7: See *Drug Release* ⟨724⟩; ▲each Tablet is glued to a suitable rod▲ (RB 1-Apr-2018) with water insoluble glue. At the end of each specified test interval, the systems are transferred to the next row of new tubes containing 50 mL of fresh *Medium*, 30 cycles/min; 2–3 cm amplitude.
Times: 4, 10, and 24 h
Calculate the percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved by using the following method.
Buffer: 4.83 g/L of monobasic sodium phosphate in water. Add 2.3 mL/L of triethylamine, and adjust with phosphoric acid to a pH of 2.2 ± 0.2.
Mobile phase: Acetonitrile and *Buffer* (25:75)
Diluent: To 1 L of water add phosphoric acid dropwise to a pH of 3.5 and mix well.
Standard stock solution: 0.5 mg/mL of USP Oxybutynin Chloride RS in acetonitrile
Standard solution: 0.05 mg/mL of USP Oxybutynin Chloride RS in *Diluent* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate. Dilute with *Diluent*, if necessary, to obtain a solution with a concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 5-cm; 5-µm packing L11
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.5–2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i), in mg/mL, of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 8*:

$$C_i = (r_U/r_S) \times C_S$$

r_U = peak response of oxybutynin from the *Sample solution*
r_S = peak response of oxybutynin from the *Standard solution*
C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at each time point shown in *Table 8*:

$$Result_1 = C_1 \times V \times D \times (1/L) \times 100$$

$$Result_2 = (C_2 + C_1) \times V \times D \times (1/L) \times 100$$

$$Result_3 = (C_1 + C_2 + C_3) \times V \times D \times (1/L) \times 100$$

C_i = concentration of oxybutynin chloride in the portion of sample withdrawn at time point i (mg/mL)
V = volume of *Medium*, 50 mL
D = dilution factor for the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved (%)
4	NMT 20
10	35–60
24	NLT 80

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 7*.
Acid stage medium: 0.1 N hydrochloric acid; 900 mL
Buffer stage medium: pH 6.0 sodium phosphate buffer with 0.2% of sodium lauryl sulfate; 900 mL
Apparatus 2: 50 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWHT-2S from www.QLA-LLC.com.]
Times: 2 h in the *Acid stage medium*; 4, 8, and 16 h (corresponding to 2, 6, 14 h after changing the medium) in the *Buffer stage medium* for 5 mg Tablets and 6, 10, 16 h (corresponding to 4, 8, 14 h after changing the medium) in the *Buffer stage medium* for 10 mg and 15 mg Tablets.
Procedure: After 2 h in the *Acid stage medium*, withdraw a sample from the solution, and filter. Replace the *Acid stage medium* with the *Buffer stage medium*, and run the test for the times specified.
Buffer: 6.94 g/L of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 3.50 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (1:1)

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Standard solution: 0.01 mg/mL of USP Oxybutynin Chloride RS in *Buffer stage medium*
Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved in the *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)
V = volume of the *Acid stage medium*, 900 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point i during the buffer stage:

$$C_i = (r_i/r_S) \times C_S$$

r_i = peak response from the *Sample solution* at time point i
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at each time point i during the buffer stage:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of oxybutynin chloride in the *Sample solution* withdrawn at time point i (mg/mL)
V = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point i during the buffer stage (mL)

Tolerances: See *Tables 9* and *10*.

Table 9. For Tablets Labeled to Contain 5 mg of Oxybutynin Chloride

Time (h)	Amount Dissolved (%)
2	NMT 10
4	15–35
8	40–70
16	NLT 70

Table 10. For Tablets Labeled to Contain 10 and 15 mg of Oxybutynin Chloride

Time (h)	Amount Dissolved (%)
2	NMT 10
6	35–60
10	60–85
16	NLT 80

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 8:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 8*.
Acid stage medium: Simulated gastric fluid, without enzymes, pH 1.2; 250 mL (first row)
Buffer stage medium: Simulated intestinal fluid, without enzymes, pH 6.8; 250 mL (rows 2–4)
Apparatus 3: 25 dips/min; 20-mesh polypropylene screen on top and bottom; 30 s drip time
Times: 2 h in the *Acid stage medium* (first row); 4, 8, and 16 h (corresponding to 2, 6, and 14 h after changing the medium) in the *Buffer stage medium* (rows 2–4)
Buffer: 4.83 g/L of monobasic sodium phosphate in water. Add 2.3 mL/L of triethylamine, and adjust with diluted phosphoric acid to a pH of 4.0.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard stock solution: 0.2 mg/mL of USP Oxybutynin Chloride RS in *Acid stage medium*
Standard solution: Transfer volume of the *Standard stock solution* specified in *Table 11* to a 100-mL volumetric flask and dilute with *Buffer stage medium* to volume.

Table 11

Tablet Strength (mg)	Volume of Standard stock solution (mL)	Final Volume (mL)
5	5.0	100.0
10	10.0	100.0
15	15.0	100.0

Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size, discarding the first few milliliters.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 5-cm; 5-µm packing L7
Column temperature: 35°

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Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the total percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at each time point (C_{T2}, C_{T4}, C_{T8}, C_{T16}):

$$C_i = (r_U/r_S) \times (C_S/L) \times V \times 100$$

C_i = percentage of oxybutynin chloride in the *Sample solution* withdrawn at time point *i*
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of ▲USP Oxybutynin Chloride RS▲ (ERR 1-Dec-2018) in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 250 mL
C_{T2} = percentage dissolved at 2 h, C_{T2}
C_{T4} = percentage dissolved at 4 h, $C_{T2} + C_{T4}$
C_{T8} = percentage dissolved at 8 h, $C_{T2} + C_{T4} + C_{T8}$
C_{T16} = percentage dissolved at 16 h, $C_{T2} + C_{T4} + C_{T8} + C_{T16}$

Tolerances: See *Table 12*.

Table 12

Time (h)	Amount Dissolved (%)
2	NMT 10
4	5–25
8	34–59
16	NLT 80

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Apr-2018)

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Diluent, Solution A, Mobile phase, Impurity stock solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Impurity standard solution: 1 µg/mL of USP Oxybutynin Related Compound A RS in *Diluent* from the *Impurity stock solution*
Analysis
Samples: *Impurity standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

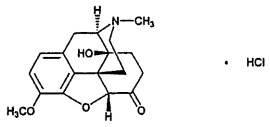
r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Impurity standard solution*
C_S = concentration of USP Oxybutynin Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

[NOTE—Disregard any peak less than 0.1%.]
Acceptance criteria
Individual impurities: NMT 1% of oxybutynin related compound A is found.
Total impurities: NMT 2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxybutynin Chloride RS
USP Oxybutynin Related Compound A RS
Phenylcyclohexylglycolic acid.
$C_{14}H_{18}O_3$ 234.30

Oxycodone Hydrochloride



$C_{18}H_{21}NO_4 \cdot HCl$ 351.82
Morphinan-6-one, 4,5-epoxy-14-hydroxy-3-methoxy-17-methyl-, hydrochloride, (5α)-;
4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride [124-90-3].

DEFINITION

Oxycodone Hydrochloride contains NLT 97.0% and NMT 103.0% of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

Sample: Dissolve 250 mg of Oxycodone Hydrochloride in 25 mL of water. Render 25 mL of the resulting solution with 6 N ammonium hydroxide. Allow the mixture to stand until a precipitate is formed. Filter, wash the precipitate with 50 mL of cold water, and dry at 105° for 2 h.
Acceptance criteria: Meets the requirements

- **B.** The retention time of the oxycodone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 0.005 M sodium 1-hexanesulfonate, methanol, triethylamine, and phosphoric acid (900:100:2:5). Adjust with 50% sodium hydroxide solution to a pH of 2.5 ± 0.1 and filter.
System suitability solution: 13 µg/mL of USP Codeine Phosphate RS and 9 µg/mL of USP Oxycodone RS in *Mobile phase*

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Standard solution: 0.9 mg/mL of USP Oxycodone RS in *Mobile phase*
Sample solution: 1 mg/mL of Oxycodone Hydrochloride in *Mobile phase*. [NOTE—Pass a portion of this solution through a filter of 0.5-µm or finer pore size, and use the filtrate as the *Sample solution*.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 206 nm
Column: 3.9-mm × 15-cm; 4-µm packing L7
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of oxycodone
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for codeine and oxycodone are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between codeine and oxycodone, *System suitability solution*
Tailing factor: 0.75–1.25, *Standard solution*
Relative standard deviation: NMT 1.10%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxycodone hydrochloride, 351.82
M_{r2} = molecular weight of oxycodone base, 315.37

Acceptance criteria: 97.0%–103.0% on the anhydrous, solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.05%
[NOTE—Use of sulfuric acid is omitted.]
[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*.*Procedure 1* is recommended if 8β-hydroxyoxycodone (7,8-dihydro-8β-14-dihydroxycodeinone) is a potential impurity.]

• **ORGANIC IMPURITIES, PROCEDURE 1**
Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxymorphone	0.31	0.15
Noroxymorphone[a]	0.33	0.15
10-Hydroxyoxycodone[b]	0.53	0.15
6-α Oxycodol[c]	0.67	0.25
8β-Hydroxyoxycodone (7,8-dihydro-8β-14-dihydroxycodeinone)[d]	0.71	0.15
Oxycodone	1.00	—
Hydrocodone	1.19	0.15
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

[a] 4,5α-Epoxy-3,14-dihydroxymorphinan-6-one.
[b] 4,5α-Epoxy-10α,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one.
[c] 4,5α-Epoxy-3-methoxy-17-methylmorphinan-6α,14-diol.
[d] 4,5α-Epoxy-8β,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one.

• **ORGANIC IMPURITIES, PROCEDURE 2**
Buffer: Mix 4.0 mL of heptafluorobutyric acid with 2000 mL of water and adjust with ammonium hydroxide to a pH of 2.3 ± 0.1.
Solution A: Methanol and *Buffer* (23:77)
Solution B: Methanol, tetrahydrofuran, and *Buffer* (20:3:77)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
30	0	100
55	0	100
55.1	100	0
65	100	0

Diluent: Trifluoroacetic acid and water (3:1000)
System suitability solution: 0.0067 mg/mL each of USP Hydrocodone RS and USP Oxycodone Related Compound A RS, and 3.0 mg/mL of USP Oxycodone Hydrochloride RS in *Diluent*
Standard solution: 0.0067 mg/mL of USP Hydrocodone RS in *Diluent*
Sample solution: 3.0 mg/mL of Oxycodone Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Column temperature: 38°
Flow rate: 0.8 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between oxycodone and hydrocodone; NLT 1.0 between hydrocodone and

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oxycodone related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of hydrocodone from the *Standard solution*

C_S = concentration of USP Hydrocodone RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of hydrocodone hydrochloride, 335.83

M_{r2} = molecular weight of hydrocodone, 299.36

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any peaks below 0.03%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxymorphone hydrochloride	0.54	0.93	0.15
1-Hydroxyoxycodone hydrochloride[a]	0.69	1.00	0.15
6-Oxycodol hydrochloride[b]	0.79	1.16	0.25
Oxycodone hydrochloride	1.00	—	—
Hydrocodone hydrochloride	1.14	1.00	0.50
14-Hydroxycodeinone hydrochloride (oxycodone related compound A hydrochloride)[c]	1.18	0.99	0.25
Noroxycodone hydrochloride[d]	1.26	0.94	0.50
Individual unspecified impurity	—	—	0.10
Total impurities	—	—	1.5

[a] 4,5α-Epoxy-1,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

[b] 4,5α-Epoxy-3-methoxy-17-methylmorphinan-6,14-diol hydrochloride.

[c] 4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one hydrochloride (oxycodone related compound A hydrochloride salt).

[d] 4,5α-Epoxy-14-hydroxy-3-methoxymorphinan-6-one hydrochloride.

SPECIFIC TESTS

- **CONTENT OF CHLORIDE**

Sample solution: 6 mg/mL of Oxycodone Hydrochloride in methanol

Analysis: To 50 mL of the *Sample solution*, add 5 mL of glacial acetic acid and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each milliliter of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.

Acceptance criteria: 9.8%–10.4% on the anhydrous, solvent-free basis

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 25 mg/mL of Oxycodone Hydrochloride in water on the anhydrous, solvent-free basis

Acceptance criteria: −137° to −149°

- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 7.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Codeine Phosphate RS
USP Hydrocodone RS
USP Oxycodone RS
USP Oxycodone Hydrochloride RS
USP Oxycodone Related Compound A RS
Also known as 14-Hydroxycodeinone; 4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one.
$C_{18}H_{19}NO_4$ 313.35

Oxycodone Hydrochloride Oral Solution

DEFINITION

Oxycodone Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$).

IDENTIFICATION

- **A.**

Sample solution: Transfer an amount equivalent to 15 mg of oxycodone from Oral Solution to a separatory funnel, add 10 mL of 0.01 N hydrochloric acid, and extract with four 40-mL portions of chloroform, collecting the chloroform extracts in a second separator. Wash the combined chloroform extracts with 5 mL of 0.01 N hydrochloric acid, and discard the chloroform layer. Combine the acidic wash with the aqueous solution remaining in the first separator and adjust with 6 N ammonium hydroxide to a pH of 9.5 ± 0.5. Extract with one 50-mL and two 20-mL portions of chloroform, and filter the chloroform extracts through chloroform-washed cotton, collecting the filtrate in a 100-mL volumetric flask. Dilute with water-saturated chloroform to volume, and mix.

Standard solution: Prepare a solution using 12 mg of USP Oxycodone RS and 25 mL of 0.01 N hydrochloric acid, and proceed as directed above, beginning with "extract with four 40-mL portions of chloroform".

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, concomitantly measured.

- **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: Evaporate 5 mL of the *Standard solution* obtained from *Identification* test *A* just to dryness. Dissolve the residue in 1.0 mL of chloroform.

Sample solution: Evaporate 5 mL of the *Sample solution* obtained from *Identification* test *A* just to dryness. Dissolve the residue in 1.0 mL of chloroform.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Acetone, toluene, ether, and ammonium hydroxide (6: 4: 1: 0.3)

Analysis

Samples: *Standard solution* and *Sample solution*

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Develop the plate until the solvent front has moved about three-fourths of the length of the plate, remove it, mark the solvent front, allow the solvent to evaporate, and spray with iodoplatinate TS.

Acceptance criteria: The principal spot from the *Sample solution* corresponds in color, size, and R_F value to that from the solution from the *Standard solution*, and no other spots are observed.

- **C.** The retention time of the oxycodone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, 0.01 M sodium 1-hexanesulfonate, and glacial acetic acid (25:74:1). Adjust with 5 N sodium hydroxide to a pH of 3.5.

Standard solution: 0.045 mg/mL of USP Oxycodone RS in *Mobile phase*

Sample solution: Transfer an amount equivalent to 5 mg of oxycodone hydrochloride from Oral Solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this mixture through a filter having a 0.5-µm or finer pore size, and use the clear filtrate as the *Sample solution*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.7 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxycodone hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxycodone hydrochloride, 351.82
M_{r2} = molecular weight of oxycodone base, 315.37

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩**:** If present, 85.0%–115.0% of the labeled amount of alcohol (C_2H_5OH), determined by the gas–liquid chromatographic method, using acetone as the internal standard

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for oral solution packaged in single-unit containers
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for oral solution packaged in multiple-unit containers

SPECIFIC TESTS

- **PH** ⟨791⟩**:** 1.4–4.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxycodone RS

Oxycodone Hydrochloride Tablets

DEFINITION

Oxycodone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the oxycodone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, 0.01 M sodium 1-heptanesulfonate, and glacial acetic acid (25:74:1). Adjust this mixture with 5 N sodium hydroxide to a pH of 3.5.

Standard solution: 0.045 mg/mL of USP Oxycodone RS in *Mobile phase*

Sample solution: 0.05 mg/mL of oxycodone hydrochloride in *Mobile phase* from NLT 20 finely powdered Tablets. Initially add 50% of the volume of the flask of *Mobile phase*, sonicate for about 5 min, and shake by mechanical means for about 15 min. Dilute with *Mobile phase* to volume, and pass a portion of this mixture through a filter of 0.5-µm or finer pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.7 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the oxycodone peak
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxycodone hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxycodone hydrochloride, 351.82
M_{r2} = molecular weight of the oxycodone base, 315.37

Acceptance criteria: 90.0%–110.0%

USP Monographs

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PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: Prepare a known concentration of USP Oxycodone RS in 0.1 N hydrochloric acid.
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* as needed.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Maximum at about 225 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 70% (*Q*) of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxycodone RS

Oxycodone Hydrochloride Extended-Release Tablets

DEFINITION

Oxycodone Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer solution: 2 g/L of sodium heptanesulfonate in water. Add 13.3 mL/L of glacial acetic acid, and adjust with 5 N sodium hydroxide solution to a pH of 3.50 ± 0.05.
Mobile phase: Acetonitrile and *Buffer solution* (1:3)
Standard solution: 0.036 mg/mL of USP Oxycodone RS in *Mobile phase*
Sample stock solution: Transfer 10 Tablets into an appropriate volumetric flask, and add a volume of a mixture of methanol and acetonitrile (1:1) equivalent to 50% of the volumetric flask volume. Sonicate for 10 min, and stir for 20 min. Dilute with *Buffer solution* to volume.
Sample solution: 0.04 mg/mL of oxycodone hydrochloride from the *Sample stock solution*, diluted with *Mobile phase* to volume. Pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 4000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxycodone hydrochloride, 351.82
M_{r2} = molecular weight of oxycodone base, 315.37

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Simulated gastric fluid (without enzymes); 900 mL
Apparatus 1: 100 rpm
Times: 1, 2, 4, 6, and 8 h for Tablets labeled to contain 10, 20, or 40 mg; 1, 2, 4, and 6 h for Tablets labeled to contain 80 mg
Standard stock solution
Tablets labeled to contain 10 mg: 398 µg/mL of USP Oxycodone RS in *Medium*
Tablets labeled to contain 20, 40, or 80 mg: 796 µg/mL of USP Oxycodone RS in *Medium*
Standard solution: Dilute the appropriate *Standard stock solution* with *Medium* to obtain solutions containing (*L*/900) mg/mL, with *L* as the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: About 226 nm (shoulder)
Cell
For Tablets labeled to contain 10, 20, or 40 mg: 1.0 cm
For Tablets labeled to contain 80 mg: 0.5 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (A_U/A_S) \times C_S \times D \times (M_{r1}/M_{r2})$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*
M_{r1} = molecular weight of oxycodone hydrochloride, 351.82
M_{r2} = molecular weight of oxycodone base, 315.37

For Tablets labeled to contain 10, 20, or 40 mg, calculate the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) released at each time point (*i*):

$$\text{Result}_i = C_i \times V \times (1/L) \times 100$$

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$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_5 = (\{C_5 \times [V - (4 \times V_S)]\} + [(C_4 + C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of oxycodone hydrochloride in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

For Tablets labeled to contain 80 mg, calculate the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) released at each time point (*i*):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$

$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of oxycodone hydrochloride in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 1* for Tablets labeled to contain 10, 20, or 40 mg; see *Table 2* for Tablets labeled to contain 80 mg.

Table 1

Time Point (*i*)	Time (h)	Amount Released (%)
1	1	20–40
2	2	35–55
3	4	55–75
4	6	70–90
5	8	NLT 80

Table 2

Time Point (*i*)	Time (h)	Amount Released (%)
1	1	25–45
2	2	45–65
3	4	65–85
4	6	NLT 80

The percentages of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$), released at the times specified, conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Simulated gastric fluid (without enzymes); 900 mL
Apparatus 1: 100 rpm
Times: 1, 4, and 12 h
0.85% Phosphoric acid: 10 mL/L of phosphoric acid in water
Mobile phase: Weigh 23.1 g of monobasic potassium phosphate into a 4-L flask, and dissolve with 3400 mL of water. Add 4 mL of triethylamine, and adjust with *0.85% Phosphoric acid* to a pH of 3.0 ± 0.1. Add 600 mL of methanol and 20 mL of *tert*-butyl methyl ether, and mix well.
Standard stock solution: 0.9 mg/mL of USP Oxycodone RS in *0.85% Phosphoric acid*
Standard solution: Dilute the *Standard stock solution*, quantitatively and stepwise, with *Medium* to obtain a solution having a concentration of 40% of the Tablet label claim. [NOTE—This solution is stable for two weeks at room temperature.]
Sample solution: Pass the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: NLT 0.5
Tailing factor: 0.75–1.5
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$Result_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
M_{r1} = molecular weight of oxycodone hydrochloride, 351.82
M_{r2} = molecular weight of oxycodone base, 315.37

Calculate the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) released at each time point (*i*):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$

$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of oxycodone hydrochloride in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL

USP Monographs

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L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 3* for Tablets labeled to contain 10 mg; see *Table 4* for Tablets labeled to contain 20 mg; see *Table 5* for Tablets labeled to contain 40 mg; see *Table 6* for Tablets labeled to contain 80 mg.

Table 3

Time Point (*i*)	Time (h)	Amount Released (%)
1	1	29–49
2	4	58–78
3	12	NLT 85

Table 4

Time Point (*i*)	Time (h)	Amount Released (%)
1	1	33–53
2	4	63–83
3	12	NLT 85

Table 5

Time Point (*i*)	Time (h)	Amount Released (%)
1	1	37–57
2	4	68–88
3	12	NLT 85

Table 6

Time Point (*i*)	Time (h)	Amount Released (%)
1	1	31–51
2	4	61–81
3	12	NLT 85

The percentages of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$), released at the times specified, conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

- **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

- **Limit of Oxycodone Related Compound B (Oxycodone *N*-Oxide)**

Diluent: 10 mL/L of phosphoric acid in water
Buffer: 6.8 g/L of monobasic potassium phosphate. Add 1.2 mL of triethylamine, and adjust with *Diluent* to a pH of 3.0 ± 0.1.
Mobile phase: Methanol, *tert*-butyl methyl ether, and *Buffer* (30:1:170)
Standard solution: 0.18 mg/mL of USP Oxycodone RS and 0.002 mg/mL of USP Oxycodone Related Compound B RS in *Diluent*. [Note—Prepare fresh daily.]
Sample stock solution: Transfer 10 Tablets into a 500-mL volumetric flask, add 50 mL of *Diluent* and 50 mL of alcohol, and sonicate for 90 min to extract the active ingredient. Dilute with *Diluent* to volume.
Sample solution: 0.2 mg/mL of oxycodone hydrochloride from the *Sample stock solution* in *Diluent*. Pass a portion of the solution through a suitable filter, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Column temperature: 60°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4.5 between the oxycodone and oxycodone related compound B peaks
Relative standard deviation: NMT 3.0% for oxycodone related compound B
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxycodone related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of oxycodone related compound B from the *Sample solution*
r_S = peak area of oxycodone related compound B from the *Standard solution*
C_S = concentration of USP Oxycodone Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxycodone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **Labeling:** When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.
- **USP Reference Standards** ⟨11⟩
USP Oxycodone RS
USP Oxycodone Related Compound B RS
4,5a-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one *N*-oxide.
$C_{18}H_{21}NO_5$ 331.36

Delete the following:

▲Oxycodone and Acetaminophen Capsules

DEFINITION

Oxycodone and Acetaminophen Capsules contain Oxycodone Hydrochloride and Acetaminophen, or Oxycodone Hydrochloride, Oxycodone Terephthalate, and Acetaminophen. Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of oxycodone hydrochloride or oxycodone hydrochloride and oxycodone terephthalate, calculated as total oxycodone ($C_{18}H_{21}NO_4$), and NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

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IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 0.5 mg/mL of USP Oxycodone RS in a mixture of methanol and water (4:1)

Standard solution B: 0.5*J* mg/mL of USP Acetaminophen RS in a mixture of methanol and water (4:1). [NOTE—*J* is the ratio of the labeled amount, in mg, of acetaminophen to the labeled amount, in mg, of oxycodone per Capsule.]

Sample solution: Nominally equivalent to 0.5 mg/mL of oxycodone from Capsules in a mixture of methanol and water (4:1). Sonicate for 5 min, and shake by mechanical means for 15 min. Allow to settle, and use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of silica gel mixture

Application volume: 20 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (4:1:2)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Mark the solvent front, and allow the plate to air-dry for about 30 min. Expose the plate to iodine vapors in a closed chamber, and locate the spots.

Acceptance criteria: The R_F values of the principal spots from the *Sample solution* correspond to those from the respective *Standard solutions*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Methanol and 0.05 M dibasic potassium phosphate (1:9). Adjust with phosphoric acid to a pH of 4.0.

Buffer: 0.95 mg/mL of monobasic potassium phosphate in water, phosphoric acid, and *n*-nonylamine (1000:1:1). Prepare as follows: Add 950 mg of monobasic potassium phosphate to 1000 mL of water. Add 1 mL of phosphoric acid, and stir until dissolved. While stirring, add 1 mL of *n*-nonylamine, and stir until a clear solution is obtained. Adjust with potassium hydroxide TS to a pH of 4.9 ± 0.1.

Mobile phase: Methanol and *Buffer* (1:9)

Oxycodone standard stock solution: 0.075 mg/mL of USP Oxycodone RS in *Solution A*

Standard stock solution: 0.03*J* mg/mL of USP Acetaminophen RS and 0.03 mg/mL of USP Oxycodone RS in *Solution A*. Prepare by adding 40% of the flask volume of *Solution A* to the appropriate quantity of USP Acetaminophen RS, and then adding 40% of the flask volume of *Oxycodone standard stock solution* and diluting with *Solution A* to volume. [NOTE—*J* is the ratio of the labeled amount of acetaminophen, in mg, to that of oxycodone equivalent.]

Standard solution: 0.003*J* mg/mL of USP Acetaminophen RS and 0.003 mg/mL of USP Oxycodone RS in *Mobile phase* from *Standard stock solution*

Sample stock solution: Nominal equivalent of 0.03 mg/mL of oxycodone, from Capsules (NLT 20), in *Solution A* in a suitable container. Shake by mechanical means for 1 h.

Sample solution: 0.003 mg/mL of oxycodone in *Mobile phase* from *Sample stock solution*. Pass the resulting solution through a membrane filter of 0.5-µm or finer pore size, discarding the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for oxycodone and acetaminophen are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.4 between acetaminophen and oxycodone

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxycodone ($C_{18}N_{21}NO_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxycodone from the *Sample solution*

r_S = peak response of oxycodone from the *Standard solution*

C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxycodone in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of oxycodone hydrochloride or oxycodone hydrochloride and oxycodone terephthalate, calculated as total oxycodone ($C_{18}H_{21}NO_4$), and 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* as needed.

Analysis: Determine the amounts of oxycodone ($C_{18}H_{21}NO_4$) and acetaminophen ($C_8H_9NO_2$) dissolved, using the procedure in the *Assay*, making any necessary

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volumetric adjustments, including adjusting the solution under test to a pH of about 5.5 before injecting.

Tolerances: NLT 75% (*Q*) of the labeled amounts of oxycodone ($C_{18}H_{21}NO_4$) and acetaminophen ($C_8H_9NO_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards** ⟨11⟩
USP Acetaminophen RS
USP Oxycodone RS▲ (USP 1-May-2020)

Oxycodone and Acetaminophen Tablets

DEFINITION

Oxycodone and Acetaminophen Tablets contain Oxycodone Hydrochloride and Acetaminophen. Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of oxycodone ($C_{18}H_{21}NO_4$), and NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution A: 0.5 mg/mL of USP Oxycodone RS in a mixture of methanol and water (4:1)

Standard solution B: 0.5*J* mg/mL of USP Acetaminophen RS in a mixture of methanol and water (4:1). [Note—*J* is the ratio of the labeled amount, in mg, of acetaminophen to the labeled amount, in mg, of oxycodone per Tablet.]

Sample solution: Nominally equivalent to 2.5 mg of oxycodone from powdered Tablets in a 5-mL mixture of methanol and water (4:1). Sonicate for 5 min, and shake by mechanical means for 15 min. Allow to settle, and use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of silica gel mixture
Application volume: 20 µL
Developing solvent system: Butyl alcohol, glacial acetic acid, and water (4:1:2)

Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Proceed as directed in the chapter. Develop the chromatographic plate until the solvent front has moved about three-fourths of the length of the plate. Mark the solvent front, and allow the plate to air-dry for about 30 min. Expose the plate to iodine vapors in a closed chamber, and locate the spots.

Acceptance criteria: The R_F values of the principal spots from the *Sample solution* correspond to those from the respective *Standard solutions*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: Methanol and 0.05 M dibasic potassium phosphate (1:9). Adjust with phosphoric acid to a pH of 4.0.

Buffer: 0.95 mg/mL of monobasic potassium phosphate in water, phosphoric acid, and *n*-nonylamine (1000:1:1). Prepare as follows: Add 950 mg of monobasic potassium phosphate to 1000 mL of water. Add 1 mL of phosphoric acid, and stir until dissolved. While stirring, add 1 mL of *n*-nonylamine, and stir until a clear solution is obtained. Adjust with potassium hydroxide TS to a pH of 4.9 ± 0.1.

Mobile phase: Methanol and *Buffer* (1:9)

Oxycodone standard stock solution: 0.075 mg/mL of USP Oxycodone RS in *Solution A*

Standard stock solution: 0.03*J* mg/mL of USP Acetaminophen RS and 0.03 mg/mL of USP Oxycodone RS in *Solution A*. Prepare by adding 40% of the flask volume of *Solution A* to the appropriate quantity of USP Acetaminophen RS, and then adding 40% of the flask volume of *Oxycodone standard stock solution* and diluting with *Solution A* to volume. [Note—*J* is the ratio of the labeled amount, in mg, of acetaminophen to that of oxycodone equivalent.]

Standard solution: 0.003*J* mg/mL of USP Acetaminophen RS and 0.003 mg/mL of USP Oxycodone RS in *Mobile phase* from *Standard stock solution*

Sample stock solution: Nominal equivalent of 0.03 mg/mL of oxycodone, from powdered Tablets (NLT 20), in *Solution A* in a suitable container. Shake by mechanical means for 1 h.

Sample solution: 0.003 mg/mL of oxycodone in *Mobile phase* from *Sample stock solution*. Pass the resulting solution through a membrane filter of 0.5-µm or finer pore size, discarding the first 10 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 2 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution*
[Note—The relative retention times for oxycodone and acetaminophen are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.4 between acetaminophen and oxycodone
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxycodone ($C_{18}N_{21}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxycodone from the *Sample solution*
r_S = peak response of oxycodone from the *Standard solution*
C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxycodone in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

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r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of oxycodone ($C_{18}H_{21}NO_4$), and 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* as needed.
Analysis: Determine the amounts of oxycodone ($C_{18}H_{21}NO_4$) and acetaminophen ($C_8H_9NO_2$) dissolved, using the procedure in the *Assay*, and making any necessary volumetric adjustments, including adjusting the solution under test to a pH of about 5.5 before injecting.
Tolerances: NLT 75% (*Q*) of the labeled amounts of oxycodone ($C_{18}H_{21}NO_4$) and acetaminophen ($C_8H_9NO_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** The Tablets may be labeled to indicate the content of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) equivalent. Each mg of oxycodone is equivalent to 1.116 mg of oxycodone hydrochloride.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Oxycodone RS

Oxycodone and Aspirin Tablets

» Oxycodone and Aspirin Tablets contain Oxycodone Hydrochloride and Aspirin, or Oxycodone Hydrochloride, Oxycodone Terephthalate, and Aspirin. Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of oxycodone ($C_{18}H_{21}NO_4$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ($C_9H_8O_4$).

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label the Tablets to state both the content of the oxycodone active moiety and the content or contents of the salt or salts of oxycodone used in formulating the article.
USP Reference standards ⟨11⟩—
USP Aspirin RS
USP Oxycodone RS
USP Salicylic Acid RS
Identification—The retention times of the oxycodone peak and the aspirin peak in the chromatograms of the respective *Assay preparations* correspond to those of the corresponding analytes of the respective *Standard preparations*, as obtained in the *Assay for oxycodone* and the *Assay for aspirin*, respectively.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05; 500 mL.
Apparatus 1: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{18}H_{21}NO_4$ dissolved using the method for *Assay for oxycodone*, making any necessary volumetric adjustments. Determine the amount of $C_9H_8O_4$ dissolved from UV absorbances at the wavelength of the isobestic point of aspirin and salicylic acid at about 265 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same medium. [NOTE—Prepare the Standard solution at the time of use. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Medium*.]
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{18}H_{21}NO_4$ is dissolved in 30 minutes and not less than 75% *(Q)* of the labeled amount of $C_9H_8O_4$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Salicylic acid—
Mobile phase—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluting solution—Prepare a mixture of acetonitrile and formic acid (99:1).
Standard preparation—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Diluting solution* to obtain a solution having a known concentration of about 0.008 mg per mL.
Test preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 380 mg of aspirin, to a 100-mL volumetric flask, add about 20 mL of *Diluting solution*, and sonicate for about 15 minutes. Dilute with *Diluting solution* to volume, and mix. Centrifuge a portion of this mixture, and use the clear supernatant as the *Test preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 299-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 4.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the salicylic acid peaks. Calculate the percentage of salicylic acid in the portion of Tablets taken by the formula:

$$10{,}000(C/a)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*, a is the quantity, in mg, of aspirin in the portion of Tablets taken, as determined in the *Assay for aspirin*, and r_U and r_S are the salicylic acid peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 3.0% is found.

USP Monographs

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Assay for aspirin— [NOTE—Volumetric flasks should be dried at 105° for not less than 1 hour, and cooled in a desiccator before use.]

Mobile phase—Prepare a mixture of *n*-heptane and glacial acetic acid (96:4), and filter through a filter of 0.5 µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare solution of 1-naphthol in chloroform containing about 1 mg per mL. [NOTE—Protect this solution from light.]

Standard preparation—Transfer about 163 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask. Add 2.5 mL of glacial acetic acid, and swirl. Add 25 mL of chloroform, and shake for 10 minutes. Add 5.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix. [NOTE—Protect this solution from light.]

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a 100-mL volumetric flask, add 5 mL of glacial acetic acid, and swirl. Add 50 mL of chloroform, and shake for 10 minutes. Add 10.0 mL of the *Internal standard solution*, dilute with chloroform to volume, mix, and filter. [NOTE—Prepare the *Assay preparation* and the *Standard preparation* concomitantly, and protect from light.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 300-nm detector and a 4.6-mm × 25-cm column containing packing L3. The flow rate is about 4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the 1-naphthol peak and the aspirin peak is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.65 for 1-naphthol and 1.0 for aspirin. Calculate the quantity, in mg, of Aspirin ($C_9H_8O_4$) in the portion of Tablets taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*, and R_U and R_S are the ratios of the aspirin peak response to the 1-naphthol peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for oxycodone—

Mobile phase—Dissolve 2.2 g of sodium 1-octanesulfonate in 740 mL of water, add 260 mL of methanol, 10 mL of glacial acetic acid, and 0.1 mL of triethylamine. Mix, and adjust with 5 N sodium hydroxide to a pH of 6.5 ± 0.1. Filter through a suitable filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solution—Use 0.1 N hydrochloric acid.

Internal standard solution—Transfer about 50 mg of ethylparaben to a 500-mL volumetric flask, add 10 mL of methanol, and swirl to dissolve. Dilute with *Diluting solution* to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Oxycodone RS in *Diluting solution*, and dilute quantitatively with *Diluting solution* to obtain a stock solution having a known concentration of about 0.75 mg per mL. Transfer 15.0 mL of this stock solution to a second 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Diluting solution* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.112 mg of USP Oxycodone RS per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 11.2 mg of oxycodone, to a suitable glass-stoppered conical flask, add 50.0 mL of *Diluting solution*, and shake by mechanical means for about 30 minutes. Filter this solution, transfer 25.0 mL of the clear filtrate to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Diluting solution* to volume, and mix. Use this solution as the *Assay preparation.*

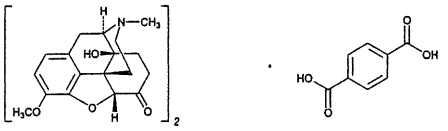
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 15-cm column that contains packing L1 and is maintained at a temperature of 50 ± 1.0°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the column efficiency, determined from the oxycodone peak, is not less than 1800 theoretical plates, the resolution, *R*, between the oxycodone and the ethylparaben peaks is not less than 6, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for oxycodone and 1.0 for ethylparaben. Calculate the quantity, in mg, of oxycodone ($C_{18}H_{21}NO_4$) in the portion of Tablets taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Oxycodone RS in the *Standard preparation*, and R_U and R_S are the ratios of the responses of the oxycodone peak and the ethylparaben peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oxycodone Terephthalate



$(C_{18}H_{21}NO_4)_2 \cdot C_8H_6O_4$ 796.86

Morphinan-6-one, 4,5-epoxy-14-hydroxy-3-methoxy-17-methyl-, 1,4-benzenedicarboxylate (2:1 salt), (5α); 4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one 1,4-benzenedicarboxylate (2:1 salt) [64336-55-6].

DEFINITION

Oxycodone Terephthalate contains NLT 97.0% and NMT 103.0% of oxycodone terephthalate $(C_{18}H_{21}NO_4)_2 \cdot C_8H_6O_4$, calculated on the dried basis.

IDENTIFICATION

• **A. MELTING RANGE OR TEMPERATURE** ⟨741⟩

Sample solution: Transfer 50 mL of the filtrate retained from the test for *Content of Terephthalate Acid* to a 125-mL conical flask. Render the solution alkaline with 6 N ammonium hydroxide. Allow the mixture to stand until a precipitate is formed. Filter, wash the precipitate with 50 mL of cold water, and dry for 2 h at 105°.

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Acceptance criteria: The precipitate melts between 218° and 223°, but the range between the beginning and end of the melting does not exceed 2°.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Use a portion of the dried precipitate obtained in *Identification* test *A*.
Acceptance criteria: Meets the requirements

Change to read:

• **C. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 150 µg/mL in 0.1 N hydrochloric acid
Acceptance criteria: Exhibits a maxima at 280 nm

ASSAY

• **PROCEDURE**
Mobile phase: To 2.2 g of sodium 1-octanesulfonate in 740 mL of water add 260 mL of methanol, 10 mL of glacial acetic acid, and 0.1 mL of triethylamine. Mix, and adjust with 5 N sodium hydroxide to a pH of 6.5 ± 0.1. Pass through a filter of 0.5-µm or finer pore size.
Diluent: 0.1 N hydrochloric acid
Internal standard solution: 0.1 mg/mL of ethylparaben prepared by dissolving in 2% of the flask volume of methanol and diluting with *Diluent* to volume
Standard stock solution: 0.75 mg/mL of USP Oxycodone RS in *Diluent*
Standard solution: 0.11 mg/mL of USP Oxycodone RS prepared as follows. Transfer 15.0 mL of *Standard stock solution* to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.
Sample stock solution: 0.71 mg/mL of Oxycodone Terephthalate in *Diluent*. Filter, discarding the first 5 mL.
Sample solution: 0.14 mg/mL of Oxycodone Terephthalate prepared as follows. Transfer 10.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 15-cm; packing L1
Column temperature: 50 ± 1.0°
Flow rate: 1 mL/min
Run time: Twice the retention time of the main oxycodone peak
Injection size: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 6 between oxycodone and ethylparaben
Column efficiency: NLT 1800 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxycodone terephthalate $(C_{18}H_{21}NO_4)_2 \cdot C_8H_6O_4$ in the portion of Oxycodone Terephthalate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of oxycodone to ethylparaben from the *Sample solution*
R_S = peak response ratio of oxycodone to ethylparaben from the *Standard solution*
C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)
C_U = concentration of oxycodone in the *Sample solution* (mg/mL)
M_{r1} = one-half of the molecular weight of oxycodone terephthalate, 398.43
M_{r2} = molecular weight of oxycodone, 315.37

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 1%
• **ORGANIC IMPURITIES**
Solution A: 2.2 g of sodium 1-octanesulfonate in 850 mL of water. Add 150 mL of methanol, 20 mL of glacial acetic acid, and 1.0 mL of triethylamine. Pass through a filter of 0.5-µm or finer pore size.
Solution B: 2.2 g of sodium 1-octanesulfonate in 500 mL of water. Add 500 mL of methanol, 20 mL of glacial acetic acid, and 1.0 mL of triethylamine. Pass through a filter of 0.5-µm or finer pore size.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	80	20
50	0	100
55	0	100

Diluent: 0.1 N hydrochloric acid
Standard stock solution: 0.9 mg/mL of USP Oxycodone RS in *Diluent*
Standard solution: 0.09 mg/mL of USP Oxycodone RS from the *Standard stock solution*, prepared by adding to 20% of the flask volume of methanol, and diluting with *Diluent* to volume
System suitability stock solution: 0.05 mg/mL of 4-hydroxybenzoic acid isopropyl ester in methanol
System suitability solution: 0.01 mg/mL of 4-hydroxybenzoic acid isopropyl ester and 0.09 mg/mL of USP Oxycodone RS in *Diluent* from the *System suitability stock solution* and *Standard stock solution*, respectively
Sample solution: 11 mg/mL of Oxycodone Terephthalate in methanol prepared as follows. Transfer the required amount of sample to a suitable volumetric flask. Add 80% of the flask volume of methanol, and shake by mechanical means for about 20 min to dissolve. Dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 15-cm; packing L1
Column temperature: 45 ± 1°
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Samples: *Standard solution* and *System suitability solution*

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Suitability requirements
Resolution: NLT 8 between the oxycodone and 4-hydroxybenzoic acid isopropyl ester peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of an individual impurity from the *Sample solution*
r_S = peak area of oxycodone from the *Standard solution*
C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxycodone Terephthalate in the *Sample solution* (mg/mL)
M_{r1} = one-half of the molecular weight of oxycodone terephthalate, 398.43
M_{r2} = molecular weight of oxycodone, 315.37

[NOTE—If any impurity is found having a retention time of about 2 in relation to that of the oxycodone peak, divide its apparent percentage by 4.8.]
Acceptance criteria
Individual impurities: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **CONTENT OF TEREPHTHALIC ACID**
Sample solution: Transfer 1 g into a 50-mL beaker. Add 25 mL of 0.2 N hydrochloric acid, and heat to boiling with continuous stirring. Cover the beaker with a watch glass, and allow to cool to room temperature. Pass the suspension through a tared, medium-porosity filtering crucible. Transfer any material remaining in the beaker to the crucible with the aid of small portions of cold 0.2 N hydrochloric acid. Wash the material in the crucible with several portions of cold 0.2 N hydrochloric acid. [NOTE—Reserve the combined filtrates for use in *Identification* test *A*.]
Analysis: Dry the material in the crucible at 105° for 1 h, allow to cool, and reweigh. The material in the crucible is terephthalic acid. Determine the weight of terephthalic acid, and calculate the percentage of terephthalic acid.
Acceptance criteria: Between 20.2% and 21.5% of terephthalic acid ($C_8H_6O_4$) in Oxycodone Terephthalate on the dried basis
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 4 h: it loses NMT 1.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxycodone RS

Oxygen

O_2 32.00
Oxygen [7782-44-7].

DEFINITION
Oxygen contains NLT 99.0% of oxygen (O_2) by volume.

IDENTIFICATION
• **A.** The paramagnetic signal exhibited by the *Sample gas* in the *Assay* confirms the presence of oxygen.
• **B.** The *Sample gas* in the *Assay* meets the *Acceptance criteria*.

ASSAY
• **PROCEDURE**
The certified standards called for in the following test are listed in *Reagents, Indicators, and Solutions*.
Zero gas: Nitrogen certified standard
Span gas: Oxygen certified standard
Sample gas: Oxygen under test
Mode: Paramagnetic oxygen measurement (see *Medical Gases Assay* ⟨415⟩)
Analysis: Determine the concentration of oxygen in percentage by volume in Oxygen using a suitable paramagnetic analyzer.
Acceptance criteria: NLT 99.0% by volume

IMPURITIES
See *Impurities Testing in Medical Gases Assay* ⟨413⟩. The detector tubes called for in the following tests are listed in *Reagents, Indicators, and Solutions*. If the label indicates that Oxygen is produced by the air-liquefaction process, then the *Impurities* tests are not required.
• **LIMIT OF CARBON DIOXIDE**
Sample: Detector tube manufacturer's recommended volume, ±5% of Oxygen
Analysis: Pass the *Sample* through a carbon dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.
Acceptance criteria: NMT 300 ppm
• **LIMIT OF CARBON MONOXIDE**
Sample: Detector tube manufacturer's recommended volume, ±5% of Oxygen
Analysis: Pass the *Sample* through a carbon monoxide detector tube at the rate specified for the tube by the detector tube manufacturer.
Acceptance criteria: NMT 10 ppm

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in pressurized containers. Container connections shall be appropriate for Oxygen. Adaptors shall not be used to connect containers to patient-use supply system piping or equipment.
• **LABELING:** Label states if Oxygen was produced by the air-liquefaction process. Where it is piped directly from the cylinder or storage tank to the patient point of use, label each outlet "Oxygen".

Oxygen 93 Percent

DEFINITION
Oxygen 93 Percent is Oxygen produced from air by the molecular sieve process. It contains NLT 90.0% and NMT 96.0% by volume of oxygen (O_2), the remainder consisting mostly of argon and nitrogen.

IDENTIFICATION
• **A.** The paramagnetic signal exhibited by the *Sample gas* in the *Assay* confirms the presence of oxygen.
• **B.** The *Sample gas* in the *Assay* meets the *Acceptance criteria*.

ASSAY
• **PROCEDURE**
The certified standards called for in the following test are listed in *Reagents, Indicators, and Solutions*.
Zero gas: Nitrogen certified standard

USP Monographs

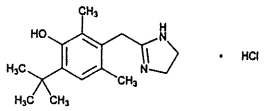
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Span gas: 93% Oxygen certified standard
Sample gas: Oxygen 93 Percent under test
Mode: Paramagnetic oxygen measurement (see *Medical Gases Assay* ⟨415⟩)
Analysis: Determine the concentration of oxygen in percentage by volume in Oxygen 93 Percent using a suitable paramagnetic analyzer.
Acceptance criteria: 90.0%–96.0% oxygen by volume

IMPURITIES
See *Impurities Testing in Medical Gases Assay* ⟨413⟩. The detector tubes called for in the following tests are listed in *Reagents, Indicators, and Solutions*.
• **CARBON DIOXIDE**
Sample: Detector tube manufacturer's recommended volume ±5% of Oxygen 93 Percent
Analysis: Pass the *Sample* through a carbon dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.
Acceptance criteria: NMT 300 ppm
• **CARBON MONOXIDE**
Sample: Detector tube manufacturer's recommended volume ±5% of Oxygen 93 Percent
Analysis: Pass the *Sample* through a carbon monoxide detector tube at the rate specified for the tube by the detector tube manufacturer.
Acceptance criteria: NMT 10 ppm

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in pressurized containers. Container connections shall be appropriate for Oxygen 93 Percent. Adaptors shall not be used to connect containers to patient use supply system piping or equipment.
• **LABELING:** If Oxygen 93 Percent is piped from a remote location to the patient point of use, label each outlet "Oxygen 93 Percent".

Oxymetazoline Hydrochloride



$C_{16}H_{24}N_2O \cdot HCl$ 296.84
Phenol, 3-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethyl-, monohydrochloride;
6-*tert*-Butyl-3-(2-imidazolin-2-ylmethyl)-2,4-dimethylphenol monohydrochloride [2315-02-8].

DEFINITION
Oxymetazoline Hydrochloride contains NLT 98.5% and NMT 101.5% of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: 50 mg in 3 mL of water
Analysis: To the *Sample solution* add 1 mL of 6 N ammonium hydroxide. Filter, and acidify the filtrate with nitric acid.
Acceptance criteria: The filtrate meets the requirements.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, 1 M sodium acetate, glacial acetic acid, and water (40:10:4:46)
Standard solution: 0.5 mg/mL of USP Oxymetazoline Hydrochloride RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Oxymetazoline Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L9
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.55% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$) in the portion of Oxymetazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxymetazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxymetazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: See *Table 1*. Return to the original conditions, and re-equilibrate the system.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	70	30
5	70	30
20	15	85
35	15	85

System suitability solution: 1.0 mg/mL of USP Oxymetazoline Hydrochloride RS and 1.5 µg/mL of USP Oxymetazoline Related Compound A RS in water
Standard solution: 1.0 µg/mL of USP Oxymetazoline Hydrochloride RS and 1.5 µg/mL of USP Oxymetazoline Related Compound A RS in water
Sample solution: 1.0 mg/mL of Oxymetazoline Hydrochloride in water

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between oxymetazoline related compound A and oxymetazoline peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxymetazoline related compound A in the portion of Oxymetazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxymetazoline related compound A from the *Sample solution*
r_S = peak response of oxymetazoline related compound A from the *Standard solution*
C_S = concentration of USP Oxymetazoline Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxymetazoline Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Oxymetazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of oxymetazoline from the *Standard solution*
C_S = concentration of USP Oxymetazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxymetazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxymetazoline related compound A[a]	0.9	0.15
Oxymetazoline	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] *N*-(2-Aminoethyl)-2-[4-(*tert*-butyl)-3-hydroxy-2,6-dimethylphenyl] acetamide.

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 50 mg/mL in water
Acceptance criteria: 4.0–6.5
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Oxymetazoline Hydrochloride RS
USP Oxymetazoline Related Compound A RS
N-(2-Aminoethyl)-2-[4-(*tert*-butyl)-3-hydroxy-2,6-dimethylphenyl]acetamide hydrochloride.
$C_{16}H_{26}N_2O_2 \cdot HCl$ 314.85

Oxymetazoline Hydrochloride Nasal Solution

DEFINITION
Oxymetazoline Hydrochloride Nasal Solution is a solution of Oxymetazoline Hydrochloride in water adjusted to a suitable tonicity. It contains NLT 90.0% and NMT 110.0% of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Methanol, water, 1 M sodium acetate, and glacial acetic acid (40:46:10:4)
Standard solution: Prepare a solution of USP Oxymetazoline Hydrochloride RS in *Mobile phase*, having a known concentration approximately equal to the labeled concentration of the Nasal Solution.
Sample solution: Nasal Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L9
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of oxymetazoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 4.0–6.5

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxymetazoline Hydrochloride RS

Oxymetazoline Hydrochloride Ophthalmic Solution

DEFINITION
Oxymetazoline Hydrochloride Ophthalmic Solution is a sterile, buffered solution of Oxymetazoline Hydrochloride in water adjusted to a suitable tonicity. It contains NLT 90.0% and NMT 110.0% of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$). It contains a suitable preservative.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Methanol, 1 M sodium acetate, glacial acetic acid, and water (40:10:4:46)
Standard solution: Prepare a solution of USP Oxymetazoline Hydrochloride RS in *Mobile phase* having a known concentration approximately equal to the labeled concentration of the Ophthalmic Solution.
Sample solution: Use the Ophthalmic Solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm or diode array. [NOTE—Use diode array detector to perform *Identification* test *B*.]
Column: 4.6-mm × 25-cm; packing L9
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

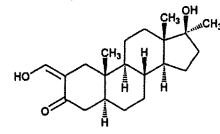
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Oxymetazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxymetazoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.8–6.8
- **STERILITY TESTS** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxymetazoline Hydrochloride RS

Oxymetholone



$C_{21}H_{32}O_3$ 332.48
Androstan-3-one, 17-hydroxy-2-(hydroxymethylene)-17-methyl-, (5α,17β)-;
17β-Hydroxy-2-(hydroxymethylene)-17-methyl-5α-androstan-3-one [434-07-1].

DEFINITION
Oxymetholone contains NLT 97.0% and NMT 103.0% of oxymetholone ($C_{21}H_{32}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲ (CN 1-May-2020)

Sample solution: 10 µg/mL in 0.01 N methanolic sodium hydroxide
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**

Diluent: Alcohol and chloroform (50:50)
Standard solution: Prepare as directed in *Single-Steroid Assay* ⟨511⟩, using USP Oxymetholone RS.
Sample solution: Dissolve 20 mg of Oxymetholone, previously dried, in *Diluent*, and dilute with *Diluent* to 10.0 mL.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum at about 315 nm
Cell: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in *Single-Steroid Assay* ⟨511⟩, *Procedure* through the third sentence of the second paragraph (ending with "50-mL centrifuge tube"), except use the *Standard solution* and the *Sample solution* in place of the *Standard Preparation* and the *Assay Preparation*, respectively. Use a mixture of benzene and alcohol (98:2) as the *Solvent*. To each tube add 25.0 mL of 0.01 N alcoholic sodium hydroxide, and shake for NLT 2 min. Centrifuge the tubes for 5 min, and determine the absorbances of the supernatants against the blank.
Calculate the percentage of oxymetholone ($C_{21}H_{32}O_3$) in the portion of Oxymetholone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

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A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Oxymetholone RS in the *Standard solution* (mg/mL)
C_U = concentration of Oxymetholone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 172°–180°
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 20 mg/mL in dioxane
  Acceptance criteria: +34° to +38°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum over phosphorus pentoxide for 4 h.
  Acceptance criteria: NMT 1.0%
- **COMPLETENESS OF SOLUTION**
  Sample solution: 20 mg/mL in dioxane
  Acceptance criteria: The solution is clear and free from undissolved solid.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxymetholone RS

Oxymetholone Tablets

DEFINITION
Oxymetholone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxymetholone ($C_{21}H_{32}O_3$).

IDENTIFICATION
- **A. INFRARED ABSORPTION**
  Sample: Nominally 50 mg of oxymetholone from powdered Tablets
  Analysis: Mix the *Sample* with 15 mL of solvent hexane, and stir occasionally for 15 min. Centrifuge the mixture, and decant and discard the solvent hexane. Extract the residue with two 10-mL portions of solvent hexane, centrifuging and decanting as before, and discard the solvent hexane. Add 25 mL of chloroform to the residue, mix by shaking for 1–2 min, and filter. Evaporate the filtrate to about 3 mL, add a few mL of solvent hexane to induce crystallization, and evaporate to dryness.
  Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the oxymetholone so obtained, and previously dried, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Oxymetholone RS, crystallized from the same solvent mixture.

ASSAY
- **PROCEDURE**
  Solution A: 4 g/L of sodium hydroxide in methanol
  Solution B: 0.4 g/L of sodium hydroxide in methanol from *Solution A*
  Standard solution: 10 µg/mL of USP Oxymetholone RS in *Solution B*. [NOTE—The solution is freshly prepared.]
  Sample solution: Nominally 10 µg/mL of Oxymetholone prepared as follows. Transfer the equivalent of 20 mg of oxymetholone, from NLT 20 powdered Tablets, to a separator. Add 10 mL of water, and extract with three 25-mL portions of chloroform, filtering each extract through chloroform-washed cotton. Evaporate the combined chloroform extracts on a steam bath to dryness, reducing the application of heat as dryness is approached. Dissolve the residue in methanol, transfer to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of the solution to a 100-mL volumetric flask, add 10 mL of *Solution A*, and dilute with methanol to volume.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum at about 315 nm
  Cell: 1 cm
  Blank: *Solution B*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Without delay, concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.
  Calculate the percentage of the labeled amount of oxymetholone ($C_{21}H_{32}O_3$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Oxymetholone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of oxymetholone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: 0.05 M alkaline borate buffer, pH 8.5; 900 mL. [NOTE—See *Reagents, Indicators, and Solutions—Buffer Solutions*.]
  Apparatus 1: 100 rpm
  Time: 45 min
  Standard solution: USP Oxymetholone RS in *Medium*. [NOTE—An amount of acetonitrile not to exceed 5% of the total volume of the *Standard solution* may be used to bring the Reference Standard into solution before dilution with the *Medium*.]
  Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum at about 313
  Tolerances: NLT 75% (*Q*) of the labeled amount of oxymetholone ($C_{21}H_{32}O_3$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  Procedure for content uniformity
  Solution A and **Solution B:** Prepare as directed in the *Assay*.
  Standard solution: 10 µg/mL of USP Oxymetholone RS in *Solution B*. [NOTE—The solution is freshly prepared.]
  Sample solution: Transfer 1 finely powdered Tablet to a 100-mL volumetric flask with the aid of about 75 mL of methanol. Heat the methanol to boiling, and allow to remain at a temperature just below the boiling point for 15 min with occasional swirling. Cool to room temperature, dilute with methanol to volume, and mix. Centrifuge a portion of the mixture until the solution becomes clear. Transfer a portion of the supernatant, nominally equivalent to 1 mg of oxymetholone, to a 100-mL volumetric flask. Add 10 mL of *Solution A*, and dilute with methanol to volume.

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Instrumental conditions
Mode: UV
Analytical wavelength: Maximum at about 315 nm
Cell: 1 cm
Blank: *Solution B*
Analysis
Samples: *Standard solution* and *Sample solution*
Without delay, concomitantly determine the absorbances of the *Standard solution* and the *Sample solution.*
Calculate the percentage of the labeled amount of oxymetholone ($C_{21}H_{32}O_3$) in the Tablet taken:

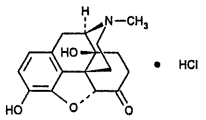
$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Oxymetholone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of oxymetholone in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxymetholone RS

Oxymorphone Hydrochloride



$C_{17}H_{19}NO_4 \cdot HCl$ 337.80
Morphinan-6-one, 4,5-epoxy-3,14-dihydroxy-17-methyl-, hydrochloride, (5α)-;
4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one hydrochloride [357-07-3].

DEFINITION
Oxymorphone Hydrochloride contains NLT 97.0% and NMT 102.0% of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: Dissolve about 250 mg in 25 mL of water, and render the solution alkaline with a saturated solution of sodium bicarbonate. Extract the liberated oxymorphone with two 15-mL portions of chloroform. Reserve the chloroform extracts for *Identification* test *B.* Acidify the aqueous phase with 2 N nitric acid.
Acceptance criteria: Meets the requirements
- **B.**
Standard solution: Similarly prepared as directed for the *Sample solution* by using USP Oxymorphone RS
Sample solution: Wash the combined chloroform extracts from *Identification* test *A* with 5 mL of water, and filter. Evaporate the chloroform solution on a steam bath nearly to dryness, then add a few mL of ether, and continue the evaporation with stirring until the solvent is removed. Dissolve the oxymorphone so obtained with alcohol-free chloroform to obtain a 1 in 50 solution.
Acceptance criteria: The IR absorption spectrum of the *Sample solution,* determined in a 0.5-mm cell, exhibits maxima only at the same wavelengths as those of the *Standard solution.*
- **C.**
Standard solution: Dissolve about 20 mg of USP Oxymorphone RS in 10 mL of 1 N hydrochloric acid, and dilute with water to 100.0 mL.
Sample solution: A solution (1 in 6500) in 0.1 N hydrochloric acid
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution.* The ratio A_{281}/A_{264} is 1.75 ± 0.2.
- **D.**
Sample solution: Dissolve 10 mg in 1 mL of water.
Analysis: To the *Sample solution* add a few drops of ferric chloride TS.
Acceptance criteria: A blue color is produced immediately.

ASSAY
- **PROCEDURE**
Sample solution: Transfer about 700 mg of Oxymorphone Hydrochloride to a glass-stoppered flask containing 50 mL of glacial acetic acid and 10 mL of mercuric acetate TS. Add 3 mL of acetic anhydride and 1 drop of methyl violet TS.
Analysis: Titrate the *Sample solution* with 0.1 N perchloric acid VS to a clear blue color. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.78 mg of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$).
Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.3%
- **ORDINARY IMPURITIES** ⟨466⟩
Sample solution: Methanol
Standard solution: Methanol
Eluant: Dehydrated alcohol, cyclohexane, and ammonium hydroxide (10:5:1)
Visualization: 1
Acceptance criteria: Meets the requirements
- **LIMIT OF NONPHENOLIC SUBSTANCES**
Analysis: Dissolve 1 g in 15 mL of water. Add 5 mL of sodium hydroxide solution (2 in 25), and extract with three 10-mL portions of chloroform. Filter the combined extracts through a small chloroform-moistened filter paper, and wash the filtrate with 5 mL of water. Filter the chloroform layer through chloroform-moistened filter paper into a tared, 50-mL beaker, and evaporate on a steam bath with the aid of a gentle current of filtered air to dryness. Dry the beaker and residue at 105° for 1 h, and weigh.
Acceptance criteria: The residue so obtained does not exceed 15 mg.

SPECIFIC TESTS
- **CONTENT OF CHLORIDE**
Sample solution: Dissolve about 300 mg in 50 mL of methanol in a glass-stoppered flask. Add 5 mL of glacial acetic acid and 3 drops of eosin Y TS.
Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.
Acceptance criteria: 10.2%–10.8% on the dried basis
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 100 mg/mL in water
Acceptance criteria: −145° to −155°
- **ACIDITY**
Analysis: Dissolve 300 mg in 10 mL of water. Add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide.

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Acceptance criteria: NMT 0.30 mL is required to produce a yellow color.

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 18 h.
Acceptance criteria: NMT 8.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxymorphone RS

Oxymorphone Hydrochloride Injection

DEFINITION

Oxymorphone Hydrochloride Injection is a sterile solution of Oxymorphone Hydrochloride in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

Solution A: Dissolve 2.02 g of sodium 1-heptanesulfonate in 900 mL of water. Add 100 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Solution B: Dissolve 2.02 g of sodium 1-heptanesulfonate in 750 mL of water. Add 250 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
35	0	100
40	0	100
40.1	100	0
50.1	100	0

Diluent: Dissolve 2.02 g of anhydrous sodium 1-heptanesulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.1.

Standard solution: 0.14 mg/mL of USP Oxymorphone RS prepared as follows. Transfer a suitable amount of USP Oxymorphone RS to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*. Sonicate to dissolve, if necessary. Add 9% of the flask volume of acetonitrile. Cool to room temperature and dilute with *Diluent* to volume.

Sample solution: Nominally 0.15 mg/mL of oxymorphone hydrochloride from Injection prepared as follows. Transfer a suitable volume of the composite sample from NLT 20 ampules to a suitable volumetric flask. Dilute with *Solution A* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector
Assay: UV 230 nm
Identification test B: Diode array UV 200–360 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*
r_S = peak response of oxymorphone from the *Standard solution*
C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80
M_{r2} = molecular weight of oxymorphone, 301.34

Acceptance criteria: 93.0%–107.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability stock solution A: 0.2 mg/mL of USP Oxymorphone Related Compound A RS prepared as follows. Transfer a suitable amount of USP Oxymorphone Related Compound A RS to a suitable volumetric flask. Dissolve with 24% of the flask volume of 0.1 N hydrochloric acid and dilute with acetonitrile to volume.

System suitability stock solution B: 0.02 mg/mL of USP Oxymorphone Related Compound A RS in acetonitrile from *System suitability stock solution A*

System suitability stock solution C: 0.14 mg/mL of USP Oxymorphone RS prepared as follows. Transfer a suitable amount of USP Oxymorphone RS to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*. Sonicate to dissolve if necessary. Add 9% of the flask volume of acetonitrile. Cool to room temperature and dilute with *Diluent* to volume.

System suitability solution: 0.0008 mg/mL of USP Oxymorphone Related Compound A RS in *System suitability stock solution C* from *System suitability stock solution B*

Standard solution: 0.00014 mg/mL of USP Oxymorphone RS prepared as follows. Dilute *System suitability stock solution C* with *Solution A*.

System suitability
Samples: *System suitability solution* and *Standard solution*

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Suitability requirements
Resolution: NLT 2 between oxymorphone related compound A and oxymorphone, *System suitability solution*
Relative standard deviation: NMT 10%, *Standard solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual degradation product in the portion of Injection taken:

$$\text{Result} = \{(r_U/F)/[r_S + \Sigma(r_U/F)]\} \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*
F = relative response factor of each individual degradation product (see *Table 2*)
r_S = peak response of oxymorphone from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Hydroxyoxymorphone[a]	0.59	1.0	0.20
Oxymorphone related compound A (oxymorphone *N*-oxide)	0.82	1.1	0.30
Oxymorphone	1.00	1.0	—
10-Ketooxymorphone[b]	1.37	0.83	0.30
Oxycodone[c]	1.97	1.0	—
1-Bromooxymorphone[c, d]	2.05	1.0	—
2,2′-Bisoxymorphone[e]	2.08	1.7	1.00
Any individual unspecified degradation product	—	1.0	0.50
Total degradation products	—	—	2.00

[a] 4,5α-Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.
[b] 4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.
[c] Process impurities, not included in the total degradation products.
[d] 1-Bromo-4,5α-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.
[e] 2,2′-Bioxymorphone.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 238.1 USP Endotoxin Units/mg of oxymorphone hydrochloride
- **PH** ⟨791⟩: 2.7–4.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers of Type I glass. Store at 25°, excursions permitted between 15° and 30°, and protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxymorphone RS
  USP Oxymorphone Related Compound A RS
  4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one *N*-oxide.
  $C_{17}H_{19}NO_5$ 317.34

Oxymorphone Hydrochloride Tablets

DEFINITION
Oxymorphone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$).

IDENTIFICATION
- **A.** The retention time of the oxymorphone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the oxymorphone peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.
Solution A: Dissolve 2.02 g of sodium 1-heptanesulfonate in 900 mL of water and add 100 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.
Solution B: Dissolve 2.02 g of sodium 1-heptanesulfonate in 750 mL of water and add 250 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
35	0	100
40	0	100
40.1	100	0
50.1	100	0

Standard solution: 0.14 mg/mL of USP Oxymorphone RS in *Solution A*. Sonicate to dissolve if necessary.
Sample solution: Nominally 0.16 mg/mL of oxymorphone hydrochloride in *Solution A* prepared as follows. Transfer NLT 8 Tablets to a suitable volumetric flask and add about 50% of the final volume of *Solution A*. Sonicate for at least 15 min with occasional vigorous shaking until the Tablets disintegrate completely. Then shake for at least 20 min. Immediately dilute with *Solution A* to volume, and mix well. Immediately pass the solution through a suitable filter of 0.45-µm pore size, discard the first 5 mL of the filtrate, and use the filtrate for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Assay: UV 230 nm
Identification test B: Diode array UV 200–360 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

USP Monographs

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*
r_S = peak response of oxymorphone from the *Standard solution*
C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80
M_{r2} = molecular weight of oxymorphone, 301.34

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: Dissolve 2.02 g of sodium 1-heptanesulfonate in 800 mL of water and add 200 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.
Standard stock solution: 0.1 mg/mL of USP Oxymorphone RS in 0.1 N hydrochloric acid
Standard solution: (L/1000) mg/mL of USP Oxymorphone RS in water from the *Standard stock solution*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 60 µL
Run time: NLT 2.7 times the retention time of oxymorphone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*
r_S = peak response of oxymorphone from the *Standard solution*
C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80
M_{r2} = molecular weight of oxymorphone, 301.34
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 20 min
Buffer: Dissolve 26.4 g of dibasic ammonium phosphate in 2 L of water.
Mobile phase: Acetonitrile, methanol, and *Buffer* (5:25:70)
Diluent: *Buffer*
Standard stock solution: 0.05 mg/mL of USP Oxymorphone RS in *Medium*
Standard solution: 0.0025 mg/mL of USP Oxymorphone RS in *Diluent* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute this solution with *Diluent* to obtain a solution with a similar concentration as that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 1.6 times the retention time of oxymorphone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*
r_S = peak response of oxymorphone from the *Standard solution*
C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80
M_{r2} = molecular weight of oxymorphone, 301.34
L = label claim (mg/Tablet)

Tolerances: NLT 85% (Q) of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

USP Monographs

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Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution A: 0.2 mg/mL of USP Oxymorphone Related Compound A RS prepared as follows. Transfer an amount of USP Oxymorphone Related Compound A RS to a suitable volumetric flask. Dissolve with 24% of the flask volume of 0.1 N hydrochloric acid and dilute with acetonitrile to volume.

System suitability stock solution B: 0.02 mg/mL of USP Oxymorphone Related Compound A RS in acetonitrile from *System suitability stock solution A*

System suitability stock solution C: 0.14 mg/mL of USP Oxymorphone RS in *Solution A*

System suitability solution: 0.0008 mg/mL of USP Oxymorphone Related Compound A RS in *System suitability stock solution C* from *System suitability stock solution B*

Standard solution: 0.00014 mg/mL of USP Oxymorphone RS in *Solution A* from *System suitability stock solution C*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between oxymorphone related compound A and oxymorphone, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*

r_T = sum of peak responses from the *Sample solution*

F = relative response factor of each individual degradation product (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Hydroxyoxymorphone[a]	0.58	1.00	0.2
Oxymorphone related compound A (oxymorphone *N*-oxide)	0.81	1.09	0.2
Oxymorphone	1.00	1.00	—
10-Ketooxymorphone[b]	1.42	0.93	0.2
Oxycodone[c]	2.11	—	—
1-Bromooxymorphone[c,d]	2.22	—	—
2,2′-Bisoxymorphone[e]	2.36	1.61	0.2
Any individual unspecified degradation product	—	1.00	0.2
Total degradation products	—	—	1.5

[a] 4,5α-Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.
[b] 4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.
[c] Process impurities, not included in the total degradation products.
[d] 1-Bromo-4,5α-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.
[e] 2,2′-Bioxymorphone.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Oxymorphone RS
  USP Oxymorphone Related Compound A RS
  4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one *N*-oxide.
  $C_{17}H_{19}NO_5$ 317.34

Oxymorphone Hydrochloride Extended-Release Tablets

DEFINITION

Oxymorphone Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 2.34 g of sodium 1-octanesulfonate monohydrate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.80.

Solution B: Acetonitrile and methanol (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.00	77.0	23.0
2.50	77.0	23.0
17.50	54.0	46.0
25.00	31.0	69.0
25.05	1.5	98.5
32.50	1.5	98.5
32.55	77.0	23.0
38.00	77.0	23.0

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Diluent: Methanol and phosphoric acid (1000:1)
Standard stock solution: 1.78 mg/mL of USP Oxymorphone RS in *Diluent*
Standard solution: 0.357 mg/mL of USP Oxymorphone RS in *Solution A* from the *Standard stock solution*
Sample stock solution: Nominally 2 mg/mL of oxymorphone hydrochloride in *Diluent* prepared as follows. Take NLT 8 Tablets, cut each into small pieces, and transfer to a suitable flask. Add a suitable volume of *Diluent* and shake for at least 16 h. Centrifuge at 3500 rpm for 5 min or until a clear supernatant is obtained.
Sample solution: Nominally 0.4 mg/mL of oxymorphone hydrochloride in *Solution A* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Assay: UV 230 nm
Identification test B: Diode array UV 200–360 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Column temperature: 50°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*
r_S = peak response of oxymorphone from the *Standard solution*
C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80
M_{r2} = molecular weight of oxymorphone, 301.34

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1
Medium: 45 mM phosphate buffer, pH 4.50 (dissolve 6.16 g of monobasic potassium phosphate in 1 L of water. Adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 4.50); 900 mL
Apparatus 2: 50 rpm, with sinker. [Note—The Sotax Helix sinker can be used.]
Times: 1, 2, and 8 h
Mobile phase: Dissolve 1.54 g of ammonium acetate in 925 mL of water and mix well. Add 75 mL of acetonitrile and adjust with trifluoroacetic acid to a pH of 4.50.
Standard stock solution: 0.2 mg/mL of USP Oxymorphone RS in *Medium*
Standard solution: [(*L*/900) × (301.34/337.80)] mg/mL of USP Oxymorphone RS in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Withdraw 1.5 mL of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 7.5-cm; 4-µm packing L11
Column temperature: 60°
Flow rate: 2.0 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of oxymorphone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*
r_S = peak response of oxymorphone from the *Standard solution*
C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80
M_{r2} = molecular weight of oxymorphone, 301.34
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	20–40
2	2	35–55
3	8	NLT 80

The percentages of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Apparatus 2, Mobile phase, Standard stock solution, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in *Test 1*.
Medium: 50 mM phosphate buffer, pH 4.50 (dissolve 6.8 g of monobasic potassium phosphate in 1 L of water. Adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 4.50); 900 mL
Times: 1, 4, and 10 h
Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	30–50

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Table 3 *(continued)*

Time Point (*i*)	Time (h)	Amount Dissolved (%)
2	4	65–85
3	10	NLT 85

The percentages of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: 50 mM phosphate buffer, pH 4.50 (dissolve 40.8 g of monobasic potassium phosphate in 6 L of water. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 4.50); 900 mL

Apparatus 2: 50 rpm

Times: 1, 4, and 14 h

Buffer: 0.1 M ammonium phosphate prepared as follows. Dissolve 13.2 g of dibasic ammonium phosphate in 1 L of water, and mix well.

Mobile phase: Acetonitrile, methanol, and *Buffer* (5:25:70)

Standard solution: 0.0112 mg/mL of USP Oxymorphone RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 212 nm

Column: 4.6-mm × 15.0-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

Run time: NLT 2 times the retention time of oxymorphone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response of oxymorphone from the *Sample solution*

r_S = peak response of oxymorphone from the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

M_{r2} = molecular weight of oxymorphone, 301.34

Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of oxymorphone hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point (*i*) (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	15–40
2	4	45–70
3	14	NLT 80

The percentages of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.

Medium: 50 mM phosphate buffer, pH 6.8 (dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, and add 77 mL of 0.2 N sodium hydroxide and 500 mL of water. Adjust with 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of 6.8 and dilute with water to 1 L); 900 mL

Apparatus 2: 50 rpm

Times: 1, 4, and 10 h

Buffer: Triethylamine and water (2:1000). Adjust with phosphoric acid or 5 N sodium hydroxide to a pH of 6.8.

Mobile phase: Acetonitrile and *Buffer* (14:86)

Standard solution: [(*L*/900) × (301.34/337.80)] mg/mL of USP Oxymorphone RS in *Medium*, where *L* is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 15.0-cm; 5-µm packing L11

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 µL for 10-, 15-, 20-, 30-, and 40-mg strengths; 20 µL for 5- and 7.5-mg strengths

Run time: NLT 1.5 times the retention time of oxymorphone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

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r_U = peak response of oxymorphone from the *Sample solution*
r_S = peak response of oxymorphone from the *Standard solution*
C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80
M_{r2} = molecular weight of oxymorphone, 301.34

Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_1 = concentration of oxymorphone hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (*i*) (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)		
		For 5-, 7.5-, 10-, 15-, and 20-mg Strengths	For 30-mg Strengths	For 40-mg Strengths
1	1	25–45	25–45	15–40
2	4	65–90	60–80	50–70
3	10	NLT 80	NLT 80	NLT 80

The percentages of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.357 µg/mL of USP Oxymorphone RS from the *Standard solution* prepared as follows. Add 20% of the total volume of *Diluent* and dilute with *Solution A* to volume.

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*
r_T = sum of peak responses from the *Sample solution*
F = relative response factor of each individual degradation product (see *Table 6*)

Acceptance criteria: See *Table 6*. Disregard any peaks less than 0.05%.

Table 6

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxymorphone related compound A[a] (oxymorphone *N*-oxide)	0.57	1.11	0.2
10-Hydroxyoxymorphone[b]	0.70	1.14	0.2
Oxymorphone	1.00	—	—
10-Ketooxymorphone[c]	1.33	0.97	0.2
Oxycodone[d]	1.82	—	—
14-Hydroxycodeinone[d, e]	1.89	—	—
1-Bromooxymorphone[d, f]	1.89	—	—
2,2′-Bisoxymorphone[g]	2.28	2.36	0.2
Any individual unspecified degradation product	—	1.00	0.2
Total degradation products	—	—	1.0

[a] 4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one *N*-oxide.
[b] 4,5α-Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.
[c] 4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.
[d] Process impurities, not included in the total degradation products.
[e] 4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one.
[f] 1-Bromo-4,5α-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.
[g] 2,2′-Bioxymorphone.

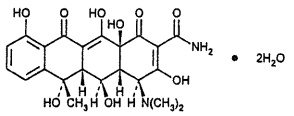
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP Reference Standards** ⟨11⟩
USP Oxymorphone RS

Oxytetracycline



$C_{22}H_{24}N_2O_9 \cdot 2H_2O$ 496.46

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-, [4*S*-(4α,4aα,5α,5aα,6β,12aα)]-, dihydrate.

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4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide dihydrate [6153-64-6]. Anhydrous 460.44 [79-57-2].

» Oxytetracycline has a potency equivalent to not less than 832 µg of $C_{22}H_{24}N_2O_9$ per mg.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Oxytetracycline RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivity at 353 nm, calculated on the anhydrous basis, is between 96.0% and 104.0% of that of USP Oxytetracycline RS, the potency of the Reference Standard being taken into account.
B: To 1 mg add 2 mL of sulfuric acid: a light red color is produced.
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 4.5 and 7.0, in an aqueous suspension containing 10 mg per mL.
Water Determination, *Method I* ⟨921⟩: between 6.0% and 9.0%.
Other requirements—Where the label states that Oxytetracycline is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Oxytetracycline for Injection.* Where the label states that Oxytetracycline must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Oxytetracycline for Injection.*

Assay—
Tetrabutylammonium hydrogen sulfate solution—Dissolve 1 g of tetrabutylammonium hydrogen sulfate in 100 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5.
Edetate disodium solution—Dissolve 0.04 g of edetate disodium in 100 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5.
pH 7.5 Phosphate buffer—Prepare a mixture of 0.33 M dibasic potassium phosphate and 0.33 M monobasic sodium phosphate (85:15). Adjust, if necessary, by adding more of the appropriate component to a pH of 7.5.
Mobile phase—Transfer, with the aid of 200 mL of water, 50 g of tertiary butyl alcohol to a 1000-mL volumetric flask. Add 60 mL of *pH 7.5 Phosphate buffer,* 50 mL of *Tetrabutylammonium hydrogen sulfate solution,* and 10 mL of *Edetate disodium solution,* and dilute with water to volume. Degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Oxytetracycline RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 0.22 mg per mL.
System suitability solution—Prepare a solution of tetracycline hydrochloride in 0.01 N hydrochloric acid containing about 0.2 mg per mL. Mix 3 mL of this solution and 1.5 mL of the *Standard preparation,* and dilute with water to 25 mL.
Assay preparation—Transfer about 44 mg of Oxytetracycline to a 200-mL volumetric flask, add about 25 mL of 0.01 N hydrochloric acid, swirl to dissolve, dilute with 0.01 N hydrochloric acid to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L21 and is maintained at 60 ± 2°. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for oxytetracycline and 1.0 for tetracycline; and the resolution, *R,* between the oxytetracycline peak and the tetracycline peak is not less than 5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.25; and the relative standard deviation for replicate injections is not more than 1.0%.
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of $C_{22}H_{24}N_2O_9$ in each mg of the Oxytetracycline taken by the formula:

$$200(CP/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Oxytetracycline RS in the *Standard preparation; P* is the assigned potency, in µg per mg, of USP Oxytetracycline RS; W is the weight, in mg, of the Oxytetracycline taken to prepare the *Assay preparation;* and r_U and r_S are the oxytetracycline peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Oxytetracycline Injection

» Oxytetracycline Injection is a sterile solution of Oxytetracycline with or without one or more suitable anesthetics, antioxidants, buffers, complexing agents, preservatives, and solvents. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of Oxytetracycline ($C_{22}H_{24}N_2O_9$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, protected from light.
USP Reference standards ⟨11⟩—
USP Oxytetracycline RS
Identification—To an accurately measured volume of Injection, equivalent to about 50 mg of oxytetracycline, add 50 mL of methanol, and shake. Using the clear solution so obtained as the *Test Solution,* proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.4 USP Endotoxin Unit per mg of oxytetracycline.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩**:** between 8.0 and 9.0.

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Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Oxytetracycline.*

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of oxytetracycline, to a 500-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Oxytetracycline.* Calculate the quantity, in mg, of $C_{22}H_{24}N_2O_9$ in each mL of the Injection taken by the formula:

$$0.5(CP/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Oxytetracycline RS in the *Standard preparation; V* is the volume, in mL, of Injection taken to prepare the *Assay preparation;* and the other terms are as defined therein.

Oxytetracycline Tablets

» Oxytetracycline Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{22}H_{24}N_2O_9$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Oxytetracycline RS

Identification—Shake a suitable quantity of finely powdered Tablets with methanol to obtain a solution containing about 1 mg of oxytetracycline per mL, and filter. Using the filtrate as the *Test Solution,* proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.

Procedure—Determine the amount of $C_{22}H_{24}N_2O_9$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 353 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Oxytetracycline RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{22}H_{24}N_2O_9$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Water Determination, *Method I* ⟨921⟩: not more than 7.5%.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Oxytetracycline.*

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of oxytetracycline, to a 500-mL volumetric flask, add about 25 mL of 0.01 N hydrochloric acid, and mix. Dilute with 0.01 N hydrochloric acid to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the filtrate as the *Assay preparation.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Oxytetracycline.* Calculate the quantity, in mg, of $C_{22}H_{24}N_2O_9$ in the portion of Tablets taken by the formula:

$$0.5(CP)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Oxytetracycline RS in the *Standard preparation,* and the other terms are as defined therein.

Oxytetracycline Hydrochloride

$C_{22}H_{24}N_2O_9 \cdot HCl$ 496.89

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, [4*S*-(4α,4aα,5α,5aα,6β,12aα)]-.

4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [2058-46-0].

» Oxytetracycline Hydrochloride has a potency equivalent to not less than 835 µg of oxytetracycline ($C_{22}H_{24}N_2O_9$) per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Where it is intended for use in preparing injectable or ophthalmic dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or ophthalmic dosage forms.

USP Reference standards ⟨11⟩—
USP Oxytetracycline RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: 0.1 N hydrochloric acid.

Absorptivity, calculated on the dried basis, at 353 nm is between 88.2% and 96.8% of that of USP Oxytetracycline RS, the potency of the Reference Standard being taken into account.

B: To 1 mg add 2 mL of sulfuric acid: a light red color is produced.

Crystallinity ⟨695⟩: meets the requirements.

pH ⟨791⟩: between 2.0 and 3.0, in a solution containing 10 mg per mL.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

Other requirements—Where the label states that Oxytetracycline Hydrochloride is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Oxytetracycline for Injection.* Where the label states that Oxytetracycline Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms,

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it meets the requirements for *Bacterial endotoxins* under *Oxytetracycline for Injection*. Where it is intended for use in preparing ophthalmic dosage forms, it is exempt from the requirements for *Bacterial endotoxins*.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Oxytetracycline*.

Assay preparation—Transfer about 44 mg of Oxytetracycline Hydrochloride to a 200-mL volumetric flask, add about 25 mL of 0.01 N hydrochloric acid, swirl to dissolve, dilute with 0.01 N hydrochloric acid to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Oxytetracycline*. Calculate the quantity, in µg, of oxytetracycline ($C_{22}H_{24}N_2O_9$) in each mg of Oxytetracycline Hydrochloride taken by the formula:

$$200(CP/W)(r_U/r_S)$$

in which the terms are as defined therein.

Oxytetracycline Hydrochloride Capsules

» Oxytetracycline Hydrochloride Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Oxytetracycline RS

Identification—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing 1 mg of oxytetracycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩.

Dissolution ⟨711⟩—

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{22}H_{24}N_2O_9$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 273 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Oxytetracycline RS in the same medium, using 5 mL of 0.1 N hydrochloric acid to dissolve the Standard.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{22}H_{24}N_2O_9$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Loss on drying ⟨731⟩—Dry about 100 mg of Capsule contents, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

Assay—

Tetrabutylammonium hydrogen sulfate solution , Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Oxytetracycline*.

Assay preparation—Remove, as completely as possible, the contents of not less than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of oxytetracycline, to a 500-mL volumetric flask, add about 50 mL of 0.01 N hydrochloric acid, and swirl to dissolve. Dilute with 0.01 N hydrochloric acid to volume, mix, and filter a portion of the solution through a 0.5-µm or finer porosity filter. Use the filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Oxytetracycline*. Calculate the quantity, in mg, of oxytetracycline ($C_{22}H_{24}N_2O_9$) in the portion of Capsules taken by the formula:

$$0.5(CP)(r_U/r_S)$$

in which the terms are as defined therein.

Oxytetracycline for Injection

» Oxytetracycline for Injection contains an amount of Oxytetracycline Hydrochloride equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* protected from light.

USP Reference standards ⟨11⟩—
USP Oxytetracycline RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.4 USP Endotoxin Unit per mg of oxytetracycline.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined, Fluid D* being used instead of *Fluid A*.

pH ⟨791⟩: between 1.8 and 2.8, in a solution containing 25 mg per mL.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It responds to *Identification* test *B* under *Oxytetracycline Hydrochloride*. It also meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Oxytetracycline*.

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Oxytetracycline for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with 0.01 N hydrochloric acid to obtain a

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solution having a concentration of about 0.2 mg of oxytetracycline per mL.

Assay preparation 2 (where the label states the quantity of oxytetracycline in a given volume of constituted solution)—Constitute Oxytetracycline for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.2 mg of oxytetracycline per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Oxytetracycline*. Calculate the quantity, in mg, of oxytetracycline ($C_{22}H_{24}N_2O_9$) withdrawn from the container or in the portion of constituted solution taken by the formula:

$$(L/D)(CP)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of oxytetracycline ($C_{22}H_{24}N_2O_9$) in the container or in the portion of constituted solution taken; D is the concentration, in mg per mL, of oxytetracycline in *Assay preparation 1* or in *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein.

Chromatographic system— Proceed as directed in the *Assay* under *Oxytetracycline*.

Assay preparation—Transfer an accurately weighed portion of the Soluble Powder, equivalent to about 100 mg of oxytetracycline hydrochloride, to a 500-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity. Use the filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Oxytetracycline*. Calculate the quantity, in g, of oxytetracycline hydrochloride ($C_{22}H_{24}N_2O_9 \cdot HCl$) in each g of Soluble Powder taken by the formula:

$$0.5(496.90/460.44)(CP/W)(r_U/r_S)$$

in which 496.90 and 460.44 are the molecular weights of oxytetracycline hydrochloride and oxytetracycline, respectively; C is the concentration, in mg per mL, of USP Oxytetracycline RS in the *Standard preparation*; P is the assigned potency, in µg of oxytetracycline per mg, of USP Oxytetracycline RS; W is the weight, in g, of Soluble Powder taken to prepare the *Assay preparation*; and r_U and r_S are the oxytetracycline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oxytetracycline Hydrochloride Soluble Powder

» Oxytetracycline Hydrochloride Soluble Powder is a dry mixture of Oxytetracycline Hydrochloride and one or more suitable excipients. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of oxytetracycline hydrochloride ($C_{22}H_{24}N_2O_9 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is for oral veterinary use only.

USP Reference standards ⟨11⟩—
USP Oxytetracycline RS

Identification—

A: Shake a quantity of Soluble Powder with methanol to obtain a solution containing about 1 mg of oxytetracycline hydrochloride per mL. Filter if necessary to obtain a clear solution. Using the filtrate as the *Test solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

pH ⟨791⟩: between 1.5 and 3.0, in the solution obtained as directed in the labeling.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.

Minimum fill ⟨755⟩: meets the requirements.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and

Oxytetracycline Hydrochloride and Hydrocortisone Acetate Ophthalmic Suspension

» Oxytetracycline Hydrochloride and Hydrocortisone Acetate Ophthalmic Suspension is a sterile suspension of Oxytetracycline Hydrochloride and Hydrocortisone Acetate in a suitable oil vehicle with one or more suitable suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in tight, light-resistant containers. The containers are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Oxytetracycline RS

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined* using 0.25 mL of specimen.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 60 mL of a mixture of methanol and chloroform (2:1) being used instead of methanol in the titration vessel.

Assay for oxytetracycline—Transfer an accurately measured volume of Ophthalmic Suspension to a separator, add 50 mL of ether, and shake. Add 25 mL of 0.1 N hydrochloric acid, shake, and allow to separate. Collect the acid layer, and repeat the extraction with three additional 25-mL portions of 0.1 N hydrochloric acid. Combine the acid extracts in a 200-mL volumetric flask, dilute with 0.1 N

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hydrochloric acid to volume, and mix. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone acetate—

Mobile phase—Prepare a degassed and filtered mixture of water and methanol (50:50).

Standard preparation—Dissolve an accurately weighed quantity of USP Hydrocortisone Acetate RS in a mixture of *Mobile phase* and alcohol (80:20) to obtain a solution having a known concentration of about 0.06 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, equivalent to about 30 mg of hydrocortisone acetate, to a separator containing 25 mL of pH 9.0 alkaline borate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*). Extract with four 25-mL portions of chloroform, filtering each chloroform extract through a thin layer of chloroform-washed anhydrous sodium sulfate into a 250-mL volumetric flask. Rinse the sodium sulfate with chloroform, collecting the filtrate in the volumetric flask, dilute with chloroform to volume, and mix. Transfer 25.0 mL of the resulting solution to a 50-mL conical flask, and evaporate slowly with the aid of mild heat until about 5 mL remains. Add about 15 mL of alcohol, and evaporate slowly until about 5 mL remains. Transfer this solution to a 50-mL volumetric flask, dilute with a mixture of *Mobile phase* and alcohol (80:20) to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 235 theoretical plates, the tailing factor for the analyte peak is not more than 1.7, and the relative standard deviation of replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{32}O_6$ in each mL of the Ophthalmic Suspension taken by the formula:

$$500(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation; V* is the volume, in mL, of Ophthalmic Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Oxytetracycline Hydrochloride and Hydrocortisone Ointment

» Oxytetracycline Hydrochloride and Hydrocortisone Ointment contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone.

Packaging and storage—Preserve in collapsible tubes or in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Oxytetracycline RS

Minimum fill ⟨755⟩: meets the requirements.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for oxytetracycline—Transfer a suitable, accurately weighed quantity of Ointment to a separator, add 50 mL of ether, and shake. Add 20 mL of 0.1 N hydrochloric acid, shake, and allow to separate. Collect the acid layer, and repeat the extraction with three additional 20-mL portions of 0.1 N hydrochloric acid. Combine the acid extracts in a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Quantitatively dilute a portion of this solution with 0.1 N hydrochloric acid so that the solution so obtained contains not less than 150 µg of oxytetracycline per mL. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone—Proceed with Ointment as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment.*

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Ointment

» Oxytetracycline Hydrochloride and Polymyxin B Sulfate Ointment contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$), and not less than 90.0 percent and not more than 125.0 percent of the labeled amount of polymyxin B.

Packaging and storage—Preserve in collapsible tubes, or in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Oxytetracycline RS
USP Polymyxin B Sulfate RS

Minimum fill ⟨755⟩: meets the requirements.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 10 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for oxytetracycline—Proceed with Ointment as directed in the *Assay for oxytetracycline* under *Oxytetracycline Hydrochloride and Hydrocortisone Ointment.*

Assay for polymyxin B—Transfer an accurately weighed quantity of Ointment, equivalent to about 10,000 USP Polymyxin B Units, to a 15-mL centrifuge tube, add 10 mL of ether, stir, and centrifuge for 10 minutes. Decant and discard the clear ether. Wash the residue with 10 mL of ether, centrifuge for 10 minutes, decanting and discarding the clear ether. Wash the residue with several 10-mL portions of acetone, centrifuging, decanting, and discarding each

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washing until the yellow color is removed from the residue. [NOTE—Take care not to remove any of the residue with the washings.] Add 0.2 mL of polysorbate 80 to the residue, and mix. Transfer the mixture to a 100-mL volumetric flask with the aid of *Buffer B.6*, dilute with the same solvent to volume, and mix. Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this solution diluted quantitatively with *Buffer B.6* to yield a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Ophthalmic Ointment

DEFINITION

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Ophthalmic Ointment is a sterile ointment containing Oxytetracycline Hydrochloride and Polymyxin B Sulfate. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of oxytetracycline and NLT 90.0% and NMT 125.0% of the labeled amount of polymyxin B.

ASSAY

- **OXYTETRACYCLINE**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Transfer a suitable, weighed quantity of the Ophthalmic Ointment to a separator, add 50 mL of ether, and shake. Add 20 mL of 0.1 N hydrochloric acid, shake, and allow to separate. Collect the acid layer, and repeat the extraction with three additional 20-mL portions of 0.1 N hydrochloric acid. Combine the acid extracts in a 100-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume. Dilute a portion of this solution with 0.1 N hydrochloric acid to obtain a solution containing NLT 150 µg/mL of oxytetracycline.

Analysis: Proceed as directed in the chapter, using a suitable aliquot of the *Sample solution* diluted with water, to yield a *Test Dilution* having an oxytetracycline concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–120.0%

- **POLYMYXIN B**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Transfer nominally 10,000 USP Polymyxin B Units from the Ophthalmic Ointment to a 15-mL centrifuge tube. Add 10 mL of ether, stir, and centrifuge for 10 min. Decant, and discard the clear ether. Wash the residue with 10 mL of ether, and centrifuge for 10 min, decanting and discarding the clear ether. Wash the residue with several 10-mL portions of acetone, centrifuging, decanting, and discarding each washing until the yellow color is removed from the residue. Take care not to remove any of the residue with the washings. Add 0.2 mL of polysorbate 80 to the residue, and mix. Transfer the mixture to a 100-mL volumetric flask with the aid of *Buffer B.6*, and dilute with the same solvent to volume.

Analysis: Proceed as directed in the chapter, using a suitable aliquot of the *Sample solution* diluted with *Buffer B.6*, to yield a *Test Dilution* having a polymyxin B concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–125.0%

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Oxytetracycline RS
USP Polymyxin B Sulfate RS

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Topical Powder

» Oxytetracycline Hydrochloride and Polymyxin B Sulfate Topical Powder contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of oxytetracycline ($C_{22}H_{24}N_2O_9$) and polymyxin B in a suitable, fine powder base.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Oxytetracycline RS
USP Polymyxin B Sulfate RS

Minimum fill ⟨755⟩**:** meets the requirements.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

Assay for oxytetracycline—Transfer a suitable, accurately weighed quantity of Topical Powder to a glass blender jar containing a sufficient, accurately measured volume of 0.1 N hydrochloric acid to yield a stock solution containing not less than 150 µg of oxytetracycline per mL, and blend at high speed for 3 to 5 minutes. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration of oxytetracycline assumed to be equal to the median dose level of the Standard.

Assay for polymyxin B—Transfer an accurately weighed quantity of Topical Powder, equivalent to about 10,000 USP Polymyxin B Units, to a 50-mL centrifuge tube, add 15 mL of acetone and 0.05 mL of hydrochloric acid, and stir. Add 20 mL of acetone, and centrifuge for 10 minutes. Decant and discard the clear liquid. Add 15 mL of acetone and 0.05 mL of hydrochloric acid to the residue, stir, add 20 mL of acetone, and centrifuge for 10 minutes, decanting and discarding the clear liquid. [NOTE—Take care not to discard any of the residue with the clear liquid.] Add 5 mL of *Buffer B.6* to the residue, and mix. Transfer the mixture to a 100-mL volumetric flask with the aid of *Buffer B.6*, dilute with the same solvent to volume, and mix. Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock solution diluted quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

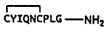
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Oxytetracycline Hydrochloride and Polymyxin B Sulfate Vaginal Inserts

» Oxytetracycline Hydrochloride and Polymyxin B Sulfate Vaginal Inserts contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of oxytetracycline ($C_{22}H_{24}N_2O_9$) and polymyxin B.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Oxytetracycline RS
USP Polymyxin B Sulfate RS
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, of powdered Vaginal Inserts in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.
Assay for oxytetracycline—Place not fewer than 5 Vaginal Inserts in a high-speed blender jar containing an accurately measured volume of 0.1 N hydrochloric acid, so that the stock solution obtained after blending for 3 to 5 minutes contains not less than 150 µg of oxytetracycline per mL. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration of oxytetracycline assumed to be equal to the median dose level of the Standard.
Assay for polymyxin B—Weigh and finely powder not fewer than 5 Vaginal Inserts. Transfer an accurately weighed portion of the powder, equivalent to about 100,000 USP Polymyxin B Units, to a filter funnel equipped with a solvent-resistant membrane filter (1-µm or finer porosity). Wash the powder with five 20-mL portions of acetone, applying vacuum, and discarding the accumulated filtrate. [NOTE—If necessary, wash the powder with additional portions of acetone to remove any yellow color.] Carefully transfer the filter and the washed powder to a beaker containing about 400 mL of *Buffer B.6* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers*), and stir. Transfer the contents of the beaker to a 500-mL volumetric flask with the aid of *Buffer B.6*, dilute with the same solvent to volume, and mix. Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this solution diluted quantitatively and stepwise with *Buffer B.6* to yield a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Oxytocin



$C_{43}H_{66}N_{12}O_{12}S_2$ 1007.19
Oxytocin.
Oxytocin [50-56-6].

» Oxytocin is a nonapeptide hormone having the property of causing the contraction of uterine smooth muscle and of the myoepithelial cells within the mammary gland. It is prepared by synthesis. Its oxytocic activity is not less than 400 USP Oxytocin Units per mg.

Packaging and storage—Preserve in tight containers, preferably of Type I glass, in a refrigerator.
USP Reference standards ⟨11⟩—
USP Oxytocin RS
USP Oxytocin Identification RS
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—The total bacterial count does not exceed 200 cfu per g. For products of animal origin, it meets also the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

Identification—
A: The retention time of the oxytocin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.
Perform one of the following two tests:
B: Nuclear Magnetic Resonance— [NOTE—Concentrations of Oxytocin in both the *Standard solution* and the *Test solution* must be the same (within 5% of each other) but can be adjusted based on the quality of the spectrum obtained. The spectra must be acquired under the same conditions for both the *Standard solution* and the *Test solution*. The spectra obtained are of sufficient quality to allow quantification of the integrals of the resonances specified below to be obtained. Integrals and spectra of both the *Standard solution* and the *Test solution* can be repeated and averaged.]
pH 5.0 Sodium phosphate buffer—Dissolve 27.6 g of monobasic sodium phosphate in 900 mL of water, adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 5.0 ± 0.1, dilute with water to 1000 mL, and mix.
Standard solution—Prepare a 10 mg per mL solution (approximately 1 mL) of USP Oxytocin Identification RS in *pH 5.0 Sodium phosphate buffer*. Lyophilize to dryness, redissolve in deuterium oxide, lyophilize again, redissolve in deuterium oxide, and lyophilize once again (to replace exchangeable hydrogens with deuterium). Dissolve in 1 mL of deuterium oxide containing 0.5% v/v (2,2,3,3-(d4)-3-(trimethylsilyl) propionic acid sodium salt (TSP) as a chemical shift reference.
Test solution—Prepare a 10 mg per mL solution (approximately 1 mL) of Oxytocin in *pH 5.0 Sodium phosphate buffer*. Proceed as directed for the *Standard solution*.
Procedure—Obtain a proton NMR spectrum of both the *Standard solution* and the *Test solution*. The spectra from both solutions are qualitatively and quantitatively similar, and all the resonances from the spectrum of the *Standard solution* are present in the spectrum of the *Test solution* and have the same chemical shift values (±0.1 ppm). Identify any other resonances in the spectrum of the *Test solution* that are not present in the spectrum of the *Standard solution*. The integrals of the acetate and deuterium oxide peaks at 1.9 ppm and 4.9 ppm can differ quantitatively in the spectra of the *Standard solution* and the *Test solution*.
C: Amino acid content—Use a suitable, validated procedure (see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩).
Standard solutions—Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. For the validation of the method, use an appropriate internal standard, such as norleucine. Prepare a separate, equimolar solution of L-tryptophan.
Test solution— [NOTE—The following hydrolysis conditions and concentrations can be modified depending on the method of analysis chosen.] Transfer about 64 mg of Oxytocin, accurately weighed, to a suitable vessel, and dissolve in 1.0 mL of water. Transfer 0.10 mL of this solution to a vacuum hydrolysis tube, add 2.0 mL of 6 N hydrochloric acid, evacuate the tube, and

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heat for 16 hours at 120°. Transfer 0.10 mL of the hydrolysate so obtained to a suitable vessel, add 1 mL of water, and lyophilize. Dissolve in and dilute to a suitable volume in a buffer solution suitable for amino acid analysis.
Procedure—Inject equal volumes of the *Standard solutions* and the *Test solution* into the amino acid analyzer, and measure and record the responses for each amino acid peak. Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine, and leucine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half-cystine: 1.4 to 2.1. Not more than traces of other amino acids are present.
Acetic acid content ⟨503⟩**:** between 6% and 10%.
Test Solution—Transfer about 15 mg of Oxytocin, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.
Ordinary impurities—The sum of the responses of impurities in the chromatogram of the *Assay preparation* obtained in the *Assay* is not more than 5% of the area of the oxytocin peak.

Assay—
Mobile phase A—Prepare a buffer solution of 0.1 M monobasic sodium phosphate.
Mobile phase B—Prepare a filtered and degassed mixture of acetonitrile in water (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Dissolve 5.0 g of chlorobutanol in 5.0 mL of glacial acetic acid, add 5.0 g of alcohol, 1.1 g of sodium acetate, and 1000 mL of water, and mix.
Standard preparation—Dissolve the entire contents of a vial of USP Oxytocin RS in a known volume of *Diluent*. [NOTE—The solution may be diluted as necessary to a working concentration range for the assay.]
Assay preparation—Dissolve an accurately weighed quantity of Oxytocin in *Diluent* to obtain a solution containing about 10 USP Oxytocin Units per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a variable wavelength detector set at 220 nm and a 12.0-cm × 4.6-mm column that contains 5-µm packing L1, and is programmed to provide variable mixtures of *Mobile phase A* and *Mobile phase B*. The column is maintained at room temperature, and the flow rate is about 1.5 mL per minute. The system is equilibrated with a mixture of 70% *Mobile phase A* and 30% *Mobile phase B*. After each injection of the *Standard preparation* and the *Assay preparation*, the composition of the mobile phase is changed linearly over the next 20 minutes so that it consists of a mixture of 50% *Mobile phase A* and 50% *Mobile phase B*. Chromatograph the *Standard preparation*, and record the chromatograms as directed for *Procedure*. Adjust the flow rate or the composition of the *Mobile phase* such that the retention time of oxytocin is approximately 10 minutes and between 15 and 17 minutes for chlorobutanol. The resolution, *R*, between oxytocin and the nearest adjacent peak is not less than 1.5, and the relative standard deviation for replicate injections is not more than 2.0% for oxytocin.
Procedure—Separately inject three equal volumes (about 100 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, and record the chromatograms as described under *Chromatographic system*. Identify the peaks, and determine the area of the oxytocin peak. Calculate the potency of oxytocin in USP Oxytocin Units per mg by the formula:

$$C(r_U/r_S)(V/W)$$

in which C is the concentration, in USP Oxytocin Units per mL, of the *Standard preparation;* and r_U and r_S are the mean peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively; V is the volume of sample solution in which the sample was dissolved; and W is the amount, in mg, of oxytocin dissolved in the sample solution.

Oxytocin Injection

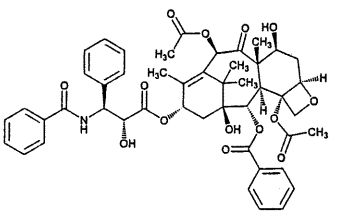
» Oxytocin Injection is a sterile solution of Oxytocin in a suitable diluent. Each mL of Oxytocin Injection possesses an oxytocic activity of not less than 90.0 percent and not more than 110.0 percent of that stated on the label in USP Oxytocin Units.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass or in suitable plastic containers.
Labeling—Label it to indicate its oxytocic activity in USP Oxytocin Units per mL. Label it also to state the animal source if naturally derived, or to state that it is synthetic.
USP Reference standards ⟨11⟩—
USP Oxytocin RS
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 35.7 Endotoxin Units per USP Oxytocin Unit.
pH ⟨791⟩**:** between 3.0 and 5.0.
Particulate Matter in Injections ⟨788⟩**:** meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed as directed for *Oxytocin* except to use undiluted Injection as the *Assay preparation* and to allow not less than 25 minutes between injections. Calculate the potency, in USP Oxytocin Units per mL, by the formula:

$$C(r_U/r_S)$$

in which C is the concentration, in USP Oxytocin Units per mL, of the *Standard preparation;* and r_U and r_S are the mean values of the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Paclitaxel



$C_{47}H_{51}NO_{14}$ 853.91
Benzenepropanoic acid, β-(benzoylamino)-α-hydroxy-, 6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H*-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester, [2a*R*-[2aα,4β,4aβ,6β,9α(α*R**,β*S**),11α,12α,12aα,12bα]]-.

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(2a*R*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]-benz[1,2-*b*]oxet-5-one 6,12b-diacetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine [33069-62-4].

» Paclitaxel contains not less than 97.0 percent and not more than 102.0 percent of $C_{47}H_{51}NO_{14}$, calculated on the anhydrous, solvent-free basis. [**CAUTION**—Paclitaxel is cytotoxic. Great care should be taken to prevent inhaling particles of Paclitaxel and exposing the skin to it.]

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.
Labeling—The labeling indicates the type of process used to produce the material and the *Related compounds* test with which the material complies.
USP Reference standards ⟨11⟩—
USP Paclitaxel RS
USP Paclitaxel Related Compound A RS
Cephalomannine.
USP Paclitaxel Related Compound B RS
10-Deacetyl-7-epipaclitaxel.
USP Paclitaxel Impurity Mixture RS
Mixture of paclitaxel and the following related compounds: propyl analog, cephalomannine, *sec*-butyl analog, *n*-butyl analog, benzyl analog, baccatin VI, pentyl analog, and 7-epipaclitaxel.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Specific rotation ⟨781S⟩: between –49.0° and –55.0° at 20°, calculated on the anhydrous, solvent-free basis.
Test solution: 10 mg per mL, in methanol.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—The total aerobic microbial count does not exceed 100 cfu per g. It meets the requirements of the tests for the absence of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* species, and *Escherichia coli.*
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.4 USP Endotoxin Unit per mg of paclitaxel.
Water Determination, *Method Ic* ⟨921⟩: not more than 4.0%.
Residue on ignition ⟨281⟩: not more than 0.2%.

Related compounds—
TEST 1 (FOR MATERIAL LABELED AS ISOLATED FROM NATURAL SOURCES)—If the material complies with this test, the labeling indicates that it meets USP *Related compounds Test 1.*
Diluent—Prepare as directed in the *Assay.*
Solution A—Prepare filtered and degassed acetonitrile.
Solution B—Prepare filtered and degassed water.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve accurately weighed quantities of USP Paclitaxel Related Compound A RS and USP Paclitaxel Related Compound B RS in methanol to obtain a solution having known concentrations of about 10 µg of each per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Standard solution—Dissolve, with the aid of sonication, an accurately weighed quantity of USP Paclitaxel RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 5 µg per mL.
Test solution—Use the *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L43. The flow rate is about 2.6 mL per minute. The column temperature is maintained at 30°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–35	35	65	isocratic
35–60	35→80	65→20	linear gradient
60–70	80→35	20→65	linear gradient
70–80	35	65	isocratic

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.78 for paclitaxel related compound A and 0.86 for paclitaxel related compound B (relative to the retention time for paclitaxel obtained from the *Test solution*); and the resolution, *R,* between paclitaxel related compound A and paclitaxel related compound B is not less than 1.0.
Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Inject a volume (about 15 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Paclitaxel taken by the formula:

$$100(Fr_i/r_U)$$

in which F is the relative response factor for each impurity peak (see *Table 1* for values); r_i is the peak area for each individual impurity; and r_U is the peak area for paclitaxel.

Table 1

Relative Retention Time	Relative Response Factor (*F*)	Name	Limit (%)
0.24	1.29	Baccatin III	0.2
0.53	1.00	10-Deacetylpaclitaxel	0.5
0.57	1.00	7-Xylosylpaclitaxel	0.2
0.78	1.26	Cephalomannine (paclitaxel related compound A)	a_1 [1]
0.78	1.26	2″,3″-Dihydrocephalomannine	a_2 [1]
0.86	1.00	10-Deacetyl-7-epipaclitaxel (paclitaxel related compound B)	0.5
1.10	1.00	Benzyl analog[3]	b_1 [2]
1.10	1.00	3″,4″-Dehydropaclitaxel C	b_2 [2]
1.40	1.00	7-Epicephalomannine	0.3

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Table 1 *(continued)*

Relative Retention Time	Relative Response Factor (*F*)	Name	Limit (%)
1.85	1.00	7-Epipaclitaxel	0.5

[1] Resolution may be incomplete for these peaks, depending upon the relative amounts present; the sum of a_1 and a_2 is not more than 0.5%.

[2] Resolution may be incomplete for these peaks, depending upon the relative amounts present; the sum of b_1 and b_2 is not more than 0.5%.

[3] The following chemical name is assigned to the related compound, benzyl analog: Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-phenyl-3-(2-phenylacetylamino)propanoic acid.

In addition to not exceeding the limits for paclitaxel related impurities in *Table 1*, not more than 0.1% of any other single impurity is found; and not more than 2.0% of total impurities is found.

TEST 2 (FOR MATERIAL LABELED AS PRODUCED BY A SEMISYNTHETIC PROCESS)—If the material complies with this test, the labeling indicates that it meets USP *Related compounds Test 2.*

Diluent—Use acetonitrile.

Solution A—Use a filtered and degassed mixture of water and acetonitrile (3:2).

Solution B—Use filtered and degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve accurately weighed quantities of USP Paclitaxel RS and USP Paclitaxel Related Compound B RS in *Diluent,* shaking and sonicating if necessary, to obtain a solution having known concentrations of about 0.96 mg and 0.008 mg per mL, respectively.

Test solution—Transfer about 10 mg of Paclitaxel, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, shaking and sonicating if necessary, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 15-cm column that contains 3-µm packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 35°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–20	100	0	isocratic
20–60	100→10	0→90	linear gradient
60–62	10→100	90→0	linear gradient
62–70	100	0	isocratic

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.94 for paclitaxel related compound B and 1.0 for paclitaxel; the resolution, *R,* between paclitaxel related compound B and paclitaxel is not less than 1.2; and the relative standard deviation for replicate injections is not more than 2.0% for the paclitaxel peak.

Procedure—Separately inject equal volumes (about 15 µL) of the *Diluent* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Disregard any peaks due to the *Diluent.* Calculate the percentage of each impurity in the portion of Paclitaxel taken by the formula:

$$100(Fr_i/r_s)$$

in which *F* is the relative response factor for each impurity (see *Table 2* for values); r_i is the peak area for each impurity obtained from the *Test solution;* and r_s is the sum of the areas of all the peaks obtained from the *Test solution.*

Table 2

Relative Retention Time	Relative Response Factor (*F*)	Name	Limit (%)
0.11	1.24	10-Deacetylbaccatin III	0.1
0.20	1.29	Baccatin III	0.2
0.42	1.39	Photodegradant[2]	0.1
0.47	1.00	10-Deacetylpaclitaxel	0.5
0.80	1.00	2-Debenzoylpaclitaxel-2-pentenoate	0.7
0.92[1]	1.00	Oxetane ring opened, acetyl and benzoyl migrated[2]	x_1
0.92[1]	1.00	10-Acetoacetylpaclitaxel	x_2
0.94[1]	1.00	10-Deacetyl-7-epipaclitaxel (paclitaxel related compound B)	x_3
1.37	1.00	7-Epipaclitaxel	0.4
1.45	1.00	10,13-Bissidechainpaclitaxel[2]	0.5
1.54	1.00	7-Acetylpaclitaxel	0.6
1.80	1.75	13-Tes-baccatin III	0.1
2.14	1.00	7-Tes-paclitaxel	0.3

[1] Resolution may be incomplete for these peaks, depending upon the relative amounts present; the sum of x_1, x_2, and x_3 is not more than 0.4%.

[2] The following chemical names are assigned to the related compounds Photodegradant; Oxetane ring opened, acetyl and benzoyl migrated; and 10,13-Bissidechainpaclitaxel:

Photodegradant
(1*R*,2*R*,4*S*,5*S*,7*R*,10*S*,11*R*,12*S*,13*S*,15*S*,16*S*)-2,10-diacetyloxy-5,13-dihydroxy-4,16,17,17-tetramethyl-8-oxa-3-oxo-12-phenylcarbonyloxypentacyclo[11.3.1.0^{1,11}.0^{4,11}.0^{7,10}]heptadec-15-yl(2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoate

Oxetane ring opened, acetyl and benzoyl migrated
(1*S*,2*S*,3*R*,4*S*,5*S*,7*S*,8*S*,10*R*,13*S*)-5,10-diacetyloxy-1,2,4,7-tetrahydroxy-8,12,15,15-tetramethyl-9-oxo-4-(phenylcarbonyloxymethyl)tricyclo[9.3.1.0^{3,8}]pentadec-11-en-13-yl(2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoate

10,13-Bissidechainpaclitaxel
Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoic acid, 10-ester with (2*S*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoic acid

In addition to not exceeding the limits for paclitaxel related impurities in *Table 2,* not more than 0.1% of any other single impurity is found; and not more than 2.0% of total impurities is found.

TEST 3 (FOR MATERIAL LABELED AS PRODUCED BY A PLANT CELL FERMENTATION PROCESS)—If the material complies with this test, the labeling indicates that it meets USP *Related compounds Test 3.*

Solution A—Prepare a filtered and degassed mixture of water and acetonitrile (3:2).

Solution B—Prepare filtered and degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve USP Paclitaxel Impurity Mixture RS in acetonitrile, sonicating if necessary, to obtain a solution having a known concentration of about 1 mg per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Paclitaxel RS in acetonitrile, sonicating if necessary, to obtain a solution having a known concentration of about 1 mg per mL.

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Test solution—Transfer about 10 mg of Paclitaxel, accurately weighed, to a 10-mL volumetric flask. Dissolve in and dilute with acetonitrile to volume, sonicating if necessary, and mix. *Chromatographic system* (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 15-cm column that contains 3-µm packing L1. The flow rate is about 1.2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–28	100	0	isocratic
28–33	100→98	0→2	linear gradient
33–58	98→10	2→90	linear gradient
58–60	10	90	isocratic
60–63	10→100	90→0	linear gradient
63–70	100	0	isocratic

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between paclitaxel and benzyl analog is not less than 1.8. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Table 3*. The relative retention times are measured versus Paclitaxel.]

Table 3

Name	Relative Retention Time	Limit (%)
Propyl analog[1]	0.54	0.2
Cephalomannine (Paclitaxel related compound A)	0.76	0.5
sec-Butyl analog[2]	0.81	0.2
n-Butyl analog[3]	0.89	0.1
Benzyl analog	1.10	0.4
Baccatin VI	1.23	0.2
Pentyl analog[4]	1.31	0.2
7-Epipaclitaxel	1.51	0.4

[1] The following chemical name is assigned to the related compound Propyl analog: Baccatin III 13-ester with (2*R*,3*S*)-3-butanoylamino-2-hydroxy-3-phenylpropanoic acid.
[2] The following chemical name is assigned to the related compound *sec*-Butyl analog: Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-(2-methylbutanoylamino)-3-phenylpropanoic acid.
[3] The following chemical name is assigned to the related compound *n*-Butyl analog: Baccatin III 13-ester with (2*S*,3*S*)-2-hydroxy-3-(pentanoylamino)-3-phenylpropanoic acid.
[4] The following chemical name is assigned to the related compound Pentyl analog: Baccatin III 13-ester with (2*R*,3*S*)-3-(hexanoylamino)-2-hydroxy-3-phenylpropanoic acid.

Procedure—Inject a volume (about 12 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for all the peaks. Calculate the percentage of each impurity in the portion of Paclitaxel taken by the formula:

$$100(r_i/r_U)$$

in which r_i is the response of each individual impurity; and r_U is the sum of the areas of all the peaks obtained from the *Test solution.* In addition to not exceeding the limits for paclitaxel related impurities in *Table 3,* not more than 0.1% of any other single impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Diluent—Prepare a mixture of methanol and acetic acid (200:1).

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (11:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve, using sonication if necessary, an accurately weighed quantity of USP Paclitaxel RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.

Assay preparation—Transfer about 10 mg of Paclitaxel, accurately weighed, to a 10-mL volumetric flask. Dissolve in *Diluent,* using sonication if necessary, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L43. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is between 0.7 and 1.3; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{47}H_{51}NO_{14}$ in the portion of Paclitaxel taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Paclitaxel RS in the *Standard preparation;* and r_U and r_S are the peak responses for paclitaxel obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Paclitaxel Injection

» Paclitaxel Injection is a sterile, stabilized solution of Paclitaxel, suitable for dilution for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of paclitaxel ($C_{47}H_{51}NO_{14}$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, at controlled room temperature.

Labeling—Label it to indicate that it is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.

USP Reference standards ⟨11⟩—
USP Paclitaxel RS
USP Paclitaxel Related Compound B RS
10-Deacetyl-7-epipaclitaxel.

Identification—

A: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the

USP Monographs

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chromatogram of the *Standard solution*, as obtained in the test for *Limit of degradation products*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.67 USP Endotoxin Unit per mg of paclitaxel.

pH ⟨791⟩: between 3.0 and 7.0, in a solution (1 in 10).

Limit of degradation products—

Solution A—Prepare a filtered and degassed mixture of water and acetonitrile (3:2).

Solution B—Use filtered and degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve accurately weighed quantities of USP Paclitaxel RS and USP Paclitaxel Related Compound B RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain solutions having known concentrations of about 1.2 mg per mL and 0.006 mg per mL, respectively.

Test solution—Quantitatively dilute an accurately measured volume of Injection with acetonitrile to obtain a solution containing about 1.2 mg of paclitaxel per mL, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 15-cm column that contains 3-µm packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 35°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–26	100	0	isocratic
26–66	100→17	0→83	linear gradient
66–67	17→100	83→0	linear gradient
67–75	100	0	isocratic

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between paclitaxel related compound B and paclitaxel is not less than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the analyte peaks. Calculate the percentage of each degradation product in the volume of Injection taken by the formula:

$$100(C_S/C_U)(r_i/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Paclitaxel Related Compound B RS in the *Standard solution*; C_U is the concentration, in mg per mL, of paclitaxel in the *Test solution*, based on the labeled amount of paclitaxel per mL of Injection; r_i is the peak area for each degradation product obtained from the *Test solution*; and r_S is the peak area for paclitaxel related compound B obtained from the *Standard solution*. In addition to not exceeding the limits stated in *Table 1*, not more than 0.1% of any other paclitaxel degradation product is found; and not more than 2.0% of total paclitaxel degradation products is found.

Table 1

Relative Retention Time	Name	Limit (%)
0.19	Baccatin III	0.8
0.21	Ethyl ester side chain	0.4
0.50	10-Deacetylpaclitaxel	0.8
0.95	10-Deacetyl-7-epipaclitaxel (paclitaxel related compound B)	0.5
1.40	7-Epipaclitaxel	0.6

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Diluent—Transfer 200 µL of glacial acetic acid to a 1-liter volumetric flask containing about 500 mL of methanol, mix, and dilute with methanol to volume.

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (11:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Paclitaxel RS in *Diluent* to obtain a solution having a known concentration of about 0.6 mg per mL.

Assay preparation—Quantitatively dilute an accurately measured volume of Injection with *Diluent* to obtain a solution containing about 0.6 mg of paclitaxel per mL.

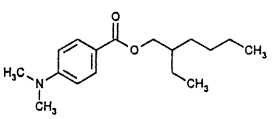
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 227-nm detector and a 4.0-mm × 25-cm column that contains 5-µm packing L43. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the retention time of the paclitaxel peak is between 6.0 and 10.0 minutes; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the paclitaxel peaks. Calculate the quantity, in mg, of paclitaxel ($C_{47}H_{51}NO_{14}$) in each mL of the Injection taken by the formula:

$$(L/D)C(r_U/r_S)$$

in which L is the labeled quantity, in mg, of paclitaxel in each mL of Injection; D is the concentration, in mg per mL, of paclitaxel in the *Assay preparation*, based on the labeled quantity; C is the concentration, in mg per mL, of USP Paclitaxel RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Padimate O



$C_{17}H_{27}NO_2$ 277.40

Benzoic acid, 4-(dimethylamino)-, 2-ethylhexyl ester; 2-Ethylhexyl *p*-(dimethylamino)benzoate [21245-02-3].

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DEFINITION
Padimate O contains NLT 97.0% and NMT 102.0% of padimate O ($C_{17}H_{27}NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Methanol, water, and glacial acetic acid (850: 150: 0.5)
Standard solution: 0.1 mg/mL of USP Padimate O RS in methanol
Sample solution: 0.1 mg/mL of Padimate O in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 308 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of padimate O ($C_{17}H_{27}NO_2$) in the portion of Padimate O taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Padimate O RS in the *Standard solution* (mg/mL)
C_U = concentration of Padimate O in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0%

IMPURITIES
- **ORGANIC IMPURITIES**

Sample solution: 10 mg/mL of Padimate O in chloroform
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 3-mm × 1.8-m stainless steel packed with 10% liquid phase G9 on support S1A
Carrier gas: Helium
Column temperature: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	10	250	10

Injection volume: 2 µL

Analysis
Sample: *Sample solution*
Calculate the percentage of the total impurities in the portion of Padimate O taken:

$$[\Sigma r_I/(\Sigma r_I + r_U)] \times 100$$

r_I = individual impurity peak response from the *Sample solution*
r_U = Padimate O peak response from the *Sample solution*

Acceptance criteria
Total impurities: NMT 2.0%

SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨841⟩: 0.990–1.000
- **REFRACTIVE INDEX** ⟨831⟩: 1.5390–1.5430
- **FATS AND FIXED OILS,** *Acid Value* ⟨401⟩: NMT 1.0
- **FATS AND FIXED OILS,** *Saponification Value* ⟨401⟩

Analysis: Proceed as directed in the chapter, except to maintain reflux for 4 h.
Acceptance criteria: 195–215

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Padimate O RS

Padimate O Lotion

» Padimate O Lotion contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{27}NO_2$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Padimate O RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Assay—
Mobile phase—Prepare a suitable filtered and degassed solution containing methanol, water, and glacial acetic acid (85: 15: 0.5).
Standard preparation—Dissolve an accurately weighed quantity of USP Padimate O RS in isopropyl alcohol and dilute quantitatively, and stepwise if necessary, with isopropyl alcohol to obtain a solution having a known concentration of about 100 µg per mL.
Assay preparation—Transfer an accurately weighed quantity of Lotion, equivalent to about 100 mg of Padimate O, to a 100-mL volumetric flask, and add about 75 mL of isopropyl alcohol. Heat gently with swirling until the specimen is dispersed. Cool to room temperature, dilute with isopropyl alcohol to volume, and mix. Pipet 10.0 mL of this solution into a 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 308-nm detector and a 4.6-mm × 25-cm column that contains 5-µm *base-deactivated* packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record

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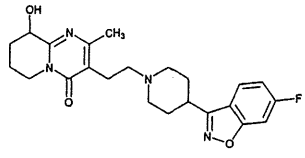
the peak responses as directed under *Procedure:* the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{27}NO_2$ in the portion of Lotion taken by the formula:

$$C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Padimate O RS in the *Standard preparation*, and r_U and r_S are the peak responses for padimate O obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Paliperidone



$C_{23}H_{27}FN_4O_3$ 426.48

4*H*-Pyrido[1,2-*a*]pyrimidin-4-one, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-;
(9*RS*)-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]] ethyl]-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [144598-75-4].

DEFINITION

Paliperidone contains NLT 98.0% and NMT 102.0% of paliperidone ($C_{23}H_{27}FN_4O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020) Do not dry the sample.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect the *System suitability solution*, *Standard solution*, and *Sample solution* from light.

Buffer: 28 mM Tetrabutylammonium hydrogen sulfate

Mobile phase: See *Table 1*.

Solution A: Methanol and *Buffer* (10:90)

Solution B: Methanol

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
35	85	15
37	100	0
45	100	0

Diluent: Dissolve 0.71 g of dibasic sodium phosphate and 0.62 g of monobasic sodium phosphate in 1 L of water.

System suitability solution: 0.5 mg/mL of USP Paliperidone Resolution Mixture RS prepared as follows. Dissolve first in methanol using 50% of the final flask volume, and dilute with *Diluent*.

Standard solution: 0.5 mg/mL of USP Paliperidone RS prepared as follows. Dissolve first in methanol using 50% of the final flask volume, and dilute with *Diluent*.

Sample solution: 0.5 mg/mL of Paliperidone prepared as follows. Dissolve first in methanol using 50% of the final flask volume, and dilute with *Diluent*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Column temperature: 40°

Flow rate: 0.9 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between paliperidone related compound B and paliperidone; NLT 2.0 between paliperidone and paliperidone hydroxybenzoyl analog, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of paliperidone ($C_{23}H_{27}FN_4O_3$) in the portion of Paliperidone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Paliperidone RS in the *Standard solution* (mg/mL)

C_U = concentration of Paliperidone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Protect the *System suitability solution*, *Standard solution*, and *Sample solution* from light.

Diluent, Mobile phase, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.5 µg/mL of USP Paliperidone RS in *Diluent* from *Standard solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between paliperidone related compound B and paliperidone; NLT 2.0 between paliperidone and paliperidone hydroxybenzoyl analog, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 5.0%, *Sensitivity solution*

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Analysis:
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Paliperidone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of paliperidone from the *Standard solution*
C_S = concentration of USP Paliperidone RS in the *Standard solution* (mg/mL)
C_U = concentration of Paliperidone in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak with area less than 0.05%.

Table 2

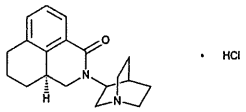
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Paliperidone related compound C[a]	0.57	1.0	0.10
Paliperidone related compound B[b]	0.83	1.0	0.10
Paliperidone	1.00	—	—
Paliperidone hydroxybenzoyl analog[c]	1.1	1.0	0.10
Paliperidone ketone[d]	1.27	0.58	0.50
Any other individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.70

[a] 3-(2-Chloroethyl)-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.
[b] 6-Fluoro-3-(piperidin-4-yl)benzoisoxazole.
[c] 3-{2-[4-(4-Fluoro-2-hydroxybenzoyl)piperidin-1-yl]ethyl}-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.
[d] 3-{2-[4-(6-Fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl]ethyl}-2-methyl-7,8-dihydro-4*H*-pyrido[1,2-*a*]pyrimidine-4,9(6*H*)-dione.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Paliperidone RS
  USP Paliperidone Resolution Mixture RS
  This contains paliperidone as the major component; it also contains paliperidone related compound B, paliperidone related compound C, paliperidone hydroxybenzoyl analog, and paliperidone ketone as minor components.

Palonosetron Hydrochloride



$C_{19}H_{24}N_2O \cdot HCl$ 332.87
1*H*-Benzo[*de*]isoquinoline-1-one, 2,3,3a,4,5,6-hexahydro-2-[(3*S*)-1-azabicyclo[2.2.2]octan-3-yl],(*S*)-, hydrochloride; (3a*S*)-2-[(3*S*)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride [135729-62-3].

DEFINITION
Palonosetron Hydrochloride contains NLT 98.0% and NMT 102.0% of palonosetron hydrochloride ($C_{19}H_{24}N_2O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, water, and trifluoroacetic acid (280: 720: 0.67)
  Standard stock solution: 0.7 mg/mL of USP Palonosetron Hydrochloride RS in methanol. Sonicate as needed to dissolve. This solution is stable for 6 weeks if stored in the refrigerator and protected from light.
  Standard solution: 0.014 mg/mL of USP Palonosetron Hydrochloride RS from the *Standard stock solution* in *Mobile phase*
  Sample stock solution: 0.7 mg/mL of Palonosetron Hydrochloride in methanol. Sonicate as needed to dissolve.
  Sample solution: 0.014 mg/mL of Palonosetron Hydrochloride from the *Sample stock solution* in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Flow rate: 1 mL/min
  Injection volume: 80 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—See *Table 2* for the relative retention times.]
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of palonosetron hydrochloride ($C_{19}H_{24}N_2O \cdot HCl$) in the portion of Palonosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

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C_S = concentration of USP Palonosetron Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Palonosetron Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **LIMIT OF SPECIFIED IMPURITIES**

Buffer: 1.5 g/L of ammonium acetate, adjusted with glacial acetic acid to a pH of 6.0

Mobile phase: Tetrahydrofuran and *Buffer* (1:9)

System suitability stock solution: 0.07 mg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound C RS, USP Palonosetron Related Compound D RS, USP Palonosetron Related Compound E RS, and USP Palonosetron Enantiomer RS in methanol. Sonicate as needed to dissolve.

System suitability solution: 3.5 µg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound C RS, USP Palonosetron Related Compound D RS, USP Palonosetron Related Compound E RS, and USP Palonosetron Enantiomer RS from the *System suitability stock solution* and 0.7 mg/mL of USP Palonosetron Hydrochloride RS in *Buffer*

Impurity standard stock solution: 0.35 mg/mL of USP Palonosetron Related Compound D RS in methanol. Sonicate as needed to dissolve.

Impurity standard solution: 0.0035 mg/mL of USP Palonosetron Related Compound D RS from the *Impurity standard stock solution* in *Buffer*

Sensitivity solution: 0.35 µg/mL of USP Palonosetron Related Compound D RS from the *Impurity standard stock solution* in *Buffer*

Sample solution: 0.7 mg/mL of Palonosetron Hydrochloride in *Buffer*. Sonicate as needed to dissolve.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 238 nm

Column: 4.6-mm × 25-cm; 5-µm packing L88

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 100 µL

Run time: At least 2.5 times the retention time of palonosetron

System suitability

Samples: *System suitability solution*, *Impurity standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.4 between palonosetron related compound D and palonosetron enantiomer; NLT 1.2 between palonosetron enantiomer and palonosetron related compound E, *System suitability solution*

Tailing factor: NMT 2.5 for palonosetron related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0% for palonosetron related compound D, *Impurity standard solution*

Signal-to-noise ratio: NLT 10 for palonosetron related compound D, *Sensitivity solution*

Analysis

Samples: *Impurity standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Palonosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of palonosetron related compound D from the *Impurity standard solution*
C_S = concentration of USP Palonosetron Related Compound D RS in the *Impurity standard solution* (mg/mL)
C_U = concentration of Palonosetron Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Palonosetron related compound A	0.45	1.0	0.1
Palonosetron related compound B	0.54	2.1	0.1
Palonosetron	1.0	—	—
Palonosetron related compound C	1.2	1.0	0.1
Palonosetron related compound D	1.3	1.0	0.5
Palonosetron enantiomer	1.4	1.0	0.1
Palonosetron related compound E	1.5	2.5	0.5

• **LIMIT OF UNSPECIFIED IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Peak identification stock solution: 0.07 mg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound D RS, and USP Palonosetron Related Compound E RS in methanol. Sonicate as needed to dissolve.

Peak identification solution: 0.07 µg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound D RS, and USP Palonosetron Related Compound E RS from the *Peak identification stock solution* and 3.5 µg/mL of USP Palonosetron Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sensitivity solution: 0.0175 µg/mL of USP Palonosetron Hydrochloride RS in *Mobile phase*

Sample stock solution: 0.175 mg/mL of Palonosetron Hydrochloride in methanol. Sonicate as needed to dissolve.

Sample solution: 0.035 mg/mL of Palonosetron Hydrochloride from the *Sample stock solution* in *Mobile phase*

System suitability

Sample: *Sensitivity solution*

Suitability requirements

Signal-to-noise ratio: NLT 10

Analysis

Samples: *Peak identification solution* and *Sample solution*

Chromatograph the *Peak identification solution* and identify specified impurities based on the relative retention times listed in *Table 2*.

Calculate the percentage of each unspecified impurity in the portion of Palonosetron Hydrochloride taken:

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$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Palonosetron related compound E[a]	0.91	—
Palonosetron related compound D[a]	0.94	—
Palonosetron	1.0	—
Palonosetron related compound B[a]	1.1	—
Palonosetron related compound A[a]	1.2	—
Any individual unspecified impurity	—	0.1
Total impurities[b]	—	1.0

[a] These impurities are controlled in the test for *Limit of Specified Impurities* and are not to be reported here.
[b] Total impurities include impurities controlled in the test for *Limit of Specified Impurities* and in the test for *Limit of Unspecified Impurities*.

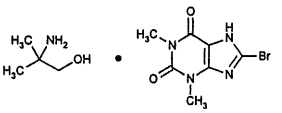
SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: 5.0–6.0
- **Loss on Drying** ⟨731⟩
  Analysis: Dry under vacuum at 80° for 3 h.
  Acceptance criteria: NMT 1.0%
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g.
- **Bacterial Endotoxins Test** ⟨85⟩**:** Where the label states that Palonosetron Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 30 USP Endotoxin Units/mg of palonosetron hydrochloride.
- **Sterility Tests** ⟨71⟩**:** Meets the requirements where the label states that Palonosetron Hydrochloride is sterile

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature, protected from light.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP Reference Standards** ⟨11⟩
  USP Palonosetron Enantiomer RS
  (3a*R*)-2-[(3*R*)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.
  $C_{19}H_{24}N_2O \cdot HCl$ 332.87
  USP Palonosetron Hydrochloride RS
  USP Palonosetron Related Compound A RS
  Palonosetron *N*-oxide;
  (3a*S*)-1-Oxo-2-[(3*S*)-quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinoline 1-oxide.
  $C_{19}H_{24}N_2O_2$ 312.41
  USP Palonosetron Related Compound B RS
  Palonosetron-3-ene *N*-oxide;
  (3*S*)-3-(1-Oxo-5,6-dihydro-1*H*-benzo[*de*]isoquinolin-2(4*H*)-yl)quinuclidine 1-oxide.
  $C_{19}H_{22}N_2O_2$ 310.39
  USP Palonosetron Related Compound C RS
  Palonosetron *S,R*-diastereomer;
  (3a*S*)-2-[(3*R*)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.
  $C_{19}H_{24}N_2O \cdot HCl$ 332.87
  USP Palonosetron Related Compound D RS
  Palonosetron *R,S*-diastereomer or palonosetron 3a-epimer;
  (3a*R*)-2-[(3*S*)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.
  $C_{19}H_{24}N_2O \cdot HCl$ 332.87
  USP Palonosetron Related Compound E RS
  Palonosetron-3-ene;
  2-[(3*S*)-Quinuclidin-3-yl]-2,4,5,6-tetrahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.
  $C_{19}H_{22}N_2O \cdot HCl$ 330.85

Pamabrom



$C_{11}H_{18}BrN_5O_3$ 348.20
8-Bromo-3,7-dihydro-1,3-dimethyl-1-*H*-purine-2,6-dione compound with 2-amino-2-methyl-1-propanol (1:1).
8-Bromotheophylline compound with 2-amino-2-methyl-1-propanol (1:1) [606-04-02].

» Pamabrom contains not less than 72.2 percent and not more than 76.6 percent of 8-bromotheophylline ($C_7H_7BrN_4O_2$), calculated on the anhydrous basis; and not less than 24.6 percent and not more than 26.6 percent of 2-amino-2-methyl-1-propanol ($C_4H_{11}NO$), calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP 8-Bromotheophylline RS
8-Bromo-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione.
$C_7H_7N_4O_2Br$ 259.06
USP Theophylline RS
Identification—It responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, using a solvent system consisting of a mixture of xylene, methanol, and glacial acetic acid (11:2:1) and a *Standard solution* and a *Test solution* prepared as directed below: the R_F value of the principal spot, which appears as a dark spot against a green background, from the *Test solution* corresponds to that obtained from the *Standard solution*.

USP Monographs

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Standard solution—Transfer an accurately weighed quantity of about 20 mg of USP 8-Bromotheophylline RS to a 100-mL volumetric flask, add 25 mL of water, 50 mL of methanol, and a small amount of dilute ammonium hydroxide. Swirl the flask to effect solution. Dilute the contents of the flask with methanol to volume, and mix.

Test solution—Transfer an accurately weighed quantity of about 25 mg of Pamabrom to a 100-mL volumetric flask, add 25 mL of water, and swirl to dissolve. Dilute the contents of the flask with methanol to volume, and mix.

Water Determination, *Method I* ⟨921⟩: not more than 3%.

Limit of theophylline—

Diluting solution, Mobile phase, and *Chromatographic system*—Proceed as directed in the *Assay for 8-bromotheophylline.*

Standard solution—Dissolve an accurately weighed quantity of USP Theophylline RS in *Diluting solution,* add a few drops of ammonium hydroxide, sonicating if necessary, to obtain a solution having a known concentration of about 1 mg of USP Theophylline RS per mL. Dilute a volume of this solution quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 5 µg per mL.

Test solution—Transfer an accurately weighed quantity of about 200 mg of Pamabrom to a 200-mL volumetric flask. Add about 50 mL of *Diluting solution,* and sonicate for 5 minutes. Cool to room temperature, dilute with *Diluting solution* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of theophylline in the portion of Pamabrom taken by the formula:

$$20(C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Theophylline RS in the *Standard solution,* W is the weight, in mg, of Pamabrom taken, and r_U and r_S are the peak responses of theophylline obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.5% is found.

Assay for 8-bromotheophylline—

Diluting solution—Prepare a mixture of water and methanol (70:30).

Mobile phase—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (69:30:1), filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve an accurately weighed quantity of caffeine in *Diluting solution,* and dilute quantitatively, and stepwise if necessary, to obtain a solution having a concentration of about 125 µg of caffeine per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP 8-Bromotheophylline RS in *Diluting solution,* add a few drops of ammonium hydroxide, sonicating if necessary, to obtain a solution having a known concentration of about 0.75 mg of USP 8-Bromotheophylline RS per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, mix, and filter.

Assay preparation—Transfer an accurately weighed quantity of about 200 mg of Pamabrom to a 200-mL volumetric flask, add about 50 mL of *Diluting solution* and two drops of ammonium hydroxide, and sonicate for 5 minutes. [NOTE—If a hazy solution is present after 5 minutes of sonication, add 1 additional drop of ammonium hydroxide.] Cool, dilute with *Diluting solution* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph 20 µL of the *Standard preparation,* and record the peak responses as directed under *Procedure:* the relative retention times are about 0.6 for caffeine and 1.0 for 8-bromotheophylline, the resolution, R, between caffeine and 8-bromotheophylline is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

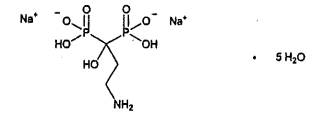
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of 8-bromotheophylline ($C_7H_7BrN_4O_2$) in the portion of Pamabrom taken by the formula:

$$4000C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP 8-Bromotheophylline RS in the *Standard preparation,* and R_U and R_S are the peak response ratios of the 8-bromotheophylline peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for 2-amino-2-methyl-1-propanol—Dissolve about 1 g of Pamabrom, accurately weighed, in 10 mL of water by warming gently on a steam bath until the solution is clear. Cool, add methyl orange TS, and titrate with 0.5 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.5 N hydrochloric acid is equivalent to 44.57 mg of $C_4H_{11}NO$.

Pamidronate Disodium



$C_3H_9NNa_2O_7P_2 \cdot 5H_2O$ 369.11

Phosphonic acid, (3-amino-1-hydroxypropylidene)bis-, disodium salt, pentahydrate.

Disodium dihydrogen (3-amino-1-hydroxypropyl idene) diphosphonate, pentahydrate [109552-15-0].

Anhydrous 279.06

[57248-88-1].

» Pamidronate Disodium contains not less than 98.0 percent and not more than 102.0 percent of $C_3H_9NNa_2O_7P_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers. Store at a temperature not exceeding 30°.

USP Reference standards ⟨11⟩—

USP Beta Alanine RS
3-Aminopropionic acid.

USP Pamidronate Disodium RS

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Clarity and color of solution—

Test preparation 1—Dissolve 1.0 g in 50 mL of water with gentle warming. Cool to room temperature.

Test preparation 2—Dissolve 1.0 g in 25 mL of 2 N sodium hydroxide solution with gentle warming. Cool to room temperature.

Procedure—Examine *Test preparation 1* and *Test preparation 2:* the solutions are clear. Separately measure the absorbance of each of these solutions at 420 nm in 4-cm cells, using water as the blank for *Test preparation 1* and using 2 N sodium hydroxide solution as the blank for *Test preparation 2:* the absorbance of each solution is not more than 0.10.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020)

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: It meets the requirements of test *A* under *Identification Tests—General ⟨191⟩, Sodium.*

***Microbial enumeration tests ⟨61⟩* and *Tests for specified microorganisms ⟨62⟩*—**The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined yeasts and molds count does not exceed 100 cfu per g.

pH ⟨791⟩: between 7.8 and 8.8, in a solution (1 in 100).

Water Determination, Method I ⟨921⟩: between 23.0% and 25.5%.

Related compounds—

TEST 1—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Transfer 30 mg of Pamidronate Disodium to a 10-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Standard solution—Dissolve an accurately weighed quantity of USP Beta Alanine RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution containing 0.006 mg of beta alanine per mL.

Application volume: 10 µL.

Developing solvent system: a mixture of methanol, diisopropyl ether, and 25% ammonia (9:8:4).

Spray reagent—Dissolve 0.2 g of ninhydrin in 100 mL of a mixture of butyl alcohol and 2 N acetic acid (95:5).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography ⟨621⟩.* Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent,* and heat between 100° and 105° for about 15 minutes. Examine the plate under white light. The spot obtained from the *Test solution* having an R_F value of about 0.5 is not greater in size or intensity than the corresponding spot obtained from the *Standard solution:* not more than 0.2% of beta alanine is found. Evaluate any other additional spot in the chromatogram of the *Test solution,* and determine the percentage of total other impurities (excluding beta alanine).

TEST 2—

Mobile phase—Proceed as directed in the *Assay.*

Impurity stock solution 1—Transfer about 300 mg of ortho-phosphoric acid, accurately weighed, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Impurity stock solution 2—Transfer about 250 mg of phosphorous acid, accurately weighed, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Impurity standard solution—Transfer 2.0 mL each of *Impurity stock solution 1* and *Impurity stock solution 2* to a 50-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Prepare as directed for the *Assay preparation.*

Chromatographic system (see *Chromatography ⟨621⟩*)—Proceed as directed in the *Assay.* Chromatograph the *Impurity standard solution,* and record the peak responses as directed for *Procedure:* the elution order is a phosphate peak followed by the phosphite peak; the resolution, *R,* between the two peaks is not less than 2.5; the relative standard deviation for replicate injections, determined from the phosphate peak, is not more than 10%; and the relative standard deviation for replicate injections, determined from the phosphite peak, is not more than 20%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Impurity standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of phosphates as ortho-phosphoric acid in the portion of Pamidronate Disodium taken by the formula:

$$0.2(W_1/W)(r_U/r_S)$$

in which W_1 is the weight, in mg, of ortho-phosphoric acid taken to prepare the *Impurity stock solution 1; W* is the weight, in mg, of Pamidronate Disodium taken to prepare the *Test solution;* and r_U and r_S are the phosphate peak responses obtained from the *Test solution* and the *Impurity standard solution,* respectively: not more than 0.5% of phosphate, determined as ortho-phosphoric acid, is found.

Calculate the percentages of phosphites as phosphorous acid in the portion of Pamidronate Disodium taken by the formula:

$$0.2(W_2/W)(r_U/r_S)$$

in which W_2 is the weight, in mg, of phosphorous acid taken to prepare the *Impurity stock solution 2; W* is as defined above; and r_U and r_S are the phosphite peak responses obtained from the *Test solution* and the *Impurity standard solution,* respectively: not more than 0.5% of phosphite, determined as phosphorous acid, is found; and not more than 0.5% of total phosphate and phosphite combined is found.

Calculate the percentage of any other impurity in the portion of Pamidronate Disodium taken by the formula:

$$0.2(W_1/W)(r_I/r_S)$$

in which W_1 and W are as defined above; r_I is the peak response of any other impurity in the *Test solution;* and r_S is the response of the phosphate peak obtained from the *Impurity standard solution:* not more than 0.5% of total other impurities (excluding beta alanine, phosphate as ortho-phosphoric acid, and phosphite as phosphorous acid) is found, the results for *Test 1* and *Test 2* being added.

Assay—

Mobile phase—To 2500 mL of water, add 0.47 mL of anhydrous formic acid, adjust with 2 N sodium hydroxide solution to a pH of 3.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*). [NOTE—The small amounts of formic acid have a strong influence on the retention times.]

Standard preparation—Dissolve an accurately weighed quantity of USP Pamidronate Disodium RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 2 mg per mL.

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Assay preparation—Transfer about 100 mg of Pamidronate Disodium, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector and a 4.6-mm ×10-cm column that contains packing L23. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 35°. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not less than 0.3 and not more than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_3H_9NNa_2O_7P_2$ in the portion of Pamidronate Disodium taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pamidronate Disodium RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Pamidronate Disodium for Injection

» Pamidronate Disodium for Injection is a sterile, freeze-dried mixture of Pamidronate Disodium and suitable excipients. It contains not less than 93.0 percent and not more than 108.0 percent of the labeled amount of pamidronate disodium ($C_3H_9NNa_2O_7P_2$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.* Store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Beta Alanine RS
3-Aminopropionic acid.
USP Pamidronate Disodium RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.88 USP Endotoxin Units per mg of anhydrous pamidronate disodium.

Uniformity of dosage units ⟨905⟩**:** meets the requirements for *Weight Variation.*

pH ⟨791⟩**:** between 6.0 and 7.0, determined in a solution constituted as directed in the labeling.

Particulate Matter in Injections ⟨788⟩ **:** meets the requirements for small-volume injections.

Water Determination, *Method Ia* ⟨921⟩ **:** not more than 5%.

Limit of beta alanine—

Adsorbent, Application volume, Developing solvent system, and *Spray reagent*—Proceed as directed for *Related compounds, Test 1* under *Pamidronate Disodium.*

Standard solution—Dissolve an accurately weighed quantity of USP Beta Alanine RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution containing 0.0075 mg of beta alanine per mL.

Test solution—Reconstitute the vial with the appropriate amount of water to obtain a solution having a concentration of 3 mg of anhydrous pamidronate disodium per mL, based on the label claim.

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent,* and heat between 100° and 105° for about 15 minutes. Examine the plate under white light. The spot having an R_F value of about 0.5 obtained from the *Test solution* is not greater in size or intensity than the corresponding spot obtained from the *Standard solution:* not more than 0.25% of beta alanine is found.

Other requirements—It meets the requirements under *Sterility Tests* ⟨71⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Pamidronate Disodium.*

Standard preparation—Dissolve an accurately weighed quantity of USP Pamidronate Disodium RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 2.5 mg per mL. Calculate the concentration, C_S, of anhydrous pamidronate disodium, the molecular weights of anhydrous and pentahydrate pamidronate disodium being 279.06 and 369.11, respectively.

Assay preparation—Constitute a suitable number of vials of Pamidronate Disodium for Injection with the appropriate amount of water to obtain a solution having a known concentration of about 2 mg of anhydrous pamidronate disodium per mL, based on the label claim.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_3H_9NNa_2O_7P_2$ in the portion of Pamidronate Disodium for Injection taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is as defined under the *Standard preparation;* C_U is the concentration, in mg per mL, of anhydrous pamidronate disodium in the *Assay preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Pancreatin

Pancreatin.
Pancreatin [8049-47-6].

» Pancreatin is a substance containing enzymes, principally amylase, lipase, and protease, obtained from the pancreas of the hog, *Sus scrofa* Linné var. *domesticus* Gray (Fam. Suidae) or of the ox, *Bos taurus* Linné (Fam. Bovidae). Pancreatin contains, in each mg, not less than 25 USP Units of amylase activity, not less than 2.0 USP Units of lipase

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activity, and not less than 25 USP Units of protease activity. Pancreatin of a higher digestive power may be labeled as a whole-number multiple of the three minimum activities or may be diluted by admixture with lactose, or with sucrose containing not more than 3.25 percent of starch, or with pancreatin of lower digestive power. [NOTE—One USP Unit of amylase activity is contained in the amount of pancreatin that decomposes starch at an initial rate such that 0.16 µEq of glycosidic linkage is hydrolyzed per minute under the conditions of the *Assay for amylase activity*.]

One USP Unit of lipase activity is contained in the amount of pancreatin that liberates 1.0 µEq of acid per minute at a pH of 9.0 and 37° under the conditions of the *Assay for lipase activity*.

One USP Unit of protease activity is contained in the amount of pancreatin that under the conditions of the *Assay for protease activity* hydrolyzes casein at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid that gives the same absorbance at 280 nm as 15 nmol of tyrosine.

Packaging and storage—Preserve in tight containers, at a temperature not exceeding 30°.

USP Reference standards ⟨11⟩—
USP Bile Salts RS
USP Pancreatin Amylase and Protease RS
USP Pancreatin Lipase RS

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the test for absence of *Salmonella* species and *Escherichia coli*.

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

Fat—Place 2.0 g of Pancreatin in a flask of about 50-mL capacity, add 20 mL of ether, insert the stopper, and set it aside for 2 hours, mixing by rotating at frequent intervals. Decant the supernatant ether by means of a guiding rod into a plain filter about 7 cm in diameter, previously moistened with ether, and collect the filtrate in a tared beaker. Repeat the extraction with a 10-mL portion of ether, proceeding as directed before, then with another 10-mL portion of ether, and transfer the ether and the remainder of the Pancreatin to the filter. Allow to drain, evaporate the ether spontaneously, and dry the residue at 105° for 2 hours: the residue of fat obtained from Pancreatin possessing three or more times the three minimum activities weighs not more than 120 mg (6.0%); that obtained from Pancreatin possessing less than three times the three minimum activities weighs not more than 60 mg (3.0%).

Assay for amylase activity (Starch digestive power)—

pH 6.8 phosphate buffer—On the day of use, dissolve 13.6 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 14.2 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 51 mL of the monobasic potassium phosphate solution with 49 mL of the dibasic sodium phosphate solution. If necessary, adjust by the dropwise addition of the appropriate solution to a pH of 6.8.

Substrate solution—On the day of use, stir a portion of purified soluble starch equivalent to 2.0 g of dried substance with 10 mL of water, and add this mixture to 160 mL of boiling water. Rinse the beaker with 10 mL of water, add it to the hot solution, and heat to boiling, with continuous mixing. Cool to room temperature, and add water to make 200 mL.

Standard preparation—Weigh accurately about 20 mg of USP Pancreatin Amylase and Protease RS into a suitable mortar. Add about 30 mL of *pH 6.8 phosphate buffer*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *pH 6.8 phosphate buffer* to a 50-mL volumetric flask, dilute with *pH 6.8 phosphate buffer* to volume, and mix. Calculate the activity, in USP Units of amylase activity per mL, of the resulting solution from the declared potency on the label of the USP Reference Standard.

Assay preparation—For Pancreatin having about the same amylase activity as the USP Pancreatin Amylase and Protease RS, weigh accurately about 40 mg of Pancreatin into a suitable mortar. [NOTE—For Pancreatin having a different amylase activity, weigh accurately the amount necessary to obtain an *Assay preparation* having amylase activity per mL corresponding approximately to that of the *Standard preparation*.] Add about 3 mL of *pH 6.8 phosphate buffer*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *pH 6.8 phosphate buffer* to a 100-mL volumetric flask, dilute with *pH 6.8 phosphate buffer* to volume, and mix.

Procedure—Prepare four stoppered, 250-mL conical flasks, and mark them *S*, *U*, *BS*, and *BU*. Pipet into each flask 25 mL of *Substrate solution*, 10 mL of *pH 6.8 phosphate buffer*, and 1 mL of sodium chloride solution (11.7 in 1000), insert the stoppers, and mix. Place the flasks in a water bath maintained at 25 ± 0.1°, and allow them to equilibrate. To flasks *BU* and *BS* add 2 mL of 1 N hydrochloric acid, mix, and return the flasks to the water bath. To flasks *U* and *BU* add 1.0-mL portions of the *Assay preparation*, and to flasks *S* and *BS* add 1.0 mL of the *Standard preparation*. Mix each, and return the flasks to the water bath. After 10 minutes, accurately timed from the addition of the enzyme, add 2-mL portions of 1 N hydrochloric acid to flasks *S* and *U*, and mix. To each flask, with continuous stirring, add 10.0 mL of 0.1 N iodine VS, and immediately add 45 mL of 0.1 N sodium hydroxide. Place the flasks in the dark at a temperature between 15° and 25° for 15 minutes. To each flask add 4 mL of 2 N sulfuric acid, and titrate with 0.1 N sodium thiosulfate VS to the disappearance of the blue color. Calculate the amylase activity, in USP Units per mg, of the Pancreatin taken by the formula:

$$100(C_S/W_U)(V_{BU} - V_U)/(V_{BS} - V_S)$$

in which C_S is the amylase activity of the *Standard preparation*, in USP Units per mL, W_U is the amount, in mg, of Pancreatin taken, and V_U, V_S, V_{BU}, and V_{BS} are the volumes, in mL, of 0.1 N sodium thiosulfate consumed in the titration of the solutions in flasks *U*, *S*, *BU*, and *BS*, respectively.

Assay for lipase activity (Fat digestive power)—

Acacia solution—Centrifuge a solution of acacia (1 in 10) until clear. Use only the clear solution.

Olive oil substrate—Combine 165 mL of *Acacia solution*, 20 mL of olive oil, and 15 g of crushed ice in the cup of an electric blender. Cool the mixture in an ice bath to 5°, and homogenize at high speed for 15 minutes, intermittently cooling in an ice bath to prevent the temperature from exceeding 30°.

Test for suitability of mixing as follows. Place a drop of the homogenate on a microscope slide, and gently press a cover slide in place to spread the liquid. Examine the entire field under high power (43× objective lens and 5× ocular), using an eyepiece equipped with a calibrated micrometer. The substrate is satisfactory if 90% of the particles do not exceed 2 µm in diameter and none exceeds 10 µm in diameter.

Buffer solution—Dissolve 60 mg of tris(hydroxymethyl) aminomethane and 234 mg of sodium chloride in water to make 100 mL.

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Bile salts solution—Prepare a solution to contain 80.0 mg of USP Bile Salts RS in each mL.

Standard test dilution—Suspend about 200 mg of USP Pancreatin Lipase RS, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the declared potency on the label of the USP Reference Standard. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Assay test dilution—Suspend about 200 mg of Pancreatin, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the estimated potency of the test material. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Procedure—Mix 10.0 mL of *Olive oil substrate*, 8.0 mL of *Buffer solution*, 2.0 mL of *Bile salts solution*, and 9.0 mL of water in a jacketed glass vessel of about 50-mL capacity, the outer chamber of which is connected to a thermostatically controlled water bath. Cover the mixture, and stir continuously with a mechanical stirring device. With the mixture maintained at a temperature of 37 ± 0.1°, add 0.1 N sodium hydroxide VS, from a microburet inserted through an opening in the cover, and adjust to a pH of 9.20 potentiometrically using a calomel-glass electrode system. Add 1.0 mL of the *Assay test dilution*, and then continue adding the 0.1 N sodium hydroxide VS for 5 minutes to maintain the pH at 9.0. Determine the volume of 0.1 N sodium hydroxide VS added after each minute.

In the same manner, titrate 1.0 mL of *Standard test dilution*.

Calculation of potency—Plot the volume of 0.1 N sodium hydroxide VS titrated against time. Using only the points which fall on the straight-line segment of the curve, calculate the mean acidity released per minute by the test specimen and the Standard. Taking into consideration the dilution factors, calculate the lipase activity, in USP Units, of the Pancreatin taken by comparison to the activity of the Standard, using the lipase activity stated on the label of USP Pancreatin Lipase RS.

Assay for protease activity (Casein digestive power)—*Casein substrate*—Place 1.25 g of finely powdered casein in a 100-mL conical flask containing 5 mL of water, shake to form a suspension, add 10 mL of 0.1 N sodium hydroxide, shake for 1 minute, add 50 mL of water, and shake for about 1 hour to dissolve the casein. The resulting solution should have a pH of about 8. If necessary, adjust the pH to about 8, using 1 N sodium hydroxide or 1 N hydrochloric acid. Transfer the solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Use this substrate on the day it is prepared.

Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate and 1.8 g of sodium hydroxide in 950 mL of water in a 1000-mL volumetric flask, adjust to a pH of 7.5 ± 0.2, using 0.2 N sodium hydroxide, dilute with water to volume, and mix. Store this solution in a refrigerator.

Trichloroacetic acid solution—Dissolve 50 g of trichloroacetic acid in 1000 mL of water. Store this solution at room temperature.

Filter paper—Determine the suitability of the filter paper by filtering a 5-mL portion of *Trichloroacetic acid solution* through the paper and measuring the absorbance of the filtrate at 280 nm, using an unfiltered portion of the same *Trichloroacetic acid solution* as the blank: the absorbance is not more than 0.04. If the absorbance is more than 0.04, the filter paper may be washed repeatedly with *Trichloroacetic acid solution* until the absorbance of the filtrate, determined as above, is not more than 0.04.

Standard test dilution—Add about 100 mg of USP Pancreatin Amylase and Protease RS, accurately weighed, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for about 25 minutes. Dilute quantitatively with *Buffer solution* to obtain a concentration of about 2.5 USP Units of protease activity per mL, based on the potency declared on the label of the Reference Standard.

Assay test dilution—Weigh accurately about 100 mg of Pancreatin into a mortar. Add about 3 mL of *Buffer solution*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *Buffer solution* to a 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to that of the *Standard test dilution*.

Procedure—Label test tubes in duplicate S_1, S_2, and S_3 for the standard series, and U for the sample. Pipet into tubes S_1 2.0 mL, into S_2 and U 1.5 mL, and into S_3 1.0 mL of *Buffer solution*. Pipet into tubes S_1 1.0 mL, into S_2 1.5 mL, and into S_3 2.0 mL of the *Standard test dilution*. Pipet into tubes U 1.5 mL of the *Assay test dilution*. Pipet into one tube each of S_1, S_2, S_3, and U 5.0 mL of *Trichloroacetic acid solution*, and mix. Designate these tubes as S_{1B}, S_{2B}, S_{3B}, and U_B, respectively. Prepare a blank by mixing 3 mL of *Buffer solution* and 5 mL of *Trichloroacetic acid solution* in a separate test tube marked *B*. Place all the tubes in a 40° water bath, insert a glass stirring rod into each tube, and allow for temperature equilibration. At zero time, add to each tube, at timed intervals, 2.0 mL of the *Casein substrate*, preheated to the bath temperature, and mix. Sixty minutes, accurately timed, after the addition of the *Casein substrate* stop the reaction in tubes S_1, S_2, S_3, and U by adding 5.0 mL of *Trichloroacetic acid solution* at the corresponding time intervals, stir, and remove all the tubes from the bath. Allow to stand for 10 minutes at room temperature for complete protein precipitation, and filter. The filtrates must be free from haze. Determine the absorbances of the filtrates, in 1-cm cells, at 280 nm, with a suitable spectrophotometer, using the filtrate from the blank (tube *B*) to set the instrument.

Calculation of potency—Correct the absorbance values for the filtrates from tubes S_1, S_2, and S_3 by subtracting the absorbance values for the filtrates from tubes S_{1B}, S_{2B}, and S_{3B}, respectively, and plot the corrected absorbance values against the corresponding volumes of the *Standard test dilution* used. From the curve, using the corrected absorbance value ($U - U_B$) for the Pancreatin taken, and taking into consideration the dilution factors, calculate the protease activity, in USP Units, of the Pancreatin taken by comparison with that of the Standard, using the protease activity stated on the label of USP Pancreatin Amylase and Protease RS.

Pancreatin Tablets

» Pancreatin Tablets contain not less than 90.0 percent of the labeled amount of pancreatin.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 30°.

Labeling—Label the Tablets to indicate minimum pancreatin fat digestive power; i.e., single strength, double strength, triple strength.

USP Reference standards ⟨11⟩—
USP Bile Salts RS

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USP Pancreatin Amylase and Protease RS
USP Pancreatin Lipase RS
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Tablets meet the requirements of the test for absence of *Salmonella* species and *Escherichia coli.*
Disintegration ⟨701⟩: 60 minutes.
Assay for amylase activity (*Starch digestive power*)—Weigh and finely powder not fewer than 20 Tablets, avoiding the production of heat during the process. Proceed as directed in the *Assay for amylase activity* under *Pancreatin*, using as the assay preparation an accurately weighed portion of the powder, equivalent to 40 mg of pancreatin. (Use an inversely proportionate amount of the powder if the Tablets are labeled to contain a whole-number multiple of the minimum requirement for pancreatin digestive activity.)

Assay for lipase activity (*Fat digestive power*)—
Acacia solution, Olive oil substrate, Buffer solution, Bile salts solution, and *Standard test dilution*—Prepare as directed in the *Assay for lipase activity* under *Pancreatin.*
Assay test dilution—Proceed as directed for *Assay test dilution* in the *Assay for lipase activity* under *Pancreatin*, using as the assay preparation an accurately weighed portion of the powder, prepared as directed in the *Assay for amylase activity*, equivalent to about 200 mg of pancreatin.
Procedure and *Calculation of potency*—Proceed as directed in the *Assay for lipase activity* under *Pancreatin.*

Assay for protease activity (*Casein digestive power*)—
Casein substrate, Buffer solution, Trichloroacetic acid solution, Filter paper and *Standard test dilution*—Prepare as directed in the *Assay for protease activity* under *Pancreatin.*
Assay test dilution—Add an accurately weighed portion of the powder, prepared as directed in the *Assay for amylase activity*, equivalent to about 100 mg of pancreatin, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for 25 minutes. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to the *Standard test dilution.*
Procedure and *Calculation of potency*—Proceed as directed in the *Assay for protease activity* under *Pancreatin.*

Pancrelipase

» Pancrelipase is a substance containing enzymes, principally lipase, with amylase and protease, obtained from the pancreas of the hog, *Sus scrofa* Linné var. *domesticus* Gray (Fam. Suidae). It contains, in each mg, not less than 24 USP Units of lipase activity, not less than 100 USP Units of amylase activity, and not less than 100 USP Units of protease activity.
[NOTE—One USP Unit of amylase activity is contained in the amount of pancrelipase that decomposes starch at an initial rate such that 0.16 µEq of glycosidic linkage is hydrolyzed per minute under the conditions of the *Assay for amylase activity.*]
One USP Unit of lipase activity is contained in the amount of pancrelipase that liberates 1.0 µEq of acid per minute at pH 9.0 and 37° under the conditions of the *Assay for lipase activity.*
One USP Unit of protease activity is contained in the amount of pancrelipase that under the conditions of the *Assay for protease activity,* hydrolyzes casein at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid that gives the same absorbance at 280 nm as 15 nmol of tyrosine.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 25°.
Labeling—Label it to indicate lipase activity in USP Units.
USP Reference standards ⟨11⟩—
USP Bile Salts RS
USP Pancreatin Amylase and Protease RS
USP Pancreatin Lipase RS
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli.*
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.
Fat—Place 2.0 g of Pancrelipase in a flask of about 50-mL capacity, add 20 mL of ether, insert the stopper, and set aside for 2 hours, mixing by rotating at frequent intervals. Decant the supernatant ether by means of a guiding rod into a plain filter about 7 cm in diameter, previously moistened with ether, and collect the filtrate in a tared beaker. Repeat the extraction with a 10-mL portion of ether, then with another 10-mL portion of ether, transfer the ether and the remainder of the Pancrelipase to the filter. Allow to drain, evaporate the ether spontaneously, and dry the residue at 105° for 2 hours: the residue of fat weighs not more than 100 mg (5.0%).

Assay for amylase activity (***Starch digestive power***)—
pH 6.8 Phosphate buffer—On the day of use, dissolve 13.6 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 14.2 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 51 mL of the monobasic potassium phosphate solution with 49 mL of the dibasic sodium phosphate solution. If necessary, adjust by the dropwise addition of the appropriate solution to a pH of 6.8.
Substrate solution—On the day of use, stir a portion of purified soluble starch equivalent to 2.0 g of dried substance with 10 mL of water, and add this mixture to 160 mL of boiling water. Rinse the beaker with 10 mL of water, add it to the hot solution, and heat to boiling, with continuous mixing. Cool to room temperature, and add water to make 200 mL.
Standard preparation—Weigh accurately about 20 mg of USP Pancreatin Amylase and Protease RS into a suitable mortar. Add about 30 mL of *pH 6.8 Phosphate buffer,* and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *pH 6.8 Phosphate buffer* to a 50-mL volumetric flask, dilute with *pH 6.8 phosphate buffer* to volume, and mix. Calculate the activity, in USP Units of amylase activity per mL, of the resulting solution from the declared potency on the label of the Reference Standard.
Assay preparation—For Pancrelipase having about 4 times the amylase activity of the USP Pancreatin Amylase and Protease RS, weigh accurately about 10 mg of Pancrelipase into a suitable mortar. [NOTE—For Pancrelipase having a different amylase activity, weigh accurately the amount necessary to obtain an *Assay preparation* having amylase activity per mL corresponding approximately to that of the *Standard preparation.*] Add about 3 mL of *pH 6.8 Phosphate buffer,* and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *pH 6.8 Phosphate buffer* to a 100-mL volumetric flask, dilute with *pH 6.8 Phosphate buffer* to volume, and mix.
Procedure—Prepare four stoppered, 250-mL conical flasks, and mark them *S, U, BS,* and *BU.* Pipet into each flask 25 mL of *Substrate solution,* 10 mL of *pH 6.8 Phosphate buffer,* and 1 mL of sodium chloride solution (11.7 in 1000), insert the

USP Monographs

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stoppers, and mix. Place the flasks in a water bath maintained at 25 ± 0.1°, and allow them to equilibrate. To flasks *BU* and *BS* add 2 mL of 1 N hydrochloric acid, mix, and return the flasks to the water bath. To flasks *U* and *BU* add 1.0-mL portions of *Assay preparation*, and to flasks *S* and *BS* add 1.0 mL of *Standard preparation*. Mix each, and return the flasks to the water bath. After 10 minutes, accurately timed from the addition of the enzyme, add 2-mL portions of 1 N hydrochloric acid to flasks *S* and *U*, and mix. To each flask, with continuous stirring, add 10.0 mL of 0.1 N iodine VS, and immediately add 45 mL of 0.1 N sodium hydroxide. Place the flasks in the dark at a temperature between 15° and 25° for 15 minutes. To each flask add 4 mL of 2 N sulfuric acid, and titrate with 0.1 N sodium thiosulfate VS to the disappearance of the blue color. Calculate the amylase activity, in USP Units per mg, of the Pancrelipase taken by the formula:

$$100(C_S/W_U)(V_{BU} - V_U)/(V_{BS} - V_S)$$

in which C_S is the amylase activity of the *Standard preparation*, in USP Units per mL, W_U is the amount, in mg, of Pancrelipase taken, and V_U, V_S, V_{BU}, and V_{BS} are the volumes, in mL, of 0.1 N sodium thiosulfate consumed in the titration of the solutions in flasks *U*, *S*, *BU*, and *BS*, respectively.

Assay for lipase activity (*Fat digestive power*)—

Acacia solution—Centrifuge a solution of acacia (1 in 10) until clear. Use only the clear solution.

Olive oil substrate—Combine 165 mL of *Acacia solution*, 20 mL of olive oil, and 15 g of crushed ice in the cup of an electric blender. Cool the mixture in an ice bath to 5°, and homogenize at high speed for 15 minutes, intermittently cooling in an ice bath to prevent the temperature from exceeding 30°.

Test for suitability of mixing as follows. Place a drop of the homogenate on a microscope slide, and gently press a cover slide in place to spread the liquid. Examine the entire field under high power (43× objective lens and 5× ocular), using an eyepiece equipped with a calibrated micrometer. The substrate is satisfactory if 90% of the particles do not exceed 2 µm in diameter and none exceeds 10 µm in diameter.

Buffer solution—Dissolve 60 mg of tris(hydroxymethyl) aminomethane and 234 mg of sodium chloride in water to make 100 mL.

Bile salts solution—Prepare a solution to contain 80.0 mg of USP Bile Salts RS in each mL.

Standard test dilution—Suspend about 200 mg of USP Pancreatin Lipase RS, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the declared potency on the label of the Reference Standard. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Assay test dilution—Suspend about 200 mg of Pancrelipase, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the estimated potency of the test material. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Procedure—Mix 10.0 mL of *Olive oil substrate*, 8.0 mL of *Buffer solution*, 2.0 mL of *Bile salts solution*, and 9.0 mL of water in a jacketed glass vessel of about 50-mL capacity, the outer chamber of which is connected to a thermostatically controlled water bath. Cover the mixture, and stir continuously with a mechanical stirring device. With the mixture maintained at a temperature of 37 ± 0.1°, add 0.1 N sodium hydroxide VS, from a microburet inserted through an opening in the cover, to adjust the pH to 9.20 potentiometrically using a calomel-glass electrode system. Add 1.0 mL of *Assay test dilution*, and then continue adding the 0.1 N sodium hydroxide VS for 5 minutes to maintain the pH at 9.0. Determine the volume of 0.1 N sodium hydroxide VS added after each minute.

In the same manner titrate 1.0 mL of *Standard test dilution*.

Calculation of potency—Plot the volume of 0.1 N sodium hydroxide VS titrated against time. Using only the points which fall on the straight-line segment of the curve, calculate the mean acidity released per minute by the test specimen and the Standard. Taking into consideration the dilution factors, calculate the lipase activity, in USP Units, of the Pancrelipase taken by comparison to the activity of the Reference Standard, using the lipase activity stated on the label of USP Pancreatin Lipase RS.

Assay for protease activity (*Casein digestive power*)—

Casein substrate—Place 1.25 g of finely powdered casein in a 100-mL conical flask containing 5 mL of water, shake to form a suspension, add 10 mL of 0.1 N sodium hydroxide, shake for 1 minute, add 50 mL of water, and shake for about 1 hour to dissolve the casein. If necessary, adjust to a pH of about 8, using 1 N sodium hydroxide or 1 N hydrochloric acid. Transfer the solution quantitatively to a 100-mL volumetric flask, dilute with water to volume, and mix. Use this substrate on the day it is prepared.

Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate and 1.8 g of sodium hydroxide in 950 mL of water in a 1000-mL volumetric flask, adjust to a pH of 7.5 ± 0.2, using 0.2 N sodium hydroxide, dilute with water to volume, and mix. Store this solution in a refrigerator.

Trichloroacetic acid solution—Dissolve 50 g of trichloroacetic acid in 1000 mL of water. This solution may be stored at room temperature.

Filter paper—Determine the suitability of the filter paper by filtering a 5-mL portion of *Trichloroacetic acid solution* through the paper and measuring the absorbance of the filtrate at 280 nm, using an unfiltered portion of the same *Trichloroacetic acid solution* as the blank: the absorbance is not more than 0.04. If the absorbance is more than 0.04, wash the filter paper repeatedly with *Trichloroacetic acid solution* until the absorbance of the filtrate, determined as above, is not more than 0.04.

Standard test dilution—Add about 100 mg of USP Pancreatin Amylase and Protease RS, accurately weighed, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for about 25 minutes. Dilute quantitatively with *Buffer solution* to produce a concentration of about 2.5 USP Units of protease activity per mL, based upon the declared potency on the label of the Reference Standard.

Assay test dilution—Weigh accurately about 100 mg of Pancrelipase into a suitable mortar. Add about 3 mL of *Buffer solution*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *Buffer solution* to a 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to the *Standard test dilution*.

Procedure—Label test tubes in duplicate S_1, S_2, and S_3 for the standard series, and *U* for the sample. Pipet into tubes S_1 2.0 mL, into S_2 and *U* 1.5 mL, and into S_3 1.0 mL of *Buffer solution*. Pipet into tubes S_1 1.0 mL, into S_2 1.5 mL, and into S_3 2.0 mL of the *Standard test dilution*. Pipet into tubes *U* 1.5 mL of the *Assay test dilution*. Pipet into one tube each of S_1, S_2, S_3, and *U* 5.0 mL of *Trichloroacetic acid solution*, and mix. Designate these tubes as S_{1B}, S_{2B}, S_{3B}, and U_B, respectively.

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Prepare a blank by mixing 3 mL of *Buffer solution* and 5 mL of *Trichloroacetic acid solution* in a separate test tube marked *B*. Place all the tubes in a 40° water bath, insert a glass stirring rod into each tube, and allow for temperature equilibration. At zero time, add to each tube, at timed intervals, 2.0 mL of the *Casein substrate*, preheated to the bath temperature, and mix. Sixty minutes, accurately timed, after the addition of the *Casein substrate* stop the reaction in tubes S_1, S_2, S_3, and *U* by adding 5.0 mL of *Trichloroacetic acid solution* at the corresponding time intervals, stir, and remove all the tubes from the bath. Allow to stand at room temperature for 10 minutes for complete protein precipitation, and filter. The filtrates must be free from haze. Determine the absorbances of the filtrates, in 1-cm cells, at 280 nm, with a suitable spectrophotometer, using the filtrate from the blank (tube *B*) to set the instrument.

Calculation of potency—Correct the absorbance values for the filtrates from tubes S_1, S_2, and S_3 by subtracting the absorbance values for the filtrates from tubes S_{1B}, S_{2B}, and S_{3B}, respectively, and plot the corrected absorbance values against the corresponding volumes of the *Standard test dilution* used. From the curve, using the corrected absorbance value ($U - U_B$) for the Pancrelipase taken, and taking into consideration the dilution factors, calculate the protease activity, in USP Units, of the Pancrelipase taken by comparison with that of the Standard, using the protease activity stated on the label of USP Pancreatin Amylase and Protease RS.

Pancrelipase Capsules

» Pancrelipase Capsules contain an amount of Pancrelipase equivalent to not less than 90.0 percent and not more than 150.0 percent of the labeled lipase activity expressed in USP Units, the labeled activity being not less than 8000 USP Units per Capsule. They contain, in each Capsule, the pancrelipase equivalent of not less than 30,000 USP Units of amylase activity, and not less than 30,000 USP Units of protease activity.

Packaging and storage—Preserve in tight containers, preferably with a desiccant, at a temperature not exceeding 25°.
Labeling—Label the Capsules to indicate lipase activity in USP Units.
USP Reference standards ⟨11⟩—
USP Bile Salts RS
USP Pancreatin Amylase and Protease RS
USP Pancreatin Lipase RS
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Capsules meet the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
Loss on drying ⟨731⟩—Dry the contents of 10 Capsules in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.
Assay—Weigh the contents of not less than 10 Capsules, and determine the average weight per capsule. Mix the combined contents, and proceed as directed for *Assay for amylase activity*, *Assay for lipase activity*, and *Assay for protease activity* under *Pancrelipase*.

Pancrelipase Delayed-Release Capsules

» Pancrelipase Delayed-Release Capsules contain an amount of Pancrelipase equivalent to not less than 90.0 percent and not more than 165.0 percent of the labeled lipase. It contains not less than 90.0 percent of the labeled activities of amylase and protease expressed in the respective USP Units.

Packaging and storage—Preserve in tight containers at controlled room temperature.
Labeling—Label the Capsules to indicate lipase, amylase, and protease activities in USP Units. The label also indicates that the Capsule contents are enteric-coated.
USP Reference standards ⟨11⟩—
USP Bile Salts RS
USP Pancreatin Amylase and Protease RS
USP Pancreatin Lipase RS
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Capsules meet the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

Dissolution ⟨711⟩—
PART 1 —
Medium: simulated gastric fluid TS, without enzyme; 800 mL.
Apparatus 1: 100 rpm.
Time: 60 minutes.
PART 2 —
pH 6.0 phosphate buffer—Dissolve 8 g of sodium chloride and 36.8 g of monobasic potassium phosphate in 4000 mL of water. Adjust with 2 N sodium hydroxide to a pH of 6.0 ± 0.1.
Medium: pH 6.0 phosphate buffer; 800 mL.
Apparatus 2: 100 rpm.
Time: 30 minutes.
Standard solution—Proceed as directed under *Standard test dilution* in the *Assay for lipase activity* under *Pancrelipase*, except to use the *Dissolution Medium* in place of cold water.
Test solution—Empty the contents of 10 Capsules, and transfer an accurately weighed portion of the contents, equivalent to the concentration of USP Units of lipase activity per mL in the *Standard solution* (between 8 and 16 Units per mL), to *Apparatus 1*.
Procedure—Proceed according to the conditions for *Part 1*. After 1 hour, remove the baskets, and allow the excess *Dissolution Medium* to drain. Transfer the contents of each basket to the dissolution vessels in *Part 2* with the aid of a few mL of *Dissolution Medium*. Proceed according to the conditions for *Part 2*. After 30 minutes, remove a 10-mL portion of the solution under test, transfer to a test tube, and cool to 4°. Proceed as directed in the *Assay for lipase activity* under *Pancrelipase*.
Tolerances—Not less than 75% *(Q)* of the labeled USP Units of lipase activity per Capsule is dissolved.
Loss on drying ⟨731⟩—Dry the contents of 10 Capsules in vacuum at 60° for 4 hours: the test specimen loses not more than 5.0% of its weight.
Assay—Weigh the contents of not less than 10 Capsules, and determine the average weight per Capsule. Grind the contents, mix the combined contents, and proceed as directed in the *Assay for lipase activity*, the *Assay for amylase activity*, and the *Assay for protease activity* under *Pancrelipase*.

USP Monographs

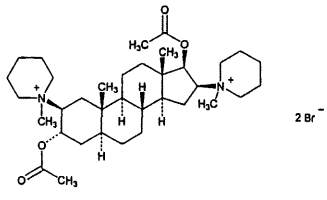
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Pancrelipase Tablets

» Pancrelipase Tablets contain an amount of Pancrelipase equivalent to not less than 90.0 percent and not more than 150.0 percent of the labeled lipase activity expressed in USP Units, the labeled activity being not less than 8000 USP Units per Tablet. They contain, in each Tablet, the pancrelipase equivalent of not less than 30,000 USP Units of amylase activity, and not less than 30,000 USP Units of protease activity.

Packaging and storage—Preserve in tight containers, preferably with a desiccant, at a temperature not exceeding 25°.
Labeling—Label the Tablets to indicate the lipase activity in USP Units.
USP Reference standards ⟨11⟩—
USP Bile Salts RS
USP Pancreatin Amylase and Protease RS
USP Pancreatin Lipase RS
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Tablets meet the requirements of the test for absence of *Salmonella* species and *Escherichia coli.*
Disintegration ⟨701⟩: 75 minutes.
Loss on drying ⟨731⟩—Dry about 5 g, accurately weighed, of finely ground Tablets in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.
Assay—Weigh and finely powder not less than 20 Tablets, avoiding the production of heat during the process, and proceed as directed for *Assay for amylase activity, Assay for lipase activity,* and *Assay for protease activity* under *Pancrelipase.*

Pancuronium Bromide



$C_{35}H_{60}Br_2N_2O_4$ 732.67
Piperidinium, 1,1′-[(2β,3α,5α,16β,17β)-3,17-bis(acetyl oxy) androstane-2,16-diyl]bis[1-methyl]-, dibromide;
1,1′-(3α,17β-Dihydroxy-5α-androstan-2β,16β-ylene)bis[1-methylpiperidinium] dibromide diacetate;
2β,16β-Dipiperidino-5α-androstane-3α,17β-diol diacetate dimethobromide [15500-66-0].

DEFINITION
Pancuronium Bromide contains NLT 98.0% and NMT 102.0% of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Bromide*: A solution (1 in 10) meets the requirements of test *B.*

ASSAY
- **PROCEDURE**

Diluent: 0.0024 M hydrochloric acid
Mobile phase: Acetonitrile, methanol, and 0.024 M hydrochloric acid (125:200:675)
Standard stock solution: 1.0 mg/mL prepared as follows. Transfer the required quantity of USP Pancuronium Bromide RS to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, dilute with *Diluent* to volume, and sonicate for 3 min.
Standard solution: 0.1 mg/mL of USP Pancuronium Bromide RS in *Diluent,* from the *Standard stock solution*
Sample stock solution: 1.0 mg/mL prepared as follows. Transfer the required quantity of Pancuronium Bromide to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, dilute with *Diluent* to volume, and sonicate for 3 min.
Sample solution: 0.1 mg/mL of Pancuronium Bromide in *Diluent,* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Conductivity with suppression
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 35°
Detector: 40°
Suppressor: 4-mm cationic membrane suppressor or equivalent
Suppression solution: 0.15 M tetrabutylammonium hydroxide
Suppressor flow rate: 1 mL/min
Flow rate: 0.75 mL/min
Injection volume: 25 µL
Run time: 2 times the retention time of pancuronium
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$) in the portion of Pancuronium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pancuronium Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Pancuronium Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Diluent, Mobile phase, Standard stock solution, and **Chromatographic system:** Proceed as directed in the *Assay.*

USP Monographs

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System suitability solution: 1 mg/mL of USP Pancuronium Bromide RS and 0.02 mg/mL each of USP Pancuronium Bromide Related Compound A RS, USP Pancuronium Bromide Related Compound B RS, USP Pancuronium Bromide Related Compound C RS, and USP Vecuronium Bromide RS, prepared as follows. Transfer the required amounts of the individual components to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, dilute with *Diluent* to volume, and sonicate for 3 min.

Standard solution: 0.01 mg/mL of USP Pancuronium Bromide RS in *Diluent*, from the *Standard stock solution*

Sample solution: 1.0 mg/mL prepared as follows. Transfer the required quantity of Pancuronium Bromide to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, dilute with *Diluent* to volume, and sonicate for 3 min.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between pancuronium related compound B and pancuronium related compound A, and NLT 1.5 between the pancuronium related compound C and vecuronium peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity, including any unspecified impurity, in the portion of Pancuronium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of pancuronium from the *Standard solution*

C_S = concentration of USP Pancuronium Bromide RS in the *Standard solution* (mg/mL)

C_U = concentration of Pancuronium Bromide in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pancuronium related compound B	0.73	1.0	0.1
Pancuronium related compound A	0.81	1.0	0.1
Vecuronium related compound F[a]	0.9	—	—
Pancuronium	1.0	—	—
Pancuronium related compound C	1.39	1.0	0.1
Vecuronium	1.53	0.48	1.0
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] Piperidinium, 1-[(2,3,5,16,17)-17-acetyloxy-3-hydroxy-2-(1-piperidinyl) androstan-16-yl]-1-methyl. This impurity is an acid degradation product of vecuronium bromide and not that of pancuronium bromide.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 30 mg/mL in water
  Acceptance criteria: +39° to +43°
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 8.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Pancuronium Bromide RS
  USP Pancuronium Bromide Related Compound A RS
  1,1′-(3α,17β-Dihydroxy-5α-androstan-2β,16β-ylene) bis(1-methylpiperidinium) dibromide.
  $C_{31}H_{56}Br_2N_2O_2$ 648.60
  USP Pancuronium Bromide Related Compound B RS
  1,1′-(17β-Acetoxy-3α-hydroxy-5α-androstan-2β,16β-ylene) bis(1-methylpiperidinium) dibromide.
  $C_{33}H_{58}Br_2N_2O_3$ 690.63
  USP Pancuronium Bromide Related Compound C RS
  1,1′-(3α-Acetoxy-17β-hydroxy-5α-androstan-2β,16β-ylene) bis(1-methylpiperidinium) dibromide.
  $C_{33}H_{58}Br_2N_2O_3$ 690.63
  USP Vecuronium Bromide RS

Pancuronium Bromide Injection

DEFINITION

Pancuronium Bromide Injection is a sterile solution containing NLT 92.0% and NMT 105.0% of the labeled amount of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$) in Water for Injection. It contains a suitable tonicity-adjusting agent.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and 0.024 M hydrochloric acid (125:220:655)

Diluent: 0.0024 M hydrochloric acid

Standard stock solution: 1.0 mg/mL of USP Pancuronium Bromide RS prepared as follows. Transfer the required quantity of USP Pancuronium Bromide RS to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile. Dilute with *Diluent* to volume. Sonicate for 3 min.

Standard solution: 0.1 mg/mL of USP Pancuronium Bromide RS from the *Standard stock solution* and *Diluent*

Sample solution: Nominally 0.1 mg/mL of pancuronium bromide from a suitable volume of Injection in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Conductivity with suppression

Column: 4.6-mm × 25-cm; 5-µm packing L1

USP Monographs

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Temperatures
Detector: 40°
Column: 35°
Suppressor: 4-mm cationic membrane suppressor or equivalent
Suppression solution: 0.15 M tetrabutylammonium hydroxide
Suppressor flow rate: 1 mL/min
Flow rate: 0.75 mL/min
Injection volume: 25 µL
Run time: 2 times the retention time of pancuronium
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pancuronium Bromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pancuronium bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent, Standard stock solution, and Chromatographic system: Proceed as directed in the *Assay*.
Mobile phase: Acetonitrile, methanol, and 0.024 M hydrochloric acid (125:180:695)
System suitability solution: 1 mg/mL of USP Pancuronium Bromide RS and 0.02 mg/mL each of USP Pancuronium Bromide Related Compound A RS, USP Pancuronium Bromide Related Compound B RS, USP Pancuronium Bromide Related Compound C RS, and USP Vecuronium Bromide RS prepared as follows. Transfer the required amounts of the individual components to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, and dilute with *Diluent* to volume. Sonicate for 3 min.
Standard solution: 0.02 mg/mL of USP Pancuronium Bromide RS from the *Standard stock solution* and *Diluent*
Sample solution: Nominally 1.0 mg/mL of pancuronium bromide from a suitable volume of Injection in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between pancuronium related compound B and pancuronium related compound A; NLT 1.5 between pancuronium related compound C and vecuronium peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity as well as any unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the pancuronium peak from the *Standard solution*
C_S = concentration of USP Pancuronium Bromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pancuronium bromide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pancuronium related compound B	0.70	3.0
Pancuronium related compound A	0.78	0.4
Vecuronium related compound F[a]	0.90	—
Pancuronium	1.0	—
Pancuronium related compound C	1.47	2.0
Vecuronium[b]	1.69	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	5.0

[a] Piperidinium, 1-[(2,3,5,16,17)-17-acetyloxy-3-hydroxy-2-(1-piperidinyl) androstan-16-yl]-1-methyl. This impurity is the acid degradation product of vecuronium bromide and may be present only in the *System suitability solution*.
[b] This process impurity is included for peak identification purposes only and is controlled in the drug substance. This is not included in the total impurities.

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.8–4.2
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 50 USP Endotoxin Units/mg of pancuronium bromide.
• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

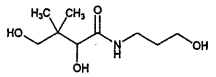
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, single-dose or multiple-dose containers for injections, as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, preferably of Type 1 glass. Store in a refrigerator between 2° and 8°, protected from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pancuronium Bromide RS
USP Pancuronium Bromide Related Compound A RS
1,1′-(3α,17β-Dihydroxy-5α-androstan-2β,16β-ylene) bis(1-methylpiperidinium) dibromide.
$C_{31}H_{56}Br_2N_2O_2$ 648.60
USP Pancuronium Bromide Related Compound B RS
1,1′-(17β-Acetoxy-3α-hydroxy-5α-androstan-2β,16β-ylene) bis(1-methylpiperidinium) dibromide.
$C_{33}H_{58}Br_2N_2O_3$ 690.63
USP Pancuronium Bromide Related Compound C RS
1,1′-(3α-Acetoxy-17β-hydroxy-5α-androstan-2β,16β-ylene) bis(1-methylpiperidinium) dibromide.
$C_{33}H_{58}Br_2N_2O_3$ 690.63
USP Vecuronium Bromide RS

USP Monographs

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Panthenol



$C_9H_{19}NO_4$ 205.25

Butanamide, 2,4-dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethyl-, (±)-;
(±)-2,4-Dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethylbutyramide;
(±)-Pantothenyl alcohol [16485-10-2].

DEFINITION

Panthenol is a racemic mixture of the dextrorotatory and levorotatory isomers of panthenol. It contains NLT 99.0% and NMT 102.0% of racemic panthenol ($C_9H_{19}NO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.**

Sample solution: 100 mg/mL of Panthenol
Analysis: To 1 mL of the *Sample solution* add 5 mL of 1 N sodium hydroxide and 1 drop of cupric sulfate TS, and shake vigorously.
Acceptance criteria: A deep blue color develops.

- **C.**

Sample solution: 10 mg/mL of Panthenol
Analysis: To 1 mL of the *Sample solution* add 1 mL of 1 N hydrochloric acid, and heat on a steam bath for 30 min. Cool, add 100 mg of hydroxylamine hydrochloride, and add 5 mL of 1 N sodium hydroxide. Allow to stand for 5 min, then adjust with 1 N hydrochloric acid to a pH of 2.5–3.0, and add 1 drop of ferric chloride TS.
Acceptance criteria: A purplish red color develops.

ASSAY

- **Procedure**

0.1 M potassium biphthalate: Transfer 20.42 g of potassium biphthalate into a 1000-mL volumetric flask, and add sufficient glacial acetic acid to dissolve. If necessary, warm the mixture on a steam bath to achieve complete solution, observing precautions against absorption of moisture. Cool to room temperature, and dilute with glacial acetic acid to volume.
Sample: 400 mg of Panthenol
Blank: Proceed as directed in the *Analysis*, omitting the *Sample*.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.1 N perchloric acid VS
Back-titrant: 0.1 M potassium biphthalate
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 300-mL flask fitted to a reflux condenser by means of a standard-taper glass joint, add 50.0 mL of *Titrant*, and reflux for 5 h. Cool, observing precautions to prevent atmospheric moisture from entering the condenser, and rinse the condenser with glacial acetic acid, collecting the rinsings in the flask. To the flask add 5 drops of crystal violet TS, and titrate with the *Back-titrant* to a blue-green endpoint. Perform the *Blank* determination.
Calculate the percentage of panthenol ($C_9H_{19}NO_4$) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)
V_S = *Back-titrant* volume consumed by the *Sample* (mL)
M = actual molarity of the *Back-titrant* (mM/mL)
F = equivalency factor, 205.3 mg/M
W = *Sample* weight (mg)

Acceptance criteria: 99.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Limit of Aminopropanol**

Sample: 10 g of Panthenol
Blank: 25 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.01 N sulfuric acid VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 25 mL of water, and add bromothymol blue TS. Titrate with the *Titrant* to a yellow endpoint. Perform the *Blank* determination.
Calculate the percentage of aminopropanol in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 75.11 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 0.10%

SPECIFIC TESTS

- **Melting Range,** *Class I* ⟨741⟩: 64.5°–68.5°
- **Optical Rotation,** *Specific Rotation* ⟨781S⟩

Sample solution: 50 mg/mL in water
Acceptance criteria: –0.05° to +0.05°

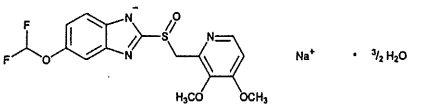
- **Loss on Drying ⟨731⟩:** Dry a sample in a vacuum over phosphorus pentoxide at 56° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards ⟨11⟩**

USP Racemic Panthenol RS

Pantoprazole Sodium



$C_{16}H_{14}F_2N_3NaO_4S \cdot 1.5H_2O$ 432.37

1*H*-Benzimidazole, 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfinyl]-, sodium salt, hydrate (2:3);
5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfinyl]benzimidazole, sodium salt, sesquihydrate [164579-32-2].

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DEFINITION

Pantoprazole Sodium contains NLT 98.0% and NMT 102.0% of pantoprazole sodium ($C_{16}H_{14}F_2N_3NaO_4S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL ⟨191⟩, *Chemical Identification Tests, Sodium*:** Meets the requirements of test *A*.

ASSAY

- **PROCEDURE**

[NOTE—Protect solutions containing pantoprazole sodium from light.]

Ammonium phosphate buffer: 1.32 g/L of dibasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 7.5

Acetonitrile–methanol mixture: Acetonitrile and methanol (7:3)

Solution A: *Acetonitrile–methanol mixture* and *Ammonium phosphate buffer* (15:85)

Solution B: *Acetonitrile–methanol mixture*

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	86	14
10	86	14
35	42	58

Diluent: Dilute 25 mL of ammonium hydroxide with water to 500 mL.

System suitability stock solution: 0.5 mg/mL each of USP Pantoprazole Sodium RS, USP Pantoprazole Related Compound A RS, and USP Pantoprazole Related Compound B RS in a mixture of acetonitrile and water (1:1)

System suitability solution: 0.005 mg/mL each of USP Pantoprazole Sodium RS, USP Pantoprazole Related Compound A RS, and USP Pantoprazole Related Compound B RS in *Diluent*, from *System suitability stock solution*

Standard stock solution: 0.4 mg/mL of USP Pantoprazole Sodium RS, dissolved first in a mixture of acetonitrile and water (1:1) using 10%–20% of the final volume, and then diluted with *Diluent* to volume

Standard solution: 0.06 mg/mL of USP Pantoprazole Sodium RS in *Diluent*, from *Standard stock solution*

Sample stock solution: Transfer about 20 mg of Pantoprazole Sodium to a 50-mL volumetric flask. Dissolve in 5–10 mL of a mixture of acetonitrile and water (1:1), and dilute with *Diluent* to volume.

Sample solution: 0.06 mg/mL of Pantoprazole Sodium in *Diluent*, from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Temperatures

Autosampler: 4°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—Identify the components based on their relative retention times (see *Table 2*).]

Suitability requirements

Resolution: NLT 10.0 between pantoprazole related compound A and pantoprazole, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pantoprazole sodium ($C_{16}H_{14}F_2N_3NaO_4S$) in the portion of Pantoprazole Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pantoprazole Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Pantoprazole Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

[NOTE—On the basis of the synthetic route, perform either *Test 1* or *Test 2*. *Test 2* is recommended when impurities C, D, E, and F are potential related compounds.]

[NOTE—Protect solutions containing pantoprazole sodium from light.]

Test 1

Ammonium phosphate buffer, Mobile phase, Diluent, System suitability solution, Standard stock solution, Sample stock solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.0004 mg/mL of USP Pantoprazole Sodium RS in *Diluent*, from *Standard stock solution*

Sample solution: *Sample stock solution*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 10.0 between pantoprazole related compound A and pantoprazole, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Pantoprazole Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of pantoprazole from the *Standard solution*

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C_S = concentration of USP Pantoprazole Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Pantoprazole Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pantoprazole sulfone (pantoprazole related compound A)	0.52	0.20
Pantoprazole	1.0	—
Pantoprazole sulfide (pantoprazole related compound B)	1.7	0.15
Any other individual impurity	—	0.10
Total impurities	—	0.5

Test 2
Solution A: 1.74 g/L of dibasic potassium phosphate adjusted with a solution of phosphoric acid (330 g/L) to a pH of 7.00 ± 0.05
Solution B: Acetonitrile
Mobile phase: See *Table 3*. Return to original conditions and re-equilibrate the system for 15 min.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
40	20	80

Diluent: Acetonitrile and 0.001 N sodium hydroxide (1:1)
System suitability solution: About 0.46 mg/mL of USP Pantoprazole Sodium RS, and about 1.3 µg/mL each of USP Pantoprazole Related Compound A RS, USP Pantoprazole Related Compound B RS, USP Pantoprazole Related Compound C RS, USP Pantoprazole Related Compounds D and F Mixture RS, and USP Pantoprazole Related Compound E RS in *Diluent*
Standard solution: 0.03 mg/mL of USP Pantoprazole Sodium RS in *Diluent*
Sample solution: 0.46 mg/mL of Pantoprazole Sodium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 and 305 nm
Column: 4-mm × 12.5-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution* at 290 nm
[NOTE—See *Table 4* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between pantoprazole related compound E and pantoprazole related compounds D and F, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
[NOTE—Pantoprazole related compound C is monitored at 305 nm, and all other compounds are monitored at 290 nm.]
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Pantoprazole Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pantoprazole sodium from the *Standard solution*
C_S = concentration of USP Pantoprazole Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Pantoprazole Sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4* for individual impurities. The reporting level for impurities is 0.05%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzimidazolethiol derivative (pantoprazole related compound C)	0.6	3.3	0.10[a]
Pantoprazole sulfone (pantoprazole related compound A)	0.9	1.0	0.20
Pantoprazole	1.0	—	—
*N*¹-Methyl pantoprazole (pantoprazole related compound D) and *N*³-Methyl pantoprazole (pantoprazole related compound F)	1.2	1.0	0.20[b]
Pantoprazole dimer (pantoprazole related compound E)	1.3	1.0	0.10
Pantoprazole sulfide (pantoprazole related compound B)	1.5	1.0	0.15
Any other individual impurity	—	—	0.10
Total impurities	—	—	0.5

[a] At 305 nm.
[b] Impurities D and F are not fully resolved and should be integrated together.

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I*: 4.5%–8.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Test 1* is used, then the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Pantoprazole Sodium RS
USP Pantoprazole Related Compound A RS
5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl) methyl]sulfonyl]-1*H*-benzimidazole.
$C_{16}H_{15}F_2N_3O_5S$ 399.37

USP Monographs

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USP Pantoprazole Related Compound B RS
5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl) methyl]thio]-1*H*-benzimidazole.
$C_{16}H_{15}F_2N_3O_3S$ 367.37

USP Pantoprazole Related Compound C RS
5-(Difluoromethoxy)-1*H*-benzimidazole-2-thiol.
$C_8H_6F_2N_2OS$ 216.21

USP Pantoprazole Related Compounds D and F Mixture RS
A mixture of 5-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1*H*-benzimidazole and 6-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1*H*-benzimidazole.
$C_{17}H_{17}F_2N_3O_4S$ 397.40

USP Pantoprazole Related Compound E RS
A mixture of the stereoisomers of 6,6′-bis(difluoromethoxy)-2,2′-bis[[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1*H*,1′*H*-5,5′-bibenzimidazolyl.
$C_{34}H_{28}F_4N_6O_8S_2$ 764.74

Pantoprazole Sodium Delayed-Release Tablets

DEFINITION

Pantoprazole Sodium Delayed-Release Tablets contain an amount of Pantoprazole Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pantoprazole ($C_{16}H_{15}F_2N_3O_4S$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 3.85 g of ammonium acetate and 1.1 g of tetrabutylammonium hydrogen sulfate in 1 L of water, and adjust with ammonium hydroxide solution diluted 1:1 with water to a pH of 7.9.

Diluent: Mixture of acetonitrile and 0.02 N sodium hydroxide (1:1)

Mobile phase: Prepare a mixture of acetonitrile and *Solution A* (35:65).

Standard solution: Transfer a weighed quantity of USP Pantoprazole Sodium RS to a suitable volumetric flask, add 0.02 N sodium hydroxide to about 60% of the final volume, sonicate for 5 min to dissolve, add about 2% of acetonitrile, and dilute with 0.02 N sodium hydroxide to volume to obtain a solution having a known concentration of about 0.2 mg/mL of pantoprazole sodium.

System suitability solution: Prepare a solution in 0.02 N sodium hydroxide, using sonication if necessary, containing about 0.2 mg/mL of pantoprazole sodium and about 0.0004 mg/mL each of USP Pantoprazole Related Compound A RS and USP Pantoprazole Related Compound B RS.

Sample solution: Transfer 5 Tablets into a suitable volumetric flask. [NOTE—Use 50- or 100-mL volumetric flasks for Tablets containing 20 or 40 mg of pantoprazole per Tablet, respectively.] Add *Diluent* to about 60% of the final volume, shake mechanically for about 60 min, and dilute with *Diluent* to volume. Pass through a suitable filter, and dilute the filtrate with 0.02 N sodium hydroxide to obtain a solution having a known concentration of about 0.2 mg/mL of pantoprazole, based on the label claim.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3 between pantoprazole and pantoprazole related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *System suitability solution*
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{15}F_2N_3O_4S$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pantoprazole Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pantoprazole in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1: Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.*

Acid stage
Acid stage medium: 0.1 N hydrochloric acid; 1000 mL
Apparatus 2: 75 rpm
Time: 120 min
Determine the amount of pantoprazole dissolved in the *Acid stage* using the following procedure.
Sample solution: After 120 min, withdraw an aliquot, pass through a suitable filter of 0.45-µm pore size, and immediately dilute a portion of the filtrate by a factor of 2 with 0.5 N sodium hydroxide. Transfer the Tablets to the vessels containing the *Buffer stage medium.*
Diluent: Prepare a mixture of pH 6.8 phosphate buffer and 0.5 N sodium hydroxide (1:1).
Mobile phase: Acetonitrile, triethylamine, and water (40:1:60). Adjust with phosphoric acid to a pH of 7.0 ± 0.05.
Standard stock solution: Transfer about 20 mg of USP Pantoprazole Sodium RS to a 50-mL volumetric flask. Add about 30 mL of 0.02 N sodium hydroxide, and sonicate until dissolved. Add 2 mL of acetonitrile, and dilute with 0.02 N sodium hydroxide to volume.
Standard solution: Transfer 1.0 mL of the *Standard stock solution* to a 20-mL volumetric flask, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 290 nm
Column: 4.6-mm × 7.5-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of pantoprazole released, as a percentage, in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (100/L) \times D$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of pantoprazole sodium in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35
V = volume of *Medium*, 1000 mL
L = Tablet label claim (mg)
D = dilution factor for the *Sample solution*, 2

Tolerances: NMT 10% of the labeled amount of pantoprazole is dissolved.
Buffer stage
Buffer stage medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 2: 75 rpm
Time: 30 min
Analysis: After 30 min, withdraw an aliquot, pass through a suitable filter of 0.45-µm pore size, and immediately dilute a portion of the filtrate by a factor of 2 with 0.5 N sodium hydroxide. Determine the amount of pantoprazole dissolved in the *Buffer stage* using the same procedure as for the *Acid stage*.
Tolerances: NLT 75% (*Q*) of the labeled amount of pantoprazole is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.
Acid stage
Acid stage medium: 0.1 N hydrochloric acid; 1000 mL
Apparatus 2: 100 rpm
Time: 2 h
Standard stock solution: Transfer a quantity of USP Pantoprazole Sodium RS to a suitable volumetric flask. Dissolve first in 0.1 N sodium hydroxide, using 10% of the final volume, then dilute with pH 6.8 phosphate buffer to volume, to obtain a solution having a known concentration of about 0.46 mg of pantoprazole sodium per mL. Mix well until a clear solution is obtained. Calculate the concentration in mg of pantoprazole per mL, the molecular weights of pantoprazole and pantoprazole sodium being 383.37 and 405.35, respectively.
Acid stage standard solution: Dilute an appropriate volume of the *Standard stock solution* to 1 L with *Acid stage medium* in such a way as to obtain a final concentration of about 10% of the Tablet label claim per L.
Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size.
Analysis:
Determine the amount of pantoprazole dissolved by using UV absorption at the wavelength of about 305 nm on portions of the *Sample solution* in comparison to the *Acid stage working standard solution* using a 4-cm path length cell and *Acid stage medium* as blank. Drain the *Acid stage medium* from each vessel and replace with *Buffer stage medium*.
Calculate the amount of pantoprazole dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of pantoprazole in the *Acid stage standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = Tablet label claim of pantoprazole (mg)

Tolerances: NMT 10% of the labeled amount of pantoprazole is dissolved.
Buffer stage
Buffer stage medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 2: 100 rpm
Time: 45 min
Buffer stage standard solution: Dilute an appropriate volume of the *Standard stock solution* as described under *Acid stage* to 250 mL with *Buffer stage medium* in such a way as to obtain a final concentration of about 100% of the Tablet label claim per L.
Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size.
Analysis: Determine the amount of pantoprazole dissolved by using UV absorption at the wavelength of maximum absorbance at about 288 nm on portions of the *Sample solution* in comparison to *Buffer stage standard solution* using a 0.5-cm path length cell and *Buffer stage medium* as blank.
Calculate the amount of pantoprazole dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Buffer stage standard solution*
C_S = concentration of pantoprazole in the *Buffer stage standard solution* (mg/mL)
V = volume of the *Buffer stage medium*, 1000 mL
L = Tablet label claim (mg)

Tolerances: NLT 75% (*Q*) of the labeled amount of pantoprazole is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets *USP Dissolution Test 3*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.
Acid stage
Acid stage medium: 0.1 N hydrochloric acid; 1000 mL
Apparatus 2: 100 rpm
Time: 2 h
Dilute ammonia solution: Transfer 40 mL of strong ammonia solution to a 100-mL volumetric flask, and dilute with water to volume.
Buffer solution: Transfer 1.5 g of ammonium acetate to a 1000-mL volumetric flask. Dissolve in and dilute with

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water to volume. Adjust the pH with *Dilute ammonia solution* to 7.0 ± 0.1.
Mobile phase: Methanol and *Buffer solution* (2:3)
Standard solution: 0.4 mg/mL. Transfer a quantity of USP Pantoprazole Sodium RS to a suitable volumetric flask, add 10% of the final volume of methanol, sonicate, and dilute with *Mobile phase* to volume.
Sample solution: After 2 h in the *Acid stage medium,* decant the medium from the vessel, remove the Tablet from the vessel, and dry it with tissue paper. Transfer the Tablet to a suitable volumetric flask, add 20% of the final volume of methanol, and sonicate for about 20 min. Dilute with *Mobile phase* to volume to obtain a final concentration of about 0.4 mg/mL of pantoprazole. Mix well, centrifuge, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperature
Column: Ambient
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 7500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of pantoprazole released, as a percentage, in the *Acid stage*:

$$\text{Result} = A - [(r_U/r_S) \times C_S \times D_U \times (M_{r1}/M_{r2}) \times (100/L)]$$

A = percentage of pantoprazole as determined in the *Assay*
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of pantoprazole sodium in the *Standard solution* (mg/mL)
D_U = dilution factor of the *Sample solution*
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35
L = Tablet label claim (mg)

Tolerances: NMT 10% of the labeled amount of pantoprazole is dissolved.
Buffer stage
Buffer stage medium: pH 6.8 phosphate buffer; 1000 mL
Apparatus 2: 100 rpm
Time: 45 min
Standard solution: Further dilute an appropriate volume of the *Standard solution* prepared in the *Acid stage* with *Buffer stage medium* to obtain a solution having a known concentration of about 0.04 mg/mL.
Sample solution: Transfer a separate Tablet to the vessel containing *Acid stage medium,* and proceed as directed for the *Acid stage.* After 2 h, decant the *Acid stage medium,* add the *Buffer stage medium,* and operate the apparatus at the specified conditions. After 45 min, withdraw 10 mL of the solution under test, and pass it through a suitable filter of 0.45-µm pore size.
Analysis: Determine the amount of pantoprazole released to the *Buffer stage medium* using the same chromatographic procedure as directed for the *Acid stage,* with the exception of injecting about 50 µL of the *Standard solution* and *Sample solution.*
Calculate the amount of pantoprazole dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (100/L)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of pantoprazole sodium in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35
V = volume of *Medium,* 1000 mL
L = Tablet label claim (mg)

Tolerances: NLT 75% (Q) of the labeled amount of pantoprazole is dissolved.
Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4.* Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.*
Acid stage
Acid stage medium: 0.1 N hydrochloric acid; 1000 mL, degassed
Apparatus 2: 100 rpm, with sinkers
Time: 2 h
Determine the amount of pantoprazole remaining in the Tablet, using the following procedure.
Diluent: Water and acetonitrile (7:3)
Buffer solution: 771 mg/L of ammonium acetate in water. Adjust with acetic acid or ammonium hydroxide to a pH of 8.5 ± 0.1.
Solution A: *Buffer solution* and acetonitrile (7:3)
Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6	100	0
17	27	73
18	100	0
22	100	0

System suitability solution: Prepare a solution containing 0.0068 mg/mL of USP Pantoprazole Related Compound A in *Diluent.* Transfer 10 mL of this solution to a 100-mL volumetric flask, add 23 mg of USP Pantoprazole Sodium RS, and dilute with *Diluent* to volume.
Acid stage standard solution: 0.23 mg/mL of USP Pantoprazole Sodium RS in *Diluent*
Sample solution: After 2 h in the *Acid stage medium,* carefully remove the Tablet from the vessel and transfer to a suitable volumetric flask. Add 50% of the final volume of *Diluent,* and sonicate for 20 min (but not more than 60 min), swirling the flask every few min. Dilute with *Diluent* to volume to obtain a final concentration of about 0.2 mg/mL of pantoprazole.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)

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Mode: LC
Detector: UV 290 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Temperature
Column: 30°
Autosampler: 4°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Acid stage standard solution*
Suitability requirements
Resolution: NLT 1.5 between pantoprazole related compound A and pantoprazole, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Acid stage standard solution*
Calculate the percentage of pantoprazole released:

$$\text{Result} = A - [(r_U/r_S) \times (C_S/L) \times D_U \times (M_{r1}/M_{r2}) \times 100]$$

A = percentage of pantoprazole as determined in the *Assay*
r_U = peak area from the *Sample solution*
r_S = peak area from the *Acid stage standard solution*
C_S = concentration of the *Acid stage standard solution* (mg/mL)
L = Tablet label claim (mg)
D_U = dilution factor of the *Sample solution*
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35

Tolerances: NMT 10% (Q) of the labeled amount of pantoprazole is dissolved.
Buffer stage
Buffer stage medium: pH 6.8 phosphate buffer (76.0 g/L of tribasic sodium phosphate dodecahydrate in water. Add 250 mL of this solution to 750 mL of *Acid stage medium*, adjust with hydrochloric acid or sodium hydroxide to a pH of 6.80 ± 0.05); 1000 mL, degassed.
Apparatus 2: 100 rpm, with sinkers
Time: 45 min
Buffer stage standard solution: 1.6 mg/mL of USP Pantoprazole Sodium RS in methanol. This solution is stable for 5 days at room temperature and 7 days when refrigerated. Dilute this solution with *Buffer stage medium* to obtain a concentration of L/1000 mg/mL, where L is the Tablet label claim in mg.
Sample solution: Transfer a Tablet with the sinker to the vessel containing *Acid stage medium*, and proceed as directed for the *Acid stage*. After 2 h, remove the *Acid stage medium*, add the *Buffer stage medium*, and operate the apparatus under the specified conditions. After 45 min, withdraw 10 mL of the solution under test, and pass it through a suitable filter of 0.45-µm pore size.
Analytical wavelength: UV 289 nm
Path length cell: 1 cm
Blank: *Buffer stage medium*
Calculate the percentage of pantoprazole released:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Buffer stage standard solution*
C_S = concentration of the *Buffer stage standard solution* (mg/mL)
L = Tablet label claim (mg)
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35
V = volume of *Buffer stage medium*, 1000 mL

Tolerances: NLT 75% (Q) of the labeled amount of pantoprazole is dissolved.
▲**Test 5:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.
Acid stage
Acid stage medium: 0.1 N hydrochloric acid; 900 mL, degassed
Apparatus 1: 100 rpm
Time: 2 h
Determine the amount of pantoprazole dissolved in the *Acid stage* using the following procedure.
Diluent: Acetonitrile and 0.01 N sodium hydroxide (50:50)
Buffer solution: 8.77g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 8.0.
Mobile phase: Acetonitrile and *Buffer solution* (35:65)
Acid stage standard solution: 0.22 mg/mL of USP Pantoprazole Sodium RS in *Diluent*
Acid stage sample solution: After 2 h in the *Acid stage medium*, drain and remove the Tablet from the basket. Transfer to a suitable volumetric flask. Add 80% of the final volume of *Diluent* and swirl until the Tablet disintegrates completely. Sonicate for 25 min, shaking the flask every few minutes. Dilute with *Diluent* to volume to obtain a final concentration of about 0.2 mg/mL of pantoprazole. Centrifuge an aliquot, then pass a portion of the supernatant through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm x 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Acid stage standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Acid stage standard solution* and *Acid stage sample solution*
Calculate the labeled amount of pantoprazole released, as percentage, in the *Acid stage*:

$$\text{Result} = A - [(r_U/r_S) \times C_S \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100]$$

A = percentage of pantoprazole as determined in the *Assay*
r_U = peak area from the *Acid stage sample solution*
r_S = peak area from the *Acid stage standard solution*
C_S = concentration of USP Pantoprazole Sodium RS in the *Acid stage standard solution* (mg/mL)
D = dilution factor of the *Acid stage sample solution* (mL)
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35
L = label claim (mg/Tablet)

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Tolerances: NMT 10% of the labeled amount of pantoprazole is dissolved.

Buffer stage

Buffer stage medium: pH 6.8 phosphate buffer (see *Buffer Solutions*); 900 mL, degassed

Apparatus 1: 100 rpm

Time: 45 min

Buffer stage standard solution: Dilute the *Acid stage standard solution* with *Buffer stage medium* to obtain a concentration equivalent to (*L*/900) mg/mL, where *L* is the label claim in milligrams. Immediately mix 5 mL of the resultant solution with 1 mL of 0.1 N sodium hydroxide.

Buffer stage sample solution: After 2 h in the *Acid stage medium*, carefully drain the Tablet in the basket. Remove the *Acid stage medium*, add the *Buffer stage medium* and operate the apparatus under the specified conditions. After 45 min, withdraw 10 mL of the solution under test, and pass it through a suitable filter of 0.45-µm pore size. Mix 5 mL of the test solution with 1 mL of 0.1 N sodium hydroxide.

Buffer, Mobile phase, and Chromatographic system: Proceed as directed in *Acid stage*, except for *Injection volume*.

Injection volume: 20 µL

System suitability

Sample: *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Buffer stage standard solution* and *Buffer stage sample solution*

Calculate the percentage of the labeled amount of pantoprazole dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of the *Buffer stage sample solution*
r_S = peak response of the *Buffer stage standard solution*
C_S = concentration of USP Pantoprazole Sodium RS in the *Buffer stage standard solution* (mg/mL)
V = volume of *Buffer stage medium*, 900 mL
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of pantoprazole is dissolved. ▲ (RB 1-May-2019)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

• **Procedure**

Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Prepare as directed in the *Assay*.

Standard solution: 0.0004 mg/mL. Dilute the *Standard solution*, prepared as directed in the *Assay*, with 0.02 N sodium hydroxide.

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

[Note—Identify the components on the basis of the relative retention times shown in *Impurity Table 1*.]

Resolution: NLT 3 between pantoprazole and pantoprazole related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for pantoprazole, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms for at least three times the retention time of the pantoprazole peak.

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pantoprazole Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pantoprazole in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of pantoprazole, 383.37
M_{r2} = molecular weight of pantoprazole sodium, 405.35

Acceptance criteria: The limits are given in *Impurity Table 1*. The reporting level for impurities is 0.1%.

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pantoprazole	1.0	—
Related compounds D[a] and F[b]	1.2	0.5[c]
Pantoprazole related compound A[d]	1.3	0.5
Pantoprazole related compound B[e]	2.7	0.3
Any other individual impurity	—	0.2
Total impurities	—	1.0

[a] 5-(Difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1*H*-benzimidazole.
[b] 6-(Difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1*H*-benzimidazole.
[c] Impurities D and F are not fully resolved and should be integrated together.
[d] 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfonyl]-1*H*-benzimidazole.
[e] 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]thio]-1*H*-benzimidazole.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.

• **Labeling:** Label Tablets to indicate that they must not be split, chewed, or crushed before administration. When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP Reference Standards** ⟨11⟩

USP Pantoprazole Related Compound A RS
5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfonyl]-1*H*-benzimidazole.
$C_{16}H_{15}F_2N_3O_5S$ 399.37

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USP Pantoprazole Related Compound B RS
5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl) methyl]thio]-1*H*-benzimidazole.
$C_{16}H_{15}F_2N_3O_3S$ 367.37
USP Pantoprazole Sodium RS

Papain

Papain [9001-73-4].

DEFINITION

Papain is a purified proteolytic substance derived from *Carica papaya* Linné (Fam. Caricaceae). Papain, prepared as directed in the *Assay*, contains NLT 6000 Units/mg. Papain of a higher digestive power may be reduced to the official standard by admixture with papain of lower activity, lactose, or other suitable diluents.

One USP Unit of Papain activity is that which releases the equivalent of 1 µg of tyrosine from a specified casein substrate under the conditions of the *Assay*, using the enzyme concentration that liberates 40 µg of tyrosine per mL of *Sample solution*.

ASSAY

• **CASEIN DIGESTIVE POWER**

Dibasic sodium phosphate solution: Dissolve 7.1 g of anhydrous dibasic sodium phosphate in water to make 1 L. Add 1 drop of toluene as a preservative.

Citric acid solution: Dissolve 10.5 g of citric acid monohydrate in water to make 1 L. Add 1 drop of toluene as a preservative.

Casein substrate: Disperse 1g of Hammersten-type casein in 50 mL of *Dibasic sodium phosphate solution*. Place in a boiling water bath for 30 min with occasional stirring. Cool to room temperature, and add *Citric acid solution* to adjust to a pH of 6.0 ± 0.1. Stir the solution rapidly and continuously during the addition of the *Citric acid solution* to prevent precipitation of the casein. Dilute with water to 100 mL. Prepare fresh daily.

Buffer solution: Dissolve 3.55 g of anhydrous dibasic sodium phosphate in 400 mL of water in a 500-mL volumetric flask. Add 7.0 g of disodium edetate and 3.05 g of cysteine hydrochloride monohydrate. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 6.0 ± 0.1, dilute with water to volume, and mix. Prepare fresh daily.

Trichloroacetic acid solution: 300 mg/mL of reagent-grade trichloroacetic acid. This solution may be stored at room temperature.

Standard solution: Weigh accurately 100 mg of USP Papain RS in a 100-mL volumetric flask, and add *Buffer solution* to dissolve. Dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Use within 30 min after preparation.

Sample solution: Weigh accurately an amount of Papain equivalent to about 100 mg of USP Papain RS in a 100-mL volumetric flask, and add *Buffer solution* to dissolve. Dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Use within 30 min after preparation.

Analysis: Into each of 12 test tubes (18 × 150 mm) pipet 5.0 mL of *Casein substrate*. Place in a water bath at 40°, and allow 10 min to reach bath temperature. The tests are run in duplicate except for the blanks. Into each of two of the tubes labeled S_1 pipet 1.0 mL of the *Standard solution* and 1.0 mL of *Buffer solution*. Mix by swirling, note zero time, insert the stopper, and replace in the bath. Into each of two tubes labeled S_2 pipet 1.5 mL of the *Standard solution* and 0.5 mL of *Buffer solution*, and proceed as before. Repeat this procedure for two more tubes labeled S_3 to which 2.0 mL of the *Standard solution* is added, and for two tubes labeled U_2 to which 1.5 mL of the *Sample solution* and 0.5 mL of *Buffer solution* are added. After 60 min, accurately timed, add to all 12 tubes 3.0 mL of *Trichloroacetic acid solution*, and shake vigorously. With the four tubes to which neither the *Standard solution* nor the *Sample solution* was added, prepare blanks by pipeting, respectively, 1.0 mL of the *Standard solution* and 1.0 mL of *Buffer solution*; 1.5 mL of the *Standard solution* and 0.5 mL of *Buffer solution*; 2.0 mL of the *Standard solution*; and 1.5 mL of the *Sample solution* and 0.5 mL of *Buffer solution*. Replace all tubes in the 40° water bath for 30–40 min to allow the precipitated protein to coagulate fully. Pass through filter paper of medium pore size, discarding the first 3 mL of the filtrate (filtrates used are clear). Read the absorbances, at 280 nm, of the filtrates of all solutions against their respective blanks. Plot the absorbance readings for S_1, S_2, and S_3 against the enzyme concentration of each corresponding level.

By interpolation from this curve, taking into consideration dilution factors, calculate the potency in Units in the weight of Papain taken:

$$\text{Result} = A \times C \times F$$

A = activity of the USP Papain RS (Units/mg)
C = concentration from the standard curve (mg/mL)
F = 100 × (50/2) × (10/1.5), dilution factor

Acceptance criteria: NLT 6000 Units/mg

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 1 in 50
Acceptance criteria: 4.8–6.2

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 60° for 4 h.
Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool place.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Papain RS

Papain Tablets for Topical Solution

DEFINITION

Papain Tablets for Topical Solution contain NLT 100.0% of the labeled potency.

ASSAY

• **PROCEDURE**

Dibasic sodium phosphate solution: Dissolve 7.1 g of anhydrous dibasic sodium phosphate in water to make 1 L. Add 1 drop of toluene as a preservative.

Citric acid solution: Dissolve 10.5 g of citric acid monohydrate in water to make 1 L. Add 1 drop of toluene as a preservative.

Casein substrate: Disperse 1 g of Hammersten-type casein in 50 mL of *Dibasic sodium phosphate solution*. Place in a boiling water bath for 30 min with occasional stirring. Cool to room temperature, and add *Citric acid solution* to adjust to a pH of 6.0 ± 0.1. Stir the solution rapidly and

USP Monographs

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continuously during the addition of the *Citric acid solution* to prevent precipitation of the casein. Dilute with water to 100 mL. Prepare fresh daily.

Buffer solution: Dissolve 3.55 g of anhydrous dibasic sodium phosphate in 400 mL of water in a 500-mL volumetric flask. Add 7.0 g of disodium edetate and 3.05 g of cysteine hydrochloride monohydrate. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 6.0 ± 0.1, dilute with water to volume, and mix. Prepare fresh daily.

Trichloroacetic acid solution: 300 mg/mL of reagent-grade trichloroacetic acid. This solution may be stored at room temperature.

Standard solution: Weigh accurately 100 mg of USP Papain RS in a 100-mL volumetric flask, and add *Buffer solution* to dissolve. Dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Use within 30 min after preparation.

Sample solution: Place a counted number of Papain Tablets for Topical Solution, equivalent to about 600,000 USP Units of Papain, in a 100-mL volumetric flask, dissolve in *Buffer solution*, dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix.

Analysis: Into each of 12 test tubes (18 × 150 mm) pipet 5.0 mL of *Casein substrate*. Place in a water bath at 40°, and allow 10 min to reach bath temperature. The tests are run in duplicate except for the blanks. Into each of two of the tubes labeled S_1 pipet 1.0 mL of the *Standard solution* and 1.0 mL of *Buffer solution*. Mix by swirling, note zero time, insert the stopper, and replace in the bath. Into each of two other tubes labeled S_2 pipet 1.5 mL of the *Standard solution* and 0.5 mL of *Buffer solution*, and proceed as before. Repeat this procedure for two tubes labeled S_3 to which 2.0 mL of the *Standard solution* is added, and for two tubes labeled U_2 to which 1.5 mL of the *Sample solution* and 0.5 mL of *Buffer solution* are added. After 60 min, accurately timed, add to all 12 tubes 3.0 mL of *Trichloroacetic acid solution*, and shake vigorously. With the four tubes to which neither the *Standard solution* nor the *Sample solution* was added, prepare blanks by pipeting, respectively, 1.0 mL of the *Standard solution* and 1.0 mL of *Buffer solution*; 1.5 mL of the *Standard solution* and 0.5 mL of *Buffer solution*; 2.0 mL of the *Standard solution*; and 1.5 mL of the *Sample solution* and 0.5 mL of *Buffer solution*. Replace all tubes in the 40° water bath for 30–40 min to allow the precipitated protein to coagulate fully. Pass through filter paper of medium pore size, discarding the first 3 mL of the filtrate (filtrates used are clear). Read the absorbances, at 280 nm, of the filtrates of all solutions against their respective blanks. Plot the absorbance readings for S_1, S_2, and S_3 against the enzyme concentration of each corresponding level. By interpolation from the standard curve, calculate the potency, in USP Units, of the number of Papain Tablets for Topical Solution taken:

$$\text{Result} = A \times C \times F$$

A = activity of the USP Papain RS (Units/mg)
C = concentration from the standard curve (mg/mL)
F = 100 × (50/2) × (10/1.5), dilution factor

Acceptance criteria: NLT 100.0% of the labeled potency

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩**:** NMT 15 min at 23 ± 2°

SPECIFIC TESTS

• **COMPLETENESS OF SOLUTION** ⟨641⟩

Sample solution: Prepare a solution of 50 Papain Tablets for Topical Solution in 500.0 mL of water, and allow to stand for 4 h.

Analysis: Pass the *Sample solution* through two superimposed, matched-weight, 47-mm-diameter membrane filters of 0.8-µm pore size, and wash the residue by rinsing the flask at the sides of the holder with water. Dry both filters in a desiccator under vacuum, over phosphorus pentoxide for 6–18 h. Weigh the filters separately, and subtract the weight of the lower filter from that of the upper filter.

Acceptance criteria: NMT 50 mg (1 mg/Tablet) difference in weights

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ **AND TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

• **pH** ⟨791⟩

Sample solution: 1 Tablet in 10 mL of water

Acceptance criteria: 6.9–8.0

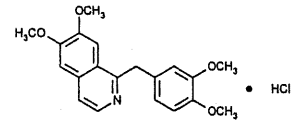
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool place.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Papain RS

Papaverine Hydrochloride



$C_{20}H_{21}NO_4 \cdot HCl$ 375.85

Isoquinoline, 1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-, hydrochloride.

6,7-Dimethoxy-1-veratrylisoquinoline hydrochloride [61-25-6].

» Papaverine Hydrochloride contains not less than 98.5 percent and not more than 100.5 percent of $C_{20}H_{21}NO_4 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩**—**

USP Papaverine Hydrochloride RS

Completeness of solution—A 1 in 15 solution in chloroform is clear and free from undissolved solid.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

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Solution: 2.5 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 251 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: A solution (1 in 50) responds to the tests for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 3.0 and 4.5, in a solution (1 in 50).
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Limit of cryptopine, thebaine, or other organic impurities—Dissolve 50 mg in 2 mL of sulfuric acid in a small test tube: the resulting solution is not more yellow-brown in color than *Matching Fluid S* (see *Readily Carbonizable Substances Test* ⟨271⟩), and it is not more pink than a standard prepared, in equal volume, by diluting 3.0 mL of 0.1 N potassium permanganate with water to 1000 mL.
Assay—Dissolve about 700 mg of Papaverine Hydrochloride, accurately weighed, in 80 mL of glacial acetic acid; add 10 mL of mercuric acetate TS and 1 drop of crystal violet TS; and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.59 mg of $C_{20}H_{21}NO_4 \cdot HCl$.

Papaverine Hydrochloride Injection

» Papaverine Hydrochloride Injection is a sterile solution of Papaverine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Papaverine Hydrochloride RS

Identification—
A: Add 2 mL of alcohol to 1 mL of Injection, and evaporate on a steam bath, with the aid of a stream of nitrogen, to dryness. Dry the residue at 105° for 2 hours: it responds to *Identification* test *A* under *Papaverine Hydrochloride.*
B: It responds to *Identification* test *C* under *Papaverine Hydrochloride.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 2.9 USP Endotoxin Units per mg of papaverine hydrochloride.
pH ⟨791⟩: not less than 3.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer 1.0 mL of Injection to a 200-mL volumetric flask, and dilute with water to volume. Pipet 3 mL of this solution into a separator, add 10 mL of water, and render alkaline with 6 N ammonium hydroxide. Extract the alkaloid with successive 5-mL portions of chloroform, and evaporate the extracts to dryness. Dissolve the residue in 0.1 N hydrochloric acid, and dilute with the same medium to 100.0 mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Papaverine Hydrochloride RS in 0.1 N hydrochloric acid having a known concentration of about 4.5 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 251 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{20}H_{21}NO_4 \cdot HCl$ in the portion of Injection taken by the formula:

$$6.67C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Papaverine Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Papaverine Hydrochloride Tablets

» Papaverine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Papaverine Hydrochloride RS
Identification—Add a portion of powdered Tablets, equivalent to about 30 mg of papaverine hydrochloride, to 10 mL of 0.1 N hydrochloric acid in a separator. Extract the mixture with 10 mL of chloroform, filter the chloroform phase through paper, evaporate the solvent on a steam bath, and dry the residue at 105° for 2 hours: it responds to *Identification* test *A* under *Papaverine Hydrochloride.*

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{20}H_{21}NO_4 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 250 nm on filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Papaverine Hydrochloride RS in the same *Medium.*
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Transfer 1 finely powdered Tablet to a 250-mL volumetric flask, add 50 mL of water and 3 mL of hydrochloric acid, mix, and allow to stand for 15 minutes with occasional agitation. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with water to provide a solution containing approximately 2.4 µg of papaverine hydrochloride per mL. Concomitantly determine the absorbances of this solution and a solution of USP Papaverine Hydrochloride RS, in the same medium at a concentration of about 2.4 µg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 250 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{20}H_{21}NO_4 \cdot HCl$ in the Tablet by the formula:

$$(TC/D)(A_U/A_S)$$

in which T is the labeled quantity, in mg, of papaverine hydrochloride in the Tablet; C is the concentration, in µg per mL, of USP Papaverine Hydrochloride RS in the Standard solution; D is the concentration, in µg per mL, of the solution from the Tablet based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

USP Monographs

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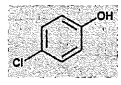
Assay—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 30 mg of papaverine hydrochloride, to a glass-stoppered conical flask, add about 100 mL of 0.1 N hydrochloric acid, and shake by mechanical means for 15 minutes. Filter the mixture into a 200-mL volumetric flask, and add 0.1 N hydrochloric acid to volume. Proceed as directed in the *Assay* under *Papaverine Hydrochloride Injection*, beginning with "Pipet 3 mL of this solution into a separator." Calculate the quantity, in mg, of $C_{20}H_{21}NO_4 \cdot HCl$ in the portion of Tablets taken by the formula:

$$6.67C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Papaverine Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

Delete the following:

▲Parachlorophenol



C_6H_5ClO 128.56
Phenol, 4-chloro-;
p-Chlorophenol [106-48-9].

DEFINITION
Parachlorophenol contains NLT 99.0% and NMT 100.5% of parachlorophenol (C_6H_5ClO).

IDENTIFICATION
- **A.**
  Sample solution: 10 mg/mL of Parachlorophenol
  Analysis: Add bromine TS dropwise to the *Sample solution*.
  Acceptance criteria: A white precipitate is formed; at first it redissolves, but then it becomes permanent as an excess of the reagent is added.
- **B.**
  Sample solution: 10 mg/mL of Parachlorophenol
  Analysis: Add 1 drop of ferric chloride TS to 10 mL of *Sample solution*.
  Acceptance criteria: The solution acquires a violet-blue color.
- **C.**
  Analysis: Heat a few crystals, held on a copper wire, in the edge of a nonluminous flame.
  Acceptance criteria: A green color is imparted to the flame.
- **D.**
  Sample: 1 g of Parachlorophenol
  Analysis: Mix the *Sample* and 5 mL of sodium hydroxide solution (1 in 3), then add 1.5 g of monochloroacetic acid. Shake, and heat on a steam bath for 1 h. Cool, dilute with 15 mL of water, and acidify with hydrochloric acid. Extract with 50 mL of ether, wash the ether solution with 10 mL of cold water, then extract the ether solution with 25 mL of sodium carbonate solution (1 in 20). Acidify the solution with hydrochloric acid, collect the resulting precipitate on a filter, and recrystallize it from hot water.
  Acceptance criteria: The resulting parachlorophenoxyacetic acid melts between 154° and 158°.

ASSAY
- **PROCEDURE**
  Sample: 1 g of Parachlorophenol
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Residual titration
  Titrant: 0.1 N bromine VS
  Back-titrant: 0.1 N sodium thiosulfate VS
  Endpoint detection: Visual
  Analysis: Transfer the *Sample* to a 500-mL volumetric flask, and dissolve and dilute with water to volume. Transfer a 25.0-mL portion of the solution to an iodine flask, cool in an ice bath to 4°, and add 20.0 mL of *Titrant*. Add 5 mL of hydrochloric acid, and immediately insert the stopper. Maintain the flask at a temperature of 4° for 30 min, shaking at frequent intervals. Allow it to stand for 15 min, remove the stopper just sufficiently to introduce quickly 5 mL of potassium iodide solution (1 in 5), taking care that no bromine vapor escapes, and at once insert the stopper in the flask. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask with a small portion of water, allowing the washings to flow into the flask. Shake the mixture, and titrate the liberated iodine with *Back-titrant*, using 3 mL of starch TS as the indicator. Perform a blank determination. Each mL of 0.1 N bromine is equivalent to 3.214 mg of parachlorophenol (C_6H_5ClO).
  Acceptance criteria: 99.0%–100.5%

IMPURITIES
- **LIMIT OF NONVOLATILE RESIDUE**
  Sample: 1 g of Parachlorophenol
  Analysis: Heat the *Sample* in a tared container on a steam bath until it is volatilized, and dry at 105° for 1 h.
  Acceptance criteria: NMT 0.1% of residue remains.
- **LIMIT OF CHLORIDE**
  Sample solution: 10 mg/mL of Parachlorophenol
  Analysis: Acidify 10 mL of *Sample solution* with 2 N nitric acid, and add a few drops of silver nitrate TS.
  Acceptance criteria: No turbidity or opalescence is produced.

SPECIFIC TESTS
- **CLARITY AND REACTION OF SOLUTION**
  Sample solution: 10 mg/mL of Parachlorophenol
  Acceptance criteria: Solution is clear and is acid to litmus.
- **CONGEALING TEMPERATURE** ⟨651⟩**:** Between 42° and 44°

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲ (USP 1-May-2020)

Camphorated Parachlorophenol

DEFINITION
Camphorated Parachlorophenol is a triturated mixture that contains NLT 33.0% and NMT 37.0% of parachlorophenol (C_6H_5ClO) and NLT 63.0% and NMT 67.0% of camphor ($C_{10}H_{16}O$). The sum of the percentages of parachlorophenol and camphor is NLT 97.0% and NMT 103.0%.

ASSAY
- **PARACHLOROPHENOL**
  Sample: 1 g
  Analysis: Add the *Sample* to a wide-mouth conical flask. Add a few glass beads, 6 mL of sodium hydroxide solution (1 in 2), and 130 mL of water. Heat the solution to boiling,

USP Monographs

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add 70 mL of potassium permanganate solution (3 in 50), and continue to boil for 20 min. To the hot solution add 40 mL of 0.1 N silver nitrate. Add 50 mL of 18 N sulfuric acid, and sodium sulfite crystals, in divided portions and with swirling until the permanganate color is discharged and no manganese dioxide remains. Boil until the vapors are no longer acid to litmus, keeping the volume nearly constant by the addition of water. Add 5 mL of nitric acid, and continue to boil for 5 min. Cool, and collect the precipitate on a tared filtering crucible, wash well with water, then with 10 mL of alcohol. Dry at 105° for 1 h, cool, and weigh. Each 1.000 g of the silver chloride so obtained is equivalent to 897.0 mg of parachlorophenol (C_6H_5ClO).

Acceptance criteria

Parachlorophenol: 33.0%–37.0% of parachlorophenol (C_6H_5ClO)

Total: The sum of the percentages of parachlorophenol and camphor is 97.0%–103.0%.

• **CAMPHOR**

Sample solution: Transfer about 300 mg of Camphorated Parachlorophenol to a 200-mL pressure bottle containing 50 mL of freshly prepared dinitrophenylhydrazine TS.

Analysis: Close the pressure bottle, immerse it in a water bath, and maintain it at about 75° for 4 h. Cool to room temperature, then transfer the contents to a beaker with the aid of 100 mL of 3 N sulfuric acid and allow it to stand overnight. Collect the precipitate on a tared filtering crucible, wash with 100 mL of 3 N sulfuric acid and then with 75 mL of cold water, in divided portions, to remove the acid. Dry at 80° for 2 h, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.4581, represents the weight of camphor ($C_{10}H_{16}O$) in the sample taken.

Acceptance criteria

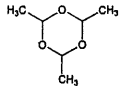
Camphor: 63.0%–67.0% of camphor ($C_{10}H_{16}O$)

Total: The sum of the percentages of parachlorophenol and camphor is 97.0%–103.0%.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Paraldehyde



$C_6H_{12}O_3$ 132.16

1,3,5-Trioxane, 2,4,6-trimethyl-;
2,4,6-Trimethyl-*s*-trioxane [123-63-7].

DEFINITION

Paraldehyde contains NLT 98.0% and NMT 102.0% of total paraldehydes, expressed as the sum of the percentages of the β and α geometric isomers of paraldehyde ($C_6H_{12}O_3$).

[NOTE—Paraldehyde is subject to oxidation to form acetic acid. It may contain a suitable stabilizer.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

• **B.** The retention times of the β-paraldehyde and α-paraldehyde geometric isomer peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Acetonitrile

System suitability solution: 20.0 mg/mL of USP Paraldehyde RS in *Diluent*

Standard solution: 4.0 mg/mL of USP Paraldehyde RS in *Diluent*

Sample solution: 4.0 mg/mL of Paraldehyde in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30.0-m; 0.25-µm coating of phase G25

Temperatures

Injection port: 150°

Detector: 250°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	8
40	10	200	—
200	—	200	6

Carrier gas: Helium

Flow rate: 1 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 1:50

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for β-paraldehyde and α-paraldehyde are 1.0 and 1.07, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the β-paraldehyde and α-paraldehyde isomers, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of total paraldehydes in the portion of Paraldehyde taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of the β-paraldehyde and α-paraldehyde isomers from the *Sample solution*

r_S = sum of the peak responses of the β-paraldehyde and α-paraldehyde isomers from the *Standard solution*

C_S = concentration of USP Paraldehyde RS in the *Standard solution* (mg/mL)

C_U = concentration of Paraldehyde in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **LIMIT OF NONVOLATILE RESIDUE**

Sample: 5.0 mL

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Analysis: Heat the *Sample* in a small, tared evaporating dish on a steam bath.
Acceptance criteria: No disagreeable odor is noticeable as the last portions evaporate, and when dried at 105° for 1 h, NMT 3 mg of residue remains (0.06%).

• **ORGANIC IMPURITIES**
Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.02 mg/mL of USP Paraldehyde RS and 0.08 mg/mL of USP Acetaldehyde RS in *Diluent*
Sample solution: 20.0 mg/mL of Paraldehyde in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between the β-paraldehyde and α-paraldehyde isomers, *System suitability solution*
Relative standard deviation: NMT 3.0% for both paraldehyde and acetaldehyde, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetaldehyde in the portion of Paraldehyde taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaldehyde from the *Sample solution*
r_S = peak response of acetaldehyde from the *Standard solution*
C_S = concentration of the USP Acetaldehyde RS in the *Standard solution* (mg/mL)
C_U = concentration of Paraldehyde in the *Sample solution* (mg/mL)

Calculate the percentage of any individual, unspecified impurity in the portion of Paraldehyde taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual, unspecified impurity from the *Sample solution*
r_S = sum of the peak responses of β-paraldehyde and α-paraldehyde isomers from the *Standard solution*
C_S = concentration of USP Paraldehyde RS in the *Standard solution* (mg/mL)
C_U = concentration of Paraldehyde in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetaldehyde	0.22	0.4
β-Paraldehyde	1.0	—
α-Paraldehyde	1.07	—
Any individual, unspecified impurity	—	0.10

SPECIFIC TESTS
• **DISTILLING RANGE** ⟨721⟩, *Method I*: It distills completely at a range of 120°–126°, a correction factor of 0.050°/mm being applied as necessary.
• **ACIDITY**
Sample solution: 6.0 mL in 100 mL of water
Analysis: To the *Sample solution*, add 5 drops of phenolphthalein TS, and titrate with 1.0 N sodium hydroxide.
Acceptance criteria: NMT 0.50 mL is required to produce a pink color (0.5% as acetic acid).

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-filled, tight, light-resistant containers, preferably of Type I or Type II glass, holding NMT 30 mL, at a temperature of NMT 25°. Paraldehyde may be shipped in bulk containers holding a minimum of 22.5 kg (50 lb) to commercial drug repackagers only.
• **LABELING:** The label of all containers of Paraldehyde, including those dispensed by the pharmacist, includes a statement directing the user to discard the unused contents of any container that has been opened for more than 24 h.
[NOTE—The label of bulk containers of Paraldehyde directs the commercial drug repackager to demonstrate compliance with the USP purity tests for Paraldehyde immediately before repackaging, and not to repackage from a container that has been opened longer than 24 h.]
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaldehyde RS
Acetaldehyde.
C_2H_4O 44.05
USP Paraldehyde RS

Paregoric

DEFINITION
Paregoric contains NLT 35 mg and NMT 45 mg of anhydrous morphine in each 100 mL.
Prepare Paregoric as follows.

Powdered Opium	4.3 g
Suitable Essential Oil(s)	—
Benzoic Acid	3.8 g
Diluted Alcohol	900 mL
Glycerin	38 mL
To make	950 mL

Prepare a diluted alcohol mixture containing 400 mg of *Benzoic Acid*, 4 mL of *Glycerin*, and sufficient *Suitable Essential Oil(s)* in each 100 mL of *Diluted Alcohol*. Separately, macerate the *Powdered Opium*, *Benzoic Acid*, and *Suitable Essential Oil(s)* for 5 days, with occasional agitation, in a mixture of *Diluted Alcohol* and *Glycerin*. Then filter, and pass enough *Diluted Alcohol* through the filter to obtain 950 mL of total filtrate. Assay a portion of this filtrate as directed herein, and dilute the remainder with a sufficient quantity of the previously prepared diluted alcohol mixture to yield a solution containing 40 mg of anhydrous morphine in each 100 mL.
Paregoric may also be prepared by using Opium or Opium Tincture instead of *Powdered Opium*, the anhydrous morphine content being adjusted to 40 mg in each 100 mL and the alcohol content being adjusted to 45%.

ASSAY
• **PROCEDURE**
Mobile phase A: 1-in-5 solution of triethylamine in water-saturated chloroform

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Mobile phase B: 1-in-100 solution of triethylamine in water-saturated chloroform

Chromatographic tubes: Prepare three similar tubes, each about 260 mm long and consisting of about 200 mm of 25-mm tubing and about 6 cm of 6-mm tubing. In each of the tubes, place a pledget of glass wool at a point where the 6-mm tubing is constricted slightly, about 2 cm from the junction.

Citrate buffer: 0.1 M sodium citrate and 0.1 M citric acid (50:50)

Standard solution: Dissolve a quantity of USP Morphine Sulfate RS equivalent to about 40 mg of anhydrous morphine in 0.5 mL of triethylamine contained in a 100-mL volumetric flask, and add methanol to volume. Pipet 10 mL of this solution into a 50-mL volumetric flask, add 1 mL of triethylamine and 1 mL of hydrochloric acid, and add water-saturated chloroform to volume.

Sample solution: Evaporate 10.0 mL of Paregoric (equivalent to about 4 mg of morphine) on a steam bath under a stream of air to about 2 mL, and cool. [NOTE—Avoid reducing the volume to less than 2 mL.] Add 0.5 mL of *Citrate buffer*.

Chromatographic columns: Fill the three tubes with adsorbent prepared as follows, using chromatographic siliceous earth as the base of the adsorbent, and tamp it firmly in place. After filling, place a small pad of glass wool above each column packing.

Column I: Pack in two layers, the lower layer consisting of 3 g of chromatographic siliceous earth mixed with 2 mL of *Citrate buffer* and the upper layer of 3 g of chromatographic siliceous earth mixed with the *Sample solution*. Dry-rinse the beaker in which the components of the two layers have been mixed with 1 g of chromatographic siliceous earth, and add it also to the top of the column.

Column II: Pack with 3 g of chromatographic siliceous earth mixed with 2 mL of dibasic potassium phosphate solution (1 in 5.75).

Column III: Pack with 3 g of chromatographic siliceous earth mixed with 2 mL of sodium hydroxide solution (1 in 50).

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 255–360 nm

Cell: 1 cm

Blank: Chloroform

Analysis

[NOTE—(1) Use water-saturated solvents throughout this procedure; (2) prepare eluants fresh daily; and (3) avoid bringing the solutions into contact with metal.]

Wash *Column I* with 100 mL of ether, followed by 100 mL of chloroform. Rinse the tip of the column with chloroform, and discard the solvents. In the following operations, rinse each column tip before discarding the column or changing receivers.

Mount the three columns vertically so that the effluent from *Column I* flows into *Column II*, and the effluent from the latter flows into *Column III*. Pass through the three columns 5 mL of *Mobile phase A*, followed by four 10-mL portions of *Mobile phase B*, allowing each portion to pass through completely before subsequent additions. Discard *Column I*.

Pass three 5-mL portions of *Mobile phase B* through the two remaining columns. Discard *Column II*.

Wash *Column III* successively with 10 mL of *Mobile phase B*, 50 mL of chloroform, 2 mL of a 1-in-10 solution of glacial acetic acid in chloroform, and 50 mL of a 1-in-100 solution of glacial acetic acid in chloroform. Discard all washings. Arrange to collect eluate from *Column III* in a 50-mL volumetric flask containing 10 mL of methanol and 1 mL of hydrochloric acid. Elute the column with 5 mL of *Mobile phase A*, followed by 33 mL of *Mobile phase B*. Dilute with chloroform to volume, and mix.

Concomitantly record the spectra of this solution and the *Standard solution*, and plot the corresponding wavelength-absorbance curves. Correct the absorbance of each solution, at the wavelength of maximum absorbance at about 285 nm, by extrapolating the portion of the base-line curve between 340 and 310 nm to this wavelength.

Calculate the weight of anhydrous morphine, in mg, in 100 mL of Paregoric taken:

$$\text{Result} = (A_U/A_S) \times W \times F$$

A_U = corrected absorbance of the *Sample solution*
A_S = corrected absorbance of the *Standard solution*
W = weight of anhydrous morphine in the 50 mL of *Standard solution* (mg)
F = dilution factor, 10

Acceptance criteria: 35–45 mg of anhydrous morphine per 100 mL of Paregoric

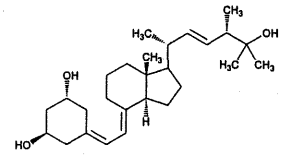
OTHER COMPONENTS

- **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩: 43.0%–47.0% of C_2H_5OH, using acetone as the internal standard

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Morphine Sulfate RS

Paricalcitol



$C_{27}H_{44}O_3$ 416.64

19-Nor-1-α,25-dihydroxyvitamin D_2;
(1α,3β,7*E*,22*E*)-19-Nor-9,10-secoergosta-5,7,22-triene-1,3,25-triol;
(7*E*,22*E*)-19-Nor-9,10-secoergosta-5,7,22-triene-1α,3β,25-triol [131918-61-1].

DEFINITION

Paricalcitol contains NLT 97.0% and NMT 103.0% of paricalcitol ($C_{27}H_{44}O_3$), calculated on the dried basis.

[**CAUTION**—Handle Paricalcitol with exceptional care because it is very potent. Care should be taken to prevent inhaling particles of Paricalcitol and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

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• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Protect paricalcitol solutions from light.]

Mobile phase: Methanol and water (4:1)

Diluent: Methanol and water (1:1)

Standard solution: Dilute USP Paricalcitol Solution RS with *Diluent* to obtain a solution containing 5.0 µg/mL of paricalcitol.

Sample solution: Transfer an accurately weighed amount of Paricalcitol to a suitable volumetric flask, add dehydrated alcohol (approximately 1 mL of dehydrated alcohol per each 0.5 mg of paricalcitol), sonicate to dissolve, and dilute with *Diluent* to volume. Further dilute this solution with *Diluent* to obtain a solution containing 5.0 µg/mL of paricalcitol.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of paricalcitol ($C_{27}H_{44}O_3$) in the portion of Paricalcitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of paricalcitol in the *Standard solution* (µg/mL)

C_U = concentration of Paricalcitol in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**

[NOTE—Unless otherwise specified, protect paricalcitol solutions from light.]

Diluent: Dehydrated alcohol and water (50:50)

Solution A: Acetonitrile and water (5:95)

Solution B: Acetonitrile and methanol (75:25)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	42	58
11[a]	42	58
20	0	100
27	0	100
27.1	42	58
30	42	58

[a] Determine the retention time of the paricalcitol peak using the *Standard solution*. Adjust the start of the gradient to be 1.0 ± 0.1 min prior to the retention time of paricalcitol and accordingly adjust the remaining gradient times.

System suitability stock solution: Prepare a 50-µg/mL solution of paricalcitol from USP Paricalcitol Solution RS in dehydrated alcohol. Using a colorless glass container, expose the solution to ultraviolet light at 254 nm. Paricalcitol undergoes partial degradation to 7*Z*-paricalcitol. A degradation of at least 0.2% of paricalcitol to 7*Z*-paricalcitol [(7*Z*,22*E*)-19-nor-9,10-secoergosta-5,7,22-triene-1α,3β,25-triol] must be obtained, based on the corresponding peaks. If it is not obtained, expose the solution to ultraviolet light again.

System suitability solution: *System suitability stock solution* and water (1:1)

Standard stock solution: 5 µg/mL of paricalcitol from USP Paricalcitol Solution RS in *Diluent*

Standard solution: 0.15 µg/mL of paricalcitol from *Standard stock solution* in *Diluent*

Sensitivity solution: 0.05 µg/mL of paricalcitol from *Standard solution* in *Diluent*

Sample stock solution: 200 µg/mL of Paricalcitol in dehydrated alcohol

Sample solution: 100 µg/mL of Paricalcitol from *Sample stock solution* in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 10-cm; 2.7-µm packing L1

Column temperature: 30°

Flow rate: 0.9 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—The relative retention times for paricalcitol and 7*Z*-paricalcitol are 1.0 and 1.06, respectively.]

Suitability requirements

Resolution: NLT 1.5 between paricalcitol and 7*Z*-paricalcitol, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Paricalcitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of paricalcitol from the *Standard solution*

C_S = concentration of paricalcitol in the *Standard solution* (µg/mL)

C_U = concentration of Paricalcitol in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

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Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Paricalcitol	1.0	—	—
22*Z*-Paricalcitol[a]	1.23	0.64	0.15
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] (7*E*,22*Z*)-19-Nor-9,10-secoergosta-5,7,22-triene-1α,3β,25-triol.

SPECIFIC TESTS

• **Loss on Drying**

(See *Thermal Analysis* ⟨891⟩.)

Sample: 8 mg of Paricalcitol

Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument. Heat at a rate of 5°/min between ambient temperature and 150° in an atmosphere of nitrogen at a flow rate of 40 mL/min. Determine the accumulated loss in weight from the thermogram.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store under argon in a freezer.

• **USP Reference Standards** ⟨11⟩

USP Paricalcitol RS

USP Paricalcitol Solution RS

Paricalcitol Capsules

DEFINITION

Paricalcitol Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of paricalcitol ($C_{27}H_{44}O_3$). They may contain butylated hydroxytoluene or other suitable antioxidant.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

[Note—Protect paricalcitol solutions from light.]

Solution A: Acetonitrile, isopropyl alcohol, and water (50:15:35)

Solution B: Acetonitrile and isopropyl alcohol (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	100	0
21	0	100
29	0	100
30	100	0
35	100	0

Internal standard solution: 0.75 µg/mL of triphenylene in acetonitrile

Standard stock solution: 10 µg/mL of paricalcitol from USP Paricalcitol Solution RS in acetonitrile

Standard solution: A mixture of 0.5 µg/mL of paricalcitol from *Standard stock solution* and 0.15 µg/mL of triphenylene from *Internal standard solution* in acetonitrile

Sample solution: Nominally 0.5 µg/mL of paricalcitol from Capsules and 0.15 µg/mL of triphenylene from *Internal standard solution*, prepared as follows. Transfer 10 Capsules to a suitable container. Transfer the volume of acetonitrile specified in *Table 2* to a separate beaker. Cut each Capsule to allow the oil to flow freely from the shell. Rinse any tools used to open the shells by pouring in portions of acetonitrile from the beaker into the sample container. When the rinsing is complete, add the remainder of the acetonitrile to the sample container, add the volume of *Internal standard solution* specified in *Table 2*, and mix well. [Note—To facilitate cutting the Capsules open, soften them in a suitable microwave oven for NMT 10 s; taking care not to melt the Capsules. A microwave oven of 600–825 watts was found to be suitable for this procedure.]

Table 2

Capsule Strength (µg/Capsule)	Volume of Acetonitrile (mL)	Volume of Internal Standard Solution (mL)	Internal Standard Correction Factor (*F*)
1	16.0	4.0	1
2	32.0	8.0	1
4	65.0	15.0	0.9375

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2.0 mL/min

Injection volume: 50 µL

Run time: 20 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for paricalcitol and triphenylene are 1.0 and 2.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between paricalcitol and triphenylene

Tailing factor: NMT 2.0 for the paricalcitol peak

Relative standard deviation: NMT 2.0% for the peak response ratios of paricalcitol to triphenylene

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of paricalcitol ($C_{27}H_{44}O_3$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

R_U = peak response ratio of paricalcitol to triphenylene from the *Sample solution*

R_S = peak response ratio of paricalcitol to triphenylene from the *Standard solution*

C_S = concentration of paricalcitol from USP Paricalcitol Solution RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of paricalcitol in the *Sample solution* (µg/mL)

F = internal standard correction factor (see *Table 2*)

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Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Rupture Test**

Apparatus: Disintegration apparatus with mesh wire, as described in *Disintegration* ⟨701⟩

Medium: Water

Procedure: Preheat disintegration apparatus water bath to 37 ± 2°. Transfer an appropriate volume of water such that at the highest point of the upward stroke the wire mesh of the basket-rack assembly remains at least 15 mm below the surface of the fluid and descends to NLT 25 mm from the bottom of the vessel on the downward stroke. Place the beakers in the water bath and allow the contents to warm to 37 ± 2°; the fluid must be maintained at this temperature for the duration of the test. Place one Capsule in each of the six tubes of the basket-rack assembly, adding a mesh wire screen to each tube, then start the action of the lifting apparatus and timing simultaneously. Observe the units at 5-min intervals.

Tolerances: The requirements are met if all of the Capsules tested rupture in NMT 15 min.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

[Note—Unless otherwise specified, protect paricalcitol solutions from light.]

Solution A: Water

Solution B: Acetonitrile

Solution C: Acetonitrile and isopropyl alcohol (50:50)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	85	15	0
55	5	95	0
56	0	0	100
60	0	0	100
61	85	15	0
70	85	15	0

Diluent: Acetonitrile and water (50:50)

Degradation stock solution: Dilute 1 mL of USP Paricalcitol Solution RS with *Diluent* to 5 mL.

Degradation solution A: Transfer 1 mL of the *Degradation stock solution* and 0.1 mL of 30% hydrogen peroxide to a suitable container, and allow to stand at room temperature for 1 h. Dilute with *Diluent* to 10 mL, and mix. This solution contains paricalcitol and related compounds A and B.

Degradation solution B: Mix 1 mL of the *Degradation stock solution* and 1 mL of 0.1 N hydrochloric acid, and heat at 70° for 1 h. Cool to room temperature, dilute with *Diluent* to 10 mL, and mix. This solution contains paricalcitol and related compounds C, D, G, H, and I.

Internal standard solution: 0.08 µg/mL of calcitriol from USP Calcitriol Solution RS in acetonitrile

Standard solution: 0.01 µg/mL of paricalcitol from USP Paricalcitol Solution RS and 0.016 µg/mL of calcitriol from *Internal standard solution* in *Diluent*

Sample solution: Nominally 1.8 µg/mL of paricalcitol from Capsules, prepared as follows. Remove the contents of a suitable number of Capsules using a syringe or cut the Capsules open with scissors and transfer the contents to a suitable container. Transfer a portion of mixed Capsule contents containing nominally 18 µg of paricalcitol to a 10-mL volumetric flask. Add acetonitrile to obtain a total mass of 5 g. Transfer 2.0 mL of *Internal standard solution* to the flask and dilute with water to volume. Mix using a vortex mixer. Centrifuge for 5-min intervals until the top layer is clear, then transfer the supernatant to a suitable chromatographic vial within 2 min.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 300 µL

Run time: 55 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for paricalcitol and calcitriol are 1.0 and 1.03, respectively. See *Table 4* for all other relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between paricalcitol and calcitriol

Relative standard deviation: NMT 7.0% for the peak response ratio of paricalcitol to calcitriol

Analysis

Samples: *Degradation solution A*, *Degradation solution B*, *Standard solution*, and *Sample solution*

Identify the impurities in the *Sample solution* on the basis of the relative retention times of the components of *Degradation solution A* and *Degradation solution B* in *Table 4*.

Table 4

Name[a]	Degradation Solution	Relative Retention Time
Related compound A	A	0.74
Related compound B	A	0.84
Related compound C	B	0.91
Related compound D	B	0.97
7*Z*-Paricalcitol[b]	—	1.01
Related compound G	B	1.47
Related compound H	B	1.50
Related compound I	B	1.51
Total impurities	—	—

[a] Related compounds A, B, C, D, G, H, and I are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.

[b] (7*Z*,22*E*)-19-Nor-9,10-secoergosta-5,7,22-triene-1α,3β,25-triol.

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of each impurity to calcitriol from the *Sample solution*

R_S = peak response ratio of paricalcitol to calcitriol from the *Standard solution*

C_S = concentration of paricalcitol from USP Paricalcitol Solution RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of paricalcitol in the *Sample solution* (µg/mL)

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Acceptance criteria
Any individual impurity: NMT 2.0%
Total impurities: NMT 3.0%

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined yeasts and molds count is NMT 10^2 cfu/g. Meet the requirements of the test for absence of *Escherichia coli.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in a tight container. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Calcitriol Solution RS
USP Paricalcitol Solution RS

Paricalcitol Injection

DEFINITION
Paricalcitol Injection is a sterile solution of Paricalcitol in a mixture of Water for Injection, Propylene Glycol, and Alcohol, or other suitable solvents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of paricalcitol ($C_{27}H_{44}O_3$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
[NOTE—Protect paricalcitol solutions from light.]
Mobile phase: Methanol and water (4:1)
Diluent: Methanol and water (1:1)
Standard solution: Dilute USP Paricalcitol Solution RS with *Diluent* to obtain a solution having a concentration of paricalcitol similar to that of the Injection.
Sample solution: Use the Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 252 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 100–200 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of paricalcitol ($C_{27}H_{44}O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of paricalcitol in the *Standard solution,* calculated on the basis of the content of paricalcitol in the USP Paricalcitol Solution RS (µg/mL)
C_U = nominal concentration of paricalcitol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS
- **CONTENT OF PROPYLENE GLYCOL AND ALCOHOL** (if present)
Mobile phase: 0.01 N sulfuric acid solution, filtered and degassed
Alcohol stock solution: Transfer 2.0 mL of dehydrated alcohol to a 10-mL volumetric flask, and dilute with water to volume.
Propylene glycol stock solution: Transfer 3.0 mL of propylene glycol to a 10-mL volumetric flask, and dilute with water to volume.
Standard solution: Transfer 5.0 mL each of *Alcohol stock solution* and *Propylene glycol stock solution* to a 50-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 5.0 mL of Injection to a 50-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Refractive index
Column: 7.8-mm × 30-cm; packing L17
Column temperature: 60°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—Elution order is propylene glycol followed by alcohol.]
Suitability requirements
Resolution: NLT 4.0 between propylene glycol and alcohol
Relative standard deviation: NMT 2.0% for each peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of propylene glycol and alcohol in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of alcohol or propylene glycol from the *Sample solution*
r_S = peak response of alcohol or propylene glycol from the *Standard solution*
C_S = concentration of alcohol or propylene glycol in the *Alcohol stock solution* or *Propylene glycol stock solution* (% v/v)
C_U = nominal concentration of alcohol or propylene glycol (if present) in the Injection (% v/v)

Acceptance criteria
Alcohol: 80.0%–120.0%
Propylene glycol: 80.0%–120.0%

IMPURITIES
- **ALUMINUM** ⟨206⟩
Diluent: Dilute 4 mL of nitric acid with water to 2000 mL.
Matrix modifier: 1.5 mg/mL of magnesium nitrate
Standard stock solution: Proceed as directed for *Standard preparations* in the chapter, beginning with "Treat some aluminum wire" and ending with "Cool, and transfer the solution, with the aid of water, to a 100-mL volumetric flask, and dilute with water to volume". Transfer 2 mL of this solution to a second 100-mL volumetric flask, and dilute with water to volume. Transfer 2 mL of this solution to a third 100-mL volumetric flask, and dilute with water to volume. This solution contains 0.4 µg/mL of aluminum.

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Standard solution A: 2.5 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*
Standard solution B: 5.0 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*
Standard solution C: 10 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*
Standard solution D: 20 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*
Standard solution E: 50 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*
Sample solution: Dilute 4.0 mL of Injection with 6.0 mL of *Diluent*, or use an appropriate dilution to obtain a solution having a concentration of NMT 0.02 µg/mL of aluminum.
System suitability solution: Dilute 9.5 mL of the *Sample solution* with 0.5 mL of the *Standard stock solution*. If the resulting solution contains more than 0.04 µg/mL of aluminum, prepare an alternate dilution having a concentration of about 0.02–0.04 µg/mL of aluminum.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry; instrument equipped with a flameless, electrically heated furnace
Lamp: Aluminum hollow-cathode
Analytical wavelength: Aluminum emission line at 309.3 nm
Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, *Sample solution*, and *System suitability solution*
Under typical conditions, the sample volume is 20 µL, the volume of the *Matrix modifier* is 5 µL, the injection temperature is 100°, and the oven conditions are as listed in *Table 1*. [NOTE—These conditions may be optimized for each instrument.]

Table 1

Step	Temperature
Drying 1	110°
Drying 2	130°
Drying 3	200°
Pyrrolysis	1100°
Read	2300°
Clean out	2450°

Determine the absorbances of the samples. Plot the absorbances of the *Standard solutions* versus the content of aluminum, in ng/mL, drawing a straight line best fitting the five points. The correlation coefficient is NLT 0.995, the recovery for the *System suitability solution* is 80%–120%, and the duplicate injections must agree within 0.0024 µg/mL. From the graph so obtained, determine the quantity of aluminum, *C*, in µg, found in each mL of the *Sample solution*.
Calculate the quantity, in µg/mL, of aluminum in the portion of Injection taken:

$$\text{Result} = C \times D$$

C = measured concentration of aluminum in the *Sample solution* (µg/mL)
D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: NMT 0.5 µg/mL

• **ORGANIC IMPURITIES**
Diluent: Acetonitrile and water (1:1)
Solution A: Acetonitrile and water (15:85)
Solution B: Acetonitrile
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	65	35
25	5	95
45	5	95

Return to the original conditions, and re-equilibrate the system.
Standard solution: Dilute USP Paricalcitol Solution RS with *Diluent* to obtain a solution having a concentration of paricalcitol equal to 0.5% of the labeled concentration of the Injection.
Degradation stock solution: Dilute 1 mL of USP Paricalcitol Solution RS with *Diluent* to 5 mL.
Degradation solution A: Transfer 1 mL of the *Degradation stock solution* and 0.1 mL of 30% hydrogen peroxide into a 10-mL container, and allow to stand at room temperature for 1 h. Dilute with *Diluent* to 10 mL, and mix.
Degradation solution B: Mix 1 mL of the *Degradation stock solution* and 1 mL of 0.1 N hydrochloric acid, and heat at 70° for 1 h. Cool to room temperature, dilute with *Diluent* to 10 mL, and mix.
Sample solution: Use the Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 252 nm
Columns
Guard: 4.6-mm × 7.5-mm or 4.6-mm × 10-mm; packing L1
Analytical: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100–200 µL
System suitability
Samples: *Standard solution* and *Degradation solution B*
Suitability requirements
Resolution: NLT 1.0 between the paricalcitol peak and the related compound D peak, *Degradation solution B*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Diluent*, *Degradation solution A*, *Degradation solution B*, *Standard solution*, and *Sample solution*
Identify the impurities in the *Sample solution* on the basis of the relative retention times of the components of *Degradation solution A* and *Degradation solution B* in *Table 3*.

Table 3

Name[a]	Degradation Solution	Relative Retention Time	Acceptance Criteria, NMT (%)
Related compound A	A	0.63	1.0
Related compound B	A	0.79	1.0
Related compound C	B	0.89	1.0
Related compound D	B	0.95	1.0
Related compound E[b]	B	1.32	1.0
Related compound F	B	1.57	1.0
Related compound G	B	1.66	1.0
Related compound H	B	1.74	1.0

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Table 3 *(continued)*

Name[a]	Degradation Solution	Relative Retention Time	Acceptance Criteria, NMT (%)
Related compound I	B	1.79	1.0
Total impurities	—	—	2.0

[a] Related compounds A–I are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.
[b] This peak is very small; the signal-to-noise ratio is approximately 3–5.

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (100/L)$$

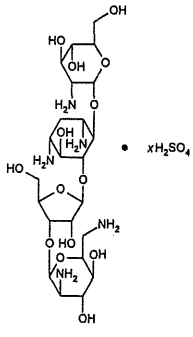
r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of paricalcitol from the *Standard solution*
C_S = concentration of paricalcitol in the *Standard solution*, calculated on the basis of the content of paricalcitol in the USP Paricalcitol Solution RS (µg/mL)
L = labeled amount of paricalcitol in the Injection (µg/mL)

Acceptance criteria: See *Table 3*. Disregard any peak observed in the *Diluent*.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 10 USP Endotoxin Units/µg of paricalcitol.
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multi-dose containers, preferably of Type I glass. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Paricalcitol Solution RS

Paromomycin Sulfate



$C_{23}H_{45}N_5O_{14} \cdot xH_2SO_4$
D-Streptamine, [*O*-2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[*O*-2,6-diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-β-D-ribofuranosyl-(1→5)]-2-deoxy-, sulfate (salt).
O-2,6-Diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-*O*-β-D-ribofuranosyl-(1→5)-*O*-[2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)]-2-deoxystreptamine sulfate (salt) [1263-89-4].
Base 615.63
[59-04-1; 7542-37-2].

» Paromomycin Sulfate is the sulfate salt of an antibiotic substance or substances produced by the growth of *Streptomyces rimosus* var. *paromomycinus*, or a mixture of two or more such salts. It has a potency equivalent to not less than 675 µg of paromomycin ($C_{23}H_{45}N_5O_{14}$) per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Paromomycin Sulfate RS

Identification—
A: Prepare a test solution in water containing 10 mg of paromomycin per mL. Apply 25 µL of this solution and 25 µL of a Standard solution of USP Paromomycin Sulfate RS containing 10 mg of paromomycin per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, place the plate in a developing chamber, and develop the chromatogram in a solvent system consisting of a mixture of freshly prepared ammonium acetate solution (4 in 100), *n*-propyl alcohol, and ammonium hydroxide (30:10:6) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to air-dry for 10 minutes. Heat the plate at 105° for 1 hour, allow to cool, and spray with a solution of ninhydrin in butanol (1 in 100). Heat the plate at 105° for 5 minutes: paromomycin appears as a red spot, and the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
B: It meets the requirements of the tests for *Sulfate* ⟨191⟩.
Specific rotation ⟨781S⟩: between +50° and +55°.
Test solution: 50 mg per mL, in water.
pH ⟨791⟩: between 5.0 and 7.5, in a solution (3 in 100).
Loss on drying ⟨731⟩—Dry about 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.
Residue on ignition ⟨281⟩: not more than 2.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.
Assay—Proceed with Paromomycin Sulfate as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Paromomycin Sulfate Capsules

» Paromomycin Sulfate Capsules contain the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of paromomycin ($C_{23}H_{45}N_5O_{14}$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Paromomycin Sulfate RS

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Identification—Mix the contents of 1 Capsule with water to obtain a solution containing the equivalent of about 20 mg of paromomycin per mL. This test solution responds to the *Identification* test under *Paromomycin Sulfate*.
Disintegration ⟨701⟩: 15 minutes, the use of disks being omitted.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Loss on drying ⟨731⟩—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 7.0% of its weight.
Assay—Proceed with Capsules as directed under *Antibiotics—Microbial Assays* ⟨81⟩, blending not less than 5 Capsules for 5 minutes in a high-speed blender with sufficient *Buffer B.3* to obtain a stock solution of convenient concentration. Dilute this stock solution quantitatively with the same buffer to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

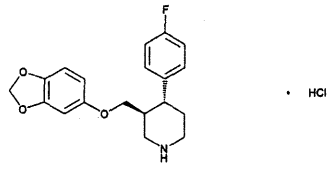
Paromomycin Oral Solution

» Paromomycin Oral Solution contains an amount of Paromomycin Sulfate equivalent to not less than 90.0 percent and not more than 130.0 percent of the labeled amount of paromomycin ($C_{23}H_{45}N_5O_{14}$). It may contain one or more suitable buffers, colors, flavors, preservatives, and solvents.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Paromomycin Sulfate RS

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 7.5 and 8.5.
Assay—Proceed with Oral Solution as directed under *Antibiotics—Microbial Assays* ⟨81⟩, diluting an accurately measured volume of Oral Solution with *Buffer B.3* to yield a stock solution of convenient concentration. Quantitatively dilute this stock solution with the same buffer to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Paroxetine Hydrochloride



$C_{19}H_{20}FNO_3 \cdot HCl$ 365.83
Hemihydrate 374.83
Piperidine, 3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)-, hydrochloride, (3*S*-*trans*)-;
(–)-(3*S*,4*R*)-4-(*p*-Fluorophenyl)-3-[(3,4-methylenedioxy) phenoxy]methyl]piperidine hydrochloride
Anhydrous [78246-49-8].
Hemihydrate [110429-35-1].

DEFINITION
Paroxetine Hydrochloride is anhydrous or contains one-half molecule of water of hydration. It contains NLT 98.5% and NMT 102.0% of paroxetine hydrochloride ($C_{19}H_{20}FNO_3 \cdot HCl$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020), ▲⟨197K⟩, or ⟨197A⟩▲ (USP 1-Aug-2019)
Standard: Dissolve USP Paroxetine Hydrochloride RS in a mixture of water and isopropyl alcohol (1 in 10). Heat to 70° to dissolve, recrystallize, and dry the residue under vacuum at 50° for 3 h.
Sample: Dissolve Paroxetine Hydrochloride in a mixture of water and isopropyl alcohol (1 in 10). Heat to 70° to dissolve, recrystallize, and dry the residue under vacuum at 50° for 3 h.
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
Sample solution: 10 mg/mL of Paroxetine Hydrochloride in methanol and water (1:1)
Acceptance criteria: Meets the requirements

Add the following:

▲• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**
Buffer: 0.05 M ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.5.
Mobile phase: Acetonitrile, *Buffer*, and triethylamine (30:70:1). [NOTE—The ratio for acetonitrile, *Buffer*, and triethylamine may be varied between 25:75:1 and 40:70:1 to meet system suitability requirements.] Adjust with glacial acetic acid to a pH of 5.5.
System suitability solution: 0.5 mg/mL each of USP Paroxetine Hydrochloride RS and USP Paroxetine Related Compound B RS
Standard solution: 0.5 mg/mL of USP Paroxetine Hydrochloride RS
Sample solution: 0.5 mg/mL of Paroxetine Hydrochloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L13
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The approximate relative retention times for paroxetine related compound B and paroxetine are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between paroxetine related compound B and paroxetine
Tailing factor: NMT 2.0 for paroxetine
Relative standard deviation: NMT 2.0% for paroxetine

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of paroxetine hydrochloride ($C_{19}H_{20}FNO_3 \cdot HCl$) in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–102.0% on the anhydrous and solvent-free basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **LIMIT OF PAROXETINE RELATED COMPOUND C**
Mobile phase: *n*-Hexane, absolute alcohol, trifluoroacetic acid, and water (900:100:2:2)
Diluent: *n*-Hexane and absolute alcohol (1:1)
System suitability solution: 0.1 mg/mL each of Paroxetine Hydrochloride and USP Paroxetine Related Compound C RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Paroxetine Related Compound C RS in *Diluent*
Sample solution: 5 mg/mL of Paroxetine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 4.6-mm × 25-cm; ▲10-µm▲ (USP 1-Aug-2019) packing L51
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for paroxetine related compound C and paroxetine are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between paroxetine and paroxetine related compound C, *System suitability solution*
Tailing factor: NMT 2.5 for the paroxetine related compound C peak, *System suitability solution*
Relative standard deviation: NMT 10.0% for paroxetine related compound C, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of paroxetine related compound C in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Paroxetine Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Paroxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1% of paroxetine related compound C

Change to read:

• **LIMIT OF 1-METHYL-4-(*p*-FLUOROPHENYL)-1,2,3,6-TETRAHYDROPYRIDINE**
▲[NOTE—Perform this test only if 1-methyl-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine is a known process impurity.]▲ (USP 1-Aug-2019)
Solution A: Dissolve 30 g of sodium perchlorate in 900 mL of water. Add 3.5 mL of phosphoric acid and 2.4 mL of triethylamine. Dilute with water to 1000 mL. Adjust with phosphoric acid or triethylamine to a pH of 2.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
20	80	20
27	55	45
36	55	45
38	85	15
45	85	15

Diluent: Acetonitrile and water (1:4)
Standard solution: 42 ng/mL of 1-methyl-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine, obtained from USP Paroxetine Related Compound E Mixture RS, in *Diluent*
Sample solution: 42 mg/mL of Paroxetine Hydrochloride in *Diluent*. Sonicate as needed to aid dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.0-mm × 25-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 75 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for 1-methyl-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine and paroxetine are about 0.6 and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 15.0% for 1-methyl-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine, determined from three replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 1-methyl-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1 \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Paroxetine Related Compound E Mixture RS in the *Standard solution* (mg/mL)

USP Monographs

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C_U = concentration of paroxetine in the *Sample solution* (mg/mL)
I = fraction of 1-methyl-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine in USP Paroxetine Related Compound E Mixture RS

Acceptance criteria: NMT 0.0001%

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 1**

Perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*, depending on the synthetic route. *Organic Impurities, Procedure 2* is recommended if paroxetine related compound F or paroxetine related compound G are potential impurities.
Solution A: Tetrahydrofuran, water, and trifluoroacetic acid (20:180:1)
Solution B: Acetonitrile, tetrahydrofuran, and trifluoroacetic acid (180:20:1)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	80	20
50	20	80
60	20	80
70	80	20

Diluent: Tetrahydrofuran and water (1:9)
System suitability solution: 1 mg/mL of USP Paroxetine System Suitability Mixture A RS in *Diluent*. Sonication may be necessary to achieve complete dissolution.
Standard solution: 0.001 mg/mL of USP Paroxetine Hydrochloride RS in *Diluent*
Sample solution: 1 mg/mL of Paroxetine Hydrochloride in *Diluent*. Sonicate to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—Identify the peaks using the relative retention times given in *Table 3*.]
Suitability requirements
Resolution: NLT 2.0 between paroxetine related compound A and paroxetine related compound B
Tailing factor: 0.8–2.0 for paroxetine related compound A
Relative standard deviation: NMT 2.0% for paroxetine related compound A
Analysis
Samples: *Diluent*, *Standard solution*, and *Sample solution*
Calculate the percentage of each impurity in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*, excluding peaks from the chromatogram of the *Diluent*
r_S = peak area of paroxetine from the *Standard solution*
C_S = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Paroxetine Hydrochloride, on the anhydrous basis, in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Paroxetine related compound A	0.66	0.1
Paroxetine related compound B	0.73	0.3
Paroxetine	1.0	—
Any unspecified impurity	—	0.1
Total impurities	—	1.0

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 2**

Buffer: Dissolve 3.4 g of monobasic potassium phosphate and 3.4 g of tetrabutylammonium hydrogen sulfate in 1.0 L of water.
Solution A: Acetonitrile and *Buffer* (2:98)
Solution B: Acetonitrile and *Buffer* (40:60)
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
70	40	60
90	0	100
95	0	100
95.1	100	0
110	100	0

Diluent: Acetonitrile and *Buffer* (10:90)
▲**Identification solution:**▲ (USP 1-May-2019) 2 mg/mL of USP Paroxetine Hydrochloride RS, 0.01 mg/mL of USP Paroxetine Related Compound B RS, 0.01 mg/mL of USP Paroxetine Related Compound F RS, and 0.004 mg/mL of USP Paroxetine Related Compound G RS*Diluent*
Standard solution: 0.004 mg/mL of USP Paroxetine Hydrochloride RS, 0.01 mg/mL of USP Paroxetine Related Compound B RS, 0.01 mg/mL of USP Paroxetine Related Compound F RS, and 0.004 mg/mL of USP Paroxetine Related Compound G RS in *Diluent*
Sample solution: 0.5 mg/mL of Paroxetine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm

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Column: 3.9-mm × 15-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 1.0 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—Identify the peaks using the relative retention times given in *Table 5*.]
Suitability requirements
Relative standard deviation: NMT 10.0% for each of paroxetine related compound B, paroxetine related compound F, paroxetine hydrochloride, and paroxetine related compound G
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of paroxetine related compound B, paroxetine related compound F, and paroxetine related compound G in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding impurity from the *Sample solution*
r_S = peak response of the corresponding impurity from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Paroxetine Hydrochloride, on the anhydrous basis, in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of paroxetine from the *Standard solution*
C_S = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Paroxetine Hydrochloride, on the anhydrous basis, in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Paroxetine related compound B	0.91	0.5
Paroxetine related compound F	0.96	0.2
Paroxetine	1.0	—
Paroxetine related compound G	1.34	0.2
Any unspecified impurity	—	0.1
Total impurities	—	1.0

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*
Anhydrous form: NMT 1.5%
Hemihydrate form: 2.2%–2.8%

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve the anhydrous form in tight containers. Preserve the hemihydrate form in well-closed containers. Store at ▲▲ (USP 1-Aug-2019) room temperature.

Change to read:

• **LABELING:** Label the article to indicate whether it is the anhydrous form or the hemihydrate form, and label it to indicate with which ▲*Organic Impurities*▲ (USP 1-Aug-2019) test the article complies.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Paroxetine Hydrochloride RS
USP Paroxetine Related Compound B RS
▲(3*S*,4*R*)-3-[(Benzodioxol-5-yloxy)methyl]-4-phenylpiperidine hydrochloride.▲ (USP 1-May-2019)
$C_{19}H_{21}NO_3 \cdot HCl$ 347.84
USP Paroxetine Related Compound C RS
(+)-*trans*-Paroxetine hydrochloride;
▲(3*R*,4*S*)-3-[(Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine hydrochloride.▲ (USP 1-May-2019)
$C_{19}H_{20}FNO_3 \cdot HCl$ 365.83
USP Paroxetine Related Compound E Mixture RS
Paroxetine hydrochloride spiked with 1-methyl-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine.
USP Paroxetine Related Compound F RS
(3*S*,4*R*)-3-[(Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)-1-methylpiperidine.
$C_{20}H_{22}FNO_3$ 343.39
USP Paroxetine Related Compound G RS
(±)*trans*-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4″-fluorophenyl-4′-phenyl)piperidine hydrochloride.
$C_{25}H_{24}FNO_3 \cdot HCl$ 405.46
USP Paroxetine System Suitability Mixture A RS
Mixture of approximately 1% paroxetine related compound A [piperidine, 3-{(1,3-benzodioxol-5-yloxy) methyl}-4-(4-methoxyphenyl)-, hydrochloride (3*S*-*trans*)]; and 1% of paroxetine related compound B ▲[(3*S*,4*R*)-3-[(benzodioxol-5-yloxy)methyl]-4-phenylpiperidine hydrochloride]▲ (USP 1-May-2019) in a matrix of paroxetine hydrochloride.

Paroxetine Tablets

DEFINITION
Paroxetine Tablets contain an amount of Paroxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer a quantity of finely powdered Tablets, equivalent to 90 mg of paroxetine, to a suitable flask. Add 100 mL of 0.1 N hydrochloric acid, and stir for 1 h. Transfer the mixture to a separatory funnel, and add 1.5 mL of

USP Monographs

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ammonium hydroxide to make the solution alkaline. Add 100 mL of ethyl ether to the funnel, and shake for 2 min. Transfer the organic layer into the necessary number of centrifuge tubes, and centrifuge for 10 min. Recombine the clarified extracts, add 1 drop of water and 0.5 mL of hydrochloric acid, stir, and evaporate to dryness under a stream of nitrogen. Dry the residue in an oven at 90° for 1 h.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Water, phosphoric acid, and triethylamine (100: 0.6: 0.3)

Mobile phase: Acetonitrile and *Buffer* (30:70)

Standard solution: 0.1 mg/mL of USP Paroxetine Hydrochloride RS in methanol

Sample stock solution: 0.5 mg/mL of paroxetine from NLT 20 Tablets in methanol prepared as follows. Transfer an amount of finely powdered Tablets equivalent to NLT 100 mg of paroxetine to a suitable volumetric flask. Dissolve in methanol. Dilute with methanol to volume. Centrifuge a portion of the solution for 6 min. Use the supernatant.

Sample solution: Nominally 0.1 mg/mL of paroxetine in methanol, from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 4.6-mm × 3.3-cm; 3-µm packing L7

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 750 theoretical plates

Tailing factor: NMT 4

Relative standard deviation: NMT 2.0% for paroxetine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of paroxetine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of paroxetine, 329.37
M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Simulated gastric fluid without enzyme; 900 mL

Apparatus 2: 60 rpm

Time: 60 min

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.

Standard stock solution: 0.63 mg/mL of USP Paroxetine Hydrochloride RS in *Medium* prepared as follows. Transfer a suitable quantity of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Add 5% of the flask volume of methanol, and dissolve. Dilute with *Medium* to volume.

Sample solution: Pass the solution under test through a suitable membrane filter of 0.45-µm pore size.

Standard solution: Quantitatively dilute the *Standard stock solution* with *Medium* to a concentration near that of the *Sample solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 4.6-mm × 3.3-cm; 3-µm packing L7

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 750 theoretical plates

Tailing factor: NMT 4

Relative standard deviation: NMT 2.0% for paroxetine

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved based on the peak responses obtained from the *Sample solution* and the *Standard solution*.

Tolerances: NLT 80% (*Q*) of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Buffer, Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: Nominally 0.1 mg/mL of paroxetine prepared as follows. Place 1 Tablet in a suitable volumetric flask, and add a volume of a hydrochloric acid solution (7 in 1000), equivalent to about 25% of the flask volume. Allow the Tablet to disintegrate. Dilute with methanol to volume. Centrifuge a portion of the solution.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
M_{r1} = molecular weight of paroxetine, 329.37
M_{r2} = molecular weight of paroxetine hydrochloride, 365.83
V = volume of the *Sample solution* (mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Paroxetine Hydrochloride RS

Paroxetine Extended-Release Tablets

DEFINITION

Paroxetine Extended-Release Tablets contain paroxetine hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$).

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IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 3.9 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.5.

Mobile phase: Acetonitrile, *Buffer*, and triethylamine (40:60:1). Adjust with glacial acetic acid to a pH of 5.5.

Standard solution: 0.5 mg/mL of USP Paroxetine Hydrochloride RS in methanol

System suitability solution: 0.5 mg/mL of USP Paroxetine Related Compound B RS in *Standard solution*

Sample solution: Nominally 0.5 mg/mL of paroxetine from NLT 10 Tablets prepared as follows. Transfer the required number of Tablets to a suitable volumetric flask. Add 80% of the flask volume of methanol. Sonicate for 30 min followed by stirring for 30 min. Dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 4.6-mm × 25-cm; 5-µm packing L13

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for paroxetine related compound B and paroxetine are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between paroxetine related compound B and paroxetine, *System suitability solution*

Tailing factor: NMT 2.0 for paroxetine, *System suitability solution*

Relative standard deviation: NMT 2.0% for paroxetine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of paroxetine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of paroxetine, 329.37

M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Acid stage medium: 0.1 N hydrochloric acid; 750 mL

Buffer stage medium: 0.05 M tris buffer prepared as follows. Dissolve 6.06 g of tris(hydroxymethyl) aminomethane in 1 L of water. Add 1.8 mL of hydrochloric acid to the resulting solution. Adjust with hydrochloric acid to a pH of 7.5; 1000 mL deaerated.

Apparatus 1: 100 rpm

Times: 2 h in *Acid stage*; 2, 4, and 12 h in *Buffer stage*

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.

Acid stage standard stock solution: 0.33 mg/mL of paroxetine prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Dissolve in 5% of the flask volume of methanol. Dilute with *Acid stage medium* to volume.

Acid stage standard solution: Dilute the *Acid stage standard stock solution* with *Acid stage medium* to obtain a final concentration of (L/7500) mg/mL, where L is the label claim in mg.

Buffer stage standard stock solution: 0.25 mg/mL of paroxetine prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Dissolve in 5% of the flask volume of methanol. Dilute with *Buffer stage medium* to volume.

Buffer stage standard solution: Dilute the *Buffer stage standard stock solution* with *Buffer stage medium* to obtain a final concentration of (L/1000) mg/mL, where L is the label claim in mg.

Acid stage sample solution: Run the *Acid stage* for 2 h. Withdraw 10 mL of the solution under test and centrifuge. Use the centrifugate for analysis.

Buffer stage sample solution: Remove the *Acid stage medium* from the vessel and replace it with the *Buffer stage medium*. At the times specified, remove 10 mL of the solution under test and centrifuge. Use the centrifugate for analysis.

Chromatographic system: Proceed as directed in the *Assay*. For *Injection volume*, use 100 µL for the *Acid stage* analysis and 10 µL for the *Buffer stage* analysis.

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0, *Acid stage standard solution* and *Buffer stage standard solution*

Relative standard deviation: NMT 3.0%, *Acid stage standard solution* and *Buffer stage standard solution*

Analysis

Samples: *Acid stage standard solution*, *Buffer stage standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*

r_S = peak response from the *Acid stage standard solution*

C_S = concentration of USP Paroxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

M_{r1} = molecular weight of paroxetine, 329.37

M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

V = volume of the *Acid stage medium*, 750 mL

L = label claim (mg/Tablet)

Calculate the concentration (C_i) of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point in the *Buffer stage*:

$$\text{Result} = (r_i/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_i = peak response from the *Buffer stage sample solution* at each time point i

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r_S = peak response from the *Buffer stage standard solution*
C_S = concentration of USP Paroxetine Hydrochloride RS in the *Buffer stage standard solution* (mg/mL)
M_{r1} = molecular weight of paroxetine, 329.37
M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Calculate the percentage of the labeled amount (Q_i) of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point (i) in the *Buffer stage medium*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of paroxetine in the *Buffer stage medium* in the portion of sample withdrawn at time point i (mg/mL)
V = volume of the *Buffer stage medium*, 1000 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Buffer stage medium* (mL)

Tolerances

Acid stage: NMT 10% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) is dissolved in 2 h.

Buffer stage: See *Table 1*.

Table 1

Time Point (i)	Time (h)	Amount Dissolved (Tablets labeled to contain 12.5 mg of paroxetine)	Amount Dissolved (Tablets labeled to contain 25 mg of paroxetine)	Amount Dissolved (Tablets labeled to contain 37.5 mg of paroxetine)
1	2	15%–35%	10%–30%	20%–45%
2	4	40%–70%	40%–70%	60%–85%
3	12	NLT 80%	NLT 80%	NLT 80%

The percentages of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2

If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 2*.

Acid stage medium: 0.1 N hydrochloric acid; 750 mL

Buffer stage medium: 0.05 M tris buffer prepared as follows. Dissolve 6.06 g of tris(hydroxymethyl) aminomethane in 900 mL of water. Add 40 mL of 1.0 M hydrochloric acid to the resulting solution. Adjust with either 1.0 M hydrochloric acid or 1.0 M sodium hydroxide to a pH of 7.5. Dilute with water to 1 L; 1000 mL deaerated.

Apparatus 2: 150 rpm with suitable sinkers

Times: 2 h in *Acid stage*; 1, 2, 4, and 6 h in *Buffer stage*

Acid stage standard stock solution: 0.04 mg/mL of USP Paroxetine Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Dissolve in 2 mL of methanol. Dilute with *Acid stage medium* to volume.

Acid stage standard solution: Dilute the *Acid stage standard stock solution* with *Acid stage medium* to obtain a final concentration of (L/7500) mg/mL of paroxetine, where L is the label claim in mg.

Buffer stage standard solution: (L/1000) mg/mL of paroxetine, where L is the label claim in mg prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Dissolve in 2 mL of methanol. Dilute with *Buffer stage medium* to volume.

Acid stage sample solution: Run the *Acid stage* for 2 h. Withdraw 10 mL of the solution under test, and filter. Use the filtrate for analysis.

Buffer stage sample solution: Remove the Tablet and sinker from the acid stage vessel and pat them dry. Introduce the Tablet and sinker into the dissolution vessel with 1000 mL of the *Buffer stage medium*. At the times specified, remove 10 mL of the solution under test and filter. Use the filtrate for analysis.

Acid stage analysis

Buffer: Add 2.9 mL of phosphoric acid to 800 mL of water. Adjust with 1 M sodium hydroxide to a pH of 6.0. Dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (40:60)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL for 12.5-mg Tablet; 20 µL for 25- and 37.5-mg Tablets

Run time: 2 times the retention time of paroxetine

System suitability

Sample: *Acid stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage standard solution* and *Acid stage sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*
r_S = peak response from the *Acid stage standard solution*
C_S = concentration of USP Paroxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)
M_{r1} = molecular weight of paroxetine, 329.37
M_{r2} = molecular weight of paroxetine hydrochloride, 365.83
V = volume of the *Acid stage medium*, 750 mL
L = label claim (mg/Tablet)

Buffer stage analysis

Instrumental conditions

Mode: UV

Analytical wavelength: 294 nm with 340 nm for background correction

Blank: *Buffer stage medium*

Analysis

Samples: *Buffer stage standard solution* and *Buffer stage sample solution*

USP Monographs

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Calculate the concentration (C_i) of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point (i) in the *Buffer stage*:

$$\text{Result} = (A_i/A_S) \times C_S \times (M_{r1}/M_{r2})$$

A_i = absorbance of the *Buffer stage sample solution* at time point i
A_S = absorbance of the *Buffer stage standard solution*
C_S = concentration of USP Paroxetine Hydrochloride RS in the *Buffer stage standard solution* (mg/mL)
M_{r1} = molecular weight of paroxetine, 329.37
M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point (i) in the *Buffer stage medium*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of paroxetine in the *Buffer stage medium* in the portion of sample withdrawn at time point i (mg/mL)
V = volume of the *Buffer stage medium*, 1000 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Buffer stage medium* (mL)

Tolerances

Acid stage: NMT 10% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) is dissolved in 2 h.

Buffer stage: See *Table 2*.

Table 2

Time Point (i)	Time (h)	Amount Dissolved
1	1	NMT 20%
2	2	20%–45%
3	4	60%–90%
4	6	NLT 85%

The percentages of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3

If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 3*.

Acid stage medium: 0.1 N hydrochloric acid; 750 mL

Buffer stage medium: 0.05 M tris buffer prepared as follows. Dissolve 42 g of tris(hydroxymethyl) aminomethane in 2000 mL of water. Pass through a suitable filter of 0.45-µm pore size. Add 5000 mL of water and adjust with hydrochloric acid to a pH of 7.5; 1000 mL deaerated.

Apparatus 2: 150 rpm with suitable sinkers

Times: 2 h in *Acid stage*; 2, 4, and 6 h in *Buffer stage*

Solution A: 1.36 g/L of potassium phosphate monobasic and 2.44 g/L of sodium 1-decanesulfonate in water. Adjust with 0.5% phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Solution A* (45:55)

Standard stock solution: 0.042 mg/mL of USP Paroxetine Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Add about 50% of the final flask volume of *Buffer stage medium* and sonicate for about 15 min to dissolve. Dilute with *Buffer stage medium* to volume.

Standard solution: 0.021 mg/mL of USP Paroxetine Hydrochloride RS prepared as follows. Mix equal portions of *Standard stock solution* and *Acid stage medium* in a suitable glass container.

Acid stage sample solution: Run the *Acid stage* for 2 h. Withdraw 10 mL of the solution under test. Centrifuge to obtain a clear supernatant. Mix equal portions of the supernatant and *Acid stage medium* in a suitable glass container. Use the resulting solution for analysis.

Buffer stage sample solution: Remove the Tablet in the sinker from the acid stage vessel. Add the Tablet in the sinker to the vessel with 1000 mL of *Buffer stage medium* and run the *Buffer stage*. At the times specified, remove 10 mL of the solution under test. Centrifuge to obtain a clear supernatant. Mix equal portions of the supernatant and *Acid stage medium* in a suitable glass container. Use the resulting solution for analysis.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: About 1.5 times the retention time of paroxetine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times D \times V \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of paroxetine, 329.37
M_{r2} = molecular weight of paroxetine hydrochloride, 365.83
D = dilution factor
V = volume of the *Acid stage medium*, 750 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point (i) in the *Buffer stage*:

$$\text{Result}_i = (r_i/r_S) \times C_S \times (M_{r1}/M_{r2}) \times D$$

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r_i = peak response from the *Buffer stage sample solution* at time point *i*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of paroxetine, 329.37
M_{r2} = molecular weight of paroxetine hydrochloride, 365.83
D = dilution factor

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point (*i*) in the *Buffer stage medium*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of paroxetine in the *Buffer stage medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of the *Buffer stage medium*, 1000 mL
L = label claim (mg/Tablet)
V_S = volume of the sample solution withdrawn from the *Buffer stage medium* (mL)

Tolerances
Acid stage: NMT 10% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) is dissolved.
Buffer stage: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	20–50
2	4	55–85
3	6	NLT 80

The percentages of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A: Tetrahydrofuran, water, and trifluoroacetic acid (20:180:1)
Solution B: Acetonitrile, tetrahydrofuran, and trifluoroacetic acid (180:20:1)
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	80	20
50	20	80
60	20	80
70	80	20
80	80	20

System suitability solution: 1 mg/mL of USP Paroxetine Hydrochloride RS, 0.1 mg/mL of USP Paroxetine System Suitability Mixture A RS, and 1 mg/mL of USP Paroxetine Related Compound F RS in methanol. [Note—Sonication may be used to aid dissolution of the individual components.]
Standard solution: 0.01 mg/mL of USP Paroxetine Hydrochloride RS in methanol
Sample solution: Nominally 1 mg/mL of paroxetine from NLT 10 Tablets prepared as follows. Transfer a suitable number of Tablets to a suitable volumetric flask. Add 50% of the flask volume of methanol. Sonicate for 30 min followed by stirring for 30 min. Dilute with methanol to volume. Mix and centrifuge. Use the clear centrifugate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution* [Note—See *Table 4* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between paroxetine related compound A and paroxetine related compound B; NLT 1.5 between paroxetine related compound F and paroxetine, *System suitability solution*
Tailing factor: NMT 2.0 for paroxetine, *Standard solution*
Relative standard deviation: NMT 5.0% for paroxetine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of paroxetine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of paroxetine 329.37
M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Paroxetine related compound A[a]	0.67	—
Paroxetine related compound B[a]	0.75	—
Paroxetine related compound F[a]	0.90	—
Paroxetine	1.0	—
Ethoxyparoxetine[b]	1.2	0.2
Any unspecified degradation product	—	0.2

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Table 5 *(continued)*

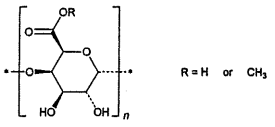
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	1.0

[a] Process impurities, included for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities of the drug product.
[b] (3*SR*,4*RS*)-3-(1,3-Benzodioxol-5-yloxy)methyl)-4-(4-ethoxyphenyl) piperidine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Paroxetine Hydrochloride RS
  USP Paroxetine Related Compound B RS
  trans-4-Phenyl-3-[(3,4-methylenedioxy)phenoxy] methylpiperidine hydrochloride;
  Also known as Piperidine, 3-[(1,3-benzodioxol-5-yloxy) methyl]-4-phenyl-, hydrochloride (3*S-trans*).
  $C_{19}H_{21}NO_3 \cdot HCl$ 347.84
  USP Paroxetine Related Compound F RS
  (3S,4R)-3-[(Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)-1-methylpiperidine.
  $C_{20}H_{22}FNO_3$ 343.39
  USP Paroxetine System Suitability Mixture A RS
  Mixture of approximately 1% paroxetine related compound A [piperidine, 3-[(1,3-benzodioxol-5-yloxy) methyl]-4-(4-methoxyphenyl)-, hydrochloride (3*S-trans*)], and 1% paroxetine related compound B [piperidine, 3-[(1,3-benzodioxol-5-yloxy)methyl]-4-phenyl-, hydrochloride (3*S-trans*)] in a matrix of paroxetine hydrochloride.

Pectin



Pectin [9000-69-5].

DEFINITION

Pectin is a purified carbohydrate polymer consisting mainly of a linear backbone of partially methoxylated alpha (1-4) linked D-galacturonic acid. It is obtained from the dilute acid extract of the rind of citrus fruits or from apple pomace. No organic solvents other than methanol, ethanol, and isopropanol are used during its production. Pectin yields NLT 74.0% of galacturonic acid ($C_6H_{10}O_7$), calculated on the dried basis.

[NOTE—Commercial pectin for the production of jellied food products is standardized to the convenient "150 jelly grade" by addition of dextrose or other sugars, and sometimes contains sodium citrate or other buffer salts. This monograph refers to the pectin to which no such additions have been made.]

IDENTIFICATION

- **PROCEDURE**

Sample stock solution: Transfer a quantity of Pectin, equivalent to 0.05 g on the dried basis, to a suitable container, and moisten with 250 µL of 2-propanol. Add 50 mL of water to the container, and mix the solution using a magnetic stirrer. Use 0.5 N sodium hydroxide to adjust the pH of the solution to 12, stop the stirrer, and allow the solution to stand undisturbed at room temperature for 15 min. Adjust with 0.5 N hydrochloric acid to a pH of 7.0, and dilute with water to 100 mL.

Tris buffer solution: Transfer 6.055 g of tris(hydroxylmethyl)aminomethane and 0.147 g of calcium chloride ($CaCl_2 \cdot 2H_2O$) to a 1000-mL volumetric flask containing 950 mL of water. Adjust with 1 N hydrochloric acid to a pH of 7.0, and dilute with water to volume.

Enzyme solution: Mix pectate lyase[1] with *Tris buffer solution* to make a solution (1 in 100).

Sample blank: Mix 0.5 mL of *Tris buffer solution*, 1.0 mL of *Sample stock solution*, and 1.0 mL of water in a quartz cuvette.

Enzyme blank: Mix 0.5 mL of *Tris buffer solution*, 1.5 mL of water, and 0.5 mL of *Enzyme solution* in a quartz cuvette.

Sample solution: Mix 0.5 mL of *Tris buffer solution*, 1.0 mL of *Sample stock solution*, 0.5 mL of water, and 0.5 mL of *Enzyme solution* in a quartz cuvette.

Analysis

Samples: *Sample blank*, *Enzyme blank*, and *Sample solution*

Perform the test with the *Samples* using a suitable UV/visible spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) and using water as a blank. Measure the absorbance at 235 nm immediately after mixing the solutions well, and record the value at time 0 for the *Enzyme blank*, $A_{0\text{-}EB}$; for the *Sample blank*, $A_{0\text{-}TB}$; and for the *Sample solution*, $A_{0\text{-}TS}$. After incubation at room temperature for 10 min, determine the absorbance again at 235 nm for the *Enzyme blank*, $A_{10\text{-}EB}$; for the *Sample blank*, $A_{10\text{-}TB}$; and for the *Sample solution*, $A_{10\text{-}TS}$. Calculate the corrected absorbance A_0 at time 0 and the corrected absorbance A_{10} at 10 min:

$$A_0 = A_{0\text{-}TS} - (A_{0\text{-}EB} + A_{0\text{-}TB})$$

$$A_{10} = A_{10\text{-}TS} - (A_{10\text{-}EB} + A_{10\text{-}TB})$$

Calculate the quantity of unsaturated product produced:

$$\text{Result} = (A_{10} - A_0)/(\varepsilon_{235} \times L)$$

ε_{235} = molar extinction coefficient of the reaction product (4600 $M^{-1} \cdot cm^{-1}$)
L = path length of the reaction cuvette, 1 cm

Acceptance criteria: The amount of unsaturated product is NLT 0.5×10^{-5} M.

ASSAY

- **DEGREE OF ESTERIFICATION**

Sample: 5.0 g

Analysis: Transfer the *Sample* to a suitable beaker, and stir for 10 min with a mixture of 5 mL of hydrochloric acid and 100 mL of 60% alcohol. Transfer to a sintered-glass filter (30- to 60-mL crucible or Büchner type, coarse), and wash with six 15-mL portions of the hydrochloric acid–60% alcohol mixture, followed by 60% alcohol until the filtrate is free from chlorides. Finally wash with 20 mL of alcohol,

[1] A suitable pure enzyme is available from Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland (www.megazyme.com).

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dry for 1 h at 105°, cool, and weigh. Transfer exactly one-tenth of the total net weight of the dried *Sample* (representing 500 mg of the original unwashed *Sample*) to a 250-mL conical flask, and moisten with 2 mL of alcohol. Add 100 mL of carbon dioxide-free water, insert the stopper, and swirl occasionally until the Pectin is completely dissolved. Add 5 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Perform a blank determination, and make any necessary correction. Record the results as the initial titer, V_I (mL). Add 20.0 mL of 0.5 N sodium hydroxide VS, insert the stopper, shake vigorously, and allow to stand for 15 min. Add 20.0 mL of 0.5 N hydrochloric acid VS, and shake until the pink color disappears. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to a faint pink color that persists after vigorous shaking. Perform a blank determination, and make any necessary correction. Record this value as the saponification titer, V_S (mL). Calculate the degree of esterification:

$$\text{Result} = [V_S/(V_I + V_S)] \times 100$$

V_S = saponification titer (mL)
V_I = initial titer (mL)

Acceptance criteria: The value for *Degree of Esterification* is within the range stated on the label.

• **Galacturonic Acid:** Each mL of 0.1 N sodium hydroxide used in the total titration (the initial titer added to the saponification titer) in the *Assay* for *Degree of Esterification* is equivalent to 19.41 mg of $C_6H_{10}O_7$. Calculate the percentage of galacturonic acid in the portion of Pectin taken:

$$\text{Result} = 19.41 \times [(V_I + V_S)/W] \times 100$$

V_I = initial titer (mL)
V_S = saponification titer (mL)
W = weight of the original unwashed and dried Pectin taken to prepare the solution for titration (mg)

Acceptance criteria: NLT 74.0%

• **Methoxy Groups:** Each mL of 0.1 N sodium hydroxide used in the saponification titer in the *Assay* for *Degree of Esterification* is equivalent to 3.10 mg of $-OCH_3$. Calculate the percentage of methoxy groups in the portion of Pectin taken:

$$\text{Result} = 3.10 \times (V_S/W) \times 100$$

V_S = saponification titer (mL)
W = weight of the original unwashed and dried Pectin taken to prepare the solution for titration (mg)

Acceptance criteria: The percentage of methoxy groups is within the range stated on the label.

IMPURITIES

• **Arsenic,** *Method II* ⟨211⟩**:** NMT 3 ppm

• **Lead**

Standard stock solution: 1000 µg/mL of lead. [Note—Use a commercially available certified solution.]

Standard solution: 2 µg/mL of lead, prepared immediately before use by pipetting 0.10 mL of *Standard stock solution* into a 50-mL volumetric flask containing 30 mL of water, 4 mL of 20% hydrochloric acid, and 4 mL of 0.1 M EDTA. Dilute with water to volume, and mix.

Reference solution: 0.4 µg/mL of lead, prepared by pipetting 5.0 mL of the *Standard solution* into a 25-mL volumetric flask containing 10 mL of water, 2 mL of 20% hydrochloric acid, and 2 mL of 0.1 M EDTA. Dilute with water to volume, and mix.

Sample solution: Transfer 2.0 g of Pectin to a clean, 100-mL glass beaker, add 25 mL of 70% nitric acid, cover with a watch glass, and heat at low to moderate heat on a hot plate in a fume hood for 2 h. Remove the watch glass, and continue to heat until the sample is dry with no visible fumes. Add 0.5 mL of 70% nitric acid, and heat to dryness. Cool to room temperature, and add 2 mL of 20% hydrochloric acid and 2 mL of 0.1 M EDTA. Quantitatively transfer the solution to a 25-mL volumetric flask, dilute with water to volume, and mix.

Blank solution: Add 30 mL of water, 4 mL of 20% hydrochloric acid, and 4 mL of 0.1 M EDTA into a 50-mL volumetric flask. Dilute with water to volume, and mix.

Analysis: Lead is determined using an inductively coupled plasma–atomic emission spectrometer (ICP–AES) (see *Plasma Spectrochemistry* ⟨730⟩) by measuring the emission at 220.35 nm with the settings optimized as directed by the manufacturer. Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Calibrate the instrument with the *Blank solution* and the *Standard solution*. Then analyze the *Reference solution* and the *Sample solution*.

Acceptance criteria: The concentration in the *Sample solution* is NMT that in the *Reference solution*, corresponding to NMT 5 ppm of lead.

• **Sulfur Dioxide,** *Method V* ⟨525⟩

Sample: 100 g

Analysis: Suspend the *Sample* in 500 mL of methanol, then transfer this mixture to the flask (C). Prepare a mixture of 20 mL of hydrochloric acid and 10 mL of water, and transfer it to the separatory funnel (B). Add 10 mL of hydrogen peroxide solution to the vessel (G). Perform the refluxing for 2 h before removing the vessel (G).

Acceptance criteria: NMT 50 ppm

• **Sugars and Organic Acids**

Sample: 1 g

Analysis: Place the *Sample* in a 500-mL flask, moisten with 3–5 mL of alcohol, rapidly pour in 100 mL of water, shake, and allow to stand until the solution is complete. To this solution add 100 mL of alcohol containing 0.3 mL of hydrochloric acid, mix, and filter rapidly. Measure 25 mL of the filtrate into a tared dish, evaporate the liquid on a steam bath, and dry the residue in a vacuum oven at 50° for 2 h.

Acceptance criteria: The weight of the residue does not exceed 20 mg, corresponding to NMT 16% of sugars and organic acids.

• **Methanol (Methyl Alcohol), Ethanol (Alcohol), and Isopropanol (2-Propanol)**

[Note—Residual alcohols are volatile. They should be stored in a cool and dry place. When preparing the *Standard stock solution*, *Standard solution*, and *Internal standard stock solution* for residual alcohols, mix thoroughly and keep the solutions at 20° when diluting with water to volume.]

Internal standard stock solution: 5000 µg/mL of USP 2-Butanol RS. [Note—This solution can be stored at 5°–8° for 3 months.]

Standard stock solution: Use USP Methyl Alcohol RS, USP 2-Propanol RS, and alcohol to prepare a solution having known concentrations of 5000 µg/mL each for methyl alcohol, 2-propanol, and alcohol.

Standard solution: To a 250-mL volumetric flask add 2.5 mL of the *Standard stock solution* and 2.5 mL of the *Internal standard stock solution*. Dilute with water to volume, and mix. This solution contains 50 µg/mL each of

USP Monographs

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methyl alcohol, 2-propanol, and alcohol. [NOTE—This solution can be stored at 5°–8° for 3 months.] Transfer 1.0 g of this solution to a 10-mL headspace vial.

Sample solution: Transfer 1.0 g of Pectin and 5 g of sucrose to a stoppered 100-mL Erlenmeyer flask containing 90 mL of water, add 1.0 mL of the *Internal standard stock solution*, and dilute with water to 100 mL. Mix the solution using a magnetic stirrer. Continue stirring until all of the Pectin has been completely dissolved; typically it takes about 1–2 h. This solution contains 50 µg/mL of USP 2-Butanol RS. Transfer 1.0 g of this solution to a 10-mL headspace vial.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: Headspace GC
Detector: Flame ionization
Column: 0.32-mm × 30-m capillary column; 1.8-µm layer of phase G43. [NOTE—An alternative column such as a 0.32-mm × 25-m capillary column bonded with a 5-µm layer of phase S3 can be used as long as the system suitability requirements are met.]
Temperatures
Detector: 280°
Column: 70°
Injection port: 200°
Carrier gas: Nitrogen
Flow rate: 1.5 mL/min
Make up gas: Nitrogen
Split flow rate: 30 mL/min
Injection volume: 1 mL (the gaseous headspace)
Injection type: Split ratio 20:1
Balanced pressure automatic headspace sampler
Equilibration time: 10 min
Equilibration temperature: 70°
Agitation speed: 500 rpm
Agitation on time: 5 s
Agitation off time: 90 s
Syringe temperature: 80°
Syringe size: 2.5 mL
Fill speed: 100 µL/s
Pull-up delay: 2.0 s
GC run time: 10.5 min
[NOTE—These GC conditions should be optimized according to the instruments used.]

System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]

Table 1

Component	Relative Retention Time
Methanol	0.5
Ethanol	0.6
2-Propanol	0.7
2-Butanol	1.0

Suitability requirements
Resolution: NLT 1.5, between each pair of analytes
Relative standard deviation: NMT 10%, determined from each analyte

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methanol, ethanol, and 2-propanol in the portion of Pectin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/W) \times F \times V \times 100$$

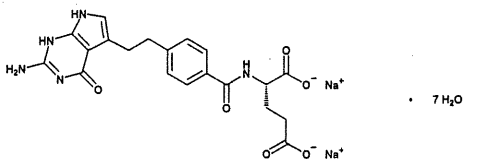
R_U = internal standard ratio (peak response of the respective alcohol/peak response of the internal standard) from the *Sample solution*
R_S = internal standard ratio (peak response of the respective alcohol/peak response of the internal standard) from the *Standard solution*
C_S = concentration of the respective residual alcohol (methanol, ethanol, or 2-propanol) in the *Standard solution* (µg/mL)
W = weight of Pectin taken to prepare the *Sample solution* (g)
F = conversion factor (10^{-6} g/µg)
V = volume of the *Sample solution*, 100 mL

Acceptance criteria: NMT 1% for total methanol, ethanol, and isopropanol

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 10.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a cool and dry place.
- **LABELING:** Label it to indicate whether it is of apple or of citrus origin. Label it to indicate the range of the degree of esterification and the range of the percentage of methoxy groups. The labeling also indicates the presence of sulfur dioxide if the residual sulfur dioxide concentration is greater than 10 ppm.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP 2-Butanol RS
USP Methyl Alcohol RS
USP 2-Propanol RS

Pemetrexed Disodium



$C_{20}H_{19}N_5Na_2O_6 \cdot 7H_2O$ 597.49
L-Glutamic acid, *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate;
Disodium *N*-{*p*-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate heptahydrate [357166-29-1].

$C_{20}H_{19}N_5Na_2O_6$ 471.38
Anhydrous [150399-23-8].

$C_{20}H_{21}N_5O_6$ 427.42
Pemetrexed (free acid) [137281-23-3].

DEFINITION
Pemetrexed Disodium contains NLT 97.5% and NMT 102.0% of pemetrexed disodium ($C_{20}H_{19}N_5Na_2O_6$), calculated on the anhydrous and solvent-free basis.

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[**CAUTION**—Handle pemetrexed disodium with great care as it alters genetic material and may be irritating to the eyes and skin.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Enantiomeric Purity* test.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*

ASSAY

- **PROCEDURE**

Buffer: 0.17% (v/v) glacial acetic acid in water. Adjust with a 50% sodium hydroxide solution to a pH of 5.3 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (11:89)
Standard solution: 0.15 mg/mL of USP Pemetrexed Disodium RS in water
Sample solution: 0.15 mg/mL of Pemetrexed Disodium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pemetrexed disodium ($C_{20}H_{19}N_5Na_2O_6$) in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pemetrexed Disodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Pemetrexed Disodium in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer: 1.45 g/L of ammonium formate in water. Adjust with formic acid to a pH of 3.5 ± 0.1.
Solution A: Acetonitrile and *Buffer* (5:95)
Solution B: Acetonitrile and *Buffer* (30:70)
Mobile phase: See *Table 1*. [NOTE—After each injection, re-equilibrate the chromatographic system at the initial condition for a minimum of 13 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
45	0	100
47	100	0

System suitability stock solution: Prepare 3 mg/mL of USP Pemetrexed Disodium RS in 0.1 N sodium hydroxide. Heat this solution at 70° for 40 min.
[NOTE—The preparation degrades pemetrexed and generates the pemetrexed *R*-dimer and pemetrexed *S*-dimer as follows:
Pemetrexed *R*-dimer: (2*S*,2′*S*)-2,2′-{[(*R*)-2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′*H*,5*H*-5,6′-bipyrrolo[2,3-*d*]pyrimidine-5,5′-diyl] bis(ethylenebenzene-4,1-diylcarbonylimino)} diglutaric acid.
Pemetrexed *S*-dimer: (2*S*,2′*S*)-2,2′-{[(*S*)-2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′*H*,5*H*-5,6′-bipyrrolo[2,3-*d*]pyrimidine-5,5′-diyl] bis(ethylenebenzene-4,1-diylcarbonylimino)} diglutaric acid.]
System suitability solution: Transfer 1 mL of the *System suitability stock solution* to a 10-mL volumetric flask and dilute with water to volume.
Sensitivity solution: 0.1 µg/mL of USP Pemetrexed Disodium RS in water
Sample solution: 0.2 mg/mL of Pemetrexed Disodium in water. Do not sonicate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Autosampler temperature: 2°–8°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for the pemetrexed *R*-dimer and pemetrexed *S*-dimer peaks are 0.87 and 0.88, respectively.]
Suitability requirements
Peak-to-valley ratio: The ratio of the height of the pemetrexed *R*-dimer peak to the height of the valley between the pemetrexed *R*-dimer and pemetrexed *S*-dimer is NLT 1.5, *System suitability solution*.
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_T = total peak areas from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Methyl pemetrexed[a]	0.82	0.15

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Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pemetrexed glutamide[b]	0.90	0.15
Pemetrexed	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.60

[a] {4-[2-(2-Amino-1-methyl-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl) ethyl]benzoyl}-L-glutamic acid.
[b] {4-[2-(2-Amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl] benzoyl}-4-L-glutamyl-L-glutamic acid.

• **ENANTIOMERIC PURITY**
Buffer: Dissolve 8 g of anhydrous beta cyclodextrin in 1 L of water. Add 15 mL of triethylamine to this solution and mix. Add about 6 mL of phosphoric acid and adjust with additional phosphoric acid to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (5:95)
Standard solution: 0.24 mg/mL of USP Pemetrexed Disodium RS in water
Sensitivity solution: 0.12 µg/mL of USP Pemetrexed Disodium RS in water from the *Standard solution*
Sample solution: 0.24 mg/mL of Pemetrexed Disodium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 2°–8°
Column: 40°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—USP Pemetrexed Disodium RS contains a small amount of pemetrexed enantiomer disodium (disodium *N*-{*p*-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl] benzoyl}-D-glutamate). The relative retention times for pemetrexed enantiomer and pemetrexed are about 0.94 and 1.0, respectively.]
Suitability requirements
Peak-to-valley ratio: The ratio of the height of the pemetrexed enantiomer peak to the height of the valley between the pemetrexed enantiomer and pemetrexed is NLT 5.0, *Standard solution*
Signal-to-noise ratio: NLT 10 for the pemetrexed peak, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of pemetrexed enantiomer in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of pemetrexed enantiomer from the *Sample solution*
r_T = total peak areas of pemetrexed enantiomer and pemetrexed from the *Sample solution*

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia* ***or*** *Method Ic*: 19.5%–22.1%
• **PH** ⟨791⟩
Sample: 56 mg/mL in water
Acceptance criteria: 7.5–8.4
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains less than 0.17 USP Endotoxin Units/mg of pemetrexed.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Endotoxin RS
USP Pemetrexed Disodium RS

Pemetrexed for Injection

DEFINITION
Pemetrexed for Injection is a sterile, lyophilized mixture of pemetrexed disodium and suitable added substances. It contains NLT 90.0% and NMT 110.0% of the labeled amount of pemetrexed ($C_{20}H_{21}N_5O_6$).
[CAUTION—Handle pemetrexed disodium with great care as it alters genetic material and may be irritating to the eyes and skin.]

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Buffer: 0.17% (v/v) glacial acetic acid in water. Adjust with a 50% sodium hydroxide solution to a pH of 5.3.
Mobile phase: Acetonitrile and *Buffer* (11:89)
Standard solution: 0.14 mg/mL of USP Pemetrexed Disodium RS in water
Sample solution: Nominally equivalent to 0.1 mg/mL of pemetrexed in water, prepared from a composite of at least three vials of Pemetrexed for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm or diode array. [NOTE—Use a diode array detector to perform *Identification B*.]
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pemetrexed ($C_{20}H_{21}N_5O_6$) in the portion of Pemetrexed for Injection taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pemetrexed Disodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pemetrexed in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of pemetrexed, 427.42
M_{r2} = molecular weight of pemetrexed disodium (anhydrous), ▲471.38▲ (ERR 1-May-2018)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Weight Variation*: Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer: 0.17% (v/v) glacial acetic acid in water. Adjust with a 50% sodium hydroxide solution to a pH of 5.5.
Solution A: Acetonitrile and *Buffer* (3:97)
Solution B: Acetonitrile and *Buffer* (12.5: 87.5)
Mobile phase: See *Table 1*. [NOTE—After each injection, re-equilibrate the chromatographic system at the initial condition for a minimum of 8 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
45	0	100
47	100	0

System suitability stock solution: 2.8 mg/mL of USP Pemetrexed Disodium RS prepared as follows. Transfer USP Pemetrexed Disodium RS to a suitable volumetric flask and add a 3% hydrogen peroxide solution equivalent to 10% of the final volume. Dilute with water to volume. Mix and heat this solution at 75° for 2–5 h and allow it to come to room temperature. [NOTE—The solution preparation forms ketopemetrexed, pemetrexed *R*-dimer, and pemetrexed *S*-dimer. Pemetrexed *R*-dimer: (2*S*,2′*S*)-2,2′-{[(*R*)-2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′*H*,5*H*-5,6′-bipyrrolo[2,3-*d*]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)}diglutaric acid. Pemetrexed *S*-dimer: (2*S*,2′*S*)-2,2′-{[(*S*)-2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′*H*,5*H*-5,6′-bipyrrolo[2,3-*d*]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)}diglutaric acid.]
System suitability solution: Transfer 5 mL of the *System suitability stock solution* to a 50-mL volumetric flask and dilute with water to volume.
Sensitivity solution: 0.14 µg/mL of USP Pemetrexed Disodium RS in water
Sample solution: Nominally equivalent to 0.2 mg/mL of pemetrexed in water, prepared from 1 vial of Pemetrexed for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Temperatures
Autosampler: 2°–8°
Column: 35°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for the pemetrexed *R*-dimer and pemetrexed *S*-dimer peaks are 0.67 and 0.71, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the pemetrexed *R*-dimer and pemetrexed *S*-dimer peaks, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Pemetrexed for Injection taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_T = total peak areas from the *Sample solution*
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ketopemetrexed[a]	0.31	0.61	0.60
Pemetrexed	1.0	—	—
Any individual unspecified impurity	—	1.0	0.24
Total Impurities	—	—	1.30

[a] (4-{2-[(*RS*)-2-Amino-4,6-dioxo-4,5,6,7-tetrahydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl}benzoyl)-L-glutamic acid.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.17 USP Endotoxin Units/mg of pemetrexed
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **pH** ⟨791⟩

Sample: A constituted solution prepared as directed in the labeling
Acceptance criteria: 6.6–7.8

- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

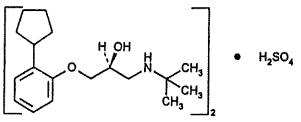
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Sterile solids packaging*. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Endotoxin RS
USP Pemetrexed Disodium RS

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Penbutolol Sulfate



$(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$ 680.94

2-Propanol, 1-(2-cyclopentylphenoxy)-3-[(1,1-dimethylethyl) amino]-, (*S*)-, sulfate (2:1) (salt).

(*S*)-1-(*tert*-Butylamino)-3-(*o*-cyclopentylphenoxy)-2-propanol sulfate (2:1) (salt) [38363-32-5].

» Penbutolol Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Penbutolol Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: A solution (10 mg per mL) responds to the tests for *Sulfate* ⟨191⟩.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Specific rotation ⟨781S⟩: between −22° and −26°, determined at 20°.

Test solution: 10 mg per mL, in methanol.

Chromatographic purity—

Organic phase—Prepare a mixture of methanol and acetonitrile (610:390). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Aqueous phase—Dissolve 11 g of sodium 1-heptanesulfonate in 1000 mL of water, add 5.0 mL of triethylamine, adjust with phosphoric acid to a pH of 2.70 ± 0.05, and filter through a filter having a porosity of 0.5 µm or finer. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Solvent mixture—Prepare a mixture of *Organic phase* and *Aqueous phase* (600:400).

Test solution—Transfer about 50 mg of Penbutolol Sulfate to a 25-mL volumetric flask, dissolve in and dilute with *Solvent mixture* to volume, and mix.

Diluted test solution—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 271-nm detector, a preinjection guard column that contains packing L4, and a 4.6-mm × 25-cm analytical column that contains packing L1 and is maintained at a constant temperature between ambient and 40°, and is programmed to provide variable mixtures of *Organic phase* and *Aqueous phase*. Before each injection, the system is equilibrated with a mobile phase consisting of a mixture of 60% *Organic phase* and 40% *Aqueous phase*. After each injection, this composition of the mobile phase is maintained for 15 minutes, then the proportion of *Organic phase* is increased linearly over the next 20 minutes so that the mobile phase consists of 80% *Organic phase* and 20% *Aqueous phase*. The proportion of *Organic phase* is then decreased to 60% over 1 minute. The flow rate is about 1 mL per minute. Chromatograph the *Diluted test solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the penbutolol peak and any impurity peak is not less than 1.5, and the tailing factor is not more than 2, when calculated by the formula:

$$W_{0.1}/2f$$

in which $W_{0.1}$ is the width of the peak at 10% of peak height.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Solvent mixture*, the *Test solution*, and the *Diluted test solution* into the chromatograph, and measure the peak responses for all the peaks. Calculate the percentage of each individual impurity in the Penbutolol Sulfate taken by the formula:

$$r_i/r_D$$

in which r_i is the peak response for an individual impurity in the chromatogram of the *Test solution*, and r_D is the penbutolol peak response obtained from the *Diluted test solution:* not more than 1.2% of any impurity is found.

Assay—

Organic phase—Prepare a mixture of methanol and acetonitrile (610:390). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Aqueous phase—Dissolve 11 g of sodium 1-heptanesulfonate in 1000 mL of water, add 5.0 mL of triethylamine, adjust with phosphoric acid to a pH of 2.70 ± 0.05, and filter through a filter having a porosity of 0.5 µm or finer. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Mobile phase—Prepare a mixture of *Organic phase* and *Aqueous phase* (650:350). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of 3,4-dimethylbenzophenone in *Mobile phase* containing about 0.01 mg per mL.

Standard preparation—Transfer about 24 mg of USP Penbutolol Sulfate RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix.

Assay preparation—Transfer about 24 mg of Penbutolol Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 271-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.9 for penbutolol and 1.0 for 3,4-dimethylbenzophenone, and the resolution, *R*, between the penbutolol peak and the 3,4-dimethylbenzophenone peak is not less than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in mg, of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$ in the portion of Penbutolol Sulfate taken by the formula:

USP Monographs

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$$100C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Penbutolol Sulfate RS in the *Standard preparation*, and R_U and R_S are the ratios of the penbutolol peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penbutolol Sulfate Tablets

» Penbutolol Sulfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Penbutolol Sulfate RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* ***197U***▲ (CN 1-May-2020)—
Solution: Sonicate a weighed portion of ground Tablets in sufficient methanol to obtain a solution containing about 0.4 mg of penbutolol sulfate per mL. Filter this solution, and dilute a portion of the filtrate with methanol to obtain a solution containing about 0.06 mg of penbutolol sulfate per mL.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Mobile phase—Dissolve 2 g of ammonium acetate in 250 mL of water, add 750 mL of acetonitrile, mix, and adjust with glacial acetic acid to a pH of 6.0. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP Penbutolol Sulfate RS quantitatively in water to obtain a stock solution having a known concentration of about 0.018 mg per mL. Mix 10.0 mL of this solution and 10.0 mL of acetonitrile, and filter through a filter having a 0.5-µm or finer porosity.
Test solution—Filter about 30 mL of the solution under test. Mix 10.0 mL of the filtrate and 10.0 mL of acetonitrile, and filter through a filter having a 0.5-µm or finer porosity.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 272-nm detector, a 4.6-mm × 15-cm column that contains 5-µm diameter packing L10. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.5%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and measure the areas of the responses for the penbutolol peaks. Calculate the quantity, in mg, of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$ dissolved by the formula:

$$1800C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Penbutolol Sulfate RS in the *Standard solution*, and r_U and r_S are the penbutolol peak responses obtained from the *Test solution* and the *Standard solution*, respectively.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$ is dissolved in 30 minutes.
Chromatographic purity—Examine the chromatogram of the *Assay preparation* obtained in the *Assay*. If an impurity peak is observed at a retention time of 0.8 relative to that of penbutolol, calculate the percentage of that impurity by the formula:

$$100r_i/r_s$$

in which r_i is the response of the impurity peak, and r_s is the sum of the responses of all of the peaks. If the percentage exceeds 1.2%, perform the following test.
Organic phase, Aqueous phase, Solvent mixture, and *Chromatographic system*—Proceed as directed in the test for *Chromatographic purity* under *Penbutolol Sulfate*.
Test solution—Transfer an accurately weighed portion of powdered Tablets, equivalent to about 100 mg of penbutolol sulfate, to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, mix, and filter.
Diluted test solution—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the test for *Chromatographic purity* under *Penbutolol Sulfate*. Calculate the percentage of each individual impurity in the portion of Tablets taken by the formula:

$$r_i/r_D$$

in which the terms are as defined therein: not more than 1.2% of any impurity is found.

Assay—
Organic phase , *Aqueous phase,* and *Mobile phase*—Proceed as directed in the *Assay* under *Penbutolol Sulfate*.
Standard preparation—Dissolve an accurately weighed quantity of USP Penbutolol Sulfate RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.
Resolution solution—Prepare a solution of 3,4-dimethyl benzophenone in *Standard preparation* containing about 0.01 mg of 3,4-dimethylbenzophenone per mL.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of penbutolol sulfate, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate for about 10 minutes, and filter a portion through a filter having a 0.5-µm or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 15-cm column that contains 5-µm diameter packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for penbutolol and 1.0 for 3,4-dimethylbenzophenone, and the tailing factor is not more than 1.4, when calculated by the formula:

$$W_{0.1}/2f$$

in which $W_{0.1}$ is the width of the peak at 10% of peak height, the resolution, R, between the penbutolol peak and the 3,4-dimethylbenzophenone peak is not less than 5, and the relative standard deviation for replicate injections is not more than 2.0%.

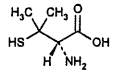
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Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$ in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Penbutolol Sulfate RS in the *Standard preparation*, and r_U and r_S are the penbutolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillamine



$C_5H_{11}NO_2S$ 149.21

D-Valine, 3-mercapto-.

D-3-Mercaptovaline [52-67-5].

» Penicillamine contains not less than 97.0 percent and not more than 102.0 percent of $C_5H_{11}NO_2S$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Penicillamine RS
USP Penicillin G Potassium RS
USP Penicillamine Disulfide RS
$C_{10}H_{20}N_2O_4S_2$

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020) (50 mg in 300 mg).

B: Dissolve 10 mg in 5 mL of water, and add 1 drop of 5 N sodium hydroxide and 20 mg of ninhydrin: a blue or violet-blue color is produced immediately.

C: Dissolve 20 mg in 4 mL of water, add 2 mL of phosphotungstic acid solution (1 in 10), and heat nearly to boiling: a deep blue color is produced immediately.

Specific rotation ⟨781S⟩: between −60.5° and −64.5°.

Test solution: 50 mg per mL, in 1.0 N sodium hydroxide.

pH ⟨791⟩: between 4.5 and 5.5, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Limit of penicillin activity—

pH 2.5 Buffer—Dissolve 100 g of monobasic potassium phosphate in water, add 0.2 mL of hydrochloric acid, dilute with water to 1000 mL, and mix. Adjust, if necessary, with phosphoric acid or with 10 N potassium hydroxide to a pH of 2.5.

Standard preparation—Prepare as directed for Penicillin G in *Table 1* under *Antibiotics—Microbial Assays* ⟨81⟩, except to prepare a final stock solution containing 100 Penicillin G Units per mL and six test dilutions ranging from 0.005 Penicillin G Unit per mL to 0.2 Penicillin G Unit per mL, and to use a median dose of the Standard of 0.050 Penicillin G Unit per mL.

Test preparation—Dissolve 1.0 g in water to make 18.0 mL, transfer 9.0 mL of this solution to a separator, add 20 mL of amyl acetate and 1 mL of *pH 2.5 Buffer*, and shake. Allow the layers to separate, and draw off the aqueous layer into a second separator, retaining the amyl acetate extract in the first separator. Check the pH of the aqueous layer, and if it is greater than 3.0 adjust it with hydrochloric acid to a pH of 2.5, and extract with 20 mL of amyl acetate. Discard the aqueous layer, and add the amyl acetate extract to the first separator. Wash the combined amyl acetate extracts with 10 mL of diluted *pH 2.5 Buffer* (1 in 10), and discard the aqueous layer. Extract the amyl acetate with 10.0 mL of *Buffer B.1* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers*). Use a portion of the buffer extract as *Test solution A*. To a 5-mL portion of the extract add 0.1 mL of penicillinase solution, and incubate at 36° to 37.5° for 60 minutes (*Test solution B*).

Preparation of inoculum—Prepare as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using *Micrococcus luteus* (ATCC 9341) as the test organism, and an inoculum that gives clear sharp zones of inhibition 17 mm to 21 mm in diameter with the median dose level of the Standard.

Procedure—Proceed as directed for the *Cylinder-Plate Method* under *Antibiotics—Microbial Assays* ⟨81⟩, using 10 mL of Medium 1 for the base layer and 4 mL of inoculated Medium 4 for the seed layer, and incubating the plates at 29° to 31°, except on each test plate to fill 2 cylinders with *Test solution A*, 2 cylinders with *Test solution B*, and 2 cylinders with the median dose of the Standard. If *Test solution A* yields no zone of inhibition, the test is negative for penicillin. If *Test solution A* yields a zone of inhibition and *Test solution B* does not, penicillin is present. Determine its level from the standard curve: not more than 0.01 Penicillin G Unit is found in each mL of *Test solution A* (0.2 Penicillin G Unit per g).

Mercury—

[NOTE—Mercuric dithizonate is light-sensitive. Perform this test in subdued light.]

Dithizone stock solution—Dissolve 40 mg of dithizone in 1000 mL of chloroform.

Dithizone titrant—Dilute 30.0 mL of *Dithizone stock solution* with chloroform to 100.0 mL. This solution contains approximately 12 mg of dithizone per L.

Standard solution—Transfer 135.4 mg of mercuric chloride to a 100-mL volumetric flask, add 0.25 N sulfuric acid to volume, and mix. This solution contains the equivalent of 100 mg of Hg in 100 mL.

Diluted standard solution—Pipet 2 mL of *Standard solution* into a 100-mL volumetric flask, add 0.25 N sulfuric acid to volume, and mix. Each mL of this solution contains the equivalent of 20 µg of Hg.

Standardization—Pipet 1 mL of *Diluted standard solution* into a 250-mL separator, and add 100 mL of 0.25 N sulfuric acid, 90 mL of water, and 10 mL of hydroxylamine hydrochloride solution (1 in 5). Then add 1 mL of edetate disodium solution (1 in 50), 1 mL of glacial acetic acid, and 5 mL of chloroform, shake for 1 minute, allow to separate, and discard the chloroform layer. To the solution add *Dithizone titrant*, in portions of 0.3 mL to 0.5 mL, from a 10-mL buret. After each addition, shake the mixture 20 times, and allow the chloroform layer to separate and discard it. Continue until an addition of *Dithizone titrant* remains green after the shaking. Calculate the quantity, in µg, of mercury equivalent to 1 mL

Supplement Two

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of *Dithizone titrant* by dividing 20 by the number of mL of *Dithizone titrant* added.

Procedure—Transfer 500 mg of Penicillamine to a 650-mL Kjeldahl flask containing a few glass beads, incline the flask at an angle of about 45°, and add 2.5 mL of nitric acid through a small funnel placed in the mouth of the flask. Allow the mixture to stand at room temperature until nitrous oxide fumes are evolved and vigorous reaction subsides (5 to 30 minutes). Add 2.5 mL of sulfuric acid through the funnel, and heat, gently at first and then to the production of fumes of sulfur trioxide, then cool. Cautiously add 2.5 mL of nitric acid, again heat to the production of sulfur trioxide fumes, and cool. Repeat the treatment with nitric acid and heat, then cool, and cautiously add 50 mL of water, rinsing the funnel and collecting the rinsings in the flask. Remove the funnel, boil the solution down to approximately half its volume (about 25 mL), and cool to room temperature. Transfer to a 250-mL separator with the aid of water, and add water to make about 50 mL. Add 1 mL of edetate disodium solution (1 in 50) and 1 mL of glacial acetic acid, and extract with small portions of chloroform until the last chloroform extract remains colorless. Discard the chloroform extract, and add 50 mL of 0.25 N sulfuric acid, 90 mL of water, and 10 mL of hydroxylamine hydrochloride solution (1 in 5). Add *Dithizone titrant*, in portions of 0.3 mL to 0.5 mL, from a 10-mL buret. After each addition, shake the mixture 20 times, and allow the chloroform layer to separate and discard it. Continue until an addition of *Dithizone titrant* remains green after the shaking. Calculate the amount of mercury present: the limit is 10 µg (0.002%).

Limit of penicillamine disulfide—

Diluent , *Mobile phase*, and *Resolution solution*—Prepare as directed in the *Assay*.

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine Disulfide RS in *Diluent* to obtain a solution having a known concentration of about 0.025 mg per mL.

Test preparation—Use the *Assay preparation*.

Chromatographic system—Proceed as directed in the *Assay*. Chromatograph the *Standard preparation*, and record the penicillamine disulfide peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the penicillamine disulfide peaks. Calculate the percentage of penicillamine disulfide ($C_{10}H_{20}N_2O_4S_2$) in the Penicillamine taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Penicillamine Disulfide RS in the *Standard preparation*, C_U is the concentration, in mg per mL, of Penicillamine in the *Test preparation*, and r_U and r_S are the penicillamine disulfide peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 1.0% of penicillamine disulfide is found.

Assay—

Diluent—Dissolve 1.0 g of edetate disodium in water to make 1000 mL of solution.

Mobile phase—Dissolve 6.9 g of monobasic sodium phosphate and 0.20 g of sodium 1-hexanesulfonate in water to make 1000 mL of solution. Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and filter through a suitable filter of 1 µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Prepare a solution in *Diluent* containing about 1 mg of USP Penicillamine RS and 0.1 mg of USP Penicillamine Disulfide RS per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine RS in *Diluent* to obtain a solution having a concentration of about 1.25 mg per mL.

Assay preparation—Transfer about 125 mg of Penicillamine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 1.6 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* the relative retention times are about 0.7 for penicillamine and 1.0 for penicillamine disulfide, and the resolution, *R*, between the penicillamine peak and the penicillamine disulfide peak is not less than 3.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of penicillamine ($C_5H_{11}NO_2S$) in the portion of Penicillamine taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Penicillamine RS in the *Standard preparation*, and r_U and r_S are the penicillamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillamine Capsules

DEFINITION

Penicillamine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of penicillamine ($C_5H_{11}NO_2S$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 100 mg of USP Penicillamine RS in 10 mL of methanol. Add 2 drops of 3 N hydrochloric acid and mix.

Sample solution: Transfer a portion of Capsule contents, containing nominally about 100 mg of penicillamine, to a 10-mL volumetric flask, and dilute with methanol to volume. Add 2 drops of 3 N hydrochloric acid, mix and filter. Use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, heated at 105° for 30 min, and allowed to cool before use

Application volume: 10 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (8:2:2)

Spray reagent: 3-mg/mL solution of ninhydrin in dehydrated alcohol

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Analysis
Samples: *Standard solution* and *Sample solution*
Separately apply the *Sample solution* and the *Standard solution* to the plate. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate, mark the solvent front, allow the solvent to evaporate, and place the plate in an atmosphere of iodine vapors. After a few minutes, spray the plate with *Spray reagent*, heat it at 105° for 10 min, allow it to cool, and examine it.
Acceptance criteria: The R_F values, colors, and intensities of the principal spots from the *Sample solution* correspond to those from the *Standard solution*.

• **B. PROCEDURE**
Solution A: 100 mg/mL of phosphotungstic acid in water
Sample solution: Dissolve a portion of Capsule contents, containing nominally about 20 mg of penicillamine, in 4 mL of water.
Analysis: To the *Sample solution*, add 2 mL of *Solution A* and heat nearly to boiling.
Acceptance criteria: A deep blue color is produced immediately.

ASSAY

• **PROCEDURE**
Mobile phase: 6.9 g/L of monobasic sodium phosphate and 0.2 g/L of sodium 1-hexanesulfonate in water. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Diluent: 1.0 g/L of edetate disodium in water
System suitability solution: 1 mg/mL of USP Penicillamine RS and 0.1 mg/mL of USP Penicillamine Disulfide RS in *Diluent*
Standard solution: 1.25 mg/mL of USP Penicillamine RS in *Diluent*
Sample solution: Nominally equivalent to 1.25 mg/mL of penicillamine in *Diluent* prepared as follows. Transfer the contents of NLT 10 Capsules to a suitable volumetric flask. Add the empty Capsule shells to the flask, and add sufficient *Diluent* to the flask to fill it to three-fourths of its capacity. Shake for 1 min, and allow the mixture to stand for 90 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 1-µm or finer porosity, and use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.6 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for penicillamine and penicillamine disulfide are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between penicillamine and penicillamine disulfide, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of penicillamine ($C_5H_{11}NO_2S$) in portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of penicillamine from the *Sample solution*
r_S = peak response of penicillamine from the *Standard solution*
C_S = concentration of USP Penicillamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of penicillamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Procedure for a pooled sample
Dilute hydrochloric acid: Dilute 37 mL of hydrochloric acid with water to 1 L.
Dilute sulfuric acid: Dilute 1 mL of sulfuric acid with water to 50 mL.
Ammonium sulfamate reagent: 2.5 mg/mL of ammonium sulfamate in *Dilute hydrochloric acid*
***N*-(1-Naphthyl)ethylenediamine dihydrochloride reagent:** 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in *Dilute hydrochloric acid*
Sulfanilamide–mercuric chloride reagent: 1 mg/mL of sulfanilamide and 1 mg/mL of mercuric chloride in *Dilute hydrochloric acid*
Sodium nitrite reagent: 2 mg/mL of sodium nitrite in *Dilute sulfuric acid*. Prepare fresh.
Standard solution: 250 µg/mL of USP Penicillamine RS in 0.1 N hydrochloric acid
Sample solution: Withdraw a portion of the solution under test, containing nominally about 278 µg of penicillamine, and pass through a suitable filter.
Blank: Volume of 0.1 N hydrochloric acid equivalent to a volume of the *Sample solution*
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 540 nm
Cell: 1 cm
Analysis: Pipet the *Sample solution* into a 100-mL volumetric flask. Into a similar flask, transfer the reagent *Blank*, and into a third 100-mL volumetric flask, pipet 1 mL of *Standard solution*. Treat each flask as follows. Add by pipet 3 mL of *Sodium nitrite reagent*, and mix by swirling occasionally. After 5 min, add 10 mL of *Ammonium sulfamate reagent*, swirl, and allow to stand for an additional 5 min. Add 5 mL of *Sulfanilamide–mercuric chloride reagent*, swirl, and immediately add 10 mL of *N-(1-Naphthyl)ethylenediamine dihydrochloride reagent*. Dilute with water to volume and mix. Determine the absorbances of both solutions against the *Blank*.
Calculate the percentage of labeled amount of penicillamine ($C_5H_{11}NO_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Penicillamine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of penicillamine in the *Sample solution* (µg/mL)
V = volume of the Medium, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (*Q*) of the labeled amount of penicillamine ($C_5H_{11}NO_2S$) is dissolved.

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Procedure for a unit sample
Buffer solution: 50 mM solution of monobasic potassium phosphate buffer, pH 3.0
Mobile phase: Methanol and *Buffer solution* (3:97)
System suitability solution: 0.002 mg/mL of USP Penicillamine Disulfide RS in 0.1 N hydrochloric acid
Sample solution: Proceed as directed in *Dissolution* ⟨711⟩, *Procedure*. After 30 min, withdraw 10 mL of solution from each vessel, and immediately pass each aliquot through a 0.45-µm polyvinylidene difluoride filter paper. Discard the first 2 mL of filtered solution, and chromatograph the remaining filtrate.
Standard solution: USP Penicillamine RS in 0.1 N hydrochloric acid at a concentration similar to *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 30 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between penicillamine and penicillamine disulfide, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of penicillamine ($C_5H_{11}NO_2S$) released:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times V \times (1/L) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Penicillamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of in the *Sample solution* (mg/mL)
V = volume of Medium, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of penicillamine ($C_5H_{11}NO_2S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **LIMIT OF PENICILLAMINE DISULFIDE**
Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.025 mg/mL of USP Penicillamine Disulfide RS in *Diluent*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for penicillamine and penicillamine disulfide are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between penicillamine and penicillamine disulfide, *System suitability solution*
Relative standard deviation: NMT 2.0% for penicillamine disulfide, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of penicillamine disulfide ($C_{10}H_{20}N_2O_4S_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of penicillamine disulfide from the *Sample solution*
r_S = peak area of penicillamine disulfide from the *Standard solution*
C_S = concentration of USP Penicillamine Disulfide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of penicillamine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS

Delete the following:

▲• **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg of Capsule contents.
Analysis: Dry *Sample* in a capillary-stoppered bottle in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
Acceptance criteria: it loses NMT 1.0% of its weight. ▲ (RB 11-Jun-2019)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Penicillamine RS
USP Penicillamine Disulfide RS
$C_{10}H_{20}N_2O_4S_2$

Penicillamine Tablets

» Penicillamine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_5H_{11}NO_2S$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Penicillamine RS
USP Penicillamine Disulfide RS
$C_{10}H_{20}N_2O_4S_2$

Identification—
A: Transfer a portion of finely powdered Tablets, equivalent to about 100 mg of penicillamine, to a 10-mL volumetric flask, dilute with methanol to volume, add 2 drops of 3 N hydrochloric acid, mix, and filter. Use the filtrate as the test solution. Prepare a Standard solution by dissolving 100 mg of USP Penicillamine RS in 10 mL of methanol, adding 2 drops of 3 N hydrochloric acid, and mixing. Separately apply 10-µL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, heated at 105° for 30 minutes, and allowed to cool before use. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butyl alcohol, glacial acetic acid, and water (8:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, mark the solvent front, allow the solvent to evaporate, and place the plate in an atmosphere of iodine vapors. After a few

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minutes, spray the plate with a 1 in 300 solution of ninhydrin in dehydrated alcohol, heat it at 105° for about 10 minutes, allow it to cool, and examine it: the R_F values, colors, and intensities of the principal spots obtained from the test solution correspond to those obtained from the Standard solution.

B: A portion of powdered Tablets responds to *Identification* test *C* under *Penicillamine.*

Dissolution ⟨711⟩—

Medium: 0.5% edetate disodium and 0.05% sodium lauryl sulfate solution; 900 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

Mobile phase—Prepare a filtered and degassed solution of 0.01 M dibasic sodium phosphate and 0.01 M monobasic potassium phosphate (60:40). If necessary, adjust the solution by the addition of 0.01 M dibasic sodium phosphate or 0.01 M monobasic potassium phosphate to a pH of 7.0 ± 0.1.

Standard solution—Prepare a solution of USP Penicillamine RS in 0.5% edetate disodium and 0.05% sodium lauryl sulfate solution having an accurately known concentration of about 0.28 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%, and the resolution factor between the solvent peak and penicillamine is not less than 1.5.

Procedure—Separately inject equal volumes (about 80 µL) of the *Standard solution* and a filtered portion of the solution under test into the chromatograph, record the chromatograms, measure the responses for the major peaks, and calculate the amount of $C_5H_{11}NO_2S$ dissolved per Tablet.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_5H_{11}NO_2S$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Loss on drying ⟨731⟩—Dry about 100 mg of finely ground Tablets, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.

Penicillamine disulfide—

Diluent—Prepare as directed in the *Assay.*

Mobile phase, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Penicillamine.*

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine Disulfide RS in *Diluent* to obtain a solution having a known concentration of about 0.025 mg per mL.

Test preparation—Use the *Assay preparation.*

Chromatographic system—Proceed as directed in the *Assay* under *Penicillamine.* Chromatograph the *Standard preparation*, and record the penicillamine disulfide peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the penicillamine disulfide peaks. Calculate the percentage of penicillamine disulfide ($C_{10}H_{20}N_2O_4S_2$) in the portion of Tablets taken by the formula:

$$20{,}000(C/L)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Penicillamine Disulfide RS in the *Standard preparation*, *L* is the quantity, in mg, of penicillamine in each Tablet based on the labeled amount, and r_U and r_S are the penicillamine disulfide peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 3.0% of penicillamine disulfide is found.

Assay—

Diluent—Dissolve 5.0 g of edetate disodium in water to make 1000 mL of solution.

Mobile phase, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Penicillamine.*

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine RS in *Diluent* to obtain a solution having a known concentration of about 1.25 mg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of penicillamine, to a 200-mL volumetric flask, add about 150 mL of *Diluent*, shake for 5 minutes, and allow the mixture to stand for 90 minutes. Dilute with *Diluent* to volume, and mix. Filter a portion of this solution through a suitable filter of 1 µm or finer porosity, and use the clear filtrate as the *Assay preparation.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Penicillamine.* Calculate the quantity, in mg, of penicillamine ($C_5H_{11}NO_2S$) in the portion of Tablets taken by the formula:

$$200C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Penicillamine RS in the *Standard preparation*, and r_U and r_S are the penicillamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillin G, Neomycin, Polymyxin B, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate Topical Suspension

» Penicillin G, Neomycin, Polymyxin B, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate Topical Suspension is a suspension of Penicillin G Procaine, Neomycin Sulfate, Polymyxin B Sulfate, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate in a suitable vehicle. It contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of neomycin, penicillin G, and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of hydrocortisone acetate ($C_{23}H_{32}O_6$) and hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label Topical Suspension to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Hydrocortisone Acetate RS

USP Hydrocortisone Hemisuccinate RS

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USP Neomycin Sulfate RS
USP Penicillin G Potassium RS
USP Polymyxin B Sulfate RS

Identification—

A: Shake a quantity of Topical Suspension, equivalent to about 50,000 USP Polymyxin B Units, with 20 mL of chloroform, add 5 mL of 0.1 N hydrochloric acid, shake vigorously, centrifuge, and use the clear supernatant liquid as the *Sample solution.* Apply separately 10 µL of the *Sample solution* and 10 µL of a *Standard solution* of USP Polymyxin B Sulfate RS in 0.1 N hydrochloric acid containing 10,000 USP Polymyxin B Units per mL to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of dimethylsilanized chromatographic silica gel mixture (see *Chromatography* ⟨621⟩). Place the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of isopropyl alcohol, water, and ammonium hydroxide (24:17:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 105° for 5 minutes. Spray the plate with a 1 in 200 solution of ninhydrin in butyl alcohol, and heat the plate at 105° for 15 minutes: the R_f value of the principal spot in the chromatogram obtained from the *Sample solution* corresponds to that of the principal spot in the chromatogram obtained from the *Standard solution.*

B: It responds to the *Identification* test under *Penicillin G Procaine Intramammary Infusion.*

C: The chromatogram of the *Assay preparation* obtained as directed in the *Assay for hydrocortisone acetate and hydrocortisone sodium succinate* exhibits major peaks for hydrocortisone acetate and hydrocortisone sodium succinate, the retention times of which, relative to that of the internal standard, correspond to those for hydrocortisone acetate and hydrocortisone hemisuccinate exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for hydrocortisone acetate and hydrocortisone sodium succinate.*

Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for penicillin G—Proceed with the Topical Suspension as directed in the *Assay for penicillin G* under *Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension.*

Assay for neomycin—Proceed with the Topical Suspension as directed in the *Assay for neomycin* under *Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension.*

Assay for polymyxin B—Proceed with the Topical Suspension as directed in the *Assay for polymyxin B* under *Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension.*

Assay for hydrocortisone acetate and hydrocortisone sodium succinate—

Mobile phase—Prepare a filtered and degassed mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (544:544:58:29:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Extraction solution—Prepare a mixture of chloroform and glacial acetic acid (1000:30).

Internal standard solution—Prepare a solution in tetrahydrofuran containing about 1.4 mg of methylprednisolone per mL.

Standard preparation—Transfer about 7.5 mg of USP Hydrocortisone Acetate RS, accurately weighed, and 7.5*J* mg of USP Hydrocortisone Hemisuccinate RS, accurately weighed, to a conical flask, *J* being the ratio of the labeled amount, in mg, of hydrocortisone sodium succinate to the labeled amount, in mg, of hydrocortisone acetate in the Topical Suspension. Add 5.0 mL of *Internal standard solution* and about 95 mL of *Extraction solution,* and mix.

Resolution solution—Dissolve about 3.7 mg of penicillin G procaine in 10 mL of *Standard preparation.*

Assay preparation—Transfer an accurately measured portion of well-mixed Topical Suspension, equivalent to about 7.5 mg of hydrocortisone acetate, to a conical flask. Add 5.0 mL of *Internal standard solution* and about 95 mL of *Extraction solution,* and shake by mechanical means for about 15 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, a guard column containing packing L3, and a 3.9-mm × 30-cm analytical column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution,* and measure the peak responses as directed for *Procedure:* the relative retention times for penicillin G procaine, hydrocortisone acetate, hydrocortisone hemisuccinate, and methylprednisone are about 0.3, 0.4, 0.7, and 1.0, respectively, and the resolution, *R,* between penicillin G procaine and hydrocortisone acetate is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation of the ratios of the hydrocortisone acetate peak to the internal standard peak and the hydrocortisone sodium succinate peak to the internal standard peak is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydrocortisone acetate in the portion of Topical Suspension taken by the formula:

$$W(R_U/R_S)$$

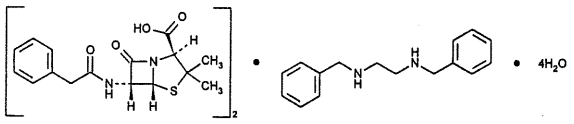
in which W is the quantity, in mg, of USP Hydrocortisone Acetate RS taken to prepare the *Standard preparation,* and R_U and R_S are the ratios of the hydrocortisone acetate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the quantity, in mg, of hydrocortisone sodium succinate in the portion of Topical Suspension taken by the formula:

$$(484.52/462.54)(W)(R_U/R_S)$$

in which 484.52 and 462.54 are the molecular weights of hydrocortisone sodium succinate and anhydrous hydrocortisone hemisuccinate, respectively, W is the quantity, in mg, of USP Hydrocortisone Hemisuccinate RS taken to prepare the *Standard preparation,* and R_U and R_S are the ratios of the hydrocortisone hemisuccinate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Penicillin G Benzathine



$(C_{16}H_{18}N_2O_4S)_2 \cdot C_{16}H_{20}N_2 \cdot 4H_2O$ 981.18

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino-], 2[*S*-(2α,5α,6β)]-, compd. with *N,N′*-bis(phenylmethyl)-1,2-ethanediamine (2:1), tetrahydrate.

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N,N′*-dibenzylethylenediamine (2:1), tetrahydrate [41372-02-5].

Anhydrous 909.15

[1538-09-6].

» Penicillin G Benzathine has a potency of not less than 1090 Penicillin G Units and not more than 1272 Penicillin G Units per mg.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 500 µg per mL.
Medium: methanol.
Absorptivity at 263 nm is between 85.0% and 110.0% of that of USP Penicillin G Benzathine RS.

Crystallinity ⟨695⟩: meets the requirements.

Bacterial Endotoxins Test ⟨85⟩ —Where the label states that Penicillin G Benzathine is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms it contains not more than 0.01 USP Endotoxin Unit per 100 Penicillin G Units.

Sterility Tests ⟨71⟩ —Where the label states that Penicillin G Benzathine is sterile it meets the requirements when tested as directed in the section *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium and Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the penicillin G in each tube, and to shake the vessels once daily.

pH ⟨791⟩: between 4.0 and 6.5, in a solution prepared by dissolving 50 mg in 50 mL of dehydrated alcohol, adding 50 mL of water, and mixing.

Water Determination, *Method I* ⟨921⟩: between 5.0% and 8.0%.

Benzathine content—To about 1 g of Pencillin G Benzathine, accurately weighed, add 30 mL of a saturated solution of sodium chloride and 10 mL of 5 N sodium hydroxide, and extract with four 50-mL portions of ether. Wash the combined ether extracts with three 10-mL portions of water. Extract the combined water washings with 25 mL of ether, and add the ether extract to the water-washed combined ether extracts. Evaporate this combined ether solution to a volume of about 5 mL, add 2 mL of dehydrated alcohol, and evaporate to dryness. Dissolve the residue in 50 mL of glacial acetic acid, add 1 mL of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 12.02 mg of benzathine ($C_{16}H_{20}N_2$): between 24.0% and 27.0% of benzathine in Penicillin G Benzathine, calculated on the anhydrous basis, is found.

Assay—

0.05 M phosphate buffer, pH 6.0—Dissolve 6.8 g of monobasic potassium phosphate in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 6.0, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a mixture of *0.05 M phosphate buffer, pH 6.0* and acetonitrile (4:1), pass through a membrane filter having a 5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 40 mg of USP Penicillin G Potassium RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile and 5 mL of methanol, and swirl to dissolve. Without delay, dilute with *0.05 M phosphate buffer, pH 6.0* to volume, and mix.

System suitability preparation—Prepare a solution of penicillin V potassium in *Mobile phase* containing about 1 mg per mL. Mix equal volumes of this solution and the *Standard preparation*.

Assay preparation—Transfer about 53 mg of Penicillin G Benzathine, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile and 5 mL of methanol, and swirl to dissolve. Without delay, dilute with *0.05 M phosphate buffer, pH 6.0* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for penicillin G and 1.0 for penicillin V; the resolution, *R*, between penicillin G and penicillin V is not less than 2.0; the column efficiency determined from the analyte peak is not less than 600 theoretical plates; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in Penicillin G Units per mg, of the Penicillin G Benzathine taken by the formula:

$$50(CP/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Penicillin G Potassium RS in the *Standard preparation; P* is the stated potency, in Penicillin G Units per mg, of USP Penicillin G Potassium RS; *W* is the quantity, in mg, of Penicillin G Benzathine taken to prepare the *Assay preparation;* and r_U and r_S are the penicillin G peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

USP Monographs

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Penicillin G Benzathine Injectable Suspension

» Penicillin G Benzathine Injectable Suspension is a sterile suspension of Penicillin G Benzathine in Water for Injection with one or more suitable buffers, dispersants, preservatives, and suspending agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of penicillin.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass, in a refrigerator.
USP Reference standards ⟨11⟩—
USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS
Identification—It responds to the *Identification* test under *Penicillin G Benzathine Oral Suspension.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.01 Endotoxin Unit per 100 Penicillin G Units.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined,* except to use Fluid Thioglycollate Medium and Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the penicillin G in each vessel, and to shake the vessels once daily.
pH ⟨791⟩: between 5.0 and 7.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.
Assay preparation—Using a suitable hypodermic needle and syringe, withdraw an accurately measured volume of Injectable Suspension, equivalent to about 300,000 Penicillin G Units, and dilute quantitatively with 1.0 N sodium hydroxide to obtain an *Assay preparation* containing about 2000 Penicillin G Units per mL. Pipet 2.0 mL of this solution into a glass-stoppered, 125-mL conical flask.
Blank preparation—Using a suitable hypodermic needle and syringe, withdraw an accurately measured volume of Injectable Suspension, equivalent to about 300,000 Penicillin G Units, and quantitatively dilute with *Buffer B.1* to obtain a suspension containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in performing the *Inactivation and Titration* to omit the addition of 1.0 N sodium hydroxide to the *Assay preparation,* and in performing the *Blank Determination* to use the *Blank preparation* in place of the *Assay preparation.* Calculate the quantity, in Penicillin G Units, in each mL of the Injectable Suspension taken by the formula:

$$(L/2D)(F)(B - I)$$

in which L is the labeled quantity, in Penicillin G Units per mL, in the Injectable Suspension taken, and D is the concentration, in Penicillin G Units per mL, in the *Assay preparation* on the basis of the labeled quantity in the Injectable Suspension and the extent of dilution, and the other terms are as defined therein.

Penicillin G Benzathine Oral Suspension

» Penicillin G Benzathine Oral Suspension contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of penicillin G. It contains one or more suitable buffers, colors, dispersants, flavors, and preservatives.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS
Identification—Mix a portion of it with methanol to obtain a solution containing about 3000 Penicillin G Units per mL. Apply 20 µL of this test solution and 20 µL of a Standard solution of USP Penicillin G Benzathine RS in methanol containing 2.5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel, and allow the spots to dry. Using an unlined developing chamber, develop the chromatogram in a solvent system consisting of a mixture of methanol, acetonitrile, and ammonium hydroxide (70:30:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow to air-dry. Spray the plate uniformly with a spray reagent prepared as follows. Dissolve 20 g of tartaric acid and 1.7 g of bismuth subnitrate in 80 mL of water. Add 2.5 mL of this solution, 2.5 mL of potassium iodide solution (4 in 10), and 10 g of tartaric acid to 50 mL of water, and mix. Examine the chromatograms: the principal spot obtained from the test solution corresponds in R_f value to that obtained from the Standard solution.

Uniformity of dosage units ⟨905⟩—
FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS : meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 6.0 and 7.0.

Assay—
Standard preparation—Using Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.
Assay preparation—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with 1.0 N sodium hydroxide to obtain a solution having a concentration of about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.
Blank preparation—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with *Buffer B.1* to obtain a suspension containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in performing the *Inactivation and titration* to omit the addition of 1.0 N sodium hydroxide to the *Assay preparation,* and in performing the *Blank determination* to use the *Blank preparation* in place of the *Assay preparation.* Calculate the quantity, in Penicillin G Units, in each mL of the Oral Suspension taken by the formula:

$$(L/2D)(F)(B - I)$$

in which L is the labeled quantity, in Penicillin G Units per mL, in the Oral Suspension taken, and D is the concentration, in Penicillin G Units per mL, of the *Assay preparation* on the basis

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of the labeled quantity in the Oral Suspension and the extent of dilution.

Penicillin G Benzathine Tablets

» Penicillin G Benzathine Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of penicillin G.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS
Identification—Shake a suitable quantity of finely powdered Tablets with methanol to obtain a solution containing about 3000 Penicillin G Units per mL, and filter: the filtrate so obtained responds to the *Identification* test under *Penicillin G Benzathine Oral Suspension*.
Disintegration ⟨701⟩: 60 minutes, simulated gastric fluid TS being used in place of water as the test medium.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 8.0%.

Assay—
Standard preparation—Using Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer a portion of the powder, accurately weighed, equivalent to about 200,000 Penicillin G Units, to a 100-mL volumetric flask, add 10 mL of 1.0 N sodium hydroxide, and mix. Allow to stand for 15 minutes, add 10 mL of 1.2 N hydrochloric acid, dilute with water to volume, and mix. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.
Blank preparation—Transfer an accurately weighed portion of the powdered Tablets remaining from the preparation of the *Assay preparation*, equivalent to about 200,000 Penicillin G Units, to a 100-mL volumetric flask, dilute with *Buffer B.1* to volume, and mix. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in performing the *Inactivation and titration* to omit the addition of 1.0 N sodium hydroxide and 1.2 N hydrochloric acid, and in the *Blank determination* to use the *Blank preparation* in place of the *Assay preparation*. Calculate the quantity, in Penicillin G Units, in the portion of Tablets taken by the formula:

$$(T/2D)(F)(B - I)$$

in which T is the labeled quantity, in Penicillin G Units, in each Tablet, and D is the concentration, in Penicillin G Units per mL, of the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution.

Penicillin G Benzathine and Penicillin G Procaine Injectable Suspension

» Penicillin G Benzathine and Penicillin G Procaine Injectable Suspension is a sterile suspension of Penicillin G Benzathine and Penicillin G Procaine or, when labeled for veterinary use only, of Penicillin G Benzathine and penicillin G procaine, in Water for Injection. It may contain one or more suitable buffers, preservatives, and suspending agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of penicillin G benzathine and penicillin G procaine.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.
Labeling—Where it is intended for veterinary use only, it is so labeled.
USP Reference standards ⟨11⟩—
USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS
USP Procaine Hydrochloride RS

Identification—
A: It responds to the *Identification* test under *Penicillin G Benzathine Oral Suspension*: the spot obtained from the test solution, corresponding in R_F value to that obtained from the Standard solution, is completely resolved from a second spot, produced by penicillin G procaine.
B: It responds to the *Identification* test under *Penicillin G Procaine*.
Crystallinity ⟨695⟩ (where it is prepared from penicillin G procaine and is labeled as intended for veterinary use only)—Dilute a portion of the Injectable Suspension, equivalent to about 300,000 Penicillin G Units, with water to obtain a volume of 10 mL, and centrifuge. Remove and discard the supernatant fluid. Resuspend the residue in 10 mL of water, and centrifuge. Remove and discard the supernatant fluid. Dry the residue in a vacuum desiccator. The dried residue meets the requirements.
pH ⟨791⟩: between 5.0 and 7.5.

Limit of soluble penicillin G and procaine (where it is prepared from penicillin G procaine and is labeled for veterinary use only)—
Mobile phase—Dissolve 4 g of sodium 1-hexanesulfonate and 5.44 g of monobasic potassium phosphate in 760 mL of water, adjust with phosphoric acid to a pH of 2.5, dilute with acetonitrile to 1000 mL, and mix. Pass through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
pH 6.0 Phosphate buffer—Dissolve 16 g of monobasic potassium phosphate and 4 g of dibasic sodium phosphate in water, dilute with water to 200 mL, adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 6.0.
Diluent—Transfer 60 mL of butyl alcohol, 100 mL of acetonitrile, and 10 mL of *pH 6.0 Phosphate buffer* to a 500-mL volumetric flask, dilute with water to volume, and mix.
Standard solution—Transfer about 24 mg of USP Penicillin G Potassium RS, accurately weighed, and about 8 mg of USP Procaine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add 12 mL of butyl alcohol and 20 mL of acetonitrile, and shake to dissolve. Add 2 mL of *pH 6.0 Phosphate buffer* and mix and bring to volume with water.
Test solution—Centrifuge about 20 mL of the Suspension. Remove the supernatant fluid, and pass it through a filter having a 5-µm or finer porosity. Transfer 5.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 204-nm detector and a 4-mm × 15-cm column that contains 5-µm packing L1.

USP Monographs

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The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 0.3%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of penicillin G ($C_{16}H_{18}N_2O_4S$) in the *Test solution* by the formula:

$$(334.40/372.48)(C)(r_U/r_S)$$

in which 334.40 and 372.48 are the molecular weights of penicillin G and penicillin G potassium respectively; C is the concentration, in mg per mL, of USP Penicillin G Potassium RS in the *Standard solution;* and r_U and r_S are the responses of the penicillin G peaks in the chromatograms of the *Test solution* and the *Standard solution*, respectively: not more than 1% is found. Calculate the percentage of procaine ($C_{13}H_{20}N_2O_2$) in the *Test solution* by the formula:

$$(236.32/272.78)(C)(r_U/r_S)$$

in which 236.32 and 272.78 are the molecular weights of procaine and procaine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Procaine Hydrochloride RS in the *Standard solution*; and r_U and r_S are the responses of the procaine peaks in the chromatograms of the *Test solution* and the *Standard solution*, respectively: not more than 1% is found.

Other requirements—It meets the requirements for *Bacterial endotoxins* and *Sterility* under *Penicillin G Procaine Injectable Suspension.* It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for penicillin G procaine—

Standard preparations—Transfer about 14*k* mg of USP Procaine Hydrochloride RS, accurately weighed, to a 500-mL volumetric flask, and dissolve in 2 mL of 0.5 N sodium hydroxide, *k* being the ratio of the labeled number of Penicillin G Procaine Units to the labeled number of Penicillin G Benzathine Units in the Injectable Suspension. After 15 minutes, add 1 mL of 1.2 N hydrochloric acid, dilute with water to volume, and mix. This stock solution contains about 28*k* µg of USP Procaine Hydrochloride RS per mL. Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 mL, respectively, of this stock solution to each of five 25-mL volumetric flasks. Transfer 4.0, 3.0, 2.0, and 1.0 mL of water to the first four flasks, respectively.

Assay preparation—Where the Injectable Suspension is represented as being in a single-dose container, withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe. Where the label states the quantities of penicillin G benzathine and penicillin G procaine in a given volume of Injectable Suspension, remove an accurately measured volume of the Injectable Suspension. For each 300,000 Penicillin G Benzathine Units in the specimen of Injectable Suspension taken, add 20 mL of 0.5 N sodium hydroxide, and mix. After 15 minutes, add 0.5 mL of 1.2 N hydrochloric acid for each mL of 0.5 N sodium hydroxide used, and dilute quantitatively with water to obtain a solution containing 36 Penicillin G Procaine Units per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask.

Procedure—To each of the flasks containing the *Standard preparations* and the *Assay preparation*, and to a seventh 50-mL volumetric flask containing 5.0 mL of water to provide the blank, add 0.5 mL of 4 N hydrochloric acid, 1.0 mL of sodium nitrite solution (1 in 1000), 1.0 mL of ammonium sulfamate solution (1 in 200), and 1.0 mL of N-(1-naph thyl) ethylenediamine dihydrochloride solution (1 in 1000), mixing and allowing 2 minutes to elapse after each addition. Dilute the contents of each flask with water to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Standard preparations* and the *Assay preparation* at the wavelength of maximum absorbance at about 550 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Plot the absorbance values of solutions from the *Standard preparations* versus concentration, in mg per mL, of procaine hydrochloride in the solutions from the *Standard preparations*, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration (C), in mg per mL, of procaine hydrochloride in the solution from the *Assay preparation.* Calculate the quantity, in Penicillin G Procaine Units in each container or in each mL of the Injectable Suspension taken by the formula:

$$(588.73/272.78)(1009.1)(CL/D)$$

in which 588.73 and 272.78 are the molecular weights of penicillin G procaine monohydrate and procaine hydrochloride, respectively; 1009.1 is the theoretical potency, in Penicillin G Units, in each mg of penicillin G procaine; L is the labeled amount, in Penicillin G Procaine Units in each container or in each mL of Injectable Suspension taken; and D is the concentration, in Penicillin G Procaine Units per mL, of the solution from the *Assay preparation*, on the basis of the labeled amount in each container or in each mL of Injectable Suspension taken and the extent of dilution.

Assay for penicillin G benzathine—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.

Assay preparation—Where the Injectable Suspension is represented as being in a single-dose container, withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe. Where the label states the quantities of penicillin G benzathine and penicillin G procaine in a given volume of Injectable Suspension, remove an accurately measured volume of Injectable Suspension, freshly mixed but free from air bubbles. Dilute the specimen of Injectable Suspension taken quantitatively with 1 N sodium hydroxide to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Blank preparation—Prepare as directed for *Assay preparation*, except to use *Buffer B.1* instead of 1.0 N sodium hydroxide.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in performing the *Inactivation and Titration* to omit the addition of 1.0 N sodium hydroxide to the *Assay preparation*, and in performing the *Blank Determination* to use the *Blank preparation* in place of the *Assay preparation.* Calculate the total quantity, T, in Penicillin G Units, in each mL of the Injectable Suspension taken by the formula:

$$(L/2D)(F)(B - I)$$

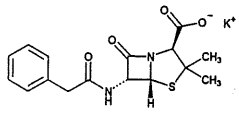
in which L is the labeled quantity, in Penicillin G Units in each container, or per mL, in the Injectable Suspension taken; and D is the concentration, in Penicillin G Units per mL, in the *Assay preparation* on the basis of the labeled quantity in the Injectable Suspension and the extent of dilution. Calculate the quantity, in Penicillin G Benzathine Units, in each container, or in each mL, of the Injectable Suspension taken by the formula:

$$T - P$$

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in which *P* is the quantity, in Penicillin G Procaine Units, in each container, or in each mL, of Injectable Suspension taken, as determined in the *Assay for penicillin G procaine.*

Penicillin G Potassium



$C_{16}H_{17}KN_2O_4S$ 372.48

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-, monopotassium salt, [2*S*-(2α,5α,6β)]-;

Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [113-98-4].

DEFINITION

Penicillin G Potassium has a potency of NLT 1440 and NMT 1680 Penicillin G Units/mg.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **C.**

Diluent: Glycerin and water (25:14)
Solution A: 106 mg/mL of sodium carbonate in water
Solution B: 120 mg/mL of sodium sulfide in *Diluent*, prepared as follows. Dissolve sodium sulfide in *Diluent*, using about 45% of the final volume and heat. Allow to cool, and dilute with *Diluent* to the final volume.
Solution C: 150 mg/mL of tartaric acid in water
Sample solution: 0.1 g of Penicillin G Potassium in 2 mL of water
Analysis
Part 1: Add 1 mL of *Solution A* to the *Sample solution* and heat.
Part 2: To the hot solution from *Part 1* add 0.05 mL of *Solution B.*
Part 3: Cool the mixture from *Part 2* in iced water and add 2 mL of *Solution C.* Allow to stand.
Acceptance criteria: Meets the requirements for *Parts 1*, *2*, and *3*
Part 1: No precipitate is formed.
Part 2: No precipitate is formed.
Part 3: A white precipitate is formed.

ASSAY

- **PROCEDURE**

Solution A: 0.01 M monobasic potassium phosphate
Mobile phase: Methanol and *Solution A* (40:60)
System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water
Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. This solution contains about 160 Penicillin G Units/mL. Shake as needed to dissolve.
Sample solution: 0.1 mg/mL of Penicillin G Potassium in water. Shake as needed to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency of penicillin G potassium, in Penicillin G Units/mg, in the portion of Penicillin G Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of penicillin G from the *Sample solution*
r_S = peak response of penicillin G from the *Standard solution*
C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)
C_U = concentration of Penicillin G Potassium in the *Sample solution* (mg/mL)
P = potency of USP Penicillin G Potassium RS (Penicillin G Units/mg)

Acceptance criteria: 1440–1680 Penicillin G Units/mg

SPECIFIC TESTS

- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **PH** ⟨791⟩
Sample solution: 60 mg/mL of Penicillin G Potassium in water
Acceptance criteria: 5.0–7.5
- **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg of Penicillin G Potassium
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.
Acceptance criteria: NMT 1.5%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Penicillin G Potassium is sterile or it must be subjected to further processing during the preparation of injectable dosage forms, it has NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.
- **STERILITY TESTS** ⟨71⟩: Where the label states that Penicillin G Potassium is sterile, it meets the requirements.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Penicillin G Potassium RS

USP Monographs

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Penicillin G Potassium Injection

DEFINITION

Penicillin G Potassium Injection is a sterile isoosmotic solution of Penicillin G Potassium in Water for Injection. It contains one or more suitable buffers and a tonicity-adjusting agent. It contains NLT 90.0% and NMT 115.0% of the labeled number of Penicillin G Units.

IDENTIFICATION

• **A.** The retention time of the penicillin G peak of *Sample solution 1* or *Sample solution 2* corresponds to that of *Standard solution 2*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer A: Dissolve 0.8 g of sodium citrate dihydrate in 150 mL of water. Adjust with 0.1 N hydrochloric acid to a pH of 6.8, and dilute with water to 200 mL.

Buffer B: Dissolve 10 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 4.15, and dilute with water to 1000 mL.

Mobile phase: Methanol and *Buffer B* (450:550)

Standard stock solution: 2000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Buffer A*

Standard solution 1: 100 Penicillin G Units/mL from *Standard stock solution* in water

Standard solution 2: 200 Penicillin G Units/mL from *Standard stock solution* in water

Standard solution 3: 300 Penicillin G Units/mL from *Standard stock solution* in water

Sample solution 1 (where it is represented as being in a single-dose container)**:** Allow 1 container of Injection to thaw, and mix. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with water to obtain a solution containing nominally about 200 Penicillin G Units/mL.

Sample solution 2 (where the label states the quantity of penicillin G in a given volume of Injection)**:** Allow 1 container of Injection to thaw, and mix. Dilute a suitable aliquot of the Injection with water to obtain a solution containing nominally about 200 Penicillin G Units/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution 2*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and *Sample solution 1* or *Sample solution 2*

Plot the peak responses from the *Standard solutions* versus concentration in Penicillin G Units/mL and draw the straight line best fitting the three plotted points.

Calculate the percentage of the labeled number of Penicillin G Units in the portion of Injection taken:

$$\text{Result} = [(N \times D)/L] \times 100$$

N = concentration of *Sample solution 1* or *Sample solution 2* determined from the graph (Penicillin G Units/mL)

D = dilution factor for *Sample solution 1* or *Sample solution 2* (mL/mL)

L = labeled number of Penicillin G Units in Injection (Penicillin G Units/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.

• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **pH** ⟨791⟩**:** 5.5–8.0

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, as described in *Packaging and Storage Requirements* ⟨659⟩. Maintain in the frozen state.

• **LABELING:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Penicillin G Potassium RS

Penicillin G Potassium for Injection

DEFINITION

Penicillin G Potassium for Injection is sterile Penicillin G Potassium or a sterile, dry mixture of Penicillin G Potassium with NLT 4.0% and NMT 5.0% of Sodium Citrate, of which NMT 0.15% may be replaced by Citric Acid. It has a potency of NLT 90.0% and NMT 120.0% of the labeled number of Penicillin G Units. In addition, where it contains Sodium Citrate it has a potency of NLT 1335 and NMT 1595 Penicillin G Units/mg.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Solution A*.

Sample solution: Nominally 12,000 Penicillin G Units/mL from Penicillin G Potassium for Injection in *Solution A*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a suitable chromatographic chamber. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Spray the plate with *Spray reagent 1* followed by *Spray*

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reagent 2. Penicillin G appears as a white spot on a purple background.

Acceptance criteria: The R_F value of the penicillin G spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Solution A: 0.01 M monobasic potassium phosphate

Mobile phase: Methanol and *Solution A* (40:60)

System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water

Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. This solution contains about 160 Penicillin G Units/mL. Shake as needed to dissolve.

Sample solution 1 (where it is represented as being in a single-dose container): Constitute Penicillin G Potassium for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and dilute with water to obtain a solution containing nominally 160 Penicillin G Units/mL.

Sample solution 2 (where the label states the quantity of penicillin G in a given volume of constituted solution): Constitute Penicillin G Potassium for Injection as directed in the labeling. Dilute a suitable aliquot of the constituted solution with water to obtain a solution containing nominally 160 Penicillin G Units/mL.

Sample solution 3 (where it contains sodium citrate): Transfer about 50 mg of the Penicillin G Potassium for Injection to a 500-mL volumetric flask. Add 400 mL of water, and shake to dissolve. Dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution*

Column efficiency: NLT 1000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution 1*, *Sample solution 2*, or *Sample solution 3*

Perform the *Assay* on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of penicillin G in a given volume of constituted solution. Use the individual results to determine the *Uniformity of Dosage Units* and the average of the results as the *Assay* value.

Calculate the percentage of the labeled number of Penicillin G Units in the container or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from *Sample solution 1* or *Sample solution 2*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of *Sample solution 1* or *Sample solution 2* (Penicillin G Units/mL)

P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Calculate the potency, in Penicillin G Units/mg, of the Penicillin G Potassium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from *Sample solution 3*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Potassium for Injection in *Sample solution 3* (mg/mL)

P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Acceptance criteria: 90.0%–120.0% of the labeled number of Penicillin G Units. Where Penicillin G Potassium for Injection contains sodium citrate it has a potency of NLT 1335 and NMT 1595 Penicillin G Units/mg.

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements. Perform the *Assay* on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of penicillin G in a given volume of constituted solution. Use the individual results to determine the *Uniformity of Dosage Units* and the average of the results as the *Assay* value.

SPECIFIC TESTS

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Specific Tests, Completeness and clarity of solutions***:** At the time of use, it meets the requirements.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.

• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements

• **PH** ⟨791⟩

Sample solution: A solution containing 60 mg/mL. Where packaged for dispensing, use the solution constituted as directed in the labeling.

Acceptance criteria: 5.0–7.5. Where it is labeled as containing sodium citrate, the pH is between 6.0 and 8.5.

• **LOSS ON DRYING** ⟨731⟩

Sample: 100 mg of Penicillin G Potassium for Injection

Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.

Acceptance criteria: NMT 1.5%

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Penicillin G Potassium RS

USP Monographs

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Penicillin G Potassium for Oral Solution

DEFINITION

Penicillin G Potassium for Oral Solution is a dry mixture of Penicillin G Potassium and one or more suitable buffers, colors, diluents, flavors, and preservatives. It contains NLT 90.0% and NMT 130.0% of the labeled number of Penicillin G Units when constituted as directed in the labeling.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Solution A*

Sample solution: Shake a portion of it, containing nominally 100,000 Penicillin G Units, with 8 mL of *Solution A*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a suitable chromatographic chamber. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background.

Acceptance criteria: The R_F value of the penicillin G spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **Procedure**

Standard solution: Prepare as directed in *Iodometric Assay—Antibiotics* ⟨425⟩, *Standard Preparation*, using USP Penicillin G Potassium RS.

Sample solution: Constitute Penicillin G Potassium for Oral Solution as directed in the labeling using *Buffer B.1* (see *Antibiotics—Microbial Assay* ⟨81⟩, *Media and Solutions, Solutions*). Dilute a suitable aliquot to obtain a solution containing nominally 2000 Penicillin G Units/mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Pipet 2 mL of the *Sample solution* into each of two glass-stoppered, 125-mL conical flasks. Proceed as directed in *Iodometric Assay—Antibiotics* ⟨425⟩, *Procedure*, using one of the flasks to perform the *Blank Determination*.

Calculate the percentage of the labeled number of Penicillin G Units in the portion of Penicillin G Potassium for Oral Solution taken:

$$\text{Result} = (B - I) \times F \times [1/(D \times V)] \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation* and *Titration* (mL)

F = equivalency factor as calculated in the chapter (Penicillin G Unit/mL of 0.01 N sodium thiosulfate consumed by the *Standard solution*)

D = nominal concentration of penicillin G in the *Sample solution* (Penicillin G Units/mL)

V = volume of *Sample solution* used for the *Inactivation* and *Titration* (mL)

Acceptance criteria: 90.0%–130.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩

For solids packaged in single-unit containers: Meets the requirements

• **Deliverable Volume** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 5.5–7.5

• **Water Determination** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards** ⟨11⟩

USP Penicillin G Potassium RS

Penicillin G Potassium Tablets

DEFINITION

Penicillin G Potassium Tablets contain NLT 90.0% and NMT 120.0% of the labeled number of Penicillin G Units.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Diluent: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution: 12,000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Diluent*

Sample solution: Nominally 12,500 Penicillin G Units/mL from Tablets in *Diluent*. Pass through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Sample solution* and the *Standard solution* to the plate, place in a suitable chromatographic chamber, and develop the chromatogram, using the *Developing solvent system*, until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background.

Acceptance criteria: The R_F value of the penicillin G spot from the *Sample solution* corresponds to that from the *Standard solution*.

USP Monographs

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ASSAY

• **PROCEDURE**

Standard solution: Prepare as directed in *Iodometric Assay—Antibiotics* ⟨425⟩, *Standard Preparation*, using USP Penicillin G Potassium RS.

Sample solution: Nominally 2000 Penicillin G Units/mL, prepared as follows. Place NLT 5 Tablets in a high-speed glass blender jar containing a measured volume of *Buffer B.1*, and blend for 4 ± 1 min. Dilute a suitable aliquot with *Buffer B.1*.

Analysis: Proceed as directed in *Iodometric Assay—Antibiotics* ⟨425⟩, *Procedure*, using glass-stoppered, 125-mL conical flasks.

Calculate the percentage of the labeled number of Penicillin G Units in the portion of Tablets taken:

$$\text{Result} = (B - I) \times (F/2) \times (1/C_U) \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in *Inactivation and Titration* of the *Sample solution* (mL)

F = factor as calculated in *Iodometric Assay—Antibiotics* ⟨425⟩, *Calculations*

C_U = nominal concentration of penicillin G in the *Sample solution* (Penicillin G Units/mL)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: pH 6.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Standard solution: 400 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Medium*

Sample solution: Use a filtered portion of the solution under test.

Solution A: A 1-in-1000 solution of polyoxyethylene (23) lauryl ether in water

Solution B: Dissolve 20 g of hydroxylamine hydrochloride in 5 mL of *Solution A*, and add water to make 1000 mL.

Buffer: 26 mg/mL of sodium hydroxide and 3.1 mg/mL of sodium acetate in water

Ferric nitrate solution: Suspend 233 g of ferric nitrate in about 600 mL of water, add 2.8 mL of sulfuric acid, stir until the ferric nitrate is dissolved, add 1 mL of polyoxyethylene (23) lauryl ether, dilute with water to 1000 mL, and mix.

Apparatus: Automatic analyzer (*Figure 1*) consisting of (1) a liquid sampler, (2) a proportioning pump, (3) suitable spectrophotometers equipped with matched flow cells and analysis capability at 480 nm, (4) a means of recording spectrophotometric readings and/or a computer for data retrieval and calculation, and (5) a manifold consisting of the components illustrated in the figure.

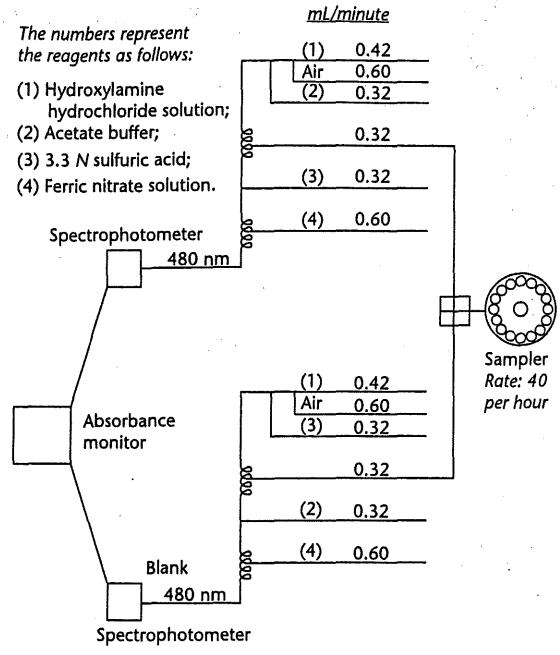


Figure 1

Analysis: With the sample line pumping water, the other lines pumping their respective reagents, and the spectrophotometer set at 480 nm, standardize the system until a steady absorbance baseline has been established. Transfer portions of the *Standard solution* and the *Sample solution* to sampler cups, and place in the sampler. Start the sampler, and conduct determinations of the *Standard solution* and the *Sample solution*, typically at the rate of 40 per h, using a ratio of about 2:1 for sample and wash time.

Calculate the percentage of the labeled amount of Penicillin G Units dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (Penicillin G Units/mL)

V = volume of *Medium*, 900 mL

L = label claim (Penicillin G Units/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of Penicillin G Units is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

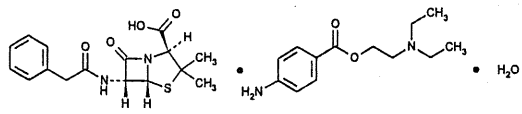
• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Penicillin G Potassium RS

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Penicillin G Procaine



$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2 \cdot H_2O$ 588.72

$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2$ 570.71

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino-], 2*S*-(2α,5α,6β)-, compound with 2-(diethylamino)ethyl 4-aminobenzoate (1:1) monohydrate;

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with 2-(diethylamino)ethyl *p*-aminobenzoate (1:1) monohydrate [6130-64-9].

Anhydrous [54-35-3].

DEFINITION

Penicillin G Procaine has a potency of NLT 900 Penicillin G Units/mg and NMT 1050 Penicillin G Units/mg.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution 1: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL, from USP Penicillin G Potassium RS in *Solution A*.

Standard solution 2: 5 mg/mL of USP Procaine Hydrochloride RS in *Solution A*

Sample solution: Nominally 12,000 Penicillin G Units/mL from Penicillin G Procaine in *Solution A*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Spray reagent 3: 50 mg/mL of *p*-dimethylaminobenzaldehyde in methanol

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram until the solvent has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Examine the plate under short- and long-wavelength UV light, noting the positions of the spots. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background. Spray the location of the spots visualized with UV light with *Spray reagent 3*. Procaine appears as a bright yellow spot.

Acceptance criteria: The R_F value of the penicillin G spot from the *Sample solution* corresponds to that from *Standard solution 1*. The R_F value of the procaine spot from the *Sample solution* corresponds to that from *Standard solution 2*.

ASSAY

• **Procedure**

Standard solution: Prepare as directed in *Iodometric Assay—Antibiotics* ⟨425⟩, *Standard Preparation*, using USP Penicillin G Potassium RS.

Sample solution: Prepare as directed in *Iodometric Assay—Antibiotics* ⟨425⟩, *Assay Preparation*, except dissolve 100 mg of Penicillin G Procaine in 2.0 mL of methanol, and dilute with *Buffer B.1* (see *Antibiotics—Microbial Assays* ⟨81⟩) to obtain a solution containing 2000 Penicillin G Units/mL.

Analysis: Pipet 2 mL of the *Sample solution* into each of two glass-stoppered, 125-mL conical flasks. Use one of these to perform the *Blank Determination*. Proceed as directed in *Iodometric Assay—Antibiotics* ⟨425⟩, *Procedure*.

Calculate the potency, in Penicillin G Units/mg, of the Penicillin G Procaine taken:

$$\text{Result} = (B - I) \times F \times 1/(D \times V) \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation* and *Titration* (mL)

F = equivalency factor as calculated in *Procedure* in the chapter (Penicillin G Units/mL of 0.01 N sodium thiosulfate consumed by the *Standard solution*)

D = nominal concentration of Penicillin G in the *Sample solution* (Penicillin G Units/mL)

V = volume of the *Sample solution* used for the *Inactivation* and *Titration* (mL)

Acceptance criteria: 900–1050 Penicillin G Units/mg

SPECIFIC TESTS

• **Content of Penicillin G and Procaine**

Solution A: Phosphoric acid diluted 1 in 10 with water

Mobile phase: Dissolve 14 g of monobasic potassium phosphate and 6.5 g of tetrabutylammonium hydroxide, 40% in water, in 700 mL of water. Adjust with 1 N potassium hydroxide to a pH of 7.0, and dilute with water to 1000 mL. Mix 500 mL of this solution, 250 mL of acetonitrile, and 250 mL of water. Adjust with 1 N potassium hydroxide or *Solution A* to a pH of 7.5 ± 0.05, and pass through a suitable filter.

Standard solution: 0.8 mg/mL of USP Penicillin G Potassium RS and 0.54 mg/mL of USP Procaine Hydrochloride RS in *Mobile phase*

System suitability solution: 2.4 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*. Mix the resultant solution with *Standard solution* (1:3).

Sample solution: Transfer 70 mg of Penicillin G Procaine to a 50-mL volumetric flask. Add 30 mL of *Mobile phase*, sonicate to dissolve, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for procaine, penicillin G, and penicillin V are about 0.4, 1.0, and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2.0 between penicillin G and penicillin V, *System suitability solution*

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Relative standard deviation: NMT 3.0% for penicillin G potassium, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of penicillin G ($C_{16}H_{18}N_2O_4S$) in the portion of Penicillin G Procaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times G_S$$

r_U = peak response of penicillin G from the *Sample solution*

r_S = peak response of penicillin G from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Procaine in the *Sample solution* (mg/mL)

G_S = content of penicillin G in USP Penicillin G Potassium RS (%)

Calculate the percentage of procaine ($C_{13}H_{20}N_2O_2$) in the portion of Penicillin G Procaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of procaine from the *Sample solution*

r_S = peak response of procaine from the *Standard solution*

C_S = concentration of USP Procaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Procaine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of procaine, 236.32

M_{r2} = molecular weight of procaine hydrochloride, 272.78

Acceptance criteria: See *Table 1*.

Table 1

Penicillin G	51.0%–59.6%
Procaine	37.5%–43.0%

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Penicillin G Procaine is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.01 USP Endotoxin Unit/100 Penicillin G Units.
- **STERILITY TESTS** ⟨71⟩: Where the label states that Penicillin G Procaine is sterile, it meets the requirements. If the test for *Membrane Filtration* is used, perform the procedure as directed in the chapter with the following exceptions. Use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G, and swirl the vessel until solution is complete before filtering.
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **PH** ⟨791⟩

Sample solution: A saturated solution containing about 300 mg/mL of Penicillin G Procaine in water

Acceptance criteria: 5.0–7.5

- **WATER DETERMINATION** ⟨921⟩, *Method I*: 2.8%–4.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Where it is intended for use in preparing injectable dosage forms, preserve as directed in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Penicillin G Potassium RS
USP Penicillin V Potassium RS
USP Procaine Hydrochloride RS

Penicillin G Procaine Intramammary Infusion

» Penicillin G Procaine Intramammary Infusion is a suspension of Penicillin G Procaine in a suitable vegetable oil vehicle. It may contain one or more buffers, dispersants, preservatives, and thickening agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of penicillin G.

Packaging and storage—Preserve in well-closed disposable syringes.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Penicillin G Potassium RS
USP Penicillin G Procaine RS

Identification—Transfer a portion of it, equivalent to about 100,000 Penicillin G Units, to a test tube, add 25 mL of methanol, and shake. Allow to separate, and use the methanol layer as the test solution. Prepare a Standard solution of USP Penicillin G Procaine RS in methanol containing about 4.5 mg per mL. Apply separately 10 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butanol, isopropyl alcohol, acetone, and water (4:4:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 15 minutes, and locate the spots: the R_F values and colors of the two principal spots obtained from the test solution correspond to those obtained from the Standard solution.

Water Determination, *Method I* ⟨921⟩: not more than 1.4%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, expelling the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B.1* and 1.0 mL of polysorbate 80, and blending for 3 to 5 minutes. Allow to stand for about 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.1* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

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Penicillin G Procaine Injectable Suspension

» Penicillin G Procaine Injectable Suspension is a sterile suspension of Penicillin G, Procaine or, where labeled for veterinary use only of sterile penicillin G procaine, in Water for Injection and contains one or more suitable buffers dispersants, or suspending agents, and a suitable preservative. It may contain procaine hydrochloride in a concentration not exceeding 2.0 percent, and may contain one or more suitable stabilizers. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of penicillin G, the labeled amount being not less than 300,000 Penicillin G Units per mL or per container.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass, in a refrigerator.
Labeling—Where it is intended for veterinary use only, the label so states.
USP Reference standards ⟨11⟩—
USP Penicillin G Potassium RS
USP Procaine Hydrochloride RS
Identification—It responds to the *Identification* test under *Penicillin G Procaine.*
Crystallinity ⟨695⟩ (where it is prepared from penicillin G procaine and is labeled for veterinary use only): meets the requirements, the dried residue prepared as directed in the test for *Pencillin G and procaine contents* being used.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.01 USP Endotoxin Unit per 100 Penicillin G Units.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* except to use a portion of specimen from each container equivalent to 300,000 Penicillin G Units, instead of the minimum volume specified in the *Table 2, Minimum Quantity to be Used for Each Medium,* and to use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G and to swirl the vessel until solution is complete before filtering. If the Injectable Suspension contains lecithin, use *Fluid D.* If it contains carboxymethylcellulose sodium, add sufficient sterile carboxymethylcellulase to *Fluid A* or *Fluid D* to dissolve the carboxymethylcellulose sodium before filtering. If it does not dissolve completely, proceed as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined,* except to use Fluid Thioglycollate Medium and Soybean–Casein Digest Medium containing an amount of sterile penicillinase sufficient to inactivate the penicillin G in each vessel.
pH ⟨791⟩: between 5.0 and 7.5.
Penicillin G and procaine contents (where it is prepared from penicillin G procaine and is labeled for veterinary use only)—Dilute a portion of it, equivalent to about 300,000 Penicillin G Units, with water to obtain a volume of 10 mL, centrifuge, and remove and discard the supernatant. Resuspend the sediment in 10 mL of water, centrifuge, and remove and discard the supernatant. Dry the sediment in a vacuum desiccator containing silica gel for 18 hours at a temperature not exceeding 25°. The dried material meets the requirements of the test for *Penicillin G and procaine contents* under *Penicillin G Procaine.* [NOTE—Reserve a portion of the dried material for the test for *Crystallinity.*]
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.
Assay preparation 1 (where it is represented as being in a single-dose container)—Withdraw all of the withdrawable contents of the Injectable Suspension, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer B.1* to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.
Assay preparation 2 (where the label states the quantity of penicillin G procaine in a given volume of Injectable Suspension)—Dilute an accurately measured volume of Injectable Suspension quantitatively with *Buffer B.1* to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2.0 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩. Calculate the quantity, in Penicillin G Units, in the container, or in the portion of Injectable Suspension taken, by the formula:

$$(L/2D)(F)(B - I)$$

in which L is the labeled quantity in Penicillin G Units, in the container, or in the volume of Injectable Suspension taken; and D is the concentration, in Penicillin G Units per mL, of *Assay preparation 1*, or of *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the portion of Injectable Suspension taken, respectively, and the extent of dilution.

Penicillin G Procaine for Injectable Suspension

» Penicillin G Procaine for Injectable Suspension is a sterile mixture of Penicillin G Procaine and one or more suitable buffers, dispersants, or suspending agents, and preservatives. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of penicillin G, the labeled amount being not less than 300,000 Penicillin G Units per container or per mL of constituted Suspension.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.
USP Reference standards ⟨11⟩—
USP Penicillin G Potassium RS
USP Procaine Hydrochloride RS
Identification—It responds to the *Identification* test under *Penicillin G Procaine.*
pH ⟨791⟩: between 5.0 and 7.5, when constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: between 2.8% and 4.2%.
Other requirements—It meets the requirements for *Bacterial endotoxins* and *Sterility* under *Penicillin G Procaine Injectable Suspension.* It meets also the requirements under

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Injections and Implanted Drug Products ⟨1⟩ and *Uniformity of Dosage Units* ⟨905⟩.

Assay—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Penicillin G Procaine for Injectable Suspension as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer B.1* to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Assay preparation 2 (where the label states the quantity of penicillin G procaine in a given volume of constituted suspension)—Constitute Penicillin G Procaine for Injectable Suspension as directed in the labeling. Dilute an accurately measured volume of the constituted injectable suspension quantitatively with *Buffer B.1* to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩. Calculate the quantity, in Penicillin G Units, in the container, or in the portion of constituted injectable suspension taken by the formula:

$$(L/2D)(F)(B - I)$$

in which L is the labeled quantity, in Penicillin G Units, in the container, or in the volume of constituted injectable suspension taken, and D is the concentration, in Penicillin G Units per mL, of *Assay preparation 1* or of *Assay preparation 2* on the basis of the labeled quantity in the container or in the portion of constituted injectable suspension taken, respectively, and the extent of dilution.

Penicillin G Procaine and Dihydrostreptomycin Sulfate Intramammary Infusion

» Penicillin G Procaine and Dihydrostreptomycin Sulfate Intramammary Infusion is a suspension of Penicillin G Procaine and Dihydrostreptomycin Sulfate in a suitable vegetable oil vehicle. It may contain suitable gelling and thickening agents. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of Penicillin G Units and of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$).

Packaging and storage—Preserve in well-closed, disposable syringes.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards ⟨11⟩—
USP Dihydrostreptomycin Sulfate RS
USP Penicillin G Potassium RS
USP Penicillin G Procaine RS

Identification—

A: It responds to the *Identification* test under *Penicillin G Procaine Intramammary Infusion.*

B: Place a portion of it, equivalent to about 100 mg of dihydrostreptomycin, in a separator, add 20 mL of chloroform and 20 mL of water, and shake by mechanical means for 15 minutes. Allow to separate, and discard the lower chloroform layer. Repeat the extraction with a 20-mL portion of chloroform, discarding the chloroform layer. Use the aqueous layer as the test solution. Prepare a Standard solution of USP Dihydrostreptomycin Sulfate RS in water containing 6.5 mg per mL. Apply separately 30 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol, water, pyridine, and glacial acetic acid (15:12:10:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a reagent prepared by dissolving 2 g of ninhydrin in 100 mL of alcohol and adding 20 mL of glacial acetic acid, heat the plate at 110° for 10 minutes, and examine the chromatograms: the R_F value and color of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

Water Determination, *Method I* ⟨921⟩: not more than 1.4%, 20 mL of a mixture of toluene and methanol (7:3) being used in the titration vessel in place of methanol.

Assay for penicillin G—Proceed as directed for penicillin G under *Antibiotics—Microbial Assays* ⟨81⟩, expelling the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B. 1* and 1.0 mL of polysorbate 80, and blending for 3 to 5 minutes. Allow to stand for about 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.1* to obtain a *Test Dilution* having a concentration of penicillin G assumed to be equal to the median dose level of the Standard.

Assay for dihydrostreptomycin—Proceed as directed for the cylinder-plate assay for dihydrostreptomycin under *Antibiotics—Microbial Assays* ⟨81⟩, expelling the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B.3* and 1.0 mL of polysorbate 80, and blending for 3 to 5 minutes. Allow to stand for about 10 minutes, and to an accurately measured volume of the aqueous phase add an accurately measured volume of penicillinase sufficient to inactivate the penicillin G contained therein. Dilute this solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration of dihydrostreptomycin assumed to be equal to the median dose level of the Standard, and store at 37° for 30 minutes before filling the cylinders.

Penicillin G Procaine and Dihydrostreptomycin Sulfate Injectable Suspension

» Penicillin G Procaine and Dihydrostreptomycin Sulfate Injectable Suspension is a sterile suspension of Penicillin G Procaine in a solution of Dihydrostreptomycin Sulfate in Water for Injection, and contains one or more suitable buffers,

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preservatives, and dispersing or suspending agents. It may contain Procaine Hydrochloride in a concentration not exceeding 2.0 percent, and it may contain one or more suitable stabilizers. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of Penicillin G Units and of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$).

Packaging and storage—Preserve in single-dose or multiple-dose, tight containers.
Labeling—Label it to indicate that it is intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Dihydrostreptomycin Sulfate RS
USP Penicillin G Potassium RS
Identification—It responds to *Identification* tests *A* and *B* under *Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate, and Dexamethasone Suspension.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.01 USP Endotoxin Unit per 100 Penicillin G Units.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* except to use a portion of specimen from each container equivalent to 300,000 Penicillin G Units, instead of the minimum volume specified in the *Table 2, Minimum Quantity to be Used for Each Medium* and to use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G and to swirl the vessel until solution is complete before filtering. If the Injectable Suspension contains lecithin, use *Fluid D* to which has been added sufficient penicillinase to inactivate the penicillin G and to swirl the vessel until solution is complete before filtering. If it contains carboxymethylcellulose sodium, add also sufficient sterile carboxymethylcellulase to *Fluid A* or *Fluid D* to dissolve the carboxymethylcellulose sodium before filtering. If it does not dissolve completely, proceed as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined,* except to use Fluid Thioglycollate Medium containing an amount of sterile penicillinase sufficient to inactivate the penicillin G in each vessel.
pH ⟨791⟩: between 5.0 and 8.0.

Assay for penicillin G—
Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.
Assay preparation—Dilute an accurately measured volume of Injectable Suspension quantitatively with *Buffer B.1* to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in the *Blank Determination* to add 0.1 mL of 1.2 N hydrochloric acid immediately before the 10.0 mL of 0.01 N iodine VS. Calculate the quantity, in Penicillin G Units, in the portion of Injectable Suspension taken by the formula:

$$(L/2D)(F)(B - I)$$

in which L is the labeled quantity, in Penicillin G Units, in the volume of Injectable Suspension taken, and D is the concentration, in Penicillin G Units per mL, of the *Assay preparation,* on the basis of the labeled quantity in the portion of Injectable Suspension taken and the extent of dilution, and the other terms are as defined therein.

Assay for dihydrostreptomycin—Proceed as directed for the turbidimetric assay for dihydrostreptomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Injectable Suspension diluted quantitatively with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate, and Dexamethasone Injectable Suspension

» Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate, and Dexamethasone Injectable Suspension is a sterile suspension of Penicillin G Procaine and Dexamethasone in a solution of Sterile Dihydrostreptomycin Sulfate and Chlorpheniramine Maleate in Water for Injection. It contains one or more suitable buffers, preservatives, and dispersing or suspending agents. It may contain Procaine Hydrochloride in a concentration not exceeding 2.0 percent, and it may contain one or more suitable stabilizers. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of Penicillin G Units and of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) and of dexamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in single-dose or multiple-dose, tight containers, in a cool place.
Labeling—Label it to indicate that it is intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Chlorpheniramine Maleate RS
USP Dexamethasone RS
USP Dihydrostreptomycin Sulfate RS
USP Penicillin G Potassium RS
USP Penicillin G Procaine RS

Identification—
A: Transfer, with the aid of water, a portion of the Injectable Suspension, freshly mixed and free from air bubbles, equivalent to about 400,000 Penicillin G Units, to a separator, add 50 mL of chloroform, and shake by mechanical means for 15 minutes. Allow to separate, and filter the lower chloroform layer through about 4 g of anhydrous sodium sulfate supported on a pledget of glass wool, collecting the filtrate in a 100-mL volumetric flask. Repeat the extraction with two 25-mL portions of chloroform, combining the filtrates in the 100-mL volumetric flask. Dilute with chloroform to volume, and mix. [NOTE—Retain the aqueous phase for *Identification* test *B.*] Prepare a Standard solution of USP Penicillin G Procaine RS in chloroform containing about 4.5 mg per mL. Apply separately 10 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated

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with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butanol, isopropyl alcohol, acetone, and water (4:4:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 15 minutes, and locate the spots: the R_F values and colors of the two principal spots obtained from the test solution correspond to those obtained from the Standard solution.

B: Dilute the aqueous phase retained from *Identification* test *A* with water to obtain a test solution containing about 5 mg of dihydrostreptomycin per mL. Prepare a Standard solution of USP Dihydrostreptomycin Sulfate RS in water containing 6.5 mg per mL. Apply separately 30 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol, water, pyridine, and glacial acetic acid (15:12:10:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a reagent prepared by dissolving 2 g of ninhydrin in 100 mL of alcohol and adding 20 mL of glacial acetic acid, heat the plate at 110° for 10 minutes, and examine the chromatograms: the R_F value and color of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

C: The chromatogram of the *Assay preparation* obtained as directed in the *Assay for chlorpheniramine maleate* exhibits a major peak for chlorpheniramine, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* similarly determined, both relative to the internal standard.

D: The chromatogram of the *Assay preparation* obtained as directed in the *Assay for dexamethasone* exhibits a major peak for dexamethasone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* similarly determined, both relative to the internal standard.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.01 Endotoxin Unit per 100 Penicillin G Units.

pH ⟨791⟩: between 5.0 and 6.0.

Other requirements—It meets the requirements of the test for *Sterility* under *Penicillin G Procaine and Dihydrostreptomycin Sulfate Injectable Suspension*, and the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for penicillin G—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.

Assay preparation—Dilute an accurately measured volume of Injectable Suspension, freshly mixed and free from air bubbles, quantitatively with *Buffer B.1* to yield a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in the *Blank Determination* to add 0.1 mL of 1.2 N hydrochloric acid immediately before the 10.0 mL of 0.01 N iodine VS. Calculate the quantity, in Penicillin G Units, in the portion of Injectable Suspension taken by the formula:

$$(L/2D)(F)(B - I)$$

in which L is the labeled quantity, in Penicillin G Units, in the volume of Injectable Suspension taken, and D is the concentration, in Penicillin G Units per mL, of the *Assay preparation*, on the basis of the labeled quantity in the portion of Injectable Suspension taken and the extent of dilution, and the other terms are as defined therein.

Assay for dihydrostreptomycin—Proceed as directed for the turbidimetric assay for dihydrostreptomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Injectable Suspension, freshly mixed and free from air bubbles, diluted quantitatively with water to yield a *Test Dilution* having a concentration of dihydrostreptomycin assumed to be equal to the median dose level of the Standard.

Assay for chlorpheniramine maleate—

Internal standard solution—Prepare a solution of brompheniramine maleate in water having a concentration of about 7 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a stock solution having a known concentration of about 6 mg per mL. Transfer 5.0 mL of this solution to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution*, and adjust with sodium hydroxide solution (1 in 2) to a pH of about 10. Add 25.0 mL of hexanes, place the cap on the tube, shake for about 2 minutes, and centrifuge. Use the upper hexanes layer as the *Standard preparation*.

Assay preparation—Transfer an accurately measured volume of Injectable Suspension, freshly mixed and free from air bubbles, equivalent to about 30 mg of chlorpheniramine maleate, to a 50-mL centrifuge tube. Proceed as directed under *Standard preparation*, beginning with "Add 5.0 mL of *Internal standard solution*." Use the upper hexanes layer as the *Assay preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, and contains a 4-mm × 1.8-m glass column packed with 1.2% liquid phase G16 and 0.5% potassium hydroxide on 100- to 120-mesh support S1A. The column is maintained isothermally at about 180°, and the injection port and the detector block are maintained at about 200°. Dry nitrogen is used as the carrier gas at a flow rate of about 50 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, R, between the analyte and internal standard peaks is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 1.5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.75 for chlorpheniramine and 1.0 for brompheniramine. Calculate the quantity, in mg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in each mL of the Injectable Suspension taken by the formula:

$$5(C/V)(R_U/R_S)$$

in which C is the concentration of USP Chlorpheniramine Maleate RS in the stock solution used to prepare the *Standard preparation*, V is the volume, in mL, of Injectable Suspension taken, and R_U and R_S are the peak response ratios of the chlorpheniramine maleate peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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Assay for dexamethasone—

Mobile phase—Prepare a suitable filtered mixture of water and acetonitrile (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve about 30 mg of beclomethasone in 2 mL of methanol in a 50-mL volumetric flask, dilute with methylene chloride to volume, and mix.

Standard preparation—Transfer about 25 mg of USP Dexamethasone RS, accurately weighed, to a 50-mL volumetric flask. Add about 1 mL of methanol, swirl to dissolve, dilute with methylene chloride to volume, and mix. Transfer 5.0 mL of this solution to a suitable flask, and add 5.0 mL of *Internal standard solution*. Heat the flask on a steam bath, and evaporate under a stream of nitrogen just to dryness. Add 10.0 mL of methanol to the flask, and swirl to dissolve the residue. This *Standard preparation* contains about 0.25 mg of USP Dexamethasone RS and 0.3 mg of beclomethasone per mL.

Assay preparation—Transfer an accurately measured volume of Injectable Suspension, freshly mixed and free from air bubbles, equivalent to about 2.5 mg of dexamethasone, to a separator containing 50 mL of 0.1 N hydrochloric acid, add 5.0 mL of *Internal standard solution*, and extract with four 25-mL portions of methylene chloride, combining the extracts in a second separator. Wash the combined extracts with 50 mL of sodium bicarbonate solution (1 in 20), filtering the lower methylene chloride layer through about 4 g of anhydrous sodium sulfate supported on a cotton pledget previously washed with methylene chloride, and collecting the filtrate in a suitable flask. Wash the aqueous layer with 25 mL of methylene chloride, and filter the lower methylene chloride layer through the same filter, collecting the filtrate in the same flask. Heat the flask on a steam bath, and evaporate under a stream of nitrogen just to dryness. Add 10.0 mL of methanol, and swirl to dissolve the residue.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, of the analyte and the internal standard peaks is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for dexamethasone and 1.0 for beclomethasone. Calculate the quantity, in mg, of dexamethasone ($C_{22}H_{29}FO_5$) in each mL of the Injectable Suspension taken by the formula:

$$10(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*, V is the volume, in mL, of Injectable Suspension taken, and R_U and R_S are the peak response ratios of the dexamethasone peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillin G Procaine, Dihydrostreptomycin Sulfate, and Prednisolone Injectable Suspension

» Penicillin G Procaine, Dihydrostreptomycin Sulfate, and Prednisolone Injectable Suspension is a sterile suspension of Penicillin G Procaine and Prednisolone in a solution of Dihydrostreptomycin Sulfate in Water for Injection. It contains one or more suitable buffers, dispersants, preservatives, and suspending agents. It may contain not more than 2.0 percent of procaine hydrochloride, and one or more suitable stabilizing agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled number of Penicillin G Units, not less than 90.0 percent and not more than 115.0 percent of the labeled amount of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone ($C_{21}H_{28}O_5$).

Packaging and storage—Preserve in single-dose or multiple-dose, tight containers.

Labeling—Label it to indicate that it is intended for veterinary use only, and is not to be used in animals to be slaughtered for human consumption.

USP Reference standards ⟨11⟩—
USP Dihydrostreptomycin Sulfate RS
USP Penicillin G Potassium RS
USP Prednisolone RS

Identification—It responds to *Identification* tests *A* and *B* under *Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate, and Dexamethasone Injectable Suspension.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.01 Endotoxin Unit per 100 Penicillin G Units.

Other requirements—It meets the requirements of the test for *Sterility*, and for *pH* under *Penicillin G Procaine and Dihydrostreptomycin Sulfate Injectable Suspension.*

Assay for penicillin G—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩.

Assay preparation—Dilute an accurately measured volume of Injectable Suspension quantitatively with *Buffer B.1* to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in the *Blank Determination* to add 0.1 mL of 1.2 N hydrochloric acid immediately before the 10.0 mL of 0.01 N iodine VS. Calculate the quantity, in Penicillin G Units, in the portion of Injectable Suspension taken by the formula:

$$(L/2D)(F)(B - I)$$

in which L is the labeled quantity, in Penicillin G Units, in the volume of Injectable Suspension taken, D is the concentration, in Penicillin G Units per mL, of the *Assay preparation*, on the basis of the labeled quantity in the portion of Injectable

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Suspension taken and the extent of dilution, and the other terms are as defined therein.

Assay for dihydrostreptomycin—Proceed as directed for the turbidimetric assay for dihydrostreptomycin as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Injectable Suspension diluted quantitatively with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for prednisolone—

Standard preparation—Prepare as directed for *Standard Preparation* under *Single-steroid Assay* ⟨511⟩, using USP Prednisolone RS.

Assay preparation—Transfer to a separator an accurately measured volume of Injectable Suspension, and add 15 mL of water. Extract with three 25-mL portions and finally with one 20-mL portion of chloroform, filtering each portion through chloroform-washed cotton into a 100-mL volumetric flask. Add chloroform to volume, and mix. Pipet 20 mL of this solution into a suitable glass-stoppered flask or tube, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 2.0 mL, accurately measured, of a mixture of equal volumes of chloroform and alcohol.

Procedure—Proceed as directed for *Single-Steroid Assay* ⟨511⟩, using *Solvent A* to develop the chromatogram. Calculate the quantity, in mg, of prednisolone ($C_{21}H_{28}O_5$) in each mL of the Injectable Suspension taken by the formula:

$$0.01(C/V)(A_U/A_S)$$

in which the terms are as defined therein.

Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension

» Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension is a suspension of Penicillin G Procaine, Neomycin Sulfate, Polymyxin B Sulfate and Hydrocortisone Acetate in Peanut Oil or Sesame Oil. It may contain one or more suitable dispersing and suspending agents. It contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of Penicillin G Units, of neomycin, and of polymyxin B Units, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Penicillin G Potassium RS
USP Polymyxin B Sulfate RS

Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for penicillin G—Proceed as directed for penicillin G under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Topical Suspension blended for 2 minutes in a high-speed glass blender jar with 499.0 mL of *Buffer B.1* and 1.0 mL of polysorbate 80. Allow to stand for 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.1* to obtain a *Test Dilution* having a concentration of penicillin G assumed to be equal to the median dose level of the Standard.

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Topical Suspension shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to an appropriate volume to obtain a stock solution. To an accurately measured volume of this stock solution add an accurately measured volume of penicillinase sufficient to inactivate the penicillin G therein, heat at 37° for 30 minutes, and dilute quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median dose level of the Standard.

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Topical Suspension blended for 2 minutes in a high-speed glass blender jar containing 499.0 mL of *Buffer B.6* and 1.0 mL of polysorbate 80. Allow to stand for 10 minutes, and to an accurately measured volume of the aqueous phase add an accurately measured volume of penicillinase sufficient to inactivate the penicillin G therein. Heat the solution at 37° for 30 minutes, and dilute quantitatively and stepwise with *Buffer B.6* to obtain a *Test Dilution* having a concentration of polymyxin assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS dissolved in *Buffer B.6* to obtain the same concentration of neomycin present in the *Test Dilution*.

Assay for hydrocortisone acetate—Using an accurately measured volume of Topical Suspension, proceed as directed in the *Assay* under *Hydrocortisone Acetate Lotion*.

Penicillin G Procaine and Novobiocin Sodium Intramammary Infusion

» Penicillin G Procaine and Novobiocin Sodium Intramammary Infusion is a suspension of Penicillin G Procaine and Novobiocin Sodium in a suitable vegetable oil vehicle. It contains a suitable preservative and suspending agent. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of Penicillin G Units and novobiocin ($C_{31}H_{36}N_2O_{11}$).

Packaging and storage—Preserve in disposable syringes that are well-closed containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Novobiocin RS
USP Penicillin G Potassium RS

USP Monographs

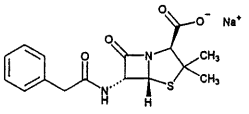
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Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for penicillin G—Proceed as directed for penicillin G under *Antibiotics—Microbial Assays* ⟨81⟩, except to use *Staphylococcus aureus* ATCC No. 12692 as the test organism. Prepare the inoculum by growing the organism at 32° to 35° for 24 hours on Medium 1 to which has been added a solution of novobiocin sodium, containing the equivalent of 2.5 mg of novobiocin per mL that has been filtered through a membrane filter having a 0.2-µm porosity, so that the medium contains the equivalent of 10 µg of novobiocin per mL. Use an inoculum composition of about 5 mL of stock suspension in each 100 mL of Medium 1. Expel the contents of a syringe of Intramammary Infusion into a high-speed glass blender jar containing 1.0 mL of polysorbate 80 and 499.0 mL of *Buffer B.1*, and blend for 3 to 5 minutes. Allow to stand for 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.1* to obtain a *Test Dilution* having a concentration of penicillin G assumed to be equal to the median dose level of the Standard.

Assay for novobiocin—Proceed as directed for novobiocin under *Antibiotics—Microbial Assays* ⟨81⟩, expelling the contents of a syringe of Intramammary Infusion into a high-speed blender jar containing 1.0 mL of polysorbate 80 and 499.0 mL of *Buffer B.3*, and blend for 3 to 5 minutes. Allow to stand for 10 minutes. To an accurately measured volume of the aqueous phase add sufficient penicillinase to inactivate the penicillin G therein, and dilute quantitatively and stepwise with *Buffer B.6* to obtain a *Test Dilution* having a concentration of novobiocin assumed to be equal to the median dose level of the Standard. [NOTE—Store this *Test Dilution* at 37° for 30 minutes and allow to cool before using it to fill the cylinders on the plates.]

Penicillin G Sodium



$C_{16}H_{17}N_2NaO_4S$ 356.37

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-, [2*S*-(2α,5α,6β)]-, monosodium salt;

Monosodium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-57-8].

DEFINITION

Penicillin G Sodium has a potency of NLT 1500 and NMT 1750 Penicillin G Units/mg.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: 0.01 M monobasic potassium phosphate

Mobile phase: Methanol and *Solution A* (40:60)

System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water

Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. Shake as needed to dissolve. This solution contains about 160 Penicillin G Units/mL.

Sample solution: 0.1 mg/mL of Penicillin G Sodium in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution*

Column efficiency: NLT 1000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency of penicillin G sodium, in Penicillin G Units/mg, in the portion of Penicillin G Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of penicillin G from the *Sample solution*

r_S = peak response of penicillin G from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Sodium in the *Sample solution* (mg/mL)

P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Acceptance criteria: 1500–1750 Penicillin G Units/mg

SPECIFIC TESTS

- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩

Sample solution: 60 mg/mL of Penicillin G Sodium in water

Acceptance criteria: 5.0–7.5

- **LOSS ON DRYING** ⟨731⟩

Sample: 100 mg of Penicillin G Sodium

Analysis: Dry the *Sample* in a capillary-stoppered bottle under a vacuum at a pressure NMT 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 1.5%

- **STERILITY TESTS** ⟨71⟩: Where the label states that Penicillin G Sodium is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Penicillin G Sodium is sterile or it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

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- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Penicillin G Potassium RS
  USP Penicillin G Sodium RS

Penicillin G Sodium for Injection

DEFINITION

Penicillin G Sodium for Injection is sterile Penicillin G Sodium or a sterile mixture of Penicillin G Sodium and NLT 4.0% and NMT 5.0% of Sodium Citrate, of which NMT 0.15% may be replaced by Citric Acid. It contains NLT 90.0% and NMT 120.0% of the labeled amount of penicillin G. In addition, where it contains Sodium Citrate, it has a potency of NLT 1420 and NMT 1667 Penicillin G Units/mg.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Solution A*.

Sample solution: Nominally 12,000 Penicillin G Units/mL from Penicillin G Sodium for Injection in *Solution A*

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)
Spray reagent 1: Starch TS
Spray reagent 2: Iodine TS diluted 1 in 10 with water

Analysis
Samples: *Standard solution* and *Sample solution*
Place the plate in a suitable chromatographic chamber. Develop the chromatogram using the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background.

Acceptance criteria: The R_F value of the penicillin G spot from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY

- **PROCEDURE**

Solution A: 0.01 M monobasic potassium phosphate
Mobile phase: Methanol and *Solution A* (40:60)
System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water
Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. Shake as needed to dissolve. This solution contains about 160 Penicillin G Units/mL.
Sample solution 1 (where it is represented as being in a single-dose container): Constitute Penicillin G Sodium for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and dilute with water to obtain a solution containing nominally 160 Penicillin G Units/mL.
Sample solution 2 (where the label states the quantity of penicillin G in a given volume of constituted solution): Constitute Penicillin G Sodium for Injection as directed in the labeling. Dilute a suitable aliquot of the constituted solution with water to obtain a solution containing nominally 160 Penicillin G Units/mL.
Sample solution 3 (where it contains sodium citrate): Transfer about 50 mg of the Penicillin G Sodium for Injection to a 500-mL volumetric flask, add about 400 mL of water, and shake to dissolve. Dilute with water to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution*
Column efficiency: NLT 1000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution 1*, *Sample solution 2*, or *Sample solution 3*
Perform the *Assay* on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of penicillin G in a given volume of constituted solution. Use the individual results to determine the *Uniformity of Dosage Units* and the average of the results as the *Assay* value.
Calculate the percentage of the labeled amount of penicillin G in the container or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from *Sample solution 1* or *Sample solution 2*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of *Sample solution 1* or *Sample solution 2* (Penicillin G Units/mL)
P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Calculate the potency, in Penicillin G Units/mg, in the portion of Penicillin G Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from *Sample solution 3*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)
C_U = concentration of Penicillin G Sodium for Injection in *Sample solution 3* (mg/mL)

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P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Acceptance criteria: 90.0%–120.0% of the labeled amount of penicillin G. Where Penicillin G Sodium for Injection contains sodium citrate, it has a potency of NLT 1420 and NMT 1667 Penicillin G Units/mg.

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements. Perform the *Assay* on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of penicillin G in a given volume of constituted solution. Use the individual results to determine the *Uniformity of Dosage Units* and the average of the results as the *Assay* value.

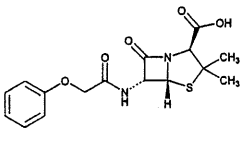
SPECIFIC TESTS

- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*: At the time of use, it meets the requirements.
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.
- **pH** ⟨791⟩
  Sample solution: A solution containing 60 mg/mL
  Acceptance criteria: 5.0–7.5. Where it is labeled as containing sodium citrate, the pH is between 6.0 and 7.5.
- **LOSS ON DRYING** ⟨731⟩
  Sample: 100 mg of Penicillin G Sodium for Injection
  Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure NMT 5 mm of mercury at 60° for 3 h.
  Acceptance criteria: NMT 1.5%
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **LABELING** ⟨7⟩, *Labels and Labeling for Injectable Products*: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Penicillin G Potassium RS

Penicillin V



$C_{16}H_{18}N_2O_5S$ 350.39

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-, [2*S*-(2α,5α,6β)]-;
(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [87-08-1].

DEFINITION

Penicillin V has a potency of NLT 1525 and NMT 1780 Penicillin V Units/mg.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Do not dry.
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)
  System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*
  Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*
  Sample solution: 2.5 mg/mL of Penicillin V in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*
  Column efficiency: NLT 1800 theoretical plates, *System suitability solution*
  Relative standard deviation: NMT 1.0% for penicillin V potassium, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the potency of penicillin V potassium, in Penicillin V Units/mg, in the portion of Penicillin V taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*

r_S = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*

C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin V in the *Sample solution* (mg/mL)

P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 1525–1780 Penicillin V Units/mg

IMPURITIES

- **LIMIT OF PHENOXYACETIC ACID**
  Mobile phase: Acetonitrile, glacial acetic acid, and water (35:1:65)
  Diluent: pH 6.6 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

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Standard solution: 0.1 mg/mL of phenoxyacetic acid in *Diluent*
Sample solution: 20.0 mg/mL of Penicillin V in *Diluent*. Use this solution on the day prepared.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenoxyacetic acid in the portion of Penicillin V taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of phenoxyacetic acid from the *Sample solution*
r_S = peak area of phenoxyacetic acid from the *Standard solution*
C_S = concentration of phenoxyacetic acid in the *Standard solution* (mg/mL)
C_U = concentration of Penicillin V in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

• **LIMIT OF *p*-HYDROXYPENICILLIN V**
Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of *p*-hydroxypenicillin V in the portion of Penicillin V taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of *p*-hydroxypenicillin V from the *Sample solution*
r_T = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V

Acceptance criteria: NMT 5.0%

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
• **PH** ⟨791⟩
Sample solution: Prepare a suspension containing 30 mg/mL of Penicillin V in water
Acceptance criteria: 2.5–4.0
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Penicillin G Potassium RS
USP Penicillin V RS
USP Penicillin V Potassium RS

Penicillin V for Oral Suspension

DEFINITION

Penicillin V for Oral Suspension is a dry mixture of Penicillin V with or without one or more suitable buffers, colors, flavors, and suspending agents. It contains NLT 90.0% and NMT 120.0% of the labeled number of Penicillin V Units when constituted as directed.

IDENTIFICATION

• **A.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)
System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*
Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*
Sample solution: Constitute Penicillin V for Oral Suspension as directed in the labeling. Transfer a suitable aliquot, freshly mixed and free from air bubbles, and containing about 400,000 Penicillin V Units to a suitable volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a solution containing nominally about 4000 Penicillin V Units/mL. Pass a portion of this solution through a suitable filter of 0.5-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*
Column efficiency: NLT 1800 theoretical plates for penicillin V, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled number of Penicillin V Units in the portion of Penicillin V for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*
r_S = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*
C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of penicillin V in the *Sample solution* (Penicillin V Units/mL)
P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

USP Monographs

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Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  For solids packaged in single-unit containers: Meets the requirements
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS
- **PH** ⟨791⟩
  Sample solution: Constitute as directed in the labeling.
  Acceptance criteria: 2.0–4.0
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** It may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Penicillin G Potassium RS
  USP Penicillin V RS
  USP Penicillin V Potassium RS

Penicillin V Tablets

DEFINITION
Penicillin V Tablets contain NLT 90.0% and NMT 120.0% of the labeled number of Penicillin V Units.

IDENTIFICATION
- **A.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)
  System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*
  Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*
  Sample solution: Nominally 4000 Penicillin V Units/mL, in *Mobile phase* dissolved from finely powdered Tablets (NLT 20). Shake for about 5 min. Pass a portion of the solution through a suitable filter of 0.5-µm or finer pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*
  Column efficiency: NLT 1800 theoretical plates, *System suitability solution*
  Relative standard deviation: NMT 1.0% for penicillin V potassium, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled number of Penicillin V Units in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*
r_S = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*
C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of penicillin V in the *Sample solution* (Penicillin V Units/mL)
P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

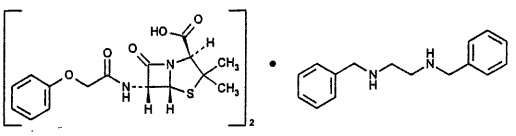
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm
  Time: 45 min
  Standard solution: USP Penicillin V Potassium RS in *Medium*
  Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.
  Tolerances: NLT 75% (*Q*) of the labeled amount of penicillin V ($C_{16}H_{18}N_2O_5S$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 3.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The Tablets may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Penicillin G Potassium RS
  USP Penicillin V RS
  USP Penicillin V Potassium RS

Penicillin V Benzathine



$(C_{16}H_{18}N_2O_5S)_2 \cdot C_{16}H_{20}N_2$ 941.12

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(2-phenoxyacetyl)amino-], [2*S*-(2α,5α,6β)]-, compd. with *N*,*N*′-bis(phenylmethyl)-1,2-ethanediamine (2:1).

USP Monographs

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(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N*,*N*′-dibenzylethylenediamine (2:1) [5928-84-7].
Tetrahydrate 1013.21
[63690-57-3].

» Penicillin V Benzathine has a potency of not less than 1060 and not more than 1240 Penicillin V Units per mg.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Penicillin V Potassium RS
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 4.0 and 6.5, in a suspension containing about 30 mg per mL.
Water Determination, *Method I* ⟨921⟩: between 5.0% and 8.0%.
Penicillin V content—Transfer about 40 mg, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Concomitantly determine the absorbances of this solution and of a similarly prepared Standard solution prepared with about 30 mg of USP Penicillin V Potassium RS at the wavelength of maximum absorbance at about 276 nm. Determine the percentage of penicillin V taken by the formula:

$$P(a_U/a_S)$$

in which P is the percentage content of penicillin V in the USP Penicillin V Potassium RS, and a_U and a_S are the absorptivities of the solution of the specimen and the Standard solution, respectively: between 62.3% and 72.5% is found.

Assay—
Standard preparation—Prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Penicillin V Potassium RS.
Assay preparations—Dissolve a quantity of Penicillin V Benzathine in 1.0 N sodium hydroxide to obtain a solution containing 2000 Penicillin V Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask, and use as the *Assay preparation* for *Inactivation and titration.* Dilute a quantity of Penicillin V Benzathine quantitatively with water to obtain a suspension containing 2000 Penicillin V Units per mL. Pipet 2 mL of this suspension into a glass-stoppered, 125-mL conical flask, and use as the *Assay preparation* for the *Blank determination.*
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in the *Inactivation and titration* of the specimen to omit the addition of 2.0 mL of 1.0 N sodium hydroxide. Calculate the potency, in Penicillin V Units per mg, in the Penicillin V Benzathine taken by the formula:

$$(F)(B - I)/(2D)$$

in which D is the concentration, in mg per mL, of the *Assay preparation* for *Inactivation and titration* on the basis of the weight of Penicillin V Benzathine taken and the extent of dilution.

Penicillin V Benzathine Oral Suspension

» Penicillin V Benzathine Oral Suspension contains not less than 90.0 percent and not more than 120.0 percent of the labeled number of Penicillin V Units per mL. It contains one or more suitable buffers, colors, dispersants, flavors, and preservatives.

Packaging and storage—Preserve in tight containers, and store in a refrigerator.
Labeling—It may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.
USP Reference standards ⟨11⟩—
USP Penicillin V Potassium RS

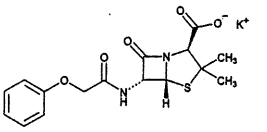
Uniformity of dosage units ⟨905⟩—
FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS : meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 6.0 and 7.0.

Assay—
Standard preparation—Prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Penicillin V Potassium RS.
Assay preparations—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with 1.0 N sodium hydroxide to obtain a solution containing 2000 Penicillin V Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask, and use as the *Assay preparation* for *Inactivation and titration.* Dilute an accurately measured volume of Oral Suspension quantitatively with water to obtain a suspension containing 2000 Penicillin V Units per mL. Pipet 2 mL of this suspension into a glass-stoppered, 125-mL conical flask, and use as the *Assay preparation* for the *Blank determination.*
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, except in the *Inactivation and titration* to omit the addition of 2.0 mL of 1.0 N sodium hydroxide. Calculate the quantity, in Penicillin V Units, in each mL of the Oral Suspension taken by the formula:

$$(T/2D)F(B - I)$$

in which T is the labeled quantity, in Penicillin V Units per mL, in the Oral Suspension, and D is the concentration, in Penicillin V Units per mL, in the *Assay preparation* for *Inactivation and titration* on the basis of the volume of Oral Suspension taken and the extent of dilution.

Penicillin V Potassium



$C_{16}H_{17}KN_2O_5S$ 388.48
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-, monopotassium salt, [2*S*-(2α,5α,6β)]-;

USP Monographs

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Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [132-98-9].

DEFINITION

Penicillin V Potassium has a potency of NLT 1380 and NMT 1610 Penicillin V Units/mg.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**

Diluent: Glycerin and water (25:14)
Solution A: 106 mg/mL of sodium carbonate in water
Solution B: 120 mg/mL of sodium sulfide in *Diluent*, prepared as follows. Dissolve sodium sulfide in *Diluent*, using about 45% of the final volume and heat. Allow to cool, and dilute with *Diluent* to the final volume.
Solution C: 150 mg/mL of tartaric acid in water
Sample solution: 0.1 g of Penicillin V Potassium in 2 mL of water
Analysis
Part 1: Add 1 mL of *Solution A* to the *Sample solution* and heat.
Part 2: To the hot solution from *Part 1* add 0.05 mL of *Solution B*.
Part 3: Cool the mixture from *Part 2* in iced water and add 2 mL of *Solution C*. Allow to stand.
Acceptance criteria: Meets the requirements for *Parts 1*, *2*, and *3*
Part 1: No precipitate is formed.
Part 2: No precipitate is formed.
Part 3: A white precipitate is formed.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)
System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*
Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*
Sample solution: 2.5 mg/mL of Penicillin V Potassium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the potency of penicillin V potassium, in Penicillin V Units/mg, in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = sum of the *p*-hydroxypenicillin V and penicillin V peak responses from the *Sample solution*
r_S = sum of the *p*-hydroxypenicillin V and penicillin V peak responses from the *Standard solution*
C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)
C_U = concentration of Penicillin V Potassium in the *Sample solution* (mg/mL)
P = potency of USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 1380–1610 Penicillin V Units/mg

IMPURITIES

- **LIMIT OF PHENOXYACETIC ACID**

Mobile phase: Acetonitrile, glacial acetic acid, and water (35:1:65)
Diluent: pH 6.6 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
Standard solution: 0.1 mg/mL of phenoxyacetic acid in *Diluent*
Sample solution: 20 mg/mL of Penicillin V Potassium in *Diluent*. Use this solution on the day prepared.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenoxyacetic acid in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = phenoxyacetic acid peak response from the *Sample solution*
r_S = phenoxyacetic acid peak response from the *Standard solution*
C_S = concentration of phenoxyacetic acid in the *Standard solution* (mg/mL)
C_U = concentration of Penicillin V Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

- **LIMIT OF *p*-HYDROXYPENICILLIN V**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of *p*-hydroxypenicillin V in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

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r_U = *p*-hydroxypenicillin V peak response from the *Sample solution*
r_T = sum of the *p*-hydroxypenicillin V and penicillin V peak responses from the *Sample solution*

Acceptance criteria: NMT 5.0%

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Specific Rotation*
  Sample solution: 10 mg/mL of Penicillin V Potassium in carbon dioxide-free water
  Acceptance criteria: +220° to +235°
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 30 mg/mL of Penicillin V Potassium in water
  Acceptance criteria: 4.0–7.5
- **LOSS ON DRYING** ⟨731⟩
  Sample: 100 mg of Penicillin V Potassium
  Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.
  Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Penicillin G Potassium RS
  USP Penicillin V Potassium RS

Penicillin V Potassium for Oral Solution

DEFINITION
Penicillin V Potassium for Oral Solution is a dry mixture of Penicillin V Potassium with or without one or more suitable buffers, colors, flavors, preservatives, and suspending agents. It contains NLT 90.0% and NMT 135.0% of the labeled number of Penicillin V Units when constituted as directed.

IDENTIFICATION
- **A.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)
  System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*
  Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*
  Sample solution: Constitute Penicillin V Potassium for Oral Solution as directed in the labeling. Transfer a suitable aliquot containing nominally 400,000 Penicillin V Units to a suitable volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a solution containing nominally 4000 Penicillin V Units/mL. Pass a portion of this solution through a suitable filter of 0.5-µm or finer pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*
  Column efficiency: NLT 1800 theoretical plates, *System suitability solution*
  Relative standard deviation: NMT 1.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled number of Penicillin V Units in the portion of Penicillin V Potassium for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*
r_S = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*
C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of penicillin V in the *Sample solution* (Penicillin V Units/mL)
P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 90.0%–135.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  For solids packaged in single-unit containers: Meets the requirements
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS
- **pH** ⟨791⟩
  Sample solution: Constitute as directed in the labeling.
  Acceptance criteria: 5.0–7.5
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** It may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Penicillin G Potassium RS
  USP Penicillin V Potassium RS

Penicillin V Potassium Tablets

DEFINITION
Penicillin V Potassium Tablets contain NLT 90.0% and NMT 120.0% of the labeled number of Penicillin V Units.

IDENTIFICATION
- **A.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

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ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)

System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*

Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*

Sample solution: Transfer a portion of finely powdered Tablets (NLT 20), containing nominally about 400,000 Penicillin V Units, to a suitable volumetric flask. Dilute with *Mobile phase* to volume, mix to obtain a solution containing nominally about 4000 Penicillin V Units/mL and shake for about 5 min. Pass a portion of this solution through a suitable filter of 0.5-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*

Column efficiency: NLT 1800 theoretical plates, *System suitability solution*

Relative standard deviation: NMT 1.0% for penicillin V potassium, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled number of Penicillin V Units in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*

r_S = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*

C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of penicillin V in the *Sample solution* (Penicillin V Units/mL)

P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: pH 6.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Penicillin V Potassium RS in *Medium*

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Analysis: Determine the percentage of the labeled number of Penicillin V Units dissolved by a suitable validated spectrophotometric analysis of a filtered portion of the solution under test.

Tolerances: NLT 75% (*Q*) of the labeled number of Penicillin V Units is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Sample: 100 mg

Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.

Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

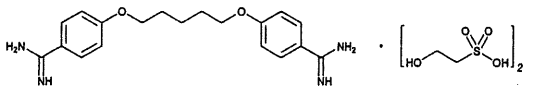
• **LABELING:** Label the chewable Tablets to indicate that they are to be chewed before swallowing. The Tablets may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Penicillin G Potassium RS

USP Penicillin V Potassium RS

Pentamidine Isethionate



$C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$ 592.68

Ethanesulfonic acid, 2-hydroxy-, compd. with 4,4′-[1,5-pentanediylbis(oxy)]bis [benzenecarboximidamide]; 4,4′-(Pentane-1,5-diylbis(oxy))dibenzimidamide bis(2-hydroxyethanesulfonate) [140-64-7].

DEFINITION

Pentamidine Isethionate contains NLT 98.5% and NMT 101.5% of $C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B. OXYGEN-FLASK COMBUSTION** ⟨471⟩

Barium chloride solution: 60 mg/mL of barium chloride in water

Analysis: Burn 150 mg, using 10 mL of 3% hydrogen peroxide as the absorbing liquid. When the process is complete, acidify with 1 mL of diluted hydrochloric acid, and add 1 mL of the *Barium chloride solution*.

Acceptance criteria: A white precipitate is formed.

• **C.** The retention time of the pentamidine isethionate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY

• **PROCEDURE**

Sample solution: 5 mg/mL in dimethylformamide. Add 0.25 mL of thymol blue TS.

Analysis: Titrate under a stream of nitrogen with 0.1 M tetrabutylammonium hydroxide VS, determining the endpoint until the color changes to intense blue. Perform a blank determination, and make any necessary correction

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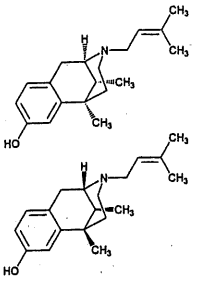
(see *Titrimetry* ⟨541⟩). Each mL of 0.1 M tetrabutylammonium hydroxide is equivalent to 29.63 mg of $C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$.
Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩
Acceptance criteria: NMT 0.1% on a 1-g sample
ORGANIC IMPURITIES
• **Procedure**
Buffer: 30 mg/mL of ammonium acetate in water, adjusted with triethylamine to a pH of 7.5
Mobile phase: Methanol and *Buffer* (65:35)
System suitability solution: Prepare 40.0 mL of a 2.5 mg/mL solution of USP Pentamidine Isethionate RS in water. Adjust with 0.2 M sodium hydroxide to a pH of 10.5, and boil under reflux for 20 min. Cool, and dilute with water to 50.0 mL. Transfer quantitatively 1 mL of this solution to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Standard solution: 2 µg/mL of USP Pentamidine Isethionate RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Pentamidine Isethionate in *Mobile phase*. [NOTE—It must be demonstrated that the final product does not contain a detectable amount of alkyl 2-hydroxyethanesulphonates, a potential in-process impurity.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: 3.5 times the retention time of pentamidine
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2 between the two major peaks. [NOTE—The chromatogram shows two major peaks.]
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria
Individual impurities: NMT 0.4%. [NOTE—Exclude any other peak producing a response of less than 0.02%.]
Total impurities: NMT 0.7%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 4.5–6.5, in a carbon dioxide-free aqueous solution containing 50 mg/mL of Pentamidine Isethionate
• **LOSS ON DRYING** ⟨731⟩**:** Dry at 105°: it loses NMT 4.0% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pentamidine Isethionate RS
$C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$

Pentazocine



$C_{19}H_{27}NO$ 285.42
2,6-Methano-3-benzazocin-8-ol, 1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-, (2α,6α,11*R**)-; (2*R**,6*R**,11*R**)-1,2,3,4,5,6-Hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol [359-83-1].

DEFINITION
Pentazocine contains NLT 98.0% and NMT 102.0% of pentazocine ($C_{19}H_{27}NO$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
• **B.** The retention time of the pentazocine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 15 mM sodium borate in water. Adjust with 10 N sodium hydroxide to a pH of 10.0.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
15	15	85
20	15	85
21	85	15
25	85	15

Diluent: Methanol, phosphoric acid, and water (500:1:500)
Standard solution: 0.025 mg/mL of USP Pentazocine RS in *Diluent*
Sample solution: 0.025 mg/mL of Pentazocine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 10-cm; 2.6-µm packing L1
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*

USP Monographs

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Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pentazocine ($C_{19}H_{27}NO$) in the portion of Pentazocine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine from the *Sample solution*
r_S = peak response of pentazocine from the *Standard solution*
C_S = concentration of USP Pentazocine RS in the *Standard solution* (mg/mL)
C_U = concentration of Pentazocine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.0006 mg/mL of USP Pentazocine RS in *Diluent*
Standard solution: 0.002 mg/mL of USP Pentazocine RS in *Diluent*
Sample solution: 2 mg/mL of Pentazocine in *Diluent*. Sonicate to dissolve, if necessary.
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Pentazocine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pentazocine from the *Standard solution*
C_S = concentration of USP Pentazocine RS in the *Standard solution* (mg/mL)
C_U = concentration of Pentazocine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Norpentazocine[a]	0.6	0.15
Pentazocine hydration product[b]	0.9	0.15
Pentazocine	1.0	—
Any unspecified impurity	—	0.10
Total impurities	—	1.0

[a] (2*R*,6*R*,11*R*)-1,2,3,4,5,6-Hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol.
[b] (2*R*,6*R*,11*R*)-3-(3-Hydroxy-3-methylbutyl)-6,11-dimethyl-1,2,3,4,5,6-hexahydro-2,6-methanobenzo[*d*]azocin-8-ol.

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** The level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Pentazocine is used can be met. Where the label states that Pentazocine must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Pentazocine is used can be met.
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at a pressure of NMT 5 mm of mercury at 60° to constant weight.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** Where Pentazocine must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pentazocine RS

Pentazocine Hydrochloride

$C_{19}H_{27}NO \cdot HCl$ 321.88
2,6-Methano-3-benzazocin-8-ol, 1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-, hydrochloride, (2α,6α,11*R**)-;
(2*R**,6*R**,11*R**)-1,2,3,4,5,6-Hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride [64024-15-3].

DEFINITION
Pentazocine Hydrochloride contains NLT 98.0% and NMT 102.0% of pentazocine hydrochloride ($C_{19}H_{27}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩**,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Sample solution: 80 µg/mL of pentazocine
Medium: 0.01 N hydrochloric acid
Analytical wavelength: 278 nm
Acceptance criteria: Absorptivities do not differ by more than 3.0%, calculated on the dried basis. [NOTE—The molecular weight of pentazocine ($C_{19}H_{27}NO$) is 285.43.]
• **B. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩
Standard solution: Dissolve 50 mg of USP Pentazocine RS in 25 mL of 0.01 N hydrochloric acid in a separator, and use this in place of the *Standard solution* specified.
Acceptance criteria: Meets the requirements

USP Monographs

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• **C. Identification Tests—General,** *Chloride* ⟨191⟩: A solution (1 in 100) meets the requirements.

ASSAY

• **Procedure**

Sample solution: Dissolve 650 mg of Pentazocine Hydrochloride in 50 mL of glacial acetic acid.

Analysis: To the *Sample solution* add 10 mL of mercuric acetate TS and 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.19 mg of pentazocine hydrochloride ($C_{19}H_{27}NO \cdot HCl$).

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.2%

• **Ordinary Impurities** ⟨466⟩

Standard solution: Methanol, USP Pentazocine RS being used

Test solution: Methanol

Eluant: Chloroform, methanol, and isopropylamine (94:3:3)

Visualization: Heat the plate in an oven at 105° for 15 min. Cool, follow with visualization technique 17, and view under short-wavelength UV light.

Acceptance criteria: The total of any ordinary impurities observed does not exceed 1.0%.

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩

Analysis: Dry at a pressure not exceeding 5 mm of mercury at 100° to constant weight.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards** ⟨11⟩

USP Pentazocine RS

Pentazocine and Acetaminophen Tablets

DEFINITION

Pentazocine and Acetaminophen Tablets contain an amount of Pentazocine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Diluent: Chloroform and methanol (1:1)

Standard solution A: 1 mg/mL of USP Pentazocine RS in *Diluent*

Standard solution B: 26 mg/mL of USP Acetaminophen RS in *Diluent*

Sample solution: Transfer a quantity of finely powdered Tablets, nominally equivalent to about 5 mg of pentazocine and 130 mg of acetaminophen, to a suitable flask. Add 5 mL of *Diluent*, shake, and allow the solids to settle. Use the supernatant.

Chromatographic system

Developing solvent system: Ethyl acetate, methanol, and formic acid (90:5:5)

Spray reagent: Dissolve 300 mg of platinic chloride in 100 mL of water and add 100 mL of potassium iodide solution (6 in 100).

Analysis: Evaporate the solvents in cool, circulating air. After developing and examining the spots, spray the plate with *Spray reagent*.

Acceptance criteria: The R_F values, size, and intensity of color of the two principal spots of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*.

ASSAY

• **Pentazocine**

Mobile phase: Chloroform, methanol, and isopropylamine (960:40:2)

Diluent: Methanol and 0.035 N sulfuric acid (1:1)

Standard stock solution: 0.5 mg/mL of USP Pentazocine RS in *Diluent*

Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a 125-mL separator. Add 30 mL of water and 5 mL of sodium carbonate solution (1:10). Extract with 60 mL of chloroform and pass the chloroform layer through filter paper, collecting the filtrate in a 100-mL volumetric flask. Dilute with chloroform to volume and mix.

Sample solution: Transfer an amount nominally equivalent to 25 mg of pentazocine, from NLT 20 finely powdered Tablets, to a 50-mL glass-stoppered cylinder. Add 50.0 mL of *Diluent* and shake intermittently for 15 min. Sonicate for about 2 min, allow the solids to settle, and transfer 10.0 mL of the supernatant to a 125-mL separator. [Note—Save the remainder of the supernatant for use in the *Assay* for *Acetaminophen*. Minimize the waiting period before this test is performed to prevent significant hydrolysis of acetaminophen to *p*-aminophenol.] Add 30 mL of water and 5 mL of sodium carbonate solution (1:10) to the separator and mix. Extract with 60 mL of chloroform and pass the chloroform layer through filter paper, collecting the filtrate in a 100-mL volumetric flask. Dilute with chloroform to volume and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 10-µm packing L3

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine from the *Sample solution*

r_S = peak response of pentazocine from the *Standard solution*

C_S = concentration of USP Pentazocine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pentazocine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

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• **ACETAMINOPHEN**

[NOTE—Minimize the time between the addition of the *Diluent* and the injection of the *Sample solution* to prevent significant hydrolysis of acetaminophen to *p*-aminophenol.]

Mobile phase: Chloroform, methanol, and isopropylamine (960:40:2)

Diluent: Methanol and 0.035 N sulfuric acid (1:1)

Standard stock solution: 13 mg/mL of USP Acetaminophen RS in *Diluent*

Standard solution: Dilute 2.0 mL of the *Standard stock solution* with ethyl acetate to 200 mL.

Sample solution: Dilute 2.0 mL of the supernatant reserved from the *Assay* for *Pentazocine* immediately with ethyl acetate to volume in a 200-mL volumetric flask to minimize hydrolysis of acetaminophen to *p*-aminophenol and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 10-µm packing L3

Flow rate: 1.4 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Diluent: Acetonitrile and 0.035 N sulfuric acid (6:4)

Mobile phase: Tetrahydrofuran, phosphoric acid, and 0.005 M monobasic sodium phosphate (50:1:950)

Pentazocine standard stock solution: 0.25 mg/mL of USP Pentazocine RS in *Diluent*

System suitability stock solution: 0.325 mg/mL of USP Acetaminophen RS in *Diluent*

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 100-mL volumetric flask. Add 5.0 mL of the *Pentazocine standard stock solution*, dilute with *Mobile phase* to volume, and mix.

Standard solution: Transfer a quantity of USP Acetaminophen RS to a suitable volumetric flask. Add a sufficient volume of *Pentazocine standard stock solution* and mix to dissolve the acetaminophen. Dilute with *Mobile phase* to volume. Mix to obtain known concentrations of 0.0125 and 0.325 mg/mL of pentazocine and acetaminophen, respectively.

Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask, add 50 mL of *Diluent*, and sonicate for 30 min. Dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a paper filter, covering the funnel with a watch glass and discarding the first few mL of the filtrate.

Sample solution: Dilute 5.0 mL of the *Sample stock solution* with *Mobile phase* to 100 mL and pass this solution through a membrane filter of 0.5-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 9.4-mm × 10-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for acetaminophen and pentazocine are 0.2 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 7 between pentazocine and acetaminophen

Relative standard deviation: NMT 2.0% for the pentazocine and acetaminophen peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine or acetaminophen from the *Sample solution*

r_S = peak response of pentazocine or acetaminophen from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard (USP Pentazocine RS or USP Acetaminophen RS) in the *Standard solution* (mg/mL)

C_U = nominal concentration of pentazocine or acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetaminophen RS

USP Pentazocine RS

Pentazocine and Aspirin Tablets

DEFINITION

Pentazocine and Aspirin Tablets contain an amount of Pentazocine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ($C_9H_8O_4$).

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IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Diluent: Chloroform and methanol (1:1)

Standard solution A: 2.5 mg/mL of USP Pentazocine RS in *Diluent*

Standard solution B: 65 mg/mL of USP Aspirin RS in *Diluent*

Sample solution: Shake a quantity of finely powdered Tablets, nominally equivalent to about 25 mg of pentazocine and 650 mg of aspirin, with 10 mL of *Diluent* in an unltrasonic bath for 2 min. Allow the solids to settle.

Chromatographic system

Developing solvent system: Ethyl acetate, methanol, and formic acid (90:5:5)

Spray reagent: Iodoplatinate spray reagent. Dissolve 300 mg of platinic chloride in 100 mL of water, and add 100 mL of potassium iodide solution (6 in 100).

Analysis: Evaporate the solvents from the spots in warm circulating air. Place the plate in the developing chamber, and after developing the plate, remove it, and mark the solvent front. Evaporate the solvents thoroughly in warm circulating air, and examine the plate under short-wavelength UV light. Expose the plate to iodine vapor for about 5 min, and observe. Then spray the plate with *Spray reagent*.

Acceptance criteria: Two principal spots from the *Sample solution* correspond in R_F values, size, and intensity of color with the pentazocine spot of *Standard solution A* and the aspirin spot of *Standard solution B*, respectively.

ASSAY

• **PROCEDURE**

Diluent A: Methanol and water (1:1)

Diluent B: Methanol and 6 N hydrochloric acid (1:1)

Diluent C: Water, methanol, and 6 N hydrochloric acid (6:1:1)

Chromatographic column: Use a 200-mm tube consisting of about a 90-mm length of 22-mm tubing fused to about a 100-mm length of 5-mm tubing having a stopcock at the bottom of this section. Place a pledget of glass wool at the bottom of the 5-mm portion just above the stopcock. Transfer a suitable quantity of sulfonic acid cation-exchange resin to a beaker, and wash three times with water, discarding the water wash each time by decantation. Cover the resin with *Diluent B*, and allow to stand for 1 h. Decant the acid wash; if it is colored yellow or orange, repeat this step until the wash is almost colorless. Then wash the resin by repeated 15-min soakings in *Diluent A* followed by decantation until the wash is neutral to wide-range indicator paper. Fill the tube to a height of 100 mm with slurry of the washed resin in *Diluent A*. Wash the column with 25 mL of *Diluent A*.

Standard solution A: 18 µg/mL of USP Salicylic Acid RS in 0.1 N sodium hydroxide

Standard solution B: 62.5 µg/mL of USP Pentazocine RS in *Diluent C*

Sample stock solution A: Transfer a portion of fine powder from NLT 20 freshly powdered Tablets, nominally equivalent to about 25 mg of pentazocine and 650 mg of aspirin from NLT 20 finely powdered Tablets, to a suitable 250-mL flask. Add 100.0 mL of *Diluent A*, and shake by mechanical means for 20 min. Centrifuge a suitable quantity for 5 min.

Sample stock solution B: Transfer 25.0 mL of the clear supernatant from *Sample stock solution A* to the prepared *Chromatographic column*, followed by five 10-mL portions of *Diluent A*, collecting the eluate in a 250-mL volumetric flask containing 10.0 mL of 2.5 N sodium hydroxide. Dilute with water to volume, and mix.

Sample solution A: Pipet 4 mL of *Sample stock solution B* into a 100-mL volumetric flask, and dilute with 0.1 N sodium hydroxide to volume, and mix.

Sample solution B: Pass through the column after *Sample stock solution B* five 5-mL portions of *Diluent B*, followed by 10 mL of water. Collect the eluate in a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 296 nm (maximum absorbance) for the analysis of aspirin; 278 nm (maximum absorbance) for the analysis of pentazocine

Cell: 1 cm

Blank: 0.1 N sodium hydroxide for the analysis of aspirin; *Diluent C* for the analysis of pentazocine

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Sample solution A*, and *Sample solution B*

Calculate the percentage of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance from *Sample solution A*
A_S = absorbance from *Standard solution A*
C_S = concentration of USP Salicylic Acid RS in *Standard solution A* (µg/mL)
C_U = nominal concentration of aspirin in *Sample solution A* (µg/mL)
M_{r1} = molecular weight of aspirin, 180.16
M_{r2} = molecular weight of salicylic acid, 138.12

Calculate the percentage of pentazocine ($C_{19}H_{27}NO$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance from *Sample solution B*
A_S = absorbance from *Standard solution B*
C_S = concentration of USP Pentazocine RS in *Standard solution B* (µg/mL)
C_U = nominal concentration of pentazocine in *Sample solution B* (µg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of aspirin ($C_9H_8O_4$) and pentazocine ($C_{19}H_{27}NO$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 80 rpm

Time: 30 min

Standard solution A: 15 µg/mL of USP Salicylic Acid RS in 0.1 N sodium hydroxide

Standard solution B: 13 µg/mL of USP Pentazocine RS in dilute glacial acetic acid (1 in 50)

Strongly basic, anion-exchange resin: Mix a suitable quantity of anion-exchange resin with 10 volumes of dilute glacial acetic acid (1 in 50), and shake for 20 min. Allow the resin to settle, and decant the supernatant. Repeat the acetic acid washing four more times. Wash with water until 5.0 mL of the water wash gives a negligible response when substituted for 5.0 mL of *Sample solution*, and carried through the *Analysis* for pentazocine.

Sample solution: To a suitable 50-mL flask add 0.4 g of *Strongly basic, anion-exchange resin* and 25 mL of the solution under test. Shake by mechanical means for 15 min. Allow to settle, and use the clear supernatant.

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Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 296 nm (maximum absorbance) for the analysis of aspirin; 408 nm (maximum absorbance) for the analysis of pentazocine
Cell: 1 cm
Blank: 0.1 N sodium hydroxide for the analysis of aspirin; the chloroform layer from the reagent blank for the analysis of pentazocine
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
For aspirin, transfer 1.0 mL of the *Sample solution* to a 25-mL volumetric flask containing 1.0 mL of sodium hydroxide solution (1 in 10), and swirl. Allow to stand for 10 min. Dilute with water to volume, and mix. Determine the absorbances of the *Sample solution* and *Standard solution A* against the *Blank* for the analysis of aspirin.
Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from *Standard solution A*
C_S = concentration of USP Salicylic Acid RS in *Standard solution A* (mg/mL)
L = labeled amount of aspirin (mg/Tablet)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of aspirin, 180.16
M_{r2} = molecular weight of salicylic acid, 138.12

For pentazocine, transfer 5.0-mL portions of the *Sample solution*, *Standard solution B*, and water to serve as the reagent blank into three separate 125-mL separators. To each separator add 10 mL of a filtered solution (1 in 4000) of bromocresol purple in dilute glacial acetic acid (1 in 50) and 20.0 mL of chloroform. Insert the stopper, and shake gently for 1 min, accurately timed. Allow the layers to separate, and determine the absorbances of the clear chloroform layers from *Standard solution B* and the *Sample solution* against the chloroform layer from the reagent blank.
Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from *Standard solution B*
C_S = concentration of USP Pentazocine RS in *Standard solution B* (mg/mL)
L = labeled amount of pentazocine (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and NLT 70% (Q) of the labeled amount of aspirin ($C_9H_8O_4$) are dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
• **NONASPIRIN SALICYLATES**
Ferric chloride–urea reagent: To a mixture of 8 mL of ferric chloride solution (6 in 10) and 42 mL of 0.05 N hydrochloric acid, add 60 g of urea. Dissolve the urea by swirling and without the aid of heat, and adjust the resulting solution, if necessary, with 6 N hydrochloric acid to a pH of 3.2. Prepare on the day of use.
Standard stock solution: 150 µg/mL of salicylic acid in chloroform
Standard solution: Pipet 5 mL of *Standard stock solution* into a 50-mL volumetric flask containing 10 mL of methanol, 2 drops of hydrochloric acid, and 10 mL of a solution (1 in 10) of glacial acetic acid in ether. Add chloroform to volume, and mix.
Sample solution
Insert a small pledget of glass wool above the stem constriction of a 20- × 2.5-cm chromatographic tube, and uniformly pack with a mixture of about 1 g of chromatographic siliceous earth and 0.5 mL of 5 M phosphoric acid. Directly above this layer, pack a similar mixture of about 3 g of chromatographic siliceous earth and 2 mL of *Ferric chloride–urea reagent*. To a quantity nominally equivalent to 50 mg of aspirin from finely powdered Tablets add 10 mL of chloroform, stir for 3 min, and transfer to the chromatographic adsorption column with the aid of 5 mL of chloroform. Pass 50 mL of chloroform in several portions through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. If the purple zone reaches the bottom of the tube, discard the column, and repeat the test with a smaller quantity of powdered Tablets.
Elute the adsorbed salicylic acid into a 100-mL volumetric flask containing 20 mL of methanol and 4 drops of hydrochloric acid by passing two 10-mL portions of a solution (1 in 10) of glacial acetic acid in water-saturated ether, and then 30 mL of chloroform, through the column, and dilute the eluate with chloroform to volume.
Instrumental conditions
Mode: UV
Analytical wavelength: 306 nm (maximum absorbance)
Cell: 1 cm
Analysis: Concomitantly determine the absorbances of both the *Sample solution* and *Standard solution*, using a solvent mixture of the same composition as that of the *Standard solution* as the blank.
Acceptance criteria: 3.0%; the absorbance of the *Sample solution* does not exceed that of the *Standard solution*, any necessary adjustment being made for having used a smaller sample.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aspirin RS
USP Pentazocine RS
USP Salicylic Acid RS

Pentazocine and Naloxone Tablets

DEFINITION
Pentazocine and Naloxone Tablets contain amounts of Pentazocine Hydrochloride and Naloxone Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amounts of pentazocine ($C_{19}H_{27}NO$) and naloxone ($C_{19}H_{21}NO_4$).

IDENTIFICATION
• **A.**
Diluent: Chloroform and methanol (1:1)
Standard solution A: 5.0 mg/mL of USP Pentazocine RS in *Diluent*
Standard solution B: 1.3 mg/mL of USP Naloxone RS in *Diluent*

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Sample solution A: Crush 1 Tablet in 10 mL of *Diluent*. Sonicate for about 2 min, and filter.
Sample solution B: Evaporate 5 mL of *Sample solution A* to dryness on a steam bath under a stream of nitrogen. Dissolve the residue in 0.2 mL of *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL, *Standard solution A* and *Sample solution A*; 5 µL, *Standard solution B* and *Sample solution B*
Developing solvent system: 1-Butanol, water, and glacial acetic acid (70:20:10)
Spray reagent: Folin-Ciocalteu Phenol TS followed by sodium hydroxide solution (1 in 10)
Analysis: Develop the chromatograms in the *Developing solvent system* until the solvent has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry under a current of warm air. Spray the plate with *Spray reagent*.
Acceptance criteria: *Sample solution A* and *Sample solution B* exhibit spots having the same R_F values and approximately the same sizes and shapes as their respective Standard solutions.

ASSAY

• **PROCEDURE**
Diluent: Methanol, water, and phosphoric acid (500:500:1)
Solution A: Prepare a filtered and degassed mixture by dissolving 675 mg of sodium 1-octanesulfonate and 426 mg of anhydrous dibasic sodium phosphate in 625 mL of water, and mix.
Mobile phase: Add 475 mL of methanol and 10 mL of phosphoric acid to *Solution A*.
Strong anion-exchange resin: Transfer 30 g of strong anion-exchange resin to a 250-mL beaker. Wash the resin with two 200-mL portions of water, decanting the water after each wash. Wash with two 200-mL portions of dilute glacial acetic acid (1 in 20), decanting the first wash, and filter with the aid of suction.
Standard stock solution: 0.2 mg/mL of USP Naloxone RS in *Diluent*
Standard solution: Transfer 100 mg of USP Pentazocine RS to a 50-mL volumetric flask. Dissolve in about 30 mL of *Diluent*. Add 5.0 mL of the *Standard stock solution*, and dilute with *Diluent* to volume.
Sample solution: Transfer an amount nominally equivalent to 100 mg of pentazocine from NLT 20 Tablets to a 100-mL volumetric flask, and add 50.0 mL of *Diluent*. Sonicate for 5 min, and shake intermittently for 15 min. Filter into a glass-stoppered conical flask. Add about 250 mg of *Strong anion-exchange resin*, and shake for 30 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 229 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for naloxone and pentazocine are about 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6 between pentazocine and naloxone
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and naloxone ($C_{19}H_{21}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine or naloxone from the *Sample solution*
r_S = peak response of pentazocine or naloxone from the *Standard solution*
C_S = concentration of the appropriate USP Pentazocine RS or USP Naloxone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pentazocine or naloxone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of pentazocine ($C_{19}H_{27}NO$) and naloxone ($C_{19}H_{21}NO_4$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Detector: UV 279 nm (corrected for absorbance at 305 nm)
Standard solution: Dissolve a suitable amount of USP Pentazocine RS in a minimum volume of 0.1 N hydrochloric acid (about 25 mg/mL), diluting quantitatively and stepwise with water.
Sample solution: Filter portions of the solution under test, suitably diluted with *Medium*, if necessary.
Analysis: Determine the labeled amount of pentazocine ($C_{19}H_{27}NO$) dissolved in the *Sample solution* in comparison with the *Standard solution*.
Tolerances: NLT 75% (*Q*) of the labeled amount of pentazocine ($C_{19}H_{27}NO$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Diluent: Methanol, water, and phosphoric acid (500:500:1)
Solution A: Prepare a filtered and degassed mixture by dissolving 675 mg of sodium 1-octanesulfonate and 426 mg of anhydrous dibasic sodium phosphate in 625 mL of water, and mix.
Mobile phase: Add 475 mL of methanol and 10 mL of phosphoric acid to *Solution A*.
Strong anion-exchange resin: Transfer 30 g of strong anion-exchange resin to a 250-mL beaker. Wash the resin with two 200-mL portions of water, decanting the water after each wash. Wash with two 200-mL portions of dilute glacial acetic acid (1 in 20), decanting the first wash, and filter with the aid of suction.
Standard stock solution: 0.2 mg/mL of USP Naloxone RS in *Diluent*
Standard solution: Transfer 100 mg of USP Pentazocine RS to a 50-mL volumetric flask. Dissolve in about 30 mL of *Diluent*. Add 5.0 mL of the *Standard stock solution*, and dilute with *Diluent* to volume.
Sample solution: Transfer 1 Tablet to a 25-mL glass-stoppered cylinder. Add 25.0 mL of *Diluent*. Sonicate for 10 min, and shake intermittently for 15 min. Filter into a glass-stoppered conical flask. Add about 125 mg of *Strong anion-exchange resin*, and shake for 30 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 229 nm

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Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution* [NOTE—The relative retention times for naloxone and pentazocine are about 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6 between pentazocine and naloxone
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and naloxone ($C_{19}H_{21}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine or naloxone from the *Sample solution*
r_S = peak response of pentazocine or naloxone from the *Standard solution*
C_S = concentration of the appropriate USP Pentazocine RS or USP Naloxone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pentazocine or naloxone in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Naloxone RS
  USP Pentazocine RS

Pentazocine Injection

DEFINITION

Pentazocine Injection is a sterile solution of Pentazocine in Water for Injection, prepared with the aid of Lactic Acid. It contains NLT 95.0% and NMT 105.0% of the labeled amount of pentazocine ($C_{19}H_{27}NO$).

IDENTIFICATION

- **A.** The retention time of the pentazocine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the pentazocine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 15 mM sodium borate in water. Adjust with 10 N sodium hydroxide to a pH of 10.0.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
15	15	85
20	15	85
21	85	15
25	85	15

Diluent: Methanol, phosphoric acid, and water (500:1:500)
Standard solution: 0.024 mg/mL of USP Pentazocine RS in *Diluent*
Sample solution: Nominally 0.024 mg/mL of pentazocine from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 10-cm; 2.6-µm packing L1
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine from the *Sample solution*
r_S = peak response of pentazocine from the *Standard solution*
C_S = concentration of USP Pentazocine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pentazocine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.5 µg/mL of USP Pentazocine RS in *Diluent*
Standard solution: 0.002 mg/mL of USP Pentazocine RS in *Diluent*
Sample solution: Nominally 1 mg/mL of pentazocine from Injection in *Diluent*
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

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r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pentazocine from the *Standard solution*
C_S = concentration of USP Pentazocine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pentazocine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

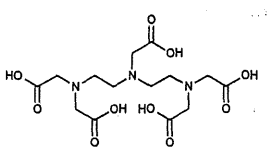
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Norpentazocine[a]	0.6	0.2
Pentazocine hydration product[b]	0.9	0.2
Pentazocine	1.0	—
Any unspecified impurity	—	0.2
Total impurities	—	0.5

[a] (2*R*,6*R*,11*R*)-1,2,3,4,5,6-Hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol.
[b] (2*R*,6*R*,11*R*)-3-(3-Hydroxy-3-methylbutyl)-6,11-dimethyl-1,2,3,4,5,6-hexahydro-2,6-methanobenzo[*d*]azocin-8-ol.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 5.8 USP Endotoxin Units/mg of pentazocine
- **PH** ⟨791⟩: 4.0–5.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at 20°–25°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Pentazocine RS

Pentetic Acid



$C_{14}H_{23}N_3O_{10}$ 393.35
Glycine, *N*,*N*-bis[2-[bis(carboxymethyl)amino]ethyl].
Diethylenetriaminepentaacetic acid [67-43-6].

» Pentetic Acid contains not less than 98.0 percent and not more than 100.5 percent of $C_{14}H_{23}N_3O_{10}$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Pentetic Acid RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩▲, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)
Residue on ignition ⟨281⟩: not more than 0.2%.

Limit of nitrilotriacetic acid—
Cupric acetate solution—Dissolve 20 g of cupric acetate in a mixture of 800 mL of water and 10 mL of glacial acetic acid. Adjust with 1 N sodium hydroxide to a pH of 4.2, dilute with water to obtain 1000 mL of solution, and filter.
Mobile phase—Prepare a mixture of 1600 mL of water, 40 mL of glacial acetic acid, 30.4 mL of 0.5 M dodecyltriethylammonium phosphate, and 20 mL of *Cupric acetate solution*. Adjust with 1 N sodium hydroxide to a pH of 4.0, dilute with water to obtain 2000 mL of solution, filter through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Stock standard solution—Transfer about 50 mg of nitrilotriacetic acid, accurately weighed, to a 100-mL volumetric flask, dilute with *Cupric acetate solution* to volume, and mix.
Standard solution—Transfer 1.0 mL of the *Stock standard solution* to a 25-mL volumetric flask, dilute with *Cupric acetate solution* to volume, and mix. This solution contains about 0.02 mg of nitrilotriacetic acid per mL.
Test solution—Transfer about 2 g of Pentetic Acid, accurately weighed, to a 100-mL volumetric flask. Add about 70 mL of *Cupric acetate solution*, and swirl to dissolve. Sonicate, if necessary, to dissolve. Dilute with *Cupric acetate solution* to volume, and mix.
Resolution solution—Transfer 1.0 mL of the *Stock standard solution* to a 25-mL volumetric flask, dilute with *Test solution* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 290-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1 that has been highly deactivated (carbon loading of about 30%). The flow rate is about 1 mL per minute. Equilibrate the column by passing, in sequence, water, methanol, and water for about 15 minutes each, and then *Mobile phase* for about 45 minutes. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between pentetic acid and nitrilotriacetic acid is not less than 2.0, and the relative retention times are about 0.6 for pentetic acid and 1.0 for nitrilotriacetic acid. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and measure the responses for the major peaks. Calculate the percentage of nitrilotriacetic acid in the portion of Pentetic Acid taken by the formula:

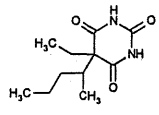
$$10{,}000(C/W)(r_U/r_S)$$

of which C is the concentration, in mg per mL, of nitrilotriacetic acid in the *Standard solution*, W is the weight, in mg, of Pentetic Acid taken to prepare the *Test solution*, and r_U and r_S are the nitrilotriacetic acid peak responses obtained from the *Test solution* and the *Standard solution*, respectively. The limit is 0.1%.
Iron—Using 1.5 g of specimen, proceed as directed in the test for *Iron* under *Edetic Acid*. The color of the test solution is not deeper than that of the solution containing the standard iron solution (0.01%).

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Assay—Transfer about 200 mg of Pentetic Acid, accurately weighed, to a 125-mL conical flask, add 50 mL of water and 1.5 mL of 1 N sodium hydroxide, and swirl to dissolve the specimen. Add 10 mL of 0.1 N ammonium thiocyanate, and mix. Add about 40 mL of methyl ethyl ketone, mix, and allow the layers to separate. Titrate with 0.05 N ferric ammonium sulfate VS, stirring continuously. As the titration proceeds, the aqueous phase turns from colorless to yellow, and the organic phase remains colorless. As the endpoint is approached, stop the titration, mix, and allow the layers to separate. Add 0.1-mL increments of 0.05 N ferric ammonium sulfate VS, mixing and allowing the layers to separate after each addition, until the organic layer turns from colorless to pink. Each mL of 0.05 N ferric ammonium sulfate consumed is equivalent to 19.668 mg of $C_{14}H_{23}N_3O_{10}$.

Pentobarbital



$C_{11}H_{18}N_2O_3$ 226.27

2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-(1-methylbutyl)-, (±)-;
(±)-5-Ethyl-5-(1-methylbutyl)barbituric acid [76-74-4].

DEFINITION

Pentobarbital contains NLT 98.0% and NMT 102.0% of $C_{11}H_{18}N_2O_3$, calculated on the dried basis. Where the material is labeled as intended solely for veterinary use, Pentobarbital contains NLT 97.0% and NMT 102.0% of $C_{11}H_{18}N_2O_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
  Sample solution: 7 in 100
  Medium: Chloroform
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 0.01 M monobasic potassium phosphate and acetonitrile (65:35). Adjust the pH to 3.5.
Standard solution: 0.1 mg/mL of USP Pentobarbital RS in *Mobile phase*
Sample stock solution: 1 mg/mL of Pentobarbital in *Mobile phase* (sonicate until dissolved)
Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 15,000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0% for pentobarbital
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{11}H_{18}N_2O_3$ in the portion of Pentobarbital taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Pentobarbital RS in the *Standard solution* (mg/mL)
C_U = concentration of Pentobarbital in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% of $C_{11}H_{18}N_2O_3$ on the dried basis; and 97.0%–102.0% of $C_{11}H_{18}N_2O_3$ on the dried basis, where the material is labeled as intended solely for veterinary use

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

- **Procedure**

Mobile phase: Prepare as directed in the *Assay*.
Standard solution: 0.001 mg/mL of USP Pentobarbital RS in *Mobile phase*
Sample solution: 1 mg/mL of Pentobarbital in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 15,000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 15.0% for pentobarbital
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any impurity in the portion of Pentobarbital taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area for any impurity from the *Sample solution*
r_S = peak area for pentobarbital from the *Standard solution*
C_S = concentration of USP Pentobarbital RS in the *Standard solution* (mg/mL)
C_U = concentration of Pentobarbital in the *Sample solution* (mg/mL)
F = relative response factor of the impurity (see *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.

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Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6-Imino-5-ethyl-5-(1-methyl butyl) barbituric acid	0.39	1.5	0.2
5-Ethyl-5-(1-ethylpropyl) barbituric acid[a]	0.93	1.0	0.1
Pentobarbital	1.0	—	—
5-Ethyl-5-(1,3-dimethylbutyl) barbituric acid	1.5	0.9	0.3
Unknown impurities	—	1.0	0.1
Total	—	—	0.5

[a] Where the material is labeled as intended solely for veterinary use, the limit of 5-ethyl-5-(1-ethylpropyl) barbituric acid is 3.0%.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Pentobarbital RS

Pentobarbital Sodium

$C_{11}H_{17}N_2NaO_3$ 248.25

2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-(1-methyl butyl)-, monosodium salt.

Sodium 5-ethyl-5-(1-methylbutyl)barbiturate [57-33-0].

» Pentobarbital Sodium contains not less than 98.0 percent and not more than 102.0 percent of $C_{11}H_{17}N_2NaO_3$, calculated on the dried basis. Where the material is labeled as intended solely for veterinary use, Pentobarbital Sodium contains not less than 97.0 percent and not more than 102.0 percent of $C_{11}H_{17}N_2NaO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Pentobarbital RS

Completeness of solution—Mix 1.0 g with 10 mL of carbon dioxide-free water: after 1 minute, the solution is clear and free from undissolved solid.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: dilute ammonium hydroxide (1 in 200).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: Ignite about 200 mg: the residue effervesces with acids, and meets the requirements of the tests for *Sodium* ⟨191⟩.

pH ⟨791⟩: between 9.8 and 11.0, in the solution prepared in the test for *Completeness of solution.*

Loss on drying ⟨731⟩—Dry it at 105° for 6 hours: it loses not more than 3.5% of its weight.

Related compounds—

Mobile phase—Prepare as directed in the *Assay.*

Standard solution—Dissolve an accurately weighed quantity of USP Pentobarbital RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.001 mg per mL.

Test solution—Transfer about 110 mg of Pentobarbital Sodium, accurately weighed, to a 100-mL volumetric flask, add about 80 mL of *Mobile phase,* and sonicate until dissolved. Dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the capacity factor, *k′,* is not less than 2.5; the column efficiency is not less than 15,000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 15.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of any impurity in the portion of Pentobarbital Sodium taken by the formula:

$$(248.25/226.27)(10{,}000/F)(C/W)(r_i/r_s)$$

in which 248.25 and 226.27 are the molecular weights of pentobarbital sodium and pentobarbital, respectively; *F* is the relative response factor of the impurity according to the table below; *C* is the concentration, in mg per mL, of USP Pentobarbital RS in the *Standard solution; W* is the weight, in mg, of Pentobarbital Sodium, on the dried basis, used to prepare the *Test solution;* r_i is the peak area for any impurity in the *Test solution;* and r_s is the peak area for pentobarbital in the *Standard solution:* the impurities meet the requirements given in the table below:

Compound Name	Relative Retention Time	Relative Response Factor	Limit (%)
6-Imino-5-ethyl-5-(1-methyl-butyl)barbituric acid	about 0.39	1.5	0.2
5-Ethyl-5-(1-ethyl-propyl) barbituric acid*	about 0.93	1.0	0.1
Pentobarbital	1.0	—	—
5-Ethyl-5-(1,3-dimethyl-butyl) barbituric acid	about 1.5	0.9	0.3
Unknown impurities	—	1.0	0.1
Total	—	—	0.5

* Where the material is labeled as intended solely for veterinary use, the limit of 5-ethyl-5-(1-ethylpropyl) barbituric acid is 3.0%.

Assay—

[NOTE—Use the value for *Loss on drying* obtained at the same time as the preparation of the *Test solution* in the test for *Related compounds* and the *Assay preparation* in the *Assay.*]

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Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as described in the *Assay* under *Pentobarbital.*

Assay preparation—Transfer about 110 mg of Pentobarbital Sodium, accurately weighed, to a 100-mL volumetric flask, add about 80 mL of *Mobile phase,* and sonicate until dissolved. Dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{11}H_{17}N_2NaO_3$ in the portion of Pentobarbital Sodium taken by the formula:

$$(248.25/226.27)1000C(r_U/r_S)$$

in which 248.25 and 226.27 are the molecular weights of pentobarbital sodium and pentobarbital, respectively; C is the concentration, in mg per mL, of USP Pentobarbital RS in the *Standard preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Pentobarbital Sodium Injection

» Pentobarbital Sodium Injection is a sterile solution of Pentobarbital Sodium in a suitable solvent. Pentobarbital may be substituted for the equivalent amount of Pentobarbital Sodium, for adjustment of the pH. The Injection contains the equivalent of not less than 92.0 percent and not more than 108.0 percent of the labeled amount of $C_{11}H_{17}N_2NaO_3$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass. The Injection may be packaged in 50-mL containers.
Labeling—The label indicates that the Injection is not to be used if it contains a precipitate.
USP Reference standards ⟨11⟩—
USP Pentobarbital RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.8 USP Endotoxin Unit per mg of pentobarbital sodium.
pH ⟨791⟩: between 9.0 and 10.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed pH 3.5 mixture of 0.01 M monobasic potassium phosphate and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Pentobarbital RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Quantitatively dilute a suitable volume of Injection with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

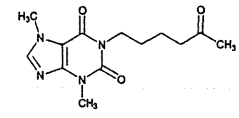
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the capacity factor, k', is not less than 2.5; the column efficiency is not less than 15,000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{11}H_{17}N_2NaO_3$ in the portion of Injection taken by the formula:

$$100(248.25/226.27)(C_S/C_U)(r_U/r_S)$$

in which 248.25 and 226.27 are the molecular weights of pentobarbital sodium and pentobarbital, respectively; C_S is the concentration, in mg per mL, of USP Pentobarbital RS in the *Standard preparation;* C_U is the final concentration, in mg per mL, of the *Assay preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Pentoxifylline



$C_{13}H_{18}N_4O_3$ 278.31
1*H*-Purine-2,6-dione, 3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)-;
1-(5-Oxohexyl)theobromine [6493-05-6].

DEFINITION
Pentoxifylline contains NLT 98.0% and NMT 102.0% of pentoxifylline ($C_{13}H_{18}N_4O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Solution A: 1 g/L of perchloric acid
Mobile phase: Methanol, tetrahydrofuran, acetonitrile, and *Solution A* (2: 2.5: 15: 80)
System suitability solution: 0.024 mg/mL of caffeine and 0.048 mg/mL of USP Pentoxifylline RS in *Mobile phase*
Standard solution: 0.05 mg/mL of USP Pentoxifylline RS in *Mobile phase*
Sample solution: 0.05 mg/mL of Pentoxifylline in *Mobile phase*

USP Monographs

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 273 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 0.7 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 10.0 between caffeine and pentoxifylline, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pentoxifylline ($C_{13}H_{18}N_4O_3$) in the portion of Pentoxifylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pentoxifylline RS in the *Standard solution* (mg/mL)
C_U = concentration of Pentoxifylline in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample: 2.0 g
Acceptance criteria: The *Sample* shows no more chloride than corresponds to 0.31 mL of 0.020 N hydrochloric acid (0.011%).
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample: 1.0 g
Acceptance criteria: The *Sample* shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.02%).
• **ORGANIC IMPURITIES**
Solution A and **Mobile phase:** Prepare as directed in the *Assay*.
System suitability solution: 0.7 µg/mL of caffeine and 350 µg/mL of USP Pentoxifylline RS in *Mobile phase*
Standard solution: 0.7 µg/mL of USP Pentoxifylline RS in *Mobile phase*
Sample solution: 350 µg/mL of Pentoxifylline in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay* except for the following:
Injection volume: 20 µL
Run time: NLT 5 times the retention time for pentoxifylline
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 10.0 between caffeine and pentoxifylline, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the areas of all the peaks in the *Sample solution*, except for that of pentoxifylline.
Calculate the percentage of each impurity in the portion of Pentoxifylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pentoxifylline from the *Standard solution*
C_S = concentration of USP Pentoxifylline RS in the *Standard solution* (µg/mL)
C_U = concentration of Pentoxifylline in the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurities: NMT 0.2%
Total impurities: NMT 0.5%

SPECIFIC TESTS
• **COMPLETENESS OF SOLUTION** ⟨641⟩
Sample solution: 1 g in 50 mL of carbon dioxide-free water
Acceptance criteria: Meets the requirements
• **ACIDITY**
Sample solution: 1 g in 50 mL of carbon dioxide-free water
Analysis: To the *Sample solution* add 1 drop of bromothymol blue TS.
Acceptance criteria: NMT 0.2 mL of 0.01 N sodium hydroxide is required to produce a color change.
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 60° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pentoxifylline RS

Pentoxifylline Compounded Oral Suspension

DEFINITION
Pentoxifylline Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of pentoxifylline ($C_{13}H_{18}N_4O_3$).
Prepare Pentoxifylline Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Pentoxifylline extended-release tablets[a] equivalent to	2 g of pentoxifylline
Purified Water, *USP*, a sufficient quantity to make	100 mL

[a] Trental 400-mg tablets, sanofi-aventis, Somerville, NJ.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Pentoxifylline extended-release tablets* in a suitable mortar, add *Purified Water* in small portions, and triturate to make a smooth paste. Add increasing volumes of *Purified Water* to make a pentoxifylline liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Purified Water* to bring to final volume, and mix well.

ASSAY
• **PROCEDURE**
Solution A: 50 mM monobasic potassium phosphate buffer, adjusted with phosphoric acid to a pH of 3.2
Mobile phase: Acetonitrile and *Solution A* (20:80). Pass through a filter of 0.45-µm pore size, and degas.

USP Monographs

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Internal standard solution: 100 µg/mL of caffeine in *Mobile phase*
Standard stock solution: 20 mg/mL of USP Pentoxifylline RS in *Mobile phase*
Standard solution: Pipet 1.0 mL of *Standard stock solution* into a 15-mL conical centrifuge tube, and add 9 mL of deionized water. Mix the sample for 30 s in a vortex mixer, and centrifuge for 30 min at 1250 × g. Pipet 50 µL of the supernatant into a separate borosilicate culture tube, dilute with 575 µL of *Mobile phase*, and add 625 µL of *Internal standard solution* to obtain a solution having a nominal concentration of 80 µg/mL of pentoxifylline and 50 µg/mL of caffeine.
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension into a 15-mL conical centrifuge tube, and add 9 mL of deionized water. Mix the sample for 30 s in a vortex mixer, and centrifuge for 30 min at 1250 × g. Pipet 50 µL of the supernatant into a separate borosilicate culture tube, dilute with 575 µL of *Mobile phase*, and add 625 µL of *Internal standard solution* to obtain a solution having a nominal concentration of 80 µg/mL of pentoxifylline and 50 µg/mL of caffeine.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for caffeine and pentoxifylline are about 0.42 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 10.0 between pentoxifylline and caffeine
Column efficiency: NLT 10,000 theoretical plates
Tailing factor: NMT 2.0 for the pentoxifylline peak
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pentoxifylline ($C_{13}H_{18}N_4O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of pentoxifylline to the internal standard from the *Sample solution*
R_S = peak response ratio of pentoxifylline to the internal standard from the *Standard solution*
C_S = concentration of USP Pentoxifylline RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pentoxifylline in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **PH** ⟨791⟩: 5.9–7.7

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Pentoxifylline RS

Pentoxifylline Extended-Release Tablets

» Pentoxifylline Extended-Release Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of pentoxifylline ($C_{13}H_{18}N_4O_3$).

Packaging and storage—Preserve in well-closed containers. Protect from light, and store between 15° and 30°.
Labeling—The labeling indicates the *Dissolution Test* with which the product complies.
USP Reference standards ⟨11⟩—
USP Pentoxifylline RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—
Test specimen—Finely powder not fewer than 5 Tablets. (A coarse screen may be used to separate the powder from the tablet film-coating if necessary.) Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of pentoxifylline, to a 15-mL centrifuge tube, add about 10 mL of methanol, cap the tube, and shake vigorously for about 5 minutes. Centrifuge for about 5 minutes to allow undissolved material to settle. Decant the supernatant into a suitable beaker, and evaporate the solution with the aid of a current of air to dryness at about 35°. Dissolve the residue in about 15 mL of methylene chloride, transfer to a separatory funnel, add about 10 mL of water, and shake. Allow the layers to separate, transfer the methylene chloride layer, and pass through a funnel partially filled with anhydrous sodium sulfate, collecting the filtrate in a small beaker. Evaporate the solution with the aid of a current of air to dryness at about 35°. Dissolve the residue so obtained in 8 to 10 mL of ether, and then chill in an ice bath, if necessary, to induce crystallization. Collect the crystals on filter paper, wash with about 2 mL of cold ether, and allow to air-dry. Prepare a mixture of about 1.5% (w/w) of the crystals in potassium bromide.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
Medium: water; 900 mL or 1000 mL.
Apparatus 2: 100 rpm.
Times: 1, 4, 8, and 12 hours.
Procedure—Determine the amount of $C_{13}H_{18}N_4O_3$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Pentoxifylline RS in the same *Medium*.
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to *Acceptance Table 2*.

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Time (hours)	Amount dissolved
1	not more than 30%
4	between 30% and 55%
8	not less than 60%
12	not less than 80%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*
Medium: water; 900 mL.
Apparatus 2: 75 rpm.
Times: 1, 6, 10, and 20 hours.
Procedure—Proceed as directed for *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 8% and 30%
6	between 35% and 60%
10	between 53% and 78%
20	not less than 80%

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3.*
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Times: 2, 8, 12, and 20 hours.
Procedure—Proceed as directed for *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
2	between 15% and 35%
8	between 55% and 75%
12	between 75% and 95%
20	not less than 85%

TEST 4—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4.*
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Times: 1, 8, and 24 hours.
Procedure—Proceed as directed for *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 0% and 20%
8	between 35% and 60%
24	not less than 80%

TEST 5—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5.*
Medium: water; 900 mL.
Apparatus 2: 75 rpm.
Times: 1, 2, 4, 6, and 20 hours.
Procedure—Proceed as directed for *Test 1,* except to use the wavelength of maximum absorbance at about 264 nm instead of 274 nm.
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 5% and 25%
2	between 10% and 35%
4	between 20% and 50%
6	between 30% and 60%
20	not less than 80%

TEST 6—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6.*
Medium: simulated gastric fluid (without enzymes); 900 mL.
Apparatus 2: 50 rpm.
Times: 2, 8, 12, and 24 hours.
Procedure—Proceed as directed for *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
2	between 10% and 30%
8	between 40% and 60%
12	between 55% and 75%
24	not less than 85%

TEST 7—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7.*
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Times: 1, 3, 8, and 18 hours.
Procedure—Proceed as directed for *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	not more than 25%
3	between 25% and 45%
8	between 55% and 75%
18	not less than 80%

TEST 8—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8.*
Medium: water; 900 mL.
Apparatus 2: 75 rpm.
Times: 1, 2, 4, 10, and 16 hours.
Procedure—Proceed as directed for *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 10% and 20%
2	between 15% and 35%

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Time (hours)	Amount dissolved
4	between 25% and 45%
10	between 55% and 75%
16	not less than 80%

TEST 9—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9.*
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Times: 1, 3, 6, 12, and 18 hours.
Procedure—Proceed as directed for *Test 1,* except to use the wavelength of maximum absorbance at about 230 nm instead of 274 nm.
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 0% and 20%
3	between 20% and 40%
6	between 30% and 60%
12	between 50% and 80%
18	not less than 80%

TEST 10—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10.*
Medium: water; 900 mL.
Apparatus 2: 75 rpm.
Times: 1, 6, 12, and 20 hours.
Procedure—Proceed as directed for *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	not more than 20%
6	between 35% and 65%
12	between 60% and 90%
20	not less than 80%

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Chromatographic purity—
Perchloric acid solution, Mobile phase, Extracting solution, and *System suitability solution*—Prepare as directed in the *Assay.*
Standard solution—Dissolve an accurately weighed quantity of USP Pentoxifylline RS in *Extracting solution* containing an amount of methanol equal to 0.8% of the total volume to be used, and dilute quantitatively, and stepwise if necessary, with *Extracting solution* to obtain a solution having a known concentration of about 0.96 µg per mL.
Test solution—Transfer 10.0 mL of the first dilution filtrate from the *Assay preparation* to a 25-mL volumetric flask, dilute with *Extracting solution* to volume, and mix. The final concentration of pentoxifylline in this solution is about 0.32 mg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* Chromatograph the *Standard solution,* and record the peak responses for pentoxifylline as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the chromatogram to run five times longer than the retention time of the pentoxifylline peak. Record the chromatograms, and measure all the peak responses from the *Test solution,* except that for pentoxifylline. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$312C(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP Pentoxifylline RS in the *Standard solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for pentoxifylline obtained from the *Standard solution:* not more than 0.3% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—
Perchloric acid solution—Dissolve 1.0 g of perchloric acid in 1000 mL of water, and mix.
Mobile phase—Prepare a filtered and degassed mixture of *Perchloric acid solution,* acetonitrile, tetrahydrofuran, and methanol (80: 15: 2.5: 2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Extracting solution—Prepare a mixture of water and alcohol (7:3).
System suitability solution—Transfer about 20 mg of USP Pentoxifylline RS and about 10 mg of caffeine, each accurately weighed, to a 25-mL volumetric flask. Add 0.2 mL of methanol, and swirl the flask to distribute the methanol. Dilute with *Extracting solution* to volume, and mix. Pipet 3.0 mL of the resulting solution into a 50-mL volumetric flask, dilute with *Extracting solution* to volume, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Pentoxifylline RS in *Extracting solution* containing an amount of methanol equal to 0.8% of the total volume to be used, and dilute quantitatively, and stepwise if necessary, with *Extracting solution* to obtain a solution having a known concentration of about 0.048 mg per mL.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of pentoxifylline, to a 50-mL volumetric flask. Pipet 0.4 mL of methanol into the flask, and swirl for at least 1 minute. Add about 30 mL of *Extracting solution,* and sonicate for 60 minutes with occasional swirling of the flask. Add an additional 15 mL of *Extracting solution,* allow to cool to room temperature, dilute with *Extracting solution* to volume, and mix. Centrifuge or pass through a suitable filter. Reserve a portion of this first dilution for preparation of the *Test solution* in the *Chromatographic purity* test. Pipet 3.0 mL of the clear solution into a 50-mL volumetric flask, dilute with *Extracting solution* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 0.7 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between caffeine and pentoxifylline is not less than 10.0. Chromatograph the *Standard preparation,* and record the peak responses for pentoxifylline as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg,

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of pentoxifylline ($C_{13}H_{18}N_4O_3$) in the portion of Tablets taken by the formula:

$$833C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pentoxifylline RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Peppermint Spirit

DEFINITION

Peppermint Spirit contains, in each 100 mL, NLT 9.0 mL and NMT 11.0 mL of peppermint oil.

Peppermint Oil	100 mL
Peppermint, in coarse powder	10 g
Alcohol, a sufficient quantity to make	1000 mL

Macerate the peppermint leaves, freed as much as possible from stems and coarsely powdered, for 1 h in 500 mL of purified water, and then strongly express them. Add the moist, macerated leaves to 900 mL of alcohol, and allow the mixture to stand for 6 h with frequent agitation. Filter, and to the filtrate add the oil, and add alcohol to make the product measure 1000 mL.

ASSAY

• **CONTENT OF PEPPERMINT OIL**

Sample: 5.0 mL of Spirit

Analysis: Transfer the *Sample* to a Babcock bottle, graduated to 8%. Add 1.0 mL of kerosene, and mix. Add a saturated calcium chloride solution, acidified with hydrochloric acid, almost to fill the bulb of the bottle. Rotate the bottle vigorously to ensure mixing, and then add a sufficient quantity of the calcium chloride solution to bring the separated oil into the neck of the bottle. Centrifuge at about 1500 rpm for 5 min, and read the volume of oil in the stem. Subtract five divisions for the kerosene added, and multiply the remaining number of divisions by 4.2 to obtain the volume, in mL, of peppermint oil in 100 mL of the Spirit.

Acceptance criteria: 9.0–11.0 mL

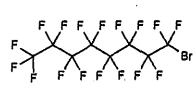
SPECIFIC TESTS

• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩: 79.0%–85.0% of C_2H_5OH

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.

Perflubron



C_8BrF_{17} 498.96

Octane, 1-bromo-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-.

1-Bromoheptadecafluorooctane.

Perfluorooctyl bromide [423-55-2].

» Perflubron contains not less than 98.0 and not more than 100.0 percent of C_8BrF_{17}.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Perflubron RS

Identification—

A: Record the IR absorption spectrum, using a gas cell. The spectrum so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Perflubron RS.

B: The retention time of the major peak in the chromatogram of the test specimen, obtained as directed in the *Assay,* corresponds to that of USP Perflubron RS, similarly chromatographed.

Specific gravity ⟨841⟩: between 1.922 and 1.925.

Chromatographic purity—

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a split injection port with a split ratio, range of 1:45 to 1:100, a flame-ionization detector, and a 0.25-mm × 60-m column coated with a 1-µm film of phase G2. Hydrogen is used as the carrier gas. The chromatograph is programmed to maintain the column temperature at 35° for 7 minutes, then to increase the temperature at a rate of 20° per minute to 185°, and held at this temperature for 4.5 minutes. The injection port is maintained between 200° and 220° and the detector at a temperature above 200°.

Procedure—Inject a volume (about 0.2 µL) of Perflubron into the chromatograph, record the chromatogram, and measure the areas of the peak responses. Calculate the percentage of each individual impurity in the portion of Perflubron taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response of the individual impurity, and r_s is the sum of the responses of all the peaks: not more than 0.20% of any individual impurity is found.

Nonvolatile residue—Transfer 75 g of Perflubron to a tared evaporating dish, evaporate to dryness, and dry the residue at 105° for 1 hour: the weight of the residue so obtained does not exceed 1.5 mg (0.002%).

Assay—

Chromatographic system—Proceed as directed in the test for *Chromatographic purity.*

Procedure—Inject about 0.2 µL of Perflubron into the chromatograph, record the chromatograms, and measure the areas of the peak responses. Calculate the percentage of C_8BrF_{17} in the portion of Perflubron taken by the formula:

$$100(r_U/r_s)$$

in which r_U is the peak response for perflubron obtained from the test specimen, and r_s is the sum of the responses of all of the peaks.

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Perflutren Protein-Type A Microspheres Injectable Suspension

» Perflutren Protein-Type A Microspheres Injectable Suspension is a sterile, nonpyrogenic suspension of microspheres produced by dispersing perflutren (octafluoropropane) gas in an aqueous solution of diluted sterile Albumin Human. It contains not less than 0.8 percent and not more than 1.2 percent protein. It may contain stabilizers, but contains no preservatives.

Packaging and storage—Preserve in single-dose, tight containers that contain perflutren gas in the headspace, and store in a refrigerator.

Labeling—Label it to indicate that perflutren gas is contained within the microspheres. The labeling also provides the following warnings: "Do not use if lower layer is cloudy or turbid, contains visible foreign matter, or if the contents do not appear as a homogeneous, opaque, milky-white suspension after mixing. Do not use if the upper white layer of product is absent. Do not inject air into the vial. Invert the vial, and gently rotate to resuspend the microspheres. Do not use if, after resuspension, the solution appears to be clear rather than opaque milky-white."

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.5 USP Endotoxin Unit per mL of Perflutren Protein-Type A Microspheres Injectable Suspension.

Safety—It meets the requirements for biologics as set forth for *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* ⟨88⟩.

Sterility Tests ⟨71⟩ **:** meets the requirements.

pH ⟨791⟩**:** between 6.4 and 7.4.

Microsphere size and concentration—

Electrolyte solution—Use filtered and buffered saline electrolyte solution.[1]

Diluent—Prepare a solution that contains, in each L of water, 1.5 g of sodium lauryl sulfate and 0.1 g of thimerosal. Prior to use, pass the solution through a 0.2-µm nylon filter. [NOTE—The *Diluent* is to be used exclusively to prepare the *Reference stock solution* described below; it must not be used to prepare the *Test solution.*]

Reference stock solution—Transfer a quantity of NIST traceable microspheres suspension containing about 0.5 g of microspheres directly into a tared centrifuge tube equipped with a cap, and weigh.[2] Using the density and concentration of the microspheres obtained from the Certificate of Analysis, calculate the volume occupied by the microspheres and the number of microspheres in the portion taken. Calculate the target total volume, in mL, by dividing the number of microspheres in the portion taken by the target concentration of 2.0×10^8 microspheres per mL. Calculate the target *Diluent* volume by subtracting the volume occupied by the microspheres from the target total volume. Transfer the target volume of *Diluent* to the centrifuge tube containing the portion of microspheres taken, and mix the tube vigorously for 1 hour. The prepared *Reference stock solution*, which contains a suspension of microspheres with a target mean particle diameter of 5.2 µm and a concentration of 2.0×10^8 microspheres per mL, is divided into smaller containers and stored at 5°.

Reference solution—Equilibrate the *Reference stock solution* to room temperature, and mix thoroughly. Immediately transfer 20 µL of the *Reference stock solution* to a beaker containing 200 mL of *Electrolyte solution*, mix, and analyze immediately.

Blank solution—Use 200 mL of *Electrolyte solution.*

Test solution—Allow the Injectable Suspension to equilibrate to room temperature. Invert the vial, and gently rotate to resuspend the microspheres. [NOTE—After resuspension, the contents should appear as a homogeneous, opaque, milky-white suspension.] Immediately withdraw a 20-µL aliquot, transfer to a beaker containing 200 mL of *Electrolyte solution*, mix, and analyze immediately.

Test apparatus—Use a multichannel particle analyzer that operates on the electrical zone-sensing principle.[3] The analyzer is fitted and calibrated with an aperture tube having a 50-µm orifice. The multichannel particle analyzer is equipped with software capable of data-smoothing, data extrapolation, distribution graphing, and data conversion. Analyze the *Blank solution*, the *Reference solution*, and the *Test solution* as directed for *Procedure:* the total count in the *Blank solution* is not more than 500; the mean particle diameter of microspheres in the *Reference solution* is within 5% of the mean particle diameter of microspheres in the *Reference stock solution;* the concentration of the *Reference solution* is within 10% of the concentration of the *Reference stock solution;* and the coincidence effect in the analysis of the *Test solution* is not more than 5%.

Procedure—Rinse the orifice of the aperture tube with *Electrolyte solution* before and after analyzing each preparation. Place the *Blank solution* in the apparatus, and adjust the vacuum on the sample stand so that the counting begins about 12 seconds after the analyzer is set to the counting position. Set the data acquisition to stop when one of the following conditions is met: preset length of time, preset volume, preset number of counts in any channel, or total counts. Collect the count versus the channel data for the *Blank solution*, and analyze using the data-smoothing, data extrapolation, distribution graphing, and data conversion features of the system software. In the same manner, analyze the *Reference solution* and the *Test solution.* The *Test solution* data are normalized and expressed as the number of microspheres per mL: the concentration of microspheres is between 5.0×10^8 and 8.0×10^8 per mL. Calculate the percentage of microspheres less than 10 µm in size in the portion of Injectable Suspension taken by the formula:

$$100(P_A/P_B)$$

in which P_A is the number of microspheres in the 1- to 10-µm size range, and P_B is the number of microsphere particles in the 1- to 32-µm size range. Not fewer than 93% of microsphere particles is smaller than 10 µm.

Container headspace content—

Reference solutions—Use 99% perflutren reference standard[4] (electronic grade perflutren gas of at least 99 molar % purity) and 60% perflutren reference standard[4] (an electronic grade gas in air mixture containing 60 molar % perflutren gas).

Blank solution—Use ambient air.

Test solution—Use gas from the container headspace.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a thermal conductivity detector and a 0.53-mm × 25-m fused-silica (porous layer

[1] Filtered and buffered saline electrolyte solution is available as ISOTON(®) II from Beckman Coulter, Inc., Fullerton, CA.

[2] Microspheres with a mean particle diameter of 5 µm are available as NIST traceable Dynospheres from Bangs Laboratories, Inc., Fishers, IN.

[3] A suitable multichannel particle analyzer is available as the Multisizer Model IIe from Beckman Coulter, Inc., Fullerton, CA.

[4] A suitable grade of perflutren (octafluoropropane) is available from Air Products and Chemicals, Inc., Allentown, PA.

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open tubular) column coated with Al_2O_3/KCl (aluminum oxide deactivated with potassium chloride).[5] The carrier gas is helium with a flow rate adjusted to obtain a retention time of about 1.5 to 1.8 minutes for perflutren. The column temperature is maintained at about 65°, the injection port temperature is maintained at about 130°, and the detector temperature is maintained at about 180°. Chromatograph the *Reference solutions* as directed for *Procedure:* the resolution, *R,* between perflutren and air is not less than 2; and the relative standard deviation for replicate injections is not more than 5%. The measured value for the 99% perflutren reference standard is within 5% of the nominal value.

Procedure—Using a gas-tight syringe, separately inject 10 µL of the *Blank solution,* the *Reference solutions,* and the *Test solution* into the chromatograph. Record the chromatograms, and measure the responses for the major peaks. The percentages of perflutren in the 99% perflutren reference standard and in the *Test solution* are calculated by comparing the peak areas in each with the peak areas obtained from the 60% perflutren reference standard. The container headspace of Injectable Suspension contains not less than 60% of perflutren gas.

Microsphere perflutren content—

Reference stock solutions—The 97% decafluorobutane reference standard is decafluorobutane gas of at least 97 molar % purity.[6] The 5% decafluorobutane–5% perflutren reference standard is a mixture containing 5 molar % decafluorobutane gas and 5 molar % perflutren gas in air.[7] The 99% perflutren reference standard is electronic grade perflutren gas of at least 99 molar % purity.[4]

Analysis vial—Transfer 100 µL of 97% decafluorobutane reference standard gas and 100 µL of glacial acetic acid to a 2-mL vial equipped with a septum cap.

Reference solution—Transfer 100 µL of 99% perflutren reference standard and 0.75 mL of 1% Albumin Human to an *Analysis vial,* and incubate by mixing for at least 3 hours.

Test solution—Allow a vial of the Injectable Suspension to equilibrate to room temperature. Invert the vial, and gently rotate to resuspend the microspheres. [NOTE—After resuspension, the contents of the vial should appear as a homogeneous, opaque, milky-white suspension.] Withdraw 0.75 mL of Injectable Suspension, and transfer to another *Analysis vial.* Incubate the *Test solution* by mixing for at least 3 hours.

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed for *Container headspace content.* The carrier gas is helium with a flow rate adjusted to obtain the following retention times: 1.0 to 1.1 minutes for air, 1.3 to 1.5 minutes for perflutren, and 1.5 to 2.5 minutes for decafluorobutane. The column temperature is maintained at about 85°, and then after elution of the perflutren the temperature is increased at a rate of 50° per minute to 120°, and maintained at 120° for 2 minutes. The injection port temperature is maintained at about 130°, and the detector temperature is maintained at about 180°. Chromatograph the 5% decafluorobutane–5% perflutren reference standard and the *Reference solution* as directed for *Procedure:* the resolution, *R,* between air and perflutren is not less than 2; the resolution, *R,* between perflutren and decafluorobutane is not less than 5; the relative standard deviation determined from the perflutren peak response for the *Reference solution* is not more than 5%; and the relative standard deviation determined from the response ratios for replicate injections of the 5% decafluorobutane–5% perflutren reference standard is not more than 5%.

Procedure—Inject 20 µL of the headspace gas from the vials containing the *Reference solution* and the *Test solution* into the gas chromatograph. Record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg per mL, of perflutren in the portion of Injectable Suspension taken by the formula:

$$(0.188M/V)(R_S/R_U)$$

in which M is the number of µmols of decafluorobutane in an *Analysis vial* after addition of the Injectable Suspension; V is the volume, in mL, of Injectable Suspension added to the *Analysis vial;* and R_S and R_U are the peak area ratios of decafluorobutane to perflutren obtained from the *Reference solution* and the *Test solution,* respectively. The quantity of perflutren in the Injectable Suspension is between 0.11 mg per mL and 0.33 mg per mL.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests* ⟨1⟩; with the exception of *Foreign and Particulate Matter.*

Assay for protein—

Diluted antifoam reagent—Transfer 100 µL of antifoam reagent[8] to a suitable container, and dilute with water to 10 mL.

Blank preparation—Transfer 500 µL of Sodium Chloride Injection to a culture tube. Dilute the contents of the tube with water to 2 mL, and add 10 µL of *Diluted antifoam reagent.*

Standard preparations—Transfer 25-, 50-, 62.5-, 75-, and 100-µL aliquots of protein standard solution[9] containing 8 g per dL into separate tubes. Dilute the contents of each tube with water to 2.00 mL, and add 10 µL of the *Diluted antifoam reagent* to each tube. During the *Procedure,* the addition of 3.0 mL of biuret reagent TS to each of the tubes produces *Standard preparations* with protein concentrations of 0.4, 0.8, 1.0, 1.2, and 1.6 mg per mL.

Assay preparation—Equilibrate each container of Injectable Suspension to room temperature, and mix each for at least 5 minutes to ensure a homogeneous suspension. Vent the container, and transfer 500-µL aliquots into separate tubes. Dilute the contents of each tube with water to 2 mL, and add 10 µL of *Diluted antifoam reagent.*

Procedure—To each of the tubes containing the *Blank preparation, Standard preparations,* and *Assay preparation,* add 3.0 mL of biuret reagent TS, mix, and allow to stand for 30 minutes, accurately timed, for maximum color development. The *Blank preparation, Standard preparations,* and *Assay preparation* are treated identically. Using the *Blank preparation,* set the absorbance equal to zero. Determine the absorbance of each of the *Standard preparations* and the *Assay preparation* in 1-cm cells with a suitable spectrophotometer at a wavelength of 540 nm. Using linear regression, analyze the data obtained for each of the *Standard preparations.* Calculate the correlation coefficient, slope, and *y*-intercept values: the correlation coefficient is not less than 0.995. Calculate the quantity, in mg, of protein in each mL of the Injectable Suspension by the formula:

$$10[(A_U - y\text{-intercept})/\text{slope}]$$

[5] The column is available from Varian U.S.A. Chrompack, Walnut Creek, CA, catalog number CP7517, as an Al_2O_3/KCl PLOT column (0.53-mm ID, 25-m length).

[6] The gas is available under product code 03047567SR-LD from Scott Medical Products, Plumsteadville, PA.

[7] The mixture of the two gases in air is available under product code 03047566SR-LD from Scott Medical Products, Plumsteadville, PA.

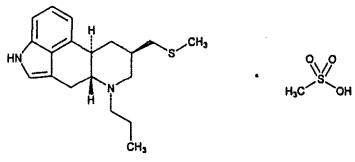
[8] Available as Antifoam Reagent, catalog number 2210, from Dow Corning Corporation, Midland, MI.

[9] Available as Bovine Serum Albumin, SRM 927c, Standard Reference Materials, National Institute of Standards and Technology, Gaithersburg, MD.

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in which 10 is the dilution factor; and A_U is the absorbance of the *Assay preparation:* the calculated quantity of protein in the Injectable Suspension is between 8 and 12 mg per mL.

Pergolide Mesylate



$C_{19}H_{26}N_2S \cdot CH_4O_3S$ 410.59

Ergoline,8-[(methylthio)methyl]-6-propyl-,monomethanesulfonate, (8β)-.

8β-[(Methylthio)methyl]-6-propylergoline monomethanesulfonate [66104-23-2].

» Pergolide Mesylate contains not less than 97.5 percent and not more than 102.0 percent of $C_{19}H_{26}N_2S \cdot CH_4O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Pergolide Mesylate RS
USP Pergolide Sulfoxide RS
(8β)-8-[(Methylsulfinyl)methyl]-6-propyl-D-ergoline.

Change to read:

Identification, ▲*Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Specific rotation ⟨781S⟩: between −17° and −23° at 20°.
Test solution: 10 mg per mL, in dimethylformamide.

Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 1 hour: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Solution A—Prepare a filtered and degassed mixture of 5.0 mL of morpholine with 995 mL of water, and adjust with phosphoric acid to a pH of 7.0.
Solution B—Prepare a filtered and degassed mixture of methanol, acetonitrile, and tetrahydrofuran (1:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system* (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution 1—Dissolve an accurately weighed quantity of USP Pergolide Mesylate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 30 µg per mL.
Standard solution 2—Dilute 10.0 mL of *Standard solution 1* to 50 mL with methanol.
Test solution—Transfer about 60 mg of Pergolide Mesylate, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains base-deactivated packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	70	30	equilibration
0–35	70→0	30→100	linear gradient

Chromatograph *Standard solution 1*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 10,000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of *Standard solution 1*, *Standard solution 2*, the *Test solution*, and a methanol blank into the chromatograph, record the chromatograms, and measure all of the peak responses. Disregard the contributions due to any peaks found in the methanol blank. The sum of the peak responses, excluding that of pergolide, from the *Test solution* is not more than the pergolide peak response obtained from *Standard solution 1* (0.5%), and no single peak response is more than the pergolide peak response obtained from *Standard solution 2* (0.1%).

Assay—
Diluent—Dissolve 5 mg of methionine in 500 mL of 0.01 N hydrochloric acid. Add 500 mL of methanol, and mix.
Mobile phase—Prepare a solution of 0.009 M sodium 1-octanesulfonate containing 1.0 mL of glacial acetic acid per L. Prepare a filtered and degassed mixture of this solution, methanol, and acetonitrile (2:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Resolution solution—Dissolve about 4 mg of USP Pergolide Sulfoxide RS and 8 mg of USP Pergolide Mesylate RS in 50 mL of *Diluent*.
Standard preparation—Dissolve an accurately weighed quantity of USP Pergolide Mesylate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.13 mg per mL.
Assay preparation—Transfer about 6.5 mg of Pergolide Mesylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains base-deactivated packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between pergolide sulfoxide and pergolide is not less than 12.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pergolide peaks. Calculate the quantity, in mg, of $C_{19}H_{26}N_2S \cdot CH_4O_3S$ in the portion of Pergolide Mesylate taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pergolide Mesylate RS in the *Standard preparation;* and r_U and

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r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pergolide Compounded Oral Suspension, Veterinary

DEFINITION

Pergolide Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of pergolide ($C_{19}H_{26}N_2S$).

Prepare Pergolide Compounded Oral Suspension, Veterinary, 1 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Pergolide (as Pergolide Mesylate, *USP*)	20 mg (26.11 mg)
Vehicle for Oral Suspension, *NF*	10 mL
Vehicle for Oral Solution, *NF*	10 mL

Connect an empty, calibrated, 35-mL Luer lock injection syringe to the port of a fluid-dispensing connector. Remove the plunger of another 35-mL Luer lock syringe, and set the plunger aside. Lock the barrel of this syringe onto the open port of the connector. Set this connected syringe apparatus in an upright, vertical position that is perpendicular to the work surface with the open syringe on top. Add the *Vehicle for Oral Suspension* and the *Pergolide Mesylate* into the open barrel. Replace the plunger on the open syringe, and invert the apparatus 180°. Apply 50 depressions to each syringe to mix. Consolidate the mixture into a single syringe. Disconnect the empty syringe. Add, via another 35-mL Luer lock injection syringe connected to the open port of the fluid-dispensing connector, a sufficient quantity of *Vehicle for Oral Solution* to bring the preparation to a final volume of 20 mL. Reattach the empty 35-mL syringe to the fluid-dispensing connector. Apply 50 depressions to each syringe to formulate a uniform suspension.

Alternatively, it may be prepared as follows. Add the *Pergolide Mesylate* to the mortar. Add the *Vehicle for Oral Suspension*, and mix to form a uniform paste. Add the *Vehicle for Oral Solution* in small portions almost to a final volume of 20 mL, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle for Oral Solution* to bring the preparation to a final volume of 20 mL, and mix well.

IDENTIFICATION

- **A.** The retention time of the pergolide peak of the *Sample solution* corresponds to that of the *Standard solutions*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.5 M sodium octanesulfonate solution and water (2:98), adjusted with glacial acetic acid to a pH of 2.2

Mobile phase: Acetonitrile and *Solution A* (50:50)

Diluent: Methanol and 0.01 N hydrochloric acid (50:50)

Standard stock solution: 1.0 mg/mL of USP Pergolide Mesylate RS in methanol prepared in low-actinic glassware

Standard solutions: Prepare five solutions of known concentrations of about 20, 10, 5, 2, and 1 µg/mL of pergolide mesylate by quantitatively diluting the *Standard stock solution* with *Mobile phase*. Use low-actinic glassware.

Sample solution: Transfer 500 µL of Oral Suspension, Veterinary to a 5-mL volumetric flask, and dilute with *Diluent* to volume. Further dilute an aliquot of the solution with *Mobile phase* to obtain a solution with a nominal concentration of 10 µg/mL of pergolide. Use low-actinic glassware.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 25 µL

System suitability
Samples: *Standard solutions* and *Sample solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution* (20 µg/mL)
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution* (20 µg/mL)
Correlation coefficient: NLT 0.995, linear regression of the *Standard solutions*
Resolution: NLT 2.0, *Sample solution*

Analysis
Samples: *Standard solutions* and *Sample solution*
Generate a regression curve of peak height versus pergolide mesylate concentration, and calculate the equation for the linear regression line.
Calculate the percentage of the labeled amount of pergolide ($C_{19}H_{26}N_2S$) in the portion of Oral Suspension, Veterinary taken. Use the molecular weights of pergolide and pergolide mesylate, 314.50 and 410.60, respectively.

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.0–4.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers, and store in a refrigerator.
- **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored in a refrigerator
- **LABELING:** Label to state that it is to be well shaken before use, protected from light, and to state the *Beyond-Use Date*. Label to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Pergolide Mesylate RS

Pergolide Tablets

» Pergolide Tablets contain an amount of Pergolide Mesylate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pergolide ($C_{19}H_{26}N_2S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Pergolide Mesylate RS
USP Pergolide Sulfoxide RS
(8β)-8-[(Methylsulfinyl)methyl]-6-propyl-D-ergoline.

Thin-layer chromatographic identification test ⟨201⟩—
Adsorbent: 0.25-mm layer of binder-free silica gel.
Test solution—Transfer a number of Tablets, equivalent to 1 mg of pergolide, to a separator containing 20 mL of

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methylene chloride and 10 mL of 0.1 N sodium hydroxide. Shake until the Tablets have disintegrated, allow the layers to separate, and drain the methylene chloride layer through a small funnel containing about 1 g of anhydrous sodium sulfate, collecting the filtrate in a suitable stoppered vessel. Wash the sodium sulfate with a few mL of methylene chloride, adding these washes to the filtrate, and evaporate to dryness under a stream of nitrogen. Redissolve the residue in 2 mL of a mixture of methylene chloride and methanol (1:1).

Standard solution: 0.65 mg per mL, in a mixture of methylene chloride and methanol (1:1).

Application volume: 20 µL.

Developing solvent system: a mixture of chloroform, methanol, and ethyl acetate (8:1:1). Allow the plate to equilibrate for about 10 minutes in the developing chamber prior to development.

Procedure—Proceed as directed in the chapter. Place the plate in a chamber containing iodine vapors, and locate the spots.

Dissolution ⟨711⟩—

Medium: simulated gastric fluid TS (without enzymes) containing 20 µg of L-cysteine per mL; 500 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{19}H_{26}N_2S$ dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and triethylamine, (21:19:0.08). Adjust with phosphoric acid to a pH of 5.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Triethylamine phosphate suspension—Add 1.0 mL of triethylamine to 500 mL of acetonitrile, mix, and adjust with phosphoric acid to a pH of 5.0. A white precipitate will form. Stir continuously during use.

Resolution solution—Prepare a solution of USP Pergolide Mesylate RS and USP Pergolide Sulfoxide RS containing a known amount of each equivalent to the labeled amount of pergolide in each 500 mL of *Medium*.

Standard solution—Transfer about 16 mg of USP Pergolide Mesylate RS, accurately weighed, to a 250-mL volumetric flask, dissolve in 10.0 mL of methanol, dilute with *Medium* to volume, and mix. Dilute this solution quantitatively and stepwise with *Medium* to obtain a solution having a known concentration equivalent to the labeled amount of pergolide in each 500 mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a fluorometer set to an excitation wavelength of 224 nm and an emission wavelength of 350 nm and with a 4.6-mm × 15-cm column that contains base-deactivated packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between pergolide sulfoxide and pergolide is not less than 1.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections, determined from the pergolide peak, is not more than 2.0%.

Procedure—Immediately before injection, pipet 2.0 mL of *Triethylamine phosphate suspension*, continuously stirred, into a suitable container containing 5.0 mL of the solution for injection, and mix to obtain a clear solution. Separately inject equal volumes (about 200 µL) of the *Standard solution* and filtered portions of the solutions under test into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the amount, in mg, of pergolide ($C_{19}H_{26}N_2S$) dissolved by the formula:

$$500C(314.50/410.60)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Pergolide Mesylate RS in the *Standard solution;* 314.50 and 410.60 are the molecular weights of pergolide and pergolide mesylate, respectively; and r_U and r_S are the peak areas obtained from the solution under test and the *Standard solution*, respectively.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{19}H_{26}N_2S$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity—

Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*.

Diluted standard preparation—Transfer 3.0 mL of the *Standard preparation* to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Test preparation—Use the *Assay preparation*.

Procedure—Separately inject equal volumes (about 100 µL) of the *Diluted standard preparation* and the *Test preparation* into the chromatograph, and measure all of the peak responses. Calculate the percentage of each impurity in the Tablets by the formula:

$$20C(314.50/410.60)(r_i/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Pergolide Mesylate RS in the *Diluted standard preparation;* 314.50 and 410.60 are the molecular weights of pergolide and pergolide mesylate, respectively; r_i is the peak response of the individual impurity obtained from the *Test preparation;* and r_S is the peak response of pergolide obtained from the *Diluted standard preparation:* not more than 6.0% of pergolide sulfoxide is found; not more than 0.5% of any individual impurity, excluding pergolide sulfoxide, is found; and not more than 1.0% of total impurities, excluding pergolide sulfoxide, is found.

Assay—

Mobile phase—Prepare a solution of 0.038 M sodium 1-octanesulfonate containing 0.0077 mg of methionine per mL and 2.45 mL of glacial acetic acid per L. Adjust with 5 N sodium hydroxide to a pH of 4.1. Prepare a filtered and degassed mixture of this solution and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution of USP Pergolide Mesylate RS and USP Pergolide Sulfoxide RS in *Mobile phase* having a known concentration of about 6.5 µg per mL of pergolide mesylate and 0.1 µg per mL of pergolide sulfoxide.

Standard preparation—Dissolve an accurately weighed quantity of USP Pergolide Mesylate RS in *Mobile phase*, and dilute quantitatively and stepwise with *Mobile phase* to obtain a solution having a known concentration of about 6.5 µg per mL.

Assay preparation—Place 20 whole Tablets into a suitable stoppered container, add *Mobile phase*, shake and sonicate until the Tablets have dissolved, and quantitatively dilute to obtain a solution containing about 5 µg per mL of pergolide.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a fluorometer set to an excitation wavelength of 280 nm and an emission wavelength of 335 nm and with a 4.6-mm × 7.5-cm column that contains base-deactivated packing L7. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 35°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between pergolide sulfoxide and pergolide is not less than 12.0; and the relative standard deviation for replicate injections is not more than 2.0%.

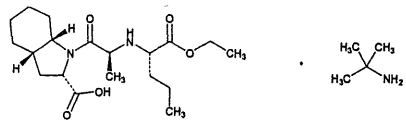
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Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Calculate the quantity, in mg, of pergolide ($C_{19}H_{26}N_2S$) in the portion of Tablets taken by the formula:

$$0.001C(314.50/410.60)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Pergolide Mesylate RS in the *Standard preparation;* 314.50 and 410.60 are the molecular weights of pergolide and pergolide mesylate, respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Perindopril Erbumine



$C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$ 441.6

1*H*-Indole-2-carboxylic acid, 1-[2-[[1-(ethoxycarbonyl)butyl] amino]-1-oxopropyl]octahydro-, [2*S*-[1[*R**(*R**)],2α,3aβ, 7aβ]]-, compound with 2-methyl-2-propanamine (1:1); (2*S*,3a*S*,7a*S*)-1-[(*S*)-*N*-[(*S*)-1-Carboxybutyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with *tert*-butylamine (1:1) [107133-36-8].

DEFINITION

Perindopril Erbumine contains NLT 98.0% and NMT 102.0% of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of *System suitability solution 1*, as obtained in the test for *Limit of Perindopril Related Compound I*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 0.92 g of sodium 1-heptanesulfonate in 1 L of water and add 1 mL of triethylamine. Adjust with a solution of perchloric acid and water (1:1) to a pH of 2.0.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard solution: 0.1 mg/mL of USP Perindopril Erbumine RS in *Buffer*. Initially add *Buffer* to about 60% of the flask volume, sonicate to dissolve, and dilute with *Buffer* to volume.
Sample solution: 0.1 mg/mL of Perindopril Erbumine in *Buffer*. Initially add *Buffer* to about 60% of the flask volume, sonicate to dissolve, and dilute with *Buffer* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of perindopril from the *Sample solution*
r_S = peak area of perindopril from the *Standard solution*
C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)
C_U = concentration of Perindopril Erbumine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A: Proceed as directed for the *Buffer* as described in the *Assay*.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
27	68	32
45	50	50
60	20	80
70	20	80
71	80	20
80	80	20

Diluent: *Solution B* and *Solution A* (20:80)
System suitability stock solution A: 0.03 mg/mL of USP Imidazole RS in *Diluent*
System suitability stock solution B: 0.03 mg/mL each of USP Perindopril Erbumine RS, USP Perindopril Related Compound B RS, USP Perindopril Related Compound C RS, USP Perindopril Related Compound D RS, and USP Perindopril Related Compound F RS in *Diluent*
System suitability solution: Transfer 5 mL each of *System suitability stock solution A* and *System suitability stock solution B* to a 50-mL volumetric flask and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size, discard the first 3 mL of filtrate, and use the clear filtrate.
Standard stock solution: 0.03 mg/mL of USP Perindopril Erbumine RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for about 5 min, and dilute with *Diluent* to volume.
Standard solution: 0.003 mg/mL of USP Perindopril Erbumine RS in *Diluent* from the *Standard stock solution*.

USP Monographs

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Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.

Sample solution: 3 mg/mL of Perindopril Erbumine in *Diluent* prepared as follows. Dissolve a suitable quantity of Perindopril Erbumine in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.0-mm × 25-cm; 4-µm packing L7
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Tailing factor: NMT 1.5 for the perindopril peak
Relative standard deviation: NMT 5.0% for the perindopril peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of perindopril erbumine from the *Standard solution*
C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)
C_U = concentration of Perindopril Erbumine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazole[a]	0.08	—	—
Perindopril related compound B[b]	0.42	1.20	0.3
Perindopril related compound C[c]	0.74	0.96	0.1
Perindopril related compound D[d]	0.85	0.98	0.1
Perindopril erbumine	1.0	—	—
Isopropyl perindopril[e]	1.22	1.00	0.40 (if present)
Perindopril related compound F[f]	1.38	0.85	0.2
Perindopril imidazolidinone analog[g]	1.65	1.00	0.15 (if present)
Any individual unspecified impurity	—	—	0.10
Total impurities[h]	—	—	1

[a] Imidazole is quantitated using the test for *Limit of Perindopril Related Compound A and Imidazole* and is included in the table for identification purposes only.
[b] (2*S*,3a*S*,7a*S*)-1-[(*S*)-2-[(*S*)-1-Carboxybutylamino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.
[c] (*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.
[d] (*S*)-2-[(3*S*,5a*S*,9a*S*,10a*R*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.
[e] (2*S*,3a*S*,7a*S*)-1-[(*S*)-2-[(*S*)-1-isopropoxy-1-oxopentan-2-ylamino]propanoyl] octahydro-1*H*-indole-2-carboxylic acid.
[f] (*S*)-Ethyl 2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*] indol-2(1*H*)-yl]pentanoate.
[g] (2*S*,3a*S*,7a*S*)-1-((2*S*)-2-((5*RS*)-3-cyclohexyl-2-(cyclohexylimino)-4-oxo-5-propylimidazolidin-1-yl)propanoyl)octahydro-1*H*-indole-2-carboxylic acid.
[h] Total impurities include all specified and unspecified impurities and imidazole from the test for *Limit of Perindopril Related Compound A and Imidazole*. Perindopril related compound A is not included.

• **LIMIT OF PERINDOPRIL RELATED COMPOUND A AND IMIDAZOLE**

Solution A: Proceed as directed in *Organic Impurities*.
Solution B: Acetonitrile
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	83	17
5	83	17
6	20	80
20	20	80
21	83	17
33	83	17

Diluent: *Solution B* and *Solution A* (17:83)
Standard stock solution: 0.25 mg/mL of USP Perindopril Related Compound A RS and 0.1 mg/mL of USP Imidazole RS in *Diluent*. Sonicate if necessary.
Standard solution: 10 mg/mL of USP Perindopril Erbumine RS, 0.025 mg/mL of USP Perindopril Related Compound A RS, and 0.01 mg/mL of USP Imidazole RS in *Diluent* prepared as follows. Transfer a weighed amount of USP Perindopril Erbumine RS and a suitable amount of *Standard stock solution* to a suitable volumetric flask, and dissolve with sonication in *Diluent* equivalent to 60% of the final volume. Dilute with *Diluent* to volume, pass through a suitable filter of 0.45-µm pore size, and discard the first 3 mL of filtrate.
Sample solution: 10 mg/mL of Perindopril Erbumine in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Perindopril related compound A: UV 210 nm
Imidazole: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Temperatures
Column: 60°
Sample cooler: 5°
Flow rate: 1 mL/min

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Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for imidazole and perindopril related compound A are 1.0 and 1.4, respectively.]
Suitability requirements
Tailing factor: NMT 1.8 for perindopril related compound A
Relative standard deviation: NMT 5.0% for perindopril related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of perindopril related compound A or imidazole in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of perindopril related compound A or imidazole from the *Sample solution*
r_S = peak response of perindopril related compound A or imidazole from the *Standard solution*
C_S = concentration of USP Perindopril Related Compound A RS or USP Imidazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Perindopril Erbumine in the *Sample solution* (mg/mL)

Acceptance criteria
Perindopril related compound A: NMT 0.25%
Imidazole: NMT 0.1%

Change to read:

• **LIMIT OF PERINDOPRIL RELATED COMPOUND I**
[NOTE—Perindopril related compound I is the epimer of perindopril: (2*S*,3a*S*,7a*S*)-1-{(*S*)-2-[(*R*)-1-ethoxy-1-oxopentan-2-ylamino]propanoyl}octahydro-1*H*-indole-2-carboxylic acid.]
Solution A: Dissolve 5 g of potassium phosphate monobasic in 1900 mL of water. Adjust with triethylamine to a pH of 6.50 and add 100 mL of acetonitrile.
Solution B: Acetonitrile
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	83	17
22	83	17
23	20	80
33	20	80
34	83	17
46	83	17

Diluent: *Solution B* and *Solution A* (17:83)
System suitability solution 1: 3 mg/mL of USP Perindopril Erbumine RS in *Diluent*. [NOTE—This solution is used in *Identification* test *B*.]
System suitability solution 2: 3 µg/mL of USP Perindopril Erbumine RS in *Diluent* from *System suitability solution 1*
Sample solution: 3 mg/mL of Perindopril Erbumine in *Diluent* prepared as follows. Dissolve a suitable quantity of Perindopril Erbumine in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution 2*
▲[NOTE—The relative retention times for perindopril and perindopril related compound I are 1.0 and 1.6, respectively.]▲ (ERR 1-Apr-2018)
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of perindopril related compound I in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of perindopril related compound I from the *Sample solution*
r_T = total of all peak responses from the *Sample solution*

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 1.0%; 3.00%–4.50% for a monohydrate
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL of Perindopril Erbumine in ethanol
Acceptance criteria: −66° to −69°, at 20°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Imidazole RS
USP Perindopril Erbumine RS
USP Perindopril Related Compound A RS
(2*S*,3a*S*,7a*S*)-Octahydro-1*H*-indole-2-carboxylic acid.
$C_9H_{15}NO_2$ 169.22
USP Perindopril Related Compound B RS
(2*S*,3a*S*,7a*S*)-1-{(*S*)-2-[(*S*)-1-Carboxybutylamino]propanoyl}octahydro-1*H*-indole-2-carboxylic acid.
$C_{17}H_{28}N_2O_5$ 340.41
USP Perindopril Related Compound C RS
(*S*)-2-{(3*S*,5a*S*,9a*S*,10a*S*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl}pentanoic acid.
$C_{17}H_{26}N_2O_4$ 322.40
USP Perindopril Related Compound D RS
(*S*)-2-{(3*S*,5a*S*,9a*S*,10a*R*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl}pentanoic acid.
$C_{17}H_{26}N_2O_4$ 322.40
USP Perindopril Related Compound F RS
(*S*)-Ethyl 2-{(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl}pentanoate.
$C_{19}H_{30}N_2O_4$ 350.45

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Perindopril Erbumine Tablets

DEFINITION

Perindopril Erbumine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$).

IDENTIFICATION

- **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 0.92 g of sodium 1-heptanesulfonate in 1 L of water and add 1 mL of triethylamine. Adjust with a solution of perchloric acid and water (1:1) to a pH of 2.0.
Mobile phase: Acetonitrile and *Solution A* (38:62)
Diluent: Acetonitrile and *Solution A* (40:60)
Standard solution: 0.08 mg/mL of USP Perindopril Erbumine RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.
Sample solution: Nominally equivalent to 0.08 mg/mL of perindopril erbumine in *Diluent* prepared as follows. Weigh and transfer the number of Tablets into a suitable volumetric flask, as indicated in *Table 1*.

Table 1

Tablet Strength (mg)	Number of Tablets (NLT)	Volumetric Flask (mL)
2	20	500
4	10	500
8	10	1000

Add *Diluent* to about 70% of the flask volume, shake mechanically for about 60 min at 180 rpm, and sonicate for 20 min. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 25-cm; 4-µm packing L7
Temperatures
Column: 60°
Sample cooler: 5°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention time of perindopril
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of perindopril erbumine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Solution A: Proceed as directed in the *Assay*.
Mobile phase: Acetonitrile and *Solution A* (350:650)
Standard stock solution: 0.55 mg/mL of USP Perindopril Erbumine RS in acetonitrile
Standard solution: Prepare solutions of USP Perindopril Erbumine RS in *Medium* from the *Standard stock solution*, with final concentrations from *Table 2*.

Table 2

Tablet Strength (mg)	Concentration (mg/mL)
2	0.0022
4	0.0044
8	0.0088

Sample solution: Pass a portion of the solution under test through a suitable filter and discard the first 1 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 50°
Flow rate: 1.2 mL/min
Injection volume: 100 µL
Run time: NLT 1.6 times the retention time of perindopril
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) dissolved.

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: Proceed as directed in the *Assay*.

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Solution B: Acetonitrile
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
27	68	32
45	50	50
60	20	80
70	20	80
71	80	20
80	80	20

Diluent: *Solution B* and *Solution A* (20:80)
System suitability stock solution A: 0.03 mg/mL of USP Imidazole RS in *Diluent*
System suitability stock solution B: 0.12 mg/mL each of USP Perindopril Related Compound C RS and USP Perindopril Related Compound D RS in *Diluent*. Initially add 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume.
System suitability solution: Accurately weigh about 1.5 mg each of USP Perindopril Related Compound B RS and USP Perindopril Related Compound F RS into a 50-mL volumetric flask. Add 30 mL of *Diluent* and sonicate for 5 min. Transfer 5.0 mL each of *System suitability stock solution A*, *System suitability stock solution B*, and *Standard stock solution*. Dilute with *Diluent* to volume.
Standard stock solution: 0.05 mg/mL of USP Perindopril Erbumine RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume.
Standard solution: 0.005 mg/mL of USP Perindopril Erbumine RS in *Diluent* from the *Standard stock solution*. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.
Sample solution: Nominally equivalent to 2 mg/mL of perindopril erbumine in *Diluent* prepared as follows. Transfer a quantity equivalent to about 20 mg of perindopril erbumine from powdered Tablets (NLT 20) into a test tube. Pipet 10.0 mL of *Diluent* into the test tube, sonicate for about 10 min, and vortex for about 1 min. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Proceed as directed in the *Assay*, except for the *Run time*. [NOTE—The run time is determined by the gradient from *Table 3*.]
System suitability
Sample: *System suitability solution*
Suitability requirements
Tailing factor: NMT 1.5 for the perindopril peak
Relative standard deviation: NMT 5.0% for the perindopril peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of perindopril erbumine from the *Standard solution*
C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of perindopril erbumine in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 4*)

Acceptance criteria: See *Table 4*. Disregard peaks less than 0.1%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazole[a]	0.08	—	—
Perindopril related compound B[b]	0.42	1.21	2.0
Perindopril related compound C[c]	0.74	0.97	0.5
Perindopril related compound D[d]	0.85	0.98	0.5
Perindopril erbumine	1.0	—	—
Perindopril related compound F[e]	1.38	0.86	3.0
Any unspecified impurity	—	—	0.2
Total impurities[f]	—	—	1.5

[a] Imidazole is given for identification only and is not quantitated using this procedure.
[b] (2*S*,3a*S*,7a*S*)-1-{(*S*)-2-[(*S*)-1-Carboxybutylamino]propanoyl}octahydro-1*H*-indole-2-carboxylic acid.
[c] (*S*)-2-{(3*S*,5a*S*,9a*S*,10a*S*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1*H*)-yl}pentanoic acid.
[d] (*S*)-2-{(3*S*,5a*S*,9a*S*,10a*R*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1*H*)-yl}pentanoic acid.
[e] (*S*)-Ethyl 2-{(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1*H*)-yl}pentanoate.
[f] Total impurities excludes perindopril related compound F and perindopril related compound B.

ADDITIONAL REQUIREMENTS

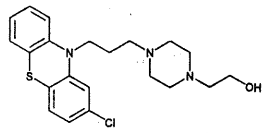
- **PACKAGING AND STORAGE:** Preserve in air-tight containers. Protect from heat and moisture.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Imidazole RS
  USP Perindopril Erbumine RS
  USP Perindopril Related Compound B RS
  (2*S*,3a*S*,7a*S*)-1-{(*S*)-2-[(*S*)-1-Carboxybutylamino]propanoyl}octahydro-1*H*-indole-2-carboxylic acid.
  $C_{17}H_{28}N_2O_5$ 340.41
  USP Perindopril Related Compound C RS
  (*S*)-2-{(3*S*,5a*S*,9a*S*,10a*S*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl}pentanoic acid.
  $C_{17}H_{26}N_2O_4$ 322.40
  USP Perindopril Related Compound D RS
  (*S*)-2-{(3*S*,5a*S*,9a*S*,10a*R*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl}pentanoic acid.
  $C_{17}H_{26}N_2O_4$ 322.40

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USP Perindopril Related Compound F RS
(*S*)-Ethyl 2-{(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl} pentanoate.
$C_{19}H_{30}N_2O_4$ 350.45

Perphenazine



$C_{21}H_{26}ClN_3OS$ 403.97
Piperazineethanol, 4-[3-(2-chloro-10*H*-phenothiazin-10-yl) propyl-];
4-[3-(2-Chlorophenothiazin-10-yl)propyl]-1-piperazineethanol [58-39-9].

DEFINITION
Perphenazine contains NLT 98.0% and NMT 102.0% of perphenazine ($C_{21}H_{26}ClN_3OS$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Diluted sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**

Sample solution: Dissolve 0.150 g of Perphenazine in 25 mL of glacial acetic acid.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis
Sample: *Sample solution*
Titrate the *Sample solution* with *Titrant*. Carry out a blank titration.
Calculate the percentage of perphenazine ($C_{21}H_{26}ClN_3OS$) in the portion of Perphenazine taken:

$$\text{Result} = [(V - B) \times N \times F/W] \times 100$$

V = *Titrant* volume consumed by the *Sample* (mL)
B = *Titrant* volume consumed by the blank (mL)
N = normality of the *Titrant* (meq/mL)
F = equivalent weight of perphenazine, 202.0 mg/meq
W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Prepare the solutions immediately before use. Carry out the test protected from light.
Solution A: Acetonitrile and 7 g/L of monobasic sodium phosphate dihydrate in water (35:65)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
10	80	20
33	30	70
48	100	0

System suitability solution: 2 mg/mL of USP Perphenazine RS, 0.002 mg/mL of USP Perphenazine Sulfoxide RS, and 0.002 mg/mL of USP Perphenazine Related Compound B RS in *Solution A*
Standard solution: 0.002 mg/mL of USP Perphenazine RS in *Solution A*
Sample solution: 2 mg/mL of Perphenazine in *Solution A*
Diluted sample solution: 0.002 mg/mL of Perphenazine in *Solution A* from the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm
Column: 4.0-mm × 25-cm; 4-µm packing L7
Column temperature: 30°
Flow rate: 1.3 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between perphenazine and perphenazine related compound B, *System suitability solution*
Tailing factor: NMT 2.5, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Diluted sample solution*. [NOTE—*Diluted sample solution* is used for *Identification* test *B*.]
Calculate the percentage of impurities in the portion of Perphenazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of perphenazine from the *Standard solution*
C_S = concentration of USP Perphenazine RS in the *Standard solution* (mg/mL)
C_U = concentration of Perphenazine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Perphenazine sulfoxide	0.3	1.6	0.2

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Perphenazine related compound B	0.8	1.0	0.5
Perphenazine	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩

Analysis: Dry a sample in a vacuum at 65° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards** ⟨11⟩

USP Perphenazine RS

USP Perphenazine Related Compound B RS

2-{4-[3-(10*H*-Phenothiazin-10-yl)propyl]piperazin-1-yl} ethanol.

$C_{21}H_{27}N_3OS$ 369.52

USP Perphenazine Sulfoxide RS

2-Chloro-10-{3-[4-(2-hydroxyethyl)piperazin-1-yl] propyl}-10*H*-phenothiazine 5-oxide.

$C_{21}H_{26}ClN_3O_2S$ 419.97

Perphenazine Tablets

DEFINITION

Perphenazine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$).

[Note—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

• **A.**

Standard solution: 1 mg/mL of USP Perphenazine RS in methanol

Sample solution: Shake a portion of finely powdered Tablets, nominally equivalent to 5 mg of perphenazine, with 10 mL of chloroform. Filter, evaporate the filtrate on a steam bath nearly to dryness, and dissolve the residue in 5 mL of methanol.

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Acetone and ammonium hydroxide (200:1)

Iodoplatinic acid: Dissolve 100 mg of chloroplatinic acid in 1 mL of 1 N hydrochloric acid. Add 25 mL of potassium iodide solution (4 in 100), dilute with water to 100 mL, and add 0.50 mL of formic acid.

Spray reagent: *Iodoplatinic acid*

Analysis

Samples: *Standard solution* and *Sample solution*

Develop using the *Developing solvent system* until the solvent front has moved 15 cm. Air-dry the plate, and spray lightly with *Spray reagent.*

Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution.*

ASSAY

• **Procedure**

Solution A: Transfer 10 mL of hydrochloric acid to a 1000-mL flask containing 500 mL of alcohol and 300 mL of water. Dilute with water to volume.

Solution B: Dissolve 100 mg of palladium chloride in a mixture of 1 mL of hydrochloric acid and 50 mL of water in a 100-mL volumetric flask, heating on a steam bath to effect solution. Cool, dilute with water to volume, and mix. Store in an amber bottle and use within 30 days. On the day of use, transfer 50 mL to a 500-mL volumetric flask, add 4 mL of hydrochloric acid and 4.1 g of anhydrous sodium acetate, dilute with water to volume, and mix.

Standard solution: 160 µg/mL of USP Perphenazine RS in *Solution A*

Sample solution: Nominally 160 µg/mL of perphenazine prepared as follows. Transfer a portion of powder, equivalent to 4 mg of perphenazine from NLT 20 finely powdered Tablets, to a glass-stoppered conical flask. Pipet into the flask 25 mL of *Solution A*, shake by mechanical means for 30 min, and centrifuge a portion of the mixture. Use the clear supernatant fluid.

Instrumental conditions

Mode: Vis

Analytical wavelength: Maximum absorbance at about 480 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Solution B*

Mix 10.0 mL each of the *Sample solution* and the *Standard solution* with 15.0 mL of *Solution B.* Filter, if necessary, and determine the absorbances of these solutions against a reagent blank.

Calculate the percentage of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Perphenazine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of perphenazine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: A known concentration of USP Perphenazine RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a concentration similar to that of the *Standard solution.*

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 257 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$) dissolved.

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Tolerances: NLT 75% (*Q*) of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$) is dissolved.

- **UNIFORMITY OF DOSAGE FORMS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Perphenazine RS

Perphenazine and Amitriptyline Hydrochloride Tablets

» Perphenazine and Amitriptyline Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of perphenazine ($C_{21}H_{26}ClN_3OS$) and amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Amitriptyline Hydrochloride RS
USP Perphenazine RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—Transfer a portion of powdered Tablets, equivalent to about 40 mg of perphenazine, to a 100-mL volumetric flask containing about 50 mL of alcohol. Agitate for 20 minutes, add alcohol to volume, mix, and filter or centrifuge. Separately prepare two Standard solutions containing 0.4 mg per mL of USP Perphenazine RS and USP Amitriptyline Hydrochloride RS, respectively, in alcohol. Separately apply 5 µL of the test solution and 5 µL of each Standard solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram using a solvent system consisting of a mixture of cyclohexane, ethyl acetate, and diethylamine (85:25:5) until the solvent front has moved about 15 cm. Remove the plate from the developing chamber, air-dry for 20 minutes, and examine the plate under short-wavelength UV light: the R_F values of the principal spots obtained from the test solution correspond to those obtained from the Standard solutions.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.
Procedure— [NOTE—Due to potential decrease in the recovery of perphenazine when multiple injections are made from a vial, no more than two withdrawals should be made from any single vial.] Determine the amounts of perphenazine and amitriptyline hydrochloride in solution in filtered portions of the solution under test, in comparison with a Standard solution having known concentrations of USP Perphenazine RS and USP Amitriptyline Hydrochloride RS in the same medium, as directed for *Procedure* in the *Assay*.
Tolerances—Not less than 75% (*Q*) of the labeled amounts of perphenazine ($C_{21}H_{26}ClN_3OS$) and amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$) is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Content uniformity* with respect to perphenazine and to amitriptyline hydrochloride.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, methanol, and methanesulfonic acid (490:310:200:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Perphenazine RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 0.8 mg per mL (*Solution P*). Transfer 4*J* mg of USP Amitriptyline Hydrochloride RS to a 50-mL volumetric flask, *J* being the ratio of the labeled amount, in mg, of amitriptyline hydrochloride to the labeled amount, in mg, of perphenazine per Tablet. Add 5.0 mL of *Solution P* and 20 mL of 0.2 N acetic acid, shake, and sonicate to dissolve the USP Reference Standards. Dilute with methanol to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, dilute with a mixture of methanol and 0.04 N acetic acid (3:2) to volume, and mix to obtain a *Standard preparation* having known concentrations of about 20 µg of USP Perphenazine RS per mL and about 20*J* µg of USP Amitriptyline Hydrochloride RS per mL.
Assay preparation—Transfer 10 Tablets to a 250-mL volumetric flask, add 100 mL of 0.2 N acetic acid, and shake the mixture until the Tablets have disintegrated. Add methanol to volume, mix, and filter. Dilute an accurately measured volume (V_F mL) of the clear filtrate quantitatively with a mixture of methanol and 0.04 N acetic acid (3:2) to obtain a solution (V_A mL) containing about 20 µg of perphenazine per mL, and filter through a membrane filter.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute, and is adjusted until the relative retention times for perphenazine and amitriptyline are about 1 and 1.5, respectively. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0% for replicate injections, and the resolution, *R,* between perphenazine and amitriptyline is not less than 4.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of perphenazine ($C_{21}H_{26}ClN_3OS$) in each Tablet taken by the formula:

$$0.25(C/10)(V_A/V_F)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Perphenazine RS in the *Standard preparation*, V_A is the volume, in mL, of the *Assay preparation*, V_F is the volume, in mL, of the filtrate taken for the *Assay preparation*, and r_U and r_S are the responses of the perphenazine peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$) taken by the same formula, reading amitriptyline hydrochloride instead of perphenazine.

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Pertussis Immune Globulin

» Pertussis Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic solution of globulins derived from the blood plasma of adult human donors who have been immunized with pertussis vaccine such that each 1.25 mL contains not less than the amount of immune globulin to be equivalent to 25 mL of human hyperimmune serum. It may contain 0.3 M glycine as a stabilizing agent, and it contains a suitable preservative.

Packaging and storage—Preserve at a temperature between 2° and 8°.
Expiration date—The expiration date is not later than 3 years after date of issue from manufacturer's cold storage (5°, 3 years).
Labeling—Label it to state that it is not intended for intravenous injection.

Petrolatum

DEFINITION
Petrolatum is a purified mixture of semisolid hydrocarbons obtained from petroleum. It may contain a suitable stabilizer.

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩
Sample: 2 g
Analysis: Heat the *Sample* in an open porcelain or platinum dish over a Bunsen flame.
Acceptance criteria: It volatilizes without emitting an acrid odor and yields NMT 0.1% of residue.
ORGANIC IMPURITIES
• **Procedure: Organic Acids**
Sample solution: 20.0 g of Petrolatum in 100 mL of a 1 in 2 mixture of neutralized alcohol and water. Agitate thoroughly, and heat to boiling.
Analysis: Add 1 mL of phenolphthalein TS, and titrate rapidly with 0.1 N sodium hydroxide VS, with vigorous agitation to the production of a sharp pink endpoint, noting the color change in the alcohol–water layer.
Acceptance criteria: NMT 400 µL of 0.1 N sodium hydroxide is required.

SPECIFIC TESTS
• **COLOR**
Standard solution: Ferric chloride CS and cobaltous chloride CS (3.8: 1.2)
Sample: 10 g
Analysis: Melt the *Sample* on a steam bath, and pour 5 mL of the liquid into a clear-glass 15-mm × 150-mm test tube, keeping the petrolatum melted.
Acceptance criteria: The warm, melted liquid is not darker than 5 mL of the *Standard solution* in a similar tube; the comparison of the two being made in reflected light against a white background, and the petrolatum tube being held directly against the background at such an angle that there is no fluorescence.
• **SPECIFIC GRAVITY** ⟨841⟩: 0.815–0.880 at 60°
• **MELTING RANGE OR TEMPERATURE,** *Class III* ⟨741⟩: 38°–60°
• **CONSISTENCY**
Apparatus: A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°, the point of the tip is truncated to a diameter of 0.381 ± 0.025 mm, the base of the tip is 8.38 ± 0.05 mm in diameter, and the length of the tip is 14.94 ± 0.05 mm.
The remaining portion of the cone has an angle of 90°, is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottom metal cylinders that are 100 ± 6 mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal, and are provided with well-fitting, water-tight covers.
Sample: Petrolatum
Analysis: Place the required number of containers in an oven, bring them and a quantity of the *Sample* to a temperature of 82 ± 2.5°, and pour the *Sample* into one or more of the containers, filling to within 6 mm of the rim. Cool to 25 ± 2.5° over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at 25 ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25 ± 0.5° by placing it in the water bath.
Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than ±3%.
Acceptance criteria: The final average of the trials is NLT 10.0 mm and NMT 30.0 mm, indicating a consistency value of 100–300.
• **ALKALINITY**
Sample: 35 g
Analysis: Introduce the *Sample* into a suitable beaker, add 100 mL of boiling water, cover, and place on a stirring hot-plate maintained at the boiling point of water. After 5 min, allow the phases to separate. Draw off the separated water into a casserole, wash the petrolatum further with two 50-mL portions of boiling water, and add the washings to the casserole. To the pooled washings, add 1 drop of phenolphthalein TS, and boil.
Acceptance criteria: The solution does not acquire a pink color.
• **ACIDITY:** If the addition of phenolphthalein TS in the test for *Alkalinity* produces no pink color, add 0.1 mL of methyl orange TS.
Acceptance criteria: No red or pink color is produced.
• **FIXED OILS, FATS, AND ROSIN**
Sample: 10 g
Analysis: Digest the *Sample* with 50 mL of 5 N sodium hydroxide at 100° for 30 min. Separate the water layer, and acidify it with 5 N sulfuric acid.
Acceptance criteria: No oily or solid matter separates.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Label it to indicate the name and proportion of any added stabilizer.

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Hydrophilic Petrolatum

DEFINITION
Prepare Hydrophilic Petrolatum as follows.

Cholesterol	30 g
Stearyl Alcohol	30 g
White Wax	80 g
White Petrolatum	860 g
To make	1000 g

Melt the *Stearyl Alcohol* and *White Wax* together on a steam bath, then add the *Cholesterol*, and stir until completely dissolved. Add the *White Petrolatum*, and mix. Remove from the bath, and stir until the mixture congeals.

White Petrolatum

DEFINITION
White Petrolatum is a purified mixture of semisolid hydrocarbons obtained from petroleum, and wholly or nearly decolorized. It may contain a suitable stabilizer.

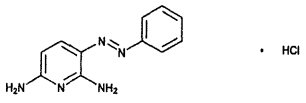
IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩
Sample: 2 g
Analysis: Heat the *Sample* in an open porcelain or platinum dish over a flame.
Acceptance criteria: It volatilizes without emitting an acrid odor and yields NMT 0.05% of residue.
ORGANIC IMPURITIES
• **Procedure: Organic Acids**
Sample solution: 20.0 g of White Petrolatum in 100 mL of a 1 in 2 mixture of neutralized alcohol and water. Agitate thoroughly and heat to boiling.
Analysis: Add 1 mL of phenolphthalein TS, and titrate rapidly with 0.1 N sodium hydroxide VS, with vigorous agitation to the production of a sharp pink endpoint, noting the color change in the alcohol–water layer.
Acceptance criteria: NMT 400 µL of 0.1 N sodium hydroxide is required.

SPECIFIC TESTS
• **COLOR**
Standard solution: Ferric chloride CS and water (1.6: 3.4)
Sample: 10 g
Analysis: Melt the *Sample* on a steam bath, and pour 5 mL of the liquid into a clear-glass 16-mm × 150-mm bacteriological test tube, keeping the petrolatum melted.
Acceptance criteria: The warm, melted liquid is not darker than 5 mL of the *Standard solution* in a similar tube; the comparison of the two being made in reflected light against a white background, and the petrolatum tube being held directly against the background at such an angle that there is no fluorescence.
• **SPECIFIC GRAVITY** ⟨841⟩: 0.815–0.880 at 60°
• **MELTING RANGE OR TEMPERATURE,** *Class III* ⟨741⟩ 38°–60°
• **CONSISTENCY**
Apparatus: A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°, the point of the tip is truncated to a diameter of 0.381 ± 0.025 mm, the base of the tip is 8.38 ± 0.05 mm in diameter, and the length of the tip is 14.94 ± 0.05 mm.
The remaining portion of the cone has an angle of 90°, is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottomed metal cylinders that are 100 ± 6 mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal and are provided with well-fitting, water-tight covers.
Sample: White Petrolatum
Analysis: Place the required number of containers in an oven, and bring them and a quantity of Petrolatum to a temperature of 82 ± 2.5°. Pour the Petrolatum into one or more of the containers, filling to within 6 mm of the rim. Cool to 25 ± 2.5° over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at 25 ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25 ± 0.5° by placing it in the water bath.
Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than ±3%.
Acceptance criteria: The final average of the trials is NLT 10.0 mm and NMT 30.0 mm, indicating a consistency value of 100–300.
• **ALKALINITY**
Sample: 35 g
Analysis: Introduce the *Sample* into a suitable beaker, add 100 mL of boiling water, cover, and place on a stirring hot-plate maintained at the boiling point of water. After 5 min, allow the phases to separate. Draw off the separated water into a casserole, wash the petrolatum further with two 50-mL portions of boiling water, and add the washings to the casserole. To the pooled washings, add 1 drop of phenolphthalein TS, and boil.
Acceptance criteria: The solution does not acquire a pink color.
• **ACIDITY**
Sample: Final solution of the test for *Alkalinity*, if the addition of phenolphthalein TS produced no pink color
Analysis: To the *Sample*, add 0.1 mL of methyl orange TS.
Acceptance criteria: No red or pink color is produced.
• **FIXED OILS, FATS, AND ROSIN**
Sample: 10 g
Analysis: Digest the *Sample* with 50 mL of 5 N sodium hydroxide at 100° for 30 min. Separate the water layer, and acidify it with 5 N sulfuric acid.
Acceptance criteria: No oily or solid matter separates.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Label it to indicate the name and proportion of any added stabilizer.

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Phenazopyridine Hydrochloride



$C_{11}H_{11}N_5 \cdot HCl$ 249.70

2,6-Pyridinediamine, 3-(phenylazo)-, monohydrochloride; 2,6-Diamino-3-(phenylazo)pyridine monohydrochloride [136-40-3].

DEFINITION

Phenazopyridine Hydrochloride contains NLT 98.0% and NMT 102.0% of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Medium: Sulfuric acid in alcohol (1 in 360)
Sample solution: 5 µg/mL of Phenazopyridine Hydrochloride in *Medium*
Acceptance criteria: Meets the requirements

- **C.** The retention time of the phenazopyridine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **D. IDENTIFICATION TESTS—GENERAL,** *Chemical Identification Tests, Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: 20 mM ammonium acetate in water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
15	50	50
20	50	50
28	30	70
33	30	70
35	95	5
40	95	5

Diluent: Acetonitrile and water (10:90)
Standard solution: 0.03 mg/mL of USP Phenazopyridine Hydrochloride RS in *Diluent*
Sample solution: 0.03 mg/mL of Phenazopyridine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) in the portion of Phenazopyridine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenazopyridine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%
- **WATER-INSOLUBLE SUBSTANCES**

Sample: 2 g of Phenazopyridine Hydrochloride
Analysis: Dissolve the *Sample* in 200 mL of water, heat to boiling, and then heat in a covered container on a steam bath for 1 h. Filter through a tared, fine-porosity, sintered-glass crucible, wash thoroughly with water, and dry at 105° to constant weight.
Acceptance criteria: The weight of the residue does not exceed 0.1% of the weight of Phenazopyridine Hydrochloride taken.

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, and **Diluent:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.25 µg/mL each of USP Phenazopyridine Hydrochloride RS and 2,6-diaminopyridine in *Diluent*
Standard solution: 0.0005 mg/mL of USP Phenazopyridine Hydrochloride RS and 0.001 mg/mL of 2,6-diaminopyridine in *Diluent*
Sample solution: 0.5 mg/mL of Phenazopyridine Hydrochloride in *Diluent*
Chromatographic system: Proceed as directed in the *Assay* except for the *Detector*.
Detector: UV 240 nm
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the phenazopyridine peak, *Standard solution*
Relative standard deviation: NMT 3.0% for the phenazopyridine peak, *Standard solution*
Signal-to-noise ratio: NLT 30 for the phenazopyridine peak, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 2,6-diaminopyridine in the portion of Phenazopyridine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2,6-diaminopyridine from the *Sample solution*

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r_S = peak response of 2,6-diaminopyridine from the *Standard solution*
C_S = concentration of 2,6-diaminopyridine in the *Standard solution* (mg/mL)
C_U = concentration of Phenazopyridine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Phenazopyridine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of phenazopyridine from the *Standard solution*
C_S = concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenazopyridine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,6-Diaminopyridine	0.37	0.2
Phenazopyridine	1.00	—
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 4 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Phenazopyridine Hydrochloride RS

Phenazopyridine Hydrochloride Tablets

DEFINITION
Phenazopyridine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$).

IDENTIFICATION
- **A.** The UV spectrum of the phenazopyridine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 20 mM ammonium acetate in water
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
15	50	50
20	50	50
28	30	70
33	30	70
35	95	5
40	95	5

Diluent: Acetonitrile and water (10:90)
Standard solution: 0.03 mg/mL of USP Phenazopyridine Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 0.3 mg/mL of phenazopyridine hydrochloride from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add *Diluent* equivalent to 75% of the flask volume and sonicate for 15 min. Allow the solution to cool to room temperature, dilute with *Diluent* to volume, and centrifuge.
Sample solution: Nominally equivalent to 0.03 mg/mL of phenazopyridine hydrochloride in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification A*, use a diode array detector in the range of 200–600 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenazopyridine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm
  Time: 45 min
  Standard solution: USP Phenazopyridine Hydrochloride RS in *Medium*

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Sample solution: Filter portions of the solution under test and suitably dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 422 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) dissolved by using UV absorption from the *Sample solution* in comparison with the *Standard solution*.
Tolerances: NLT 75% (*Q*) of the labeled amount of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, and **Diluent:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.25 µg/mL of USP Phenazopyridine Hydrochloride RS in *Diluent*
Standard solution: 0.001 mg/mL of USP Phenazopyridine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of phenazopyridine hydrochloride from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add *Diluent* equivalent to 60% of the flask volume and sonicate for 15 min. Allow the solution to cool to room temperature and dilute with *Diluent* to volume. Centrifuge the solution and dilute the supernatant with *Diluent* to obtain 0.5 mg/mL of phenazopyridine hydrochloride.
Chromatographic system: Proceed as directed in the *Assay* except for the *Detector*.
Detector: UV 240 nm
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the phenazopyridine peak, *Standard solution*
Relative standard deviation: NMT 3.0% for the phenazopyridine peak, *Standard solution*
Signal-to-noise ratio: NLT 30 for the phenazopyridine peak, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual unspecified impurity in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of phenazopyridine from the *Standard solution*
C_S = concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenazopyridine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

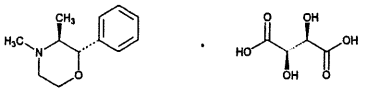
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,6-Diaminopyridine[a]	0.37	—
Phenazopyridine	1.00	—
Individual unspecified impurity	—	0.2
Total impurities	—	2.0

[a] For identification only. These are process impurities monitored in the drug substance and are not included in the total impurities.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Phenazopyridine Hydrochloride RS

Phendimetrazine Tartrate



$C_{12}H_{17}NO \cdot C_4H_6O_6$ 341.36
Morpholine, 3,4-dimethyl-2-phenyl-, (2*S*-*trans*)-, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1);
(2*S*,3*S*)-3,4-Dimethyl-2-phenylmorpholine L-(+)-tartrate (1:1) [50-58-8].

DEFINITION

Phendimetrazine Tartrate contains NLT 98.0% and NMT 102.0% of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 1 mg/mL in methanol
Acceptance criteria: Meets the requirements
• **C. IDENTIFICATION TESTS—GENERAL,** *Tartrate* ⟨191⟩

ASSAY

• **PROCEDURE**
Sample solution: Transfer an accurately weighed amount of 500 mg of Phendimetrazine Tartrate to a suitable beaker, and dissolve in 50 mL of glacial acetic acid.
Analysis: Add 1 drop of crystal violet TS to the *Sample solution*, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.14 mg of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$).
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

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• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample: 1.0 g
Acceptance criteria: 0.035%; the *Sample* shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid.
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample: 1.0 g
Acceptance criteria: 0.01%; the *Sample* shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid.
• **ORGANIC IMPURITIES**
Standard solution: An aqueous solution containing 100 mg/mL of USP Phendimetrazine Tartrate RS
Sample solution: 100 mg/mL of Phendimetrazine Tartrate in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Acetone, methanol, and ammonium hydroxide (50:50:1)
Analysis
Develop the chromatogram in a suitable chamber with the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, view under short-wavelength UV light, and observe the location of the spots. Expose the plate to iodine vapors in a closed chamber.
Acceptance criteria: Yellow spots appear at the same locations as the spots observed under UV light, and the R_F value of the spot of the *Sample solution* corresponds to that of the *Standard solution*, and no other spot is obtained.
• **L-*Erythro* Isomer**
Sample solution: Dissolve 3.0 g of Phendimetrazine Tartrate in 25 mL of sodium hydroxide solution (1 in 20) in a suitable separator. Add 25 mL of sodium hydroxide solution (1 in 2), swirl, and allow the phendimetrazine base to separate. Discard the lower, alkaline layer, and collect the upper layer, centrifuging, if necessary, to obtain a clear liquid.
Chromatographic system
Mode: GC
Detector: Flame ionization
Column: 25-m × 0.25-mm capillary column, the inside wall of which is coated with a 0.4-µm film of liquid phase G1
Carrier gas: Helium
Temperatures
Injection port: 250°
Column: 140°
Detector: 280°
Injection volume: 1.0 µL
Injection type: Split ratio, 100:1
Analysis
Sample: *Sample solution*
[NOTE—The retention times for the D-*threo* isomer and the L-*erythro* isomer are about 8.5 and 9 min, respectively.]
Preferably using an electronic integrator, determine the areas of all peaks in the chromatogram.
Calculate the percentage of the L-*erythro* isomer in the *Sample solution* taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of the L-*erythro* isomer peak
r_T = sum of the areas of the L-*erythro* isomer peak and the D-*threo* isomer peak

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 182°–188°, with decomposition, but the range between beginning and end of melting does not exceed 3°.
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 100 mg/mL in water
Acceptance criteria: +32° to +36°
• **pH** ⟨791⟩**:** 3.0–4.0, in a solution (1 in 40)
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry to constant weight at 105°.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Phendimetrazine Tartrate RS

Phendimetrazine Tartrate Capsules

DEFINITION
Phendimetrazine Tartrate Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$).

IDENTIFICATION
• **A.**
Analysis: Shake a quantity of Capsule contents, nominally equivalent to 300 mg of phendimetrazine tartrate, with 50 mL of water. Filter, and transfer the filtrate to a 200-mL separator. Add 3 mL of 12.5 N sodium hydroxide, and extract with two 50-mL portions of chloroform. Extract the combined chloroform extracts in a 250-mL separator with two 15-mL portions of 0.5 N hydrochloric acid, and evaporate the combined aqueous extracts on a steam bath to dryness. Dissolve the residue in 5 mL of acetone, and add 50 mL of anhydrous ether to the solution. On standing, phendimetrazine hydrochloride crystallizes out. Filter the precipitate, wash with anhydrous ether, and dry at 105°.
Acceptance criteria: The phendimetrazine hydrochloride crystals so obtained melt at 189°–193°, but the range between the beginning and end of melting does not exceed 2°.
• **B. IDENTIFICATION TESTS—GENERAL,** *Tartrate* ⟨191⟩

ASSAY
• **PROCEDURE**
Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 575 mL of water, add 400 mL of methanol and 25 mL of dilute acetic acid (14 in 100), and mix. Adjust with glacial acetic acid to a pH of 3.0 ± 0.1, if necessary. Pass through a membrane filter of 0.45-µm pore size, and degas.
Diluent: Dilute acetic acid (14 in 100), methanol, and water (2.5: 40: 57.5)
Internal standard solution: 0.1 mg/mL of salicylamide in *Diluent*
Standard solution: 0.7 mg/mL of USP Phendimetrazine Tartrate RS in *Internal standard solution*
Sample solution: Remove, as completely as possible, the contents of NLT 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to 35 mg of phendimetrazine tartrate, to a 50-mL volumetric flask. Add 25 mL of *Internal standard solution*, and sonicate for 15 min. Cool the solution to room temperature, dilute with *Internal standard solution* to volume, mix, and filter through a membrane filter of 0.45-µm pore size.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 256 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for salicylamide and phendimetrazine tartrate are 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the analyte and internal standard peaks
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of phendimetrazine tartrate to the internal standard from the *Sample solution*
R_S = peak response ratio of phendimetrazine tartrate to the internal standard from the *Standard solution*
C_S = concentration of USP Phendimetrazine Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phendimetrazine tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Solution A: 0.025 M monobasic potassium phosphate solution. Adjust with 1 N potassium hydroxide to a pH of 7.5.
Mobile phase: Acetonitrile and *Solution A* (65:35). Filter, and degas.
Standard solution: A known concentration of USP Phendimetrazine Tartrate RS, similarly prepared as the *Sample solution*
Sample solution: Filter a portion of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 15-cm; packing L15
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0% from three replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) dissolved by comparison of the responses of the major peaks obtained from the *Sample solution* and the *Standard solution*.
Tolerances: NLT 70% (*Q*) of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Phendimetrazine Tartrate RS

Phendimetrazine Tartrate Tablets

DEFINITION
Phendimetrazine Tartrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$).

IDENTIFICATION
• **A.**
Analysis: Shake a quantity of finely powdered Tablets, nominally equivalent to 300 mg of phendimetrazine tartrate, with 50 mL of water. Filter, and transfer the filtrate to a 200-mL separator. Add 3 mL of 12.5 N sodium hydroxide, and extract with two 50-mL portions of chloroform. Extract the combined chloroform extracts in a 250-mL separator with two 15-mL portions of 0.5 N hydrochloric acid, and evaporate the combined aqueous extracts on a steam bath to dryness. Dissolve the residue in 5 mL of acetone, and add 50 mL of anhydrous ether to the solution. On standing, phendimetrazine hydrochloride crystallizes out. Filter the precipitate, wash with anhydrous ether, and dry at 105°.
Acceptance criteria: The phendimetrazine hydrochloride crystals so obtained melt at 189°–193°, but the range between the beginning and end of melting does not exceed 2°.
• **B. IDENTIFICATION TESTS—GENERAL,** *Tartrate* ⟨191⟩

ASSAY
• **PROCEDURE**
Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 575 mL of water, add 400 mL of methanol and 25 mL of dilute acetic acid (14 in 100), and mix. Adjust with glacial acetic acid to a pH of 3.0 ± 0.1, if necessary. Pass through a membrane filter of 0.45-µm pore size, and degas.
Diluent: Dilute acetic acid (14 in 100), methanol, and water (2.5: 40: 57.5)
Internal standard solution: 0.1 mg/mL of salicylamide in *Diluent*
Standard solution: 0.7 mg/mL of USP Phendimetrazine Tartrate RS in *Internal standard solution*
Sample solution: Transfer a portion of finely powdered Tablets from NLT 20 Tablets, nominally equivalent to 35 mg of phendimetrazine tartrate, to a 50-mL volumetric flask. Add 25 mL of *Internal standard solution*, and sonicate for 15 min. Cool the solution to room temperature, dilute with *Internal standard solution* to volume, mix, and pass through a membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 256 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*

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[NOTE—The relative retention times for salicylamide and phendimetrazine tartrate are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of phendimetrazine tartrate to the internal standard from the *Sample solution*

R_S = peak response ratio of phendimetrazine tartrate to the internal standard from the *Standard solution*

C_S = concentration of USP Phendimetrazine Tartrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phendimetrazine tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Solution A: 0.025 M monobasic potassium phosphate solution. Adjust with 1 N potassium hydroxide to a pH of 7.5.

Mobile phase: Acetonitrile and *Solution A* (65:35). Filter and degas.

Standard solution: USP Phendimetrazine Tartrate RS, similarly prepared as the *Sample solution*

Sample solution: Filter a portion of the solution under test.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 15-cm; packing L15

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0% from three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) dissolved in comparison with the *Standard solution*.

Tolerances: NLT 70% (*Q*) of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

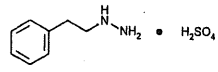
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Phendimetrazine Tartrate RS

Phenelzine Sulfate



$C_8H_{12}N_2 \cdot H_2SO_4$ 234.27

Hydrazine, (2-phenylethyl)-, sulfate (1:1).

Phenethylhydrazine sulfate (1:1). [156-51-4].

» Phenelzine Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $C_8H_{12}N_2 \cdot H_2SO_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, protected from heat and light.

USP Reference standards ⟨11⟩—

USP Phenelzine Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Dissolve 100 mg in 5 mL of water, render the solution alkaline with 1 N sodium hydroxide, and add 1 mL of alkaline cupric tartrate TS: a red to yellow-red precipitate is formed.

C: A solution (1 in 10) meets the requirements of the tests for *Sulfate* ⟨191⟩.

Melting range ⟨741⟩**:** between 164° and 168°.

pH ⟨791⟩**:** between 1.4 and 1.9, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it at a pressure not exceeding 5 mm of mercury over silica gel at 80° for 2 hours: it loses not more than 1.0% of its weight.

Limit of hydrazine—

Mobile phase—Prepare a filtered and degassed mixture of methanol and 1% monobasic ammonium phosphate solution (75:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Transfer an accurately weighed quantity of about 42.0 mg of hydrazine sulfate, equivalent to about 10 mg of hydrazine, to a 100-mL volumetric flask. Dissolve in water, sonicate for about 5 minutes, dilute with methanol to volume, and mix. Dilute an accurately measured volume of this solution quantitatively and stepwise with methanol to obtain a solution having a known concentration of 5.0 µg of hydrazine per mL. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, add 50 mL of methanol and 0.7 mL of ammonium hydroxide, and shake to mix. Add 0.5 mL of salicylaldehyde, shake by mechanical means for about 5 minutes, dilute with methanol to volume, sonicate for about 2 minutes, and mix.

Test solution—Transfer about 25.8 mg of Phenelzine Sulfate, accurately weighed, to a 200-mL volumetric flask, dissolve in about 50 mL of methanol, and sonicate. Add 0.7 mL of ammonium hydroxide, shake to mix, add 0.5 mL of salicylaldehyde, shake by mechanical means for about 5 minutes, dilute with methanol to volume, sonicate for about 2 minutes, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 340-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure:* the relative retention times for salicylaldehyde, phenelzine sulfate derivative, and hydrazine sulfate derivative are about 0.21, 0.47, and 1.0, respectively;

USP Monographs

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the column efficiency determined from the analyte peak is not less than 4500 theoretical plates; the resolution, *R*, between phenelzine and salazine (derivatized hydrazine) is not less than 1.25; and the relative standard deviation for replicate injections is not more than 7%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas for salazine (derivatized hydrazine). Calculate the percentage of hydrazine in the portion of Phenelzine Sulfate taken by the formula:

$$20(C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of hydrazine base in the *Standard solution*; W is the weight, in mg, of Phenelzine Sulfate taken to prepare the *Test solution*; and r_U and r_S are the salazine peak areas obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% is found.

Ordinary impurities ⟨466⟩—

Test solution: a mixture of methanol and water (1:1).

Standard solution: a mixture of methanol and water (1:1).

Eluant: acetone. [NOTE—Prewash the plate with *Eluant*, and dry.]

Visualization: 1.

Assay—

Ion-pair solution, Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Phenelzine Sulfate Tablets.*

Assay preparation—Transfer about 26 mg of Phenelzine Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of $C_8H_{12}N_2 \cdot H_2SO_4$ in the portion of Phenelzine Sulfate taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Phenelzine Sulfate RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenelzine Sulfate Tablets

» Phenelzine Sulfate Tablets contain an amount of phenelzine sulfate ($C_8H_{12}N_2 \cdot H_2SO_4$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of phenelzine ($C_8H_{12}N_2$).

Packaging and storage—Preserve in tight containers, protected from heat and light.

USP Reference standards ⟨11⟩—

USP Phenelzine Sulfate RS

Identification—Extract a portion of powdered Tablets, equivalent to about 30 mg of phenelzine, with 10 mL of water, and filter: the filtrate responds to *Identification* tests *B* and *C* under *Phenelzine Sulfate.*

Disintegration ⟨701⟩—Place 1 tablet in each of the 6 tubes of the basket and, if the tablets have a soluble external coating, immerse the basket in water at room temperature for 5 minutes. Then add a disk to each tube, and operate the apparatus, using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 30 minutes of operation in simulated gastric fluid TS, lift the basket from the fluid, and observe the tablets. If the tablets have not disintegrated completely, substitute simulated intestinal fluid TS maintained at 37 ± 2° as the immersion fluid. Continue the test for a total period of time, including previous exposure to water and simulated gastric fluid TS, of 1 hour and 30 minutes. Lift the basket from the fluid, and observe the tablets: all of the tablets have disintegrated completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not less than 16 of the total 18 tablets tested disintegrate completely.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Ion-pair solution—Dissolve about 6.8 g of monobasic potassium phosphate and about 2.16 g of sodium 1-octanesulfonate in 1000 mL of water, and mix. Adjust with phosphoric acid to a pH of 3.0, and filter.

Mobile phase—Prepare a filtered and degassed mixture of *Ion-pair solution* and methanol (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Phenelzine Sulfate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 258 µg per mL.

Assay preparation—Transfer not less than 20 Tablets to a suitable container, add about 300 mL of *Mobile phase*, and homogenize until dissolved. Transfer this solution to a 500-mL volumetric flask, dilute with *Mobile phase* to volume, mix, centrifuge, and filter, discarding the first 5 mL of the filtrate. Transfer a portion of the filtrate, equivalent to about 12.9 mg of phenelzine sulfate, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of phenelzine ($C_8H_{12}N_2$) in the *Assay preparation* by the formula:

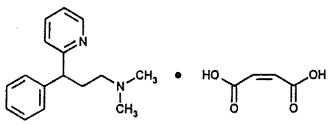
$$(136.20/234.27)(50C)(r_U/r_S)$$

in which 136.20 and 234.27 are the molecular weights of phenelzine and phenelzine sulfate, respectively, C is the concentration, in µg per mL, of USP Phenelzine Sulfate RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

USP Monographs

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Pheniramine Maleate



$C_{16}H_{20}N_2 \cdot C_4H_4O_4$ 356.42
2-[α-[2-Dimethylaminoethyl]benzyl]pyridine bimaleate.
N,N-Dimethyl-3-phenyl-3-(2-pyridyl)propylamine hydrogen maleate [132-20-7].

» Pheniramine Maleate contains not less than 98.0 percent and not more than 102.0 percent of $C_{16}H_{20}N_2 \cdot C_4H_4O_4$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Pheniramine Maleate RS

Change to read:

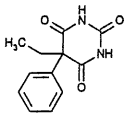
Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
Melting range, *Class I* ⟨741⟩: between 104° and 109°.
pH ⟨791⟩: between 4.5 and 5.5, in a solution (10 mg per mL).
Loss on drying ⟨731⟩—Dry it in vacuum at 65° for 6 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.5%.

Chromatographic purity—
0.005 M Octane sulfonic acid—Transfer 1.08 g of sodium 1-octane sulfonate to a 1-liter volumetric flask. Dilute with 1.5% (v/v) acetic acid solution to volume, add 5.0 mL of triethylamine, mix, and filter.
Mobile phase—Prepare a filtered and degassed mixture of *0.005 M Octane sulfonic acid* and acetonitrile (39:11). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve suitable quantities of phenylethyl alcohol and USP Pheniramine Maleate RS in water to obtain a solution containing about 3.6 and 0.24 mg per mL, respectively.
Test solution—Transfer about 24 mg of Pheniramine Maleate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 265-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 phenylethyl alcohol and 1.0 for pheniramine maleate, and the resolution, *R,* between phenylethyl alcohol and pheniramine maleate is not less than 2.0, the tailing factor is not more than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity (not including the solvent peak and maleic acid, if observed) in the portion of Pheniramine Maleate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity, and r_s is the sum of the responses of all of the peaks: not more than 0.5% of any individual impurity is found, and not more than 2.0% of total impurities is found.
Assay—Dissolve about 500 mg of Pheniramine Maleate, accurately weighed, in 25 mL of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 17.82 mg of $C_{16}H_{20}N_2 \cdot C_4H_4O_4$.

Phenobarbital



$C_{12}H_{12}N_2O_3$ 232.24
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-phenyl-.
5-Ethyl-5-phenylbarbituric acid [50-06-6].

» Phenobarbital contains not less than 98.0 percent and not more than 101.0 percent of $C_{12}H_{12}N_2O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Phenobarbital RS

Identification—
A: The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phenobarbital RS. If a difference appears, dissolve portions of both the test specimen and the USP Reference Standard in a suitable solvent, evaporate the solutions to dryness, and repeat the test on the residues.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay.*
Melting range ⟨741⟩: between 174° and 178°, but the range between beginning and end of melting does not exceed 2°.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.15%.

Assay—
pH 4.5 Buffer solution—Dissolve about 6.6 g of sodium acetate trihydrate and 3.0 mL of glacial acetic acid in 1000 mL of water, and adjust, if necessary, with glacial acetic acid to a pH of 4.5 ± 0.1.
Mobile phase—Prepare a filtered and degassed mixture of *pH 4.5 Buffer solution* and methanol (3:2), making adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Dissolve a sufficient quantity of caffeine in a mixture of methanol and *pH 4.5 Buffer solution* (1:1) to obtain a solution having a concentration of about 125 µg per mL.
Standard preparation—Dissolve about 20 mg of USP Phenobarbital RS, accurately weighed, in 15.0 mL of *Internal standard solution.* Sonicate if necessary.

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Assay preparation—Transfer about 20 mg of Phenobarbital, accurately weighed, to a conical flask, add 15.0 mL of *Internal standard solution*, mix, and sonicate for 15 minutes. Filter through a membrane filter (0.5 µm or finer porosity) before use.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R,* between the analyte and the internal standard peaks is not less than 1.2, the tailing factor for the analyte and the internal standard peaks is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for caffeine and 1.0 for phenobarbital. Calculate the quantity, in mg, of $C_{12}H_{12}N_2O_3$ in the portion of Phenobarbital taken by the formula:

$$W(R_U/R_S)$$

in which W is the weight, in mg, of USP Phenobarbital RS taken for the *Standard preparation*, and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenobarbital Oral Solution

» Phenobarbital Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Phenobarbital RS

Identification—

A: Place 10 mL of Oral Solution in a separator containing 20 mL of water, add 5 mL of 1 N sodium hydroxide, and extract with two 10-mL portions of chloroform, discarding the chloroform extracts. Add 5 mL of 3 N hydrochloric acid, and extract with two 25-mL portions of chloroform, filtering the extracts through paper into a beaker. Remove the chloroform by evaporation on a steam bath, and dry the residue at 105° for 2 hours: the residue so obtained meets the requirements for *Identification* test *A* under *Phenobarbital.*

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay.*

Alcohol Determination, *Method II* ⟨611⟩: between 12.0% and 15.0% of C_2H_5OH.

Assay—

pH 4.5 Buffer solution, Mobile phase, and *Chromatographic system*—Prepare as directed in the *Assay* under *Phenobarbital.*

Diluent—Prepare a mixture of methanol and *pH 4.5 Buffer solution* (2:1).

Internal standard solution—Dissolve a sufficient quantity of caffeine in *Diluent* to obtain a solution having a concentration of about 1.7 mg per mL.

Standard preparation—Transfer about 33 mg of USP Phenobarbital RS, accurately weighed, to a 25-mL volumetric flask containing 2.0 mL of *Internal standard solution.* Dilute with *Diluent* to volume, and mix.

Assay preparation—Transfer a quantity of Oral Solution, equivalent to about 33 mg of phenobarbital, to a 25-mL volumetric flask containing 2.0 mL of *Internal standard solution.* Dilute with *Diluent* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenobarbital.* Calculate the quantity, in mg, of phenobarbital ($C_{12}H_{12}N_2O_3$) in the portion of the Oral Solution taken by the formula:

$$W(R_U/R_S)$$

in which the terms are as defined therein.

Phenobarbital Compounded Oral Suspension

DEFINITION

Phenobarbital Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$).

Prepare Phenobarbital Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Phenobarbital tablets[a] equivalent to	1.2 g of phenobarbital
Vehicle: a 1:1 mixture of Ora-Sweet[b] (regular or sugar-free) and Ora-Plus,[b] a sufficient quantity to make	120 mL

[a] Phenobarbital 60-mg tablets, Excellium Pharmaceutical, Inc., Fairfield, NJ.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Phenobarbital tablets* in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a phenobarbital liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (30:70). Adjust with dilute sulfuric acid to a pH of 3.0.

Standard stock solution: 0.4 mg/mL of USP Phenobarbital RS in *Mobile phase*

Standard solution: 20 µg/mL of phenobarbital prepared from *Standard stock solution* and *Mobile phase*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 20 µg/mL of phenobarbital from Oral Suspension and *Mobile phase,* and centrifuge.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1

USP Monographs

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Column temperature: 60°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for phenobarbital is about 6.8 min.]
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenobarbital RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of phenobarbital in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 115 days after the date on which it was compounded, when stored at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Phenobarbital RS

Phenobarbital Tablets

» Phenobarbital Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{12}H_{12}N_2O_3$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Phenobarbital RS

Identification—
A: Triturate a quantity of finely powdered Tablets, equivalent to about 60 mg of phenobarbital, with 50 mL of chloroform, and filter. Evaporate the clear filtrate to dryness, and dry at 105° for 2 hours: the residue so obtained responds to *Identification* test *A* under *Phenobarbital.*
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{12}H_{12}N_2O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 240 nm on filtered portions of the solution under test, suitably diluted with pH 9.6 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), in comparison with a Standard solution having a known concentration of USP Phenobarbital RS in the same *Medium.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{12}H_{12}N_2O_3$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
pH 4.5 Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Phenobarbital.*
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of phenobarbital, add 15.0 mL of *Internal standard solution,* mix, and sonicate for 15 minutes. Filter through a membrane filter having a 0.5-µm or finer porosity before use.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenobarbital.* Calculate the quantity, in mg, of $C_{12}H_{12}N_2O_3$ in the portion of Tablets taken by the formula:

$$(W)(R_U/R_S)$$

in which the terms are as defined therein.

Phenobarbital Sodium

$C_{12}H_{11}N_2NaO_3$ 254.22
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-phenyl-, monosodium salt.
Sodium 5-ethyl-5-phenylbarbiturate [57-30-7].

» Phenobarbital Sodium contains not less than 98.5 percent and not more than 101.0 percent of $C_{12}H_{11}N_2NaO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Phenobarbital RS
Completeness of solution—Mix 1.0 g with 10 mL of carbon dioxide-free water: after 1 minute, the solution is clear and free from undissolved solid.

Identification—
A: Dissolve about 50 mg of Phenobarbital Sodium in 15 mL of water in a separator, add 2 mL of hydrochloric acid, shake, and extract the liberated phenobarbital with four 25-mL portions of chloroform. Filter the combined extracts through a pledget of cotton or other suitable filter into a beaker, and wash the separator and the filter with several small portions of chloroform. Evaporate a 50-mL portion of the chloroform solution of phenobarbital on a steam bath with the aid of a current of air. Add 10 mL of ether, again evaporate, and dry the residue at 105° for 2 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phenobarbital RS.

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B: Ignite about 200 mg: the residue effervesces with acids, and responds to the tests for *Sodium* ⟨191⟩.

C: The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

pH ⟨791⟩: between 9.2 and 10.2, in the solution prepared in the test for *Completeness of solution.*

Loss on drying ⟨731⟩—Dry it at 150° for 4 hours: it loses not more than 7.0% of its weight.

Other requirements—Where the label states that Phenobarbital Sodium is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and for *Bacterial endotoxins* under *Phenobarbital Sodium for Injection.* Where the label states that Phenobarbital Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Phenobarbital Sodium for Injection.*

Assay—

pH 4.5 Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Phenobarbital.*

Assay preparation—Transfer about 22 mg of Phenobarbital Sodium, accurately weighed, to a conical flask, add 15.0 mL of *Internal standard solution,* mix, and sonicate for 15 minutes. Pass through a membrane filter having a 0.5-µm or finer porosity before use.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenobarbital.* Calculate the quantity, in mg, of $C_{12}H_{11}N_2NaO_3$ in the portion of Phenobarbital Sodium taken by the formula:

$$(254.22/232.24)(W)(R_U/R_S)$$

in which 254.22 and 232.24 are the molecular weights of phenobarbital sodium and phenobarbital, respectively; and the other terms are as defined therein.

Phenobarbital Sodium Injection

» Phenobarbital Sodium Injection is a sterile solution of Phenobarbital Sodium in a suitable solvent. Phenobarbital may be substituted for the equivalent amount of Phenobarbital Sodium, for adjustment of the pH. The Injection contains the equivalent of not less than 90.0 percent and not more than 105.0 percent of the labeled amount of $C_{12}H_{11}N_2NaO_3$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

Labeling—The label indicates that the Injection is not to be used if it contains a precipitate.

USP Reference standards ⟨11⟩—
USP Phenobarbital RS

Identification—

A: Transfer to a separator a volume of Injection, equivalent to about 50 mg of phenobarbital sodium, add 15 mL of water, add 2 mL of hydrochloric acid, shake, and extract the liberated phenobarbital with four 25-mL portions of chloroform. Filter the combined extracts through a pledget of cotton or other suitable filter into a beaker, and wash the separator and the filter with several small portions of chloroform. Evaporate a 50-mL portion of the chloroform solution of phenobarbital on a steam bath with the aid of a current of air. Add 10 mL of ether, again evaporate, and dry the residue at 105° for 2 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phenobarbital RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.3 USP Endotoxin Unit per mg of phenobarbital sodium.

pH ⟨791⟩: between 9.2 and 10.2.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

pH 4.5 Buffer solution, Mobile phase, Internal standard solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Phenobarbital.*

Standard preparation—Transfer about 15 mg of USP Phenobarbital RS, accurately weighed, to a 50-mL volumetric flask, add 25 mL of *Mobile phase,* and sonicate if necessary to dissolve. Add 15.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.3 mg of USP Phenobarbital RS per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 65 mg of phenobarbital sodium, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, add 15.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenobarbital.* Calculate the quantity, in mg, of $C_{12}H_{11}N_2NaO_3$ in each mL of the Injection taken by the formula:

$$(254.22/232.24)(4W/V)(R_U/R_S)$$

in which 254.22 and 232.24 are the molecular weights of phenobarbital sodium and phenobarbital, respectively, V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Phenobarbital Sodium for Injection

» Phenobarbital Sodium for Injection is Phenobarbital Sodium suitable for parenteral use.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

USP Reference standards ⟨11⟩—
USP Phenobarbital RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.8 USP Endotoxin Unit per mg of phenobarbital sodium.

Other requirements—It conforms to the Definition, responds to the *Identification* tests, and meets the requirements for *Completeness of solution, pH, Loss on drying,* and *Assay* under *Phenobarbital Sodium.* It meets also the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units*

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⟨905⟩, and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Phenol



C_6H_6O 94.11
Phenol [108-95-2].

DEFINITION
Phenol contains NLT 99.0% and NMT 100.5% of phenol (C_6H_6O), calculated on the anhydrous basis. It may contain a suitable stabilizer.

IDENTIFICATION
[**CAUTION**—Avoid contact with skin because serious burns may result.]
- **A.**

Analysis: To a solution add bromine TS.
Acceptance criteria: A white precipitate is formed, and it dissolves at first but becomes permanent as more of the reagent is added.
- **B.**

Sample solution: 1 in 100
Analysis: To 10 mL of the *Sample solution* add 1 drop of ferric chloride TS.
Acceptance criteria: A violet color is produced.

ASSAY
- **PROCEDURE**

Sample: 2 g
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.1 N bromine VS
Back-titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Place the *Sample* in a 1000-mL volumetric flask, and dilute with water to volume. Pipet 20 mL of the solution into an iodine flask, add 30.0 mL of *Titrant*, then add 5 mL of hydrochloric acid, and immediately insert the stopper. Shake the flask repeatedly during 30 min, allow it to stand for 15 min, quickly add 5 mL of potassium iodide solution (1 in 5), taking precautions against the escape of bromine vapor, and at once insert the stopper. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask with a small quantity of water so that the washing flows into the flask. Add 1 mL of chloroform, and shake the mixture. Titrate the liberated iodine with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination. Each mL of 0.1 N bromine is equivalent to 1.569 mg of phenol (C_6H_6O).
Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES
- **LIMIT OF NONVOLATILE RESIDUE**

Sample: 5 g
Analysis: Heat the *Sample* in a tared porcelain dish on a steam bath until it has evaporated, and dry the residue at 105° for 1 h.
Acceptance criteria: NMT 0.05%

SPECIFIC TESTS
- **CLARITY OF SOLUTION AND REACTION**

Sample solution: 1 in 15
Acceptance criteria: The *Sample solution* is clear, and it is neutral or acid to litmus paper.
- **CONGEALING TEMPERATURE** ⟨651⟩**:** NLT 39°
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label it to indicate the name and amount of any substance added as a stabilizer.

Camphorated Phenol Topical Gel

DEFINITION
Camphorated Phenol Topical Gel is a mixture of Camphor and Phenol in a suitable gel vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of camphor ($C_{10}H_{16}O$) and phenol (C_6H_6O).

IDENTIFICATION
- **A.** The retention times of the camphor and phenol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Internal standard solution: 10 mg/mL of *n*-dodecane in chloroform
Standard stock solution: 9.6 mg/mL of USP Phenol RS and 22.4 mg/mL of USP Camphor RS, in chloroform
Standard solution: 0.96 mg/mL of USP Phenol RS, 2.24 mg/mL of USP Camphor RS, and 1 mg/mL of *n*-docecane in chloroform from *Standard stock solution* and *Internal standard solution*
Sample solution: Transfer 1 g of Topical Gel to a 50-mL flask. Add 5.0 mL of *Internal standard solution*, and dilute with chloroform to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m glass; packed with 100- to 120-mesh S1A, coated with 15% G44
Carrier gas: Helium
Temperatures
Detector: 200°
Column: 140°
Injection volume: 1–2 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times are 0.3 for phenol, 0.8 for camphor, and 1.0 for the internal standard.]
Suitability requirements
Resolution: NLT 2.0 between camphor and the internal standard, and NLT 5.0 between phenol and camphor
Relative standard deviation: NMT 2.0% of the peak response ratio of camphor and phenol to the internal standard for five consecutive injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of camphor (w/w) and phenol (w/w) in the portion of Topical Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the corresponding analyte to the internal standard from the *Sample solution*

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R_S = peak response ratio of the corresponding analyte to the internal standard from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the appropriate analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature, avoid excessive heat, and close the cover after each use.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Camphor RS
  USP Phenol RS

Camphorated Phenol Topical Solution

DEFINITION
Camphorated Phenol Topical Solution is a solution of Camphor and Phenol in eucalyptus oil and Light Mineral Oil. It contains NLT 90.0% and NMT 110.0% of the labeled amount of camphor ($C_{10}H_{16}O$) and phenol (C_6H_6O).

IDENTIFICATION
- **A.** The retention times of the camphor and phenol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Internal standard solution: 10 mg/mL of *n*-dodecane in chloroform
Standard stock solution: 9.6 mg/mL of USP Phenol RS and 22.4 mg/mL of USP Camphor RS, in chloroform
Standard solution: 0.96 mg/mL of USP Phenol RS, 2.24 mg/mL of of USP Camphor RS, and 1 mg/mL of *n*-dodecane in chloroform from the *Standard stock solution* and *Internal standard solution*
Sample solution: Transfer 1 g of Topical Solution to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with chloroform to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m glass; packed with 100- to 120-mesh S1A, coated with 15% G44
Carrier gas: Helium
Temperatures
Detector: 200°
Column: 140°
Injection volume: 1–2 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times are 0.3 for phenol, 0.8 for camphor, and 1.0 for the internal standard.]
Suitability requirements
Resolution: NLT 2.0 between camphor and the internal standard, and NLT 5.0 between phenol and camphor
Relative standard deviation: NMT 2.0% of the peak response ratio of camphor and phenol to the internal standard for five consecutive injections
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of camphor (w/w) and phenol (w/w) in the portion of Topical Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the corresponding analyte to the internal standard from the *Sample solution*
R_S = peak response ratio of the corresponding analyte to the internal standard from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the appropriate analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨841⟩: 0.840–0.865

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature, avoid excessive heat, and close the cover after each use.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Camphor RS
  USP Phenol RS

Liquefied Phenol

DEFINITION
Liquefied Phenol is Phenol maintained in a liquid condition by the presence of about 10% of water. It contains NLT 89.0% by weight of C_6H_6O. It may contain a suitable stabilizer.
[CAUTION—Avoid contact with skin because serious burns may result.]
[NOTE—When phenol is to be mixed with a fixed oil, mineral oil, or white petrolatum, use crystalline Phenol, not Liquefied Phenol.]

IDENTIFICATION
- **A.** To a solution add bromine TS: a white precipitate is formed, and it dissolves at first but becomes permanent as more of the reagent is added.
- **B.** To 10 mL of a solution (1 in 100) add 1 drop of ferric chloride TS: a violet color is produced.

ASSAY
- **PROCEDURE**

Sample solution: Place 2 g of Liquefied Phenol in a 1000-mL volumetric flask, and dilute with water to volume.
Analysis: Pipet 20 mL of the *Sample solution* into an iodine flask, add 30.0 mL of 0.1 N bromine VS, then add 5 mL of hydrochloric acid, and immediately insert the stopper. Shake the flask repeatedly during 30 min, allow it to stand for 15 min, add quickly 5 mL of potassium iodide solution (1 in 5), taking precautions against the escape of bromine vapor, and at once insert the stopper in the flask. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask with a small quantity of water so that the washing flows into the flask. Add 1 mL of chloroform, and shake the mixture.
Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination (see *Titrimetry* ⟨541⟩, *Residual Titrations*). Each mL of 0.1 N bromine is equivalent to 1.569 mg of C_6H_6O.

USP Monographs

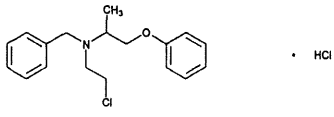
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Acceptance criteria: NLT 89.0% by weight of C_6H_6O

SPECIFIC TESTS
- **CLARITY OF SOLUTION AND REACTION:** A solution (1 in 15) is clear, and is neutral or acid to litmus paper.
- **LIMIT OF NONVOLATILE RESIDUE:** Heat 5 g in a tared porcelain dish on a steam bath until it has evaporated, and dry the residue at 105° for 1 h: NMT 0.05% of residue remains.
- **DISTILLING RANGE,** *Method I* ⟨721⟩**:** NMT 182.5°, an air-cooled condenser being used

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant glass containers.
- **LABELING:** Label it to indicate the name and amount of any substance added as a stabilizer.

Phenoxybenzamine Hydrochloride



$C_{18}H_{22}ClNO \cdot HCl$ 340.29

Benzenemethanamine, *N*-(2-chloroethyl)-*N*-(1-methyl-2-phenoxyethyl)-, hydrochloride;
N-(2-Chloroethyl)-*N*-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride [63-92-3].

DEFINITION
Phenoxybenzamine Hydrochloride contains NLT 98.0% and NMT 101.0% of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 1 mg/mL of Phenoxybenzamine Hydrochloride in methanol
Analytical wavelength: 275 nm
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY
- **PROCEDURE**

Sample: 500 mg of Phenoxybenzamine Hydrochloride
Mode: Titrimetry
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid, add 15 mL of mercuric acetate TS, and titrate with *Titrant*, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.03 mg of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$).
Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES
- **ORDINARY IMPURITIES** ⟨466⟩

Standard solution: Methanol
Sample solution: Methanol
Eluant: Toluene and acetone (1:1)
Visualization: 1
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩**:** 136°–141°
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample under vacuum at 60° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Phenoxybenzamine Hydrochloride RS

Phenoxybenzamine Hydrochloride Capsules

DEFINITION
Phenoxybenzamine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$).

IDENTIFICATION
- **A.** The UV absorption spectra of the phenoxybenzamine peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak from the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 2.2 mg/mL of anhydrous monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Filtered and degassed mixture of *Solution A* and acetonitrile (45:55)
Standard solution: 0.2 mg/mL of USP Phenoxybenzamine Hydrochloride RS in acetonitrile. [NOTE—Sonicate if necessary.]
System suitability solution: 10 mL of the *Standard solution* and 0.5 mL of 0.1 N sodium hydroxide taken in a vial. [NOTE—Basic solutions of phenoxybenzamine hydrochloride will produce the known degradant, tertiary amine phenoxybenzamine—the second major peak that elutes before the phenoxybenzamine peak and has a relative retention time of about 0.3 and an unknown related substance. Severe degradation of the drug substance will be observed if the solution is allowed to stand for more than 1 h.]
Sample solution: Nominally 0.2 mg/mL of phenoxybenzamine hydrochloride in acetonitrile prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Transfer a portion of the mixed powder, equivalent to about 10 mg of phenoxybenzamine hydrochloride, to a 50-mL volumetric flask. Add about 40 mL of acetonitrile, and sonicate for 15 min with occasional swirling. Cool, and dilute with acetonitrile to volume to obtain the concentration, based on the label claim. Allow the sample to stand undisturbed for 30 min such that the undissolved material settles to the bottom.

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Transfer the top clear solution into HPLC vials, and use as the *Sample solution.*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector
Assay: UV 268 nm
Identification A: Diode array, UV 240–340 nm
Column: 4.6-mm × 150-cm; 5 µm packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 4 between phenoxybenzamine and the unknown peak eluting after the phenoxybenzamine peak (at about 9.4 min), *System suitability solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenoxybenzamine from the *Sample solution*
r_S = peak response of phenoxybenzamine from the *Standard solution*
C_S = concentration of USP Phenoxybenzamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenoxybenzamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 1: 100 rpm
Time: 45 min
Buffer: 2.2 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05.
Mobile phase: *Buffer* and acetonitrile (9:11)
Standard solution: 0.02 mg/mL of USP Phenoxybenzamine Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 268 nm
Column: 4.6-mm × 150-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2%
Calculate the percentage of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of phenoxybenzamine from the *Sample solution*
r_S = peak response of phenoxybenzamine from the *Standard solution*
C_S = concentration of USP Phenoxybenzamine Hydrochloride RS from the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 500 mL

Tolerances: NLT 75% (Q) of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*
Analysis
Sample: *Sample solution*
Calculate the percentage of each degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1.*

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria (NMT %)
Phenoxybenzamine tertiary amine[a]	0.3	1.1	1.5
Phenoxybenzamine	1.0	—	—
Any unspecified degradation product	—	1.0	0.2
Total degradation products[b]	—	—	2.0

[a] 2-[Benzyl(1-phenoxypropan-2-yl)amino]ethan-1-ol.
[b] Includes specified and unspecified degradation products.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Phenoxybenzamine Hydrochloride RS

Phenoxybenzamine Hydrochloride Compounded Oral Suspension

DEFINITION

Phenoxybenzamine Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$).

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Prepare Phenoxybenzamine Hydrochloride Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Phenoxybenzamine Hydrochloride powder	1 g
Corn Oil, *NF*, a sufficient quantity to make	100 mL

Pour the weighed *Phenoxybenzamine Hydrochloride powder* into a suitable mortar. Wet the powder with a small amount of *Corn Oil*, and triturate to make a smooth paste. Add the *Corn Oil* to make the mortar contents pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container using the *Corn Oil* to rinse the mortar. Add sufficient *Corn Oil* to bring the preparation to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: 25 mM monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.1

Mobile phase: Acetonitrile and *Solution A* (70:30)

Diluent: Prepare a mixture of 20 mL of acetonitrile and 80 mL of isopropyl alcohol in a conical flask. Add 10 g of anhydrous sodium sulfate to the flask, shake well for 1 min, and allow the sodium sulfate to precipitate to the bottom. [NOTE—The addition of anhydrous sodium sulfate removes trace amounts of water in the solvent.]

Standard solution: 0.5 mg/mL of phenoxybenzamine hydrochloride prepared from USP Phenoxybenzamine Hydrochloride RS in acetonitrile

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 0.5 mL of the Oral Suspension into a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix well to dissolve.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for phenoxybenzamine hydrochloride is about 5.2 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenoxybenzamine hydrochloride from the *Sample solution*

r_S = peak response of phenoxybenzamine hydrochloride from the *Standard solution*

C_S = concentration of phenoxybenzamine hydrochloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenoxybenzamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

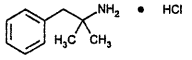
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature

• **LABELING:** Label it to indicate that it is to be well-shaken immediately before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Phenoxybenzamine Hydrochloride RS

Phentermine Hydrochloride



$C_{10}H_{15}N \cdot HCl$ 185.69

Benzeneethanamine, α,α-dimethyl-, hydrochloride; α,α-Dimethylphenethylamine hydrochloride [1197-21-3].

DEFINITION

Phentermine Hydrochloride contains NLT 98.0% and NMT 101.0% of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Analytical wavelength: 256 nm

Solution: 600 µg/mL

Medium: 0.1 N hydrochloric acid

Acceptance criteria: Absorptivities at the *Analytical wavelength*, calculated on the dried basis, do not differ by more than 2.0%.

• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY

• **PROCEDURE**

Sample: About 400 mg of Phentermine Hydrochloride

Analysis: Dissolve the *Sample* in 40 mL of glacial acetic acid, and add 10 mL of mercuric acetate TS, warming slightly to effect solution. Cool to room temperature, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.57 mg of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Standard stock solution: 2 mg/mL of USP Phentermine Hydrochloride RS in chloroform

Standard solutions: Dilute the *Standard stock solution* with chloroform to obtain the concentrations designated in *Table 1* by letter.

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Table 1

Standard solution	Dilution	Concentration (mg/mL)	Percentage (for comparison with Sample solution)
A	(undiluted)	2.0	1.0
B	(1 in 2)	1.0	0.5
C	(1 in 5)	0.4	0.2
D	(1 in 10)	0.2	0.1

Sample solution: 200 mg/mL of Phentermine Hydrochloride in chloroform
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, cyclohexane, and diethylamine (50:40:10)
Analysis
Samples: *Standard solutions* and *Sample solution*
Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in air. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots observed in the *Sample solution* with those of the principal spots in the *Standard solutions*.
Acceptance criteria: The sum of the intensities of secondary spots from the *Sample solution* corresponds to NMT 1.0% of related compounds, with no single impurity corresponding to more than 0.5%.

SPECIFIC TESTS
• **Melting Range or Temperature** ⟨741⟩: 202°–205°
• **pH** ⟨791⟩
Sample solution: 1 in 50
Acceptance criteria: 5.0–6.0
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Phentermine Hydrochloride RS

Phentermine Hydrochloride Capsules

DEFINITION
Phentermine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

IDENTIFICATION
• **A.**
Sample solution: Stir a portion of the Capsule contents in acetone to prepare a solution containing a nominal concentration at about 1 mg/mL of phentermine hydrochloride.
Analysis: Filter the *Sample solution* using an acetone resistant filter. Transfer 1 mL of the clear filtrate to a mortar containing about 200 mg of potassium bromide, triturate with a pestle, and air-dry to allow the acetone to evaporate. Place in an oven at 125° for 30 min to dry the mixture.
Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phentermine Hydrochloride RS.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: 1.1 g of sodium 1-heptanesulfonate in 575 mL of water. Add 25 mL of dilute glacial acetic acid (14 in 100) and 400 mL of methanol. Adjust dropwise, if necessary, with glacial acetic acid to a pH of 3.3 ± 0.1. Pass through a membrane filter of 0.5-µm pore size. The volume of methanol may be adjusted to provide a suitable retention time for phentermine hydrochloride (about 8 min).
Diluent: 0.04 M phosphoric acid
Standard solution: 0.4 mg/mL of USP Phentermine Hydrochloride RS in *Diluent*
Sample solution: Remove, as completely as possible, the contents of NLT 20 Capsules, and weigh. Transfer a portion of the mixed powder, nominally equivalent to about 20 mg of phentermine hydrochloride, to a 50-mL volumetric flask. Add 40 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume, and mix. Pass through a membrane filter of 0.5-µm pore size, discarding the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phentermine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phentermine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 900 mL. Use 500 mL for Capsules containing 15 mg or less of phentermine hydrochloride.
Apparatus 2: 50 rpm
Time: 45 min
Analysis: Determine the amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) dissolved, by using the *Procedure* set forth in the *Assay*, making any necessary modifications including concentration of the analyte in the volume of the *Sample solution* taken.

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Tolerances: NLT 75% (*Q*) of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Phentermine Hydrochloride RS

Phentermine Hydrochloride Tablets

DEFINITION

Phentermine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

IDENTIFICATION

• **A.**

Sample solution: Stir a portion of finely powdered Tablet contents in acetone to prepare a solution containing a nominal concentration at about 1 mg/mL of phentermine hydrochloride.

Analysis: Filter the *Sample solution* using an acetone resistant filter. Transfer 1 mL of the clear filtrate to a mortar containing about 200 mg of potassium bromide, triturate with a pestle, and air-dry to allow the acetone to evaporate. Place in an oven at 125° for 30 min to dry the mixture.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phentermine Hydrochloride RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Prepare a suitably degassed solution containing 0.03% diethylamine in methanol.

Internal standard solution: About 0.02 mg/mL of caffeine in *Mobile phase*

Standard solution: USP Phentermine Hydrochloride RS in the *Internal standard solution*, equivalent to 0.75 mg/mL of phentermine hydrochloride

Sample solution: Transfer an equivalent to 7.5 mg, from NLT 20 finely powdered Tablets, to a suitable flask. Pipet 10.0 mL of the *Internal standard solution* into the flask. Insert the stopper, mix, and sonicate for about 10 min. Pass through a filter of 0.5-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for caffeine and phentermine are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4 between caffeine and phentermine
Column efficiency: NLT 2000 theoretical plates
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of phentermine to the internal standard from the *Sample solution*
R_S = peak response ratio of phentermine to the internal standard from the *Standard solution*
C_S = concentration of USP Phentermine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phentermine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 900 mL. Use 500 mL for Tablets containing 15 mg or less of phentermine hydrochloride.
Apparatus 2: 50 rpm
Time: 45 min
Solution A: Dissolve 1.1 g of sodium 1-heptanesulfonate in 1 L of water. Add 3.5 mL of glacial acetic acid, and mix.
Mobile phase: Methanol and *Solution A* (525:475). Filter, degas, and adjust with phosphoric acid to a pH of 2.5.
Sample solution: Filtered portion of the pooled sample under test
Standard solution: Dissolve USP Phentermine Hydrochloride RS in water, and dilute with water, if necessary, to obtain a known concentration approximately equivalent to the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 208 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phentermine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL or 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) is dissolved.

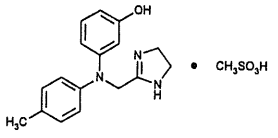
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Sample solution: Proceed as directed in the *Assay*, except prepare the *Sample solution* as follows. Transfer 1 Tablet to each of 10 suitable containers, and add 1 mL of water and 10 mL of the *Internal standard solution* to each. Mix,

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sonicate for about 10 min after each Tablet has disintegrated, and filter.
Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Phentermine Hydrochloride RS

Phentolamine Mesylate



$C_{17}H_{19}N_3O \cdot CH_4O_3S$ 377.46
Phenol, 3-[[(4,5-dihydro-1*H*-imidazol-2-yl)methyl](4-methylphenyl)amino]-, monomethanesulfonate (salt).
m-[*N*-(2-Imidazolin-2-ylmethyl)-*p*-toluidino]phenol monomethanesulfonate (salt) [65-28-1].

» Phentolamine Mesylate contains not less than 98.0 percent and not more than 102.0 percent of $C_{17}H_{19}N_3O \cdot CH_4O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Phentolamine Mesylate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 20 µg per mL.
Medium: water.
C: The R_F value of the principal spot in the chromatogram of the *Identification preparation* corresponds to that of *Standard preparation A* as obtained in the test for *Chromatographic purity.*
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Sulfate ⟨221⟩—A 0.10-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.2%).

Chromatographic purity—
Standard preparations—Dissolve USP Phentolamine Mesylate RS in methanol, and mix to obtain *Standard preparation A* having a known concentration of 50 µg per mL. Quantitatively dilute with methanol to obtain *Standard preparations,* designated below by letter, having the following compositions:

Standard preparation	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimen)
A	(undiluted)	50	0.5
B	(3 in 5)	30	0.3
C	(1 in 5)	10	0.1

Test preparation—Dissolve an accurately weighed quantity of Phentolamine Mesylate in methanol to obtain a solution containing 10 mg per mL.
Identification preparation—Dilute a portion of the *Test preparation* quantitatively with methanol to obtain a solution containing 50 µg per mL.
Detection reagent—Prepare (1) a solution of 1 g of potassium ferricyanide in 20 mL of water, and (2) a solution of 1.9 g of ferric chloride in 20 mL of water. Just prior to use, mix equal volumes of the solutions.
Procedure—Apply separately 5 µL of the *Test preparation,* 5 µL of the *Identification preparation,* and 5 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of chloroform, diethylamine, and methanol (15:3:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 100° for 1 hour. Spray the plate with *Detection reagent.* Within 15 minutes after spraying, compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations:* no secondary spot from the chromatogram of the *Test preparation* is larger or more intense than the principal spot obtained from *Standard preparation A* (0.5%), and the sum of the intensities of all secondary spots obtained from the *Test preparation* corresponds to not more than 1.0%.

Assay—
0.1 N Tetrabutylammonium hydroxide in isopropyl alcohol—Dilute with dehydrated isopropyl alcohol a commercially available 25% solution of tetrabutylammonium hydroxide in methanol, and standardize as directed under *Tetrabutylammonium Hydroxide, Tenth-Normal (0.1 N)* (see *Volumetric Solutions* in the section *Reagents, Indicators, and Solutions*), using dehydrated isopropyl alcohol instead of dimethylformamide.
Procedure—Dissolve with the aid of sonication, if necessary, about 300 mg of Phentolamine Mesylate, accurately weighed, in 100 mL of dehydrated isopropyl alcohol. Titrate in an atmosphere of nitrogen with *0.1 N Tetrabutylammonium hydroxide in isopropyl alcohol,* determining the endpoint potentiometrically, using a glass electrode and a calomel electrode containing a saturated solution of tetramethylammonium chloride in dehydrated isopropyl alcohol (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 37.75 mg of $C_{17}H_{19}N_3O \cdot CH_4O_3S$.

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Phentolamine Mesylate for Injection

» Phentolamine Mesylate for Injection is sterile Phentolamine Mesylate or a sterile mixture of Phentolamine Mesylate with a suitable buffer or suitable diluents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{19}N_3O \cdot CH_4O_3S$.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Phentolamine Mesylate RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
Identification—Mix a portion of it, equivalent to about 40 mg of phentolamine mesylate, with about 15 mL of chloroform. Filter into a beaker, and evaporate to dryness, taking precautions against introducing moisture: the residue so obtained responds to *Identification* test *A* under *Phentolamine Mesylate.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.8 USP Endotoxin Units per mg of phentolamine mesylate.
Uniformity of dosage units ⟨905⟩: meets the requirements.
Procedure for content uniformity—Dissolve the contents of 1 container in water to provide a solution containing about 20 µg of phentolamine mesylate per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Phentolamine Mesylate RS, in the same medium, at a concentration of about 20 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 278 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{17}H_{19}N_3O \cdot CH_4O_3S$ in the Phentolamine Mesylate for Injection taken by the formula:

$$(T/D)C(A_U/A_S)$$

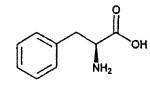
in which T is the labeled quantity, in mg, of phentolamine mesylate in the Phentolamine Mesylate for Injection, D is the concentration, in µg per mL, of phentolamine mesylate in the solution from the Phentolamine Mesylate for Injection, based on the labeled quantity per container and the extent of dilution, C is the concentration, in µg per mL, of USP Phentolamine Mesylate RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Phentolamine Mesylate for Injection and the Standard solution, respectively.
pH ⟨791⟩: between 4.5 and 6.5, in a freshly prepared solution having a concentration of about 1 in 100.
Other requirements—It meets the requirements for *Sterility Tests* ⟨71⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Standard preparation—Transfer about 25 mg of USP Phentolamine Mesylate RS, accurately weighed, to a 50-mL volumetric flask, add water to volume, and mix.
Assay preparation—Dissolve the contents of 10 containers of Phentolamine Mesylate for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Transfer an aliquot, equivalent to about 25 mg of phentolamine mesylate, to a 50-mL volumetric flask, add water to volume, and mix.
Procedure—Pipet 5-mL portions, respectively, of the *Standard preparation, Assay preparation,* and water to provide a blank, into separate 125-mL separators. Into each separator pipet 5-mL portions of 0.1 N hydrochloric acid and saturated picric acid solution. Extract with three 25-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 100-mL volumetric flask. Dilute with chloroform to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{17}H_{19}N_3O \cdot CH_4O_3S$ in the aliquot of Phentolamine Mesylate for Injection taken by the formula:

$$50C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Phentolamine Mesylate RS in the *Standard preparation,* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Phenylalanine



$C_9H_{11}NO_2$ 165.19
L-Phenylalanine [63-91-2].

DEFINITION
Phenylalanine contains NLT 98.5% and NMT 101.5% of L-phenylalanine ($C_9H_{11}NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
Sample: 160 mg of Phenylalanine
Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant.* Perform the *Blank* determination.
Calculate the percentage of phenylalanine ($C_9H_{11}NO_2$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 165.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.4%

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- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Standard solution: 0.50 mL of 0.020 N hydrochloric acid
  Sample: 0.73 g of Phenylalanine
  Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Standard solution: 0.10 mL of 0.020 N sulfuric acid
  Sample: 0.33 g of Phenylalanine
  Acceptance criteria: NMT 0.03%
- **IRON** ⟨241⟩**:** NMT 30 ppm
- **RELATED COMPOUNDS**
  Diluent: Glacial acetic acid and water (1:1)
  Standard solution: 0.05 mg/mL of USP L-Phenylalanine RS in *Diluent*. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]
  System suitability solution: 0.4 mg/mL each of USP L-Phenylalanine RS and USP L-Tyrosine RS in *Diluent*
  Sample solution: 10 mg/mL of Phenylalanine in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)
  Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
  System suitability
  Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.
  Analysis
  Samples: *Standard solution*, *System suitability solution*, and *Sample solution*
  After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
  Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
  Individual impurities: NMT 0.5%
  Total impurities: NMT 2.0%

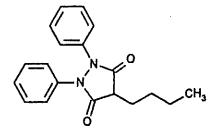
SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 20 mg/mL in water
  Acceptance criteria: −32.7° to −34.7°
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL solution
  Acceptance criteria: 5.4–6.0
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Phenylalanine RS
  USP L-Tyrosine RS

Phenylbutazone



$C_{19}H_{20}N_2O_2$ 308.37

3,5-Pyrazolidinedione, 4-butyl-1,2-diphenyl-.
4-Butyl-1,2-diphenyl-3,5-pyrazolidinedione [50-33-9].

» Phenylbutazone contains not less than 98.0 percent and not more than 102.0 percent of $C_{19}H_{20}N_2O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Phenylbutazone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: sodium hydroxide solution (1 in 2500).
Absorptivities at 264 nm, calculated on the dried basis, do not differ by more than 2.0%.
Melting range ⟨741⟩: between 104° and 107°.
Loss on drying ⟨731⟩—Dry it in vacuum at a pressure of 30 ± 10 mm of mercury at 80° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%, 2.0 g being used for the test.
Chloride ⟨221⟩—Boil 2.0 g with 60 mL of water for 5 minutes, cool, and filter. To a 30-mL portion of the filtrate add 1 mL of 2 N nitric acid and 1 mL of silver nitrate TS: the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.007%).
Sulfate ⟨221⟩—To a 30-mL portion of the filtrate obtained in the test for *Chloride* add 2 mL of barium chloride TS: the mixture shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.01%).

Assay—
Acetate buffer—Transfer 2.72 g of sodium acetate to a 1000-mL beaker, and dissolve in about 700 mL of water. Adjust with glacial acetic acid to a pH of 4.1. Filter through a 0.5-µm filter, dilute with filtered water to 1000 mL, and mix.
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile with 560 mL of *Acetate buffer* (440:560). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Dissolve 300 mg of desoxycorticosterone acetate in 200 mL of acetonitrile, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Phenylbutazone RS in acetonitrile, with the aid of sonication, and dilute quantitatively with acetonitrile to obtain a solution having a concentration of about 1.4 mg per mL. Pipet 10 mL of this solution into a 50-mL volumetric flask,

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add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. [NOTE—Use this solution within 8 hours of its preparation.]

Assay preparation—Transfer about 140 mg of Phenylbutazone, accurately weighed, to a 100-mL volumetric flask, add 75 mL of acetonitrile, and sonicate to dissolve. Dilute with acetonitrile to volume, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. [NOTE—Use this solution within 8 hours of its preparation.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7, preceded by a pre-column that contains packing L2. The flow rate is about 2.4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, of phenylbutazone and the internal standard is not less than 3.5, and the relative standard deviation of the ratio of their peak responses in replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.0 for the internal standard and 0.7 for phenylbutazone. Calculate the quantity, in mg, of $C_{19}H_{20}N_2O_2$ in the portion of Phenylbutazone taken by the formula:

$$500C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Phenylbutazone RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak response of the phenylbutazone to that of the internal standard for the *Assay preparation* and the *Standard preparation,* respectively.

Phenylbutazone Boluses

» Phenylbutazone Boluses contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of phenylbutazone ($C_{19}H_{20}N_2O_2$) and nominally not less than 1 g of phenylbutazone per bolus.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label Boluses to indicate that they are for veterinary use only.

USP Reference standards ⟨11⟩—
USP Phenylbutazone RS

Identification—

A: Transfer a portion of powdered Boluses, equivalent to about 500 mg of phenylbutazone, to a 250-mL conical flask, add 100 mL of solvent hexane, and heat the mixture under reflux for 15 minutes. Filter the hot mixture, and allow the filtrate to cool. Separate the crystals thus formed by filtration, and dry in vacuum at 80° for 30 minutes: the phenylbutazone so obtained responds to *Identification* test *A* under *Phenylbutazone.*

B: The retention time of the phenylbutazone peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Disintegration ⟨701⟩: 45 minutes with disks, determined as directed for *Uncoated Tablets,* simulated gastric fluid being used as the immersion fluid.

Uniformity of dosage units ⟨905⟩—meet the requirements for *Weight Variation.*

Assay—

Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Phenylbutazone.*

Assay preparation—Weigh and finely powder a Phenylbutazone Bolus. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of phenylbutazone, to a 250-mL volumetric flask. Transfer 10.0 mL of water to the flask, and shake by mechanical means for 15 minutes. Add about 120 mL of acetonitrile, and sonicate until insoluble material is dispersed into fine particles. Shake by mechanical means for 20 minutes, dilute with acetonitrile to volume, and mix. Transfer 7.0 mL of this solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5 µm or finer, discarding the first few mL of the filtrate. Use the clear filtrate as the *Assay preparation.* [NOTE—Use this solution within 8 hours of its preparation.]

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenylbutazone.* Calculate the quantity, in mg, of $C_{19}H_{20}N_2O_2$ in the portion of the Bolus taken by the formula:

$$1786C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Phenylbutazone RS in the *Standard preparation,* and R_U and R_S are the ratios of the peak responses of phenylbutazone to that of the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Phenylbutazone Injection

» Phenylbutazone Injection is a sterile solution of Phenylbutazone in Sterile Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{20}N_2O_2$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Protect from light, and store in a refrigerator.

Labeling—Label Injection to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Phenylbutazone RS

Clarity of solution—The Injection is essentially free from particles of foreign matter that can be observed on visual inspection.

Identification—

A: Transfer a volume of Injection, equivalent to about 500 mg of phenylbutazone, to a 250-mL conical flask, add 100 mL of solvent hexane, and heat the mixture under reflux for 15 minutes. Filter the hot mixture, and allow the filtrate to cool. Separate the crystals thus formed by filtration, and dry in vacuum at 80° for 30 minutes: the phenylbutazone so obtained responds to *Identification* test *A* under *Phenylbutazone.*

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B: The retention time of the phenylbutazone peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.1 USP Endotoxin Units per mg of phenylbutazone.

pH ⟨791⟩: between 9.5 and 10.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Change to read:

Assay—

Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Phenylbutazone*.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 200 mg of phenylbutazone, to a 100-mL volumetric flask. Dilute with acetonitrile to volume, and mix. Transfer 7.0 mL of this solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. [NOTE—Use this solution within 8 hours of its preparation.]

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenylbutazone*. Calculate the quantity, in mg, of $C_{19}H_{20}N_2O_2$ in each mL of the Injection taken by the formula:

$$\blacktriangle 714.3 \blacktriangle_{\text{(ERR 1-Jun-2019)}} (C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Phenylbutazone RS in the *Standard preparation*, V is the volume, in mL, of Injection taken to prepare the *Assay preparation*, and R_U and R_S are the ratios of the peak responses of phenylbutazone to that of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylbutazone Tablets

» Phenylbutazone Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of phenylbutazone ($C_{19}H_{20}N_2O_2$) and nominally not more than 200 mg of phenylbutazone per Tablet.

Packaging and storage—Preserve in tight containers.

Labeling—Label Tablets to indicate that they are for veterinary use only.

USP Reference standards ⟨11⟩—
USP Phenylbutazone RS

Identification—Transfer to a 250-mL conical flask a portion of powdered Tablets, equivalent to about 500 mg of phenylbutazone, add 100 mL of solvent hexane, and heat the mixture under reflux for 15 minutes. Filter the hot mixture, and allow the filtrate to cool. Separate the crystals thus formed by filtration, and dry in vacuum at 80° for 30 minutes: the phenylbutazone so obtained responds to *Identification* test *A* under *Phenylbutazone*.

Dissolution ⟨711⟩—

Medium: pH 7.5 simulated intestinal fluid TS (without the enzyme); 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{19}H_{20}N_2O_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 264 nm on filtered portions of the solution under test, suitably diluted, if necessary, with *Medium*, using a suitable spectrophotometer, 1-cm cells, and *Medium* as the blank, in comparison with a solution of known concentration of USP Phenylbutazone RS in the same *Medium*.

Tolerances—Not less than 70% *(Q)* is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Transfer 1 Tablet to a 100-mL volumetric flask, add 60 mL of methanol, and shake by mechanical means for about 20 minutes or until the tablet is completely disintegrated. Dilute with methanol to volume, and mix. Filter a portion of mixture, discarding the first 10 mL of the filtrate. Dilute an accurately measured portion of the filtrate with sodium hydroxide solution (1 in 2500) to obtain a solution containing about 10 µg per mL. Prepare a solution of USP Phenylbutazone RS in methanol having a known concentration of about 1 mg per mL. Quantitatively dilute a portion of this solution with sodium hydroxide solution (1 in 2500) to obtain a Standard solution having a final known concentration of about 10 µg per mL. Concomitantly determine the absorbances of the solution from the Tablet and the Standard solution at the wavelength of maximum absorbance at about 264 nm with a suitable spectrophotometer, using sodium hydroxide solution (1 in 2500) as the blank. Calculate the quantity, in mg, of $C_{19}H_{20}N_2O_2$ in the Tablet by the formula:

$$(TC/D)(A_U/A_S)$$

in which T is the labeled quantity, in mg, of phenylbutazone in the Tablet; C is the concentration, in µg per mL, of USP Phenylbutazone RS in the Standard solution; D is the concentration, in µg per mL, of phenylbutazone in the solution from the Tablet based on the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—

Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Phenylbutazone*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Accurately weigh a portion of the powder, equivalent to about 500 mg of phenylbutazone, and transfer to a 250-mL volumetric flask. Pipet 50 mL of water into the flask, and shake by mechanical means for 15 minutes. Add about 120 mL of acetonitrile, and sonicate until insoluble material is dispersed into fine particles. Shake by mechanical means for 20 minutes, dilute with acetonitrile to volume, and mix. Centrifuge a portion of this solution. Pipet 7 mL of the solution into a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. Pass a portion through a 0.5-µm filter, discarding the first few mL of the filtrate. [NOTE—Use this solution within 8 hours of its preparation.]

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenylbutazone*. Calculate the quantity, in mg, of phenylbutazone ($C_{19}H_{20}N_2O_2$) in the portion of Tablets taken by the formula:

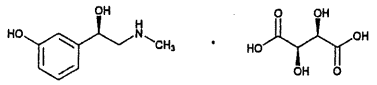
$$1786C(R_U/R_S)$$

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in which C is the concentration, in mg per mL, of USP Phenylbutazone RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak response of phenylbutazone to that of the internal standard for the *Assay preparation* and the *Standard preparation,* respectively.

Phenylephrine Bitartrate



$C_9H_{13}NO_2 \cdot C_4H_6O_6$ 317.29

Benzenemethanol, 3-hydroxy-α-[(methylamino)methyl]-, (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1) (salt); (*R*)-(-)-*m*-Hydroxy-α-[(methylamino)methyl]benzyl alcohol hydrogen tartrate [17162-39-9].

DEFINITION

Phenylephrine Bitartrate contains NLT 98.0% and NMT 102.0% of phenylephrine bitartrate ($C_9H_{13}NO_2 \cdot C_4H_6O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Tartrate* ⟨191⟩

Sample: The alkaline filtrate from the test for *Optical Rotation* ⟨781S⟩, *Specific Rotation*

Acceptance criteria: The *Sample* responds positively to the test for *Tartrate* in *Identification Tests—General* ⟨191⟩.

C. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: Dissolve 3.25 g of 1-octanesulfonic acid sodium salt monohydrate in 1 L of water, and adjust with 3 M phosphoric acid to a pH of 2.8.
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and *Buffer* (90:10)
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
10	70	30
10.1	93	7
18	93	7

Diluent: *Solution A* and *Solution B* (80:20)
Standard solution: 0.6 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
Sample solution: 0.9 mg/mL of Phenylephrine Bitartrate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.0-mm × 5.5-cm; 3-µm packing L1
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 4 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.9
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenylephrine bitartrate ($C_9H_{13}NO_2 \cdot C_4H_6O_6$) in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phenylephrine from the *Sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of phenylephrine bitartrate, 317.29
M_{r2} = molecular weight of phenylephrine hydrochloride, 203.67

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, and **Diluent:** Proceed as directed in the *Assay.*
System suitability solution: 1.0 mg/mL of USP Phenylephrine Hydrochloride RS and 0.9 µg/mL each of USP Norphenylephrine Hydrochloride RS and USP Phenylephrine Related Compound C RS in *Diluent*
Standard solution: 0.001 mg/mL each of USP Phenylephrine Hydrochloride RS, USP Norphenylephrine Hydrochloride RS, USP Phenylephrine Related Compound C RS, USP Phenylephrine Related Compound D RS, and USP Phenylephrine Related Compound E RS in *Diluent*
Blank: 0.8 mg/mL of L(+)-tartaric acid in *Diluent*
Sample solution: 1.56 mg/mL of Phenylephrine Bitartrate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4-mm × 5.5-cm; 3-µm packing L1
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 4 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between norphenylephrine and phenylephrine and NLT 1.5 between phenylephrine and phenylephrine related compound C, *System suitability solution*
Relative standard deviation: NMT 5% for norphenylephrine, phenylephrine, phenylephrine related compound C, phenylephrine related compound D, and phenylephrine related compound E, *Standard solution*

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Analysis

Samples: *Standard solution*, *Blank*, and *Sample solution*

Examine the chromatogram of the *Blank* for the peaks, and disregard any corresponding peaks observed in the chromatogram of the *Sample solution*.

Calculate the percentage of norphenylephrine as free base in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of norphenylephrine from the *Sample solution*

r_S = peak response of norphenylephrine from the *Standard solution*

C_S = concentration of USP Norphenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of norphenylephrine as free base, 153.18

M_{r2} = molecular weight of norphenylephrine as hydrochloride salt, 189.64

Calculate the percentage of phenylephrine related compound C as free base in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phenylephrine related compound C from the *Sample solution*

r_S = peak response of phenylephrine related compound C from the *Standard solution*

C_S = concentration of USP Phenylephrine Related Compound C RS in the *Standard solution* (mg/mL)

C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of phenylephrine related compound C as free base, 165.19

M_{r2} = molecular weight of phenylephrine related compound C as hydrochloride salt, 201.65

Calculate the percentage of phenylephrine related compound D in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenylephrine related compound D from the *Sample solution*

r_S = peak response of phenylephrine related compound D from the *Standard solution*

C_S = concentration of USP Phenylephrine Related Compound D RS in the *Standard solution* (mg/mL)

C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL)

Calculate the percentage of phenylephrine related compound E as free base in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phenylephrine related compound E from the *Sample solution*

r_S = peak response of phenylephrine related compound E from the *Standard solution*

C_S = concentration of USP Phenylephrine Related Compound E RS in the *Standard solution* (mg/mL)

C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of phenylephrine related compound E as free base, 255.31

M_{r2} = molecular weight of phenylephrine related compound E as hydrochloride salt, 291.77

Calculate the percentage of any individual unspecified impurity in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of phenylephrine from the *Standard solution*

C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of phenylephrine bitartrate, 317.29

M_{r2} = molecular weight of phenylephrine hydrochloride, 203.67

Acceptance criteria: See *Table 2*. Disregard any peaks below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Norphenylephrine	0.9	0.2
Phenylephrine	1.0	—
Phenylephrine related compound C	1.2	0.1
Phenylephrine related compound D	2.9	0.2
Phenylephrine related compound E	3.1	0.1
Any individual unspecified impurity	—	0.1
Total impurities	—	0.5

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

  Sample solution: Prepare a solution of about 240 mg/mL of Phenylephrine Bitartrate in water. Make the solution slightly alkaline by adding concentrated ammonium hydroxide dropwise. Rub the wall of the vessel with a glass rod so that the base precipitates out. Filter the base under suction, wash with a little water and acetone, and dry at 105° for 2 h. Prepare and measure a 50-mg/mL solution of base precipitate in 1 M hydrochloric acid.

  Acceptance criteria: −53° to −57°

- **PH** ⟨791⟩

  Sample solution: 10% w/v aqueous solution

  Acceptance criteria: 3.0–4.0

- **LOSS ON DRYING** ⟨731⟩

  Analysis: Dry at 105° to a constant weight.

  Acceptance criteria: NMT 0.5%

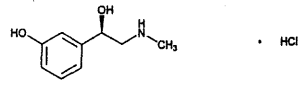
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Norphenylephrine Hydrochloride RS
3-(2-Amino-1-hydroxyethyl)phenol hydrochloride.
$C_8H_{11}NO_2 \cdot HCl$ 189.64
USP Phenylephrine Bitartrate RS
USP Phenylephrine Hydrochloride RS
USP Phenylephrine Related Compound C RS
1-(3-Hydroxyphenyl)-2-(methylamino)ethan-1-one hydrochloride.
$C_9H_{11}NO_2 \cdot HCl$ 201.65
USP Phenylephrine Related Compound D RS
(*R*)-3-{2-[Benzyl(methyl)amino]-1-hydroxyethyl}phenol.
$C_{16}H_{19}NO_2$ 257.33
USP Phenylephrine Related Compound E RS
2-[Benzyl(methyl)amino]-1-(3-hydroxyphenyl)ethan-1-one hydrochloride.
$C_{16}H_{17}NO_2 \cdot HCl$ 291.77

Phenylephrine Hydrochloride



$C_9H_{13}NO_2 \cdot HCl$ 203.67
Benzenemethanol, 3-hydroxy-α-[(methylamino)methyl]-, hydrochloride (*R*)-;
(–)-*m*-Hydroxy-α-[(methylamino)methyl]benzyl alcohol hydrochloride [61-76-7].

DEFINITION
Phenylephrine Hydrochloride contains NLT 98.0% and NMT 102.0% of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: 10 mg/mL
Acceptance criteria: Meets the requirements
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: Dissolve 3.25 g of 1-octanesulfonic acid sodium salt monohydrate in 1 L of water, and adjust with 3 M phosphoric acid to a pH of 2.8.
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and *Buffer* (90:10)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
3	93	7
13	70	30
14	93	7
16	93	7

Diluent: *Solution A* and *Solution B* (80:20)
Standard solution: 0.4 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
Sample solution: 0.4 mg/mL of Phenylephrine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.0-mm × 5.5-cm; 3-µm packing L1
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.9
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenylephrine from the *Sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard solution: 0.10 mL of 0.020 N sulfuric acid
Sample solution: 50 mg in 25 mL of water
Acceptance criteria: The *Sample solution* shows no more turbidity than corresponds to that of the *Standard solution* (0.20%).
• **ORGANIC IMPURITIES**
Buffer, Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1.0 mg/mL of USP Phenylephrine Hydrochloride RS and 10 µg/mL each of USP Norphenylephrine Hydrochloride RS and USP Phenylephrine Related Compound C RS in *Diluent*
Standard solution: 0.001 mg/mL each of USP Phenylephrine Hydrochloride RS, USP Norphenylephrine Hydrochloride RS, USP Phenylephrine Related Compound C RS, USP Phenylephrine Related Compound D RS, and USP Phenylephrine Related Compound E RS in *Diluent*
Sample solution: 1.0 mg/mL of Phenylephrine Hydrochloride in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between norphenylephrine and phenylephrine and NLT 1.5 between phenylephrine and phenylephrine related compound C, *System suitability solution*

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Relative standard deviation: NMT 5% for norphenylephrine, phenylephrine, phenylephrine related compound C, phenylephrine related compound D, and phenylephrine related compound E, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of norphenylephrine as free base in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of norphenylephrine from the *Sample solution*
r_S = peak response of norphenylephrine from the *Standard solution*
C_S = concentration of USP Norphenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of norphenylephrine as free base, 153.18
M_{r2} = molecular weight of norphenylephrine as hydrochloride salt, 189.64

Calculate the percentage of phenylephrine related compound C as free base in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phenylephrine related compound C from the *Sample solution*
r_S = peak response of phenylephrine related compound C from the *Standard solution*
C_S = concentration of USP Phenylephrine Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of phenylephrine related compound C as free base, 165.19
M_{r2} = molecular weight of phenylephrine related compound C as hydrochloride salt, 201.65

Calculate the percentage of phenylephrine related compound D in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenylephrine related compound D from the *Sample solution*
r_S = peak response of phenylephrine related compound D from the *Standard solution*
C_S = concentration of USP Phenylephrine Related Compound D RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of phenylephrine related compound E as free base in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phenylephrine related compound E from the *Sample solution*
r_S = peak response of phenylephrine related compound E from the *Standard solution*
C_S = concentration of USP Phenylephrine Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of phenylephrine related compound E as free base, 255.31
M_{r2} = molecular weight of phenylephrine related compound E as hydrochloride salt, 291.77

Calculate the percentage of any individual unspecified impurity in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peaks below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Norphenylephrine	0.9	0.10
Phenylephrine	1.0	—
Phenylephrine related compound C	1.3	0.1
Phenylephrine related compound D	3.8	0.10
Phenylephrine related compound E	4.0	0.1
Any individual unspecified impurity	—	0.10
Total impurities	—	0.2

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 50 mg/mL in water
  Acceptance criteria: –43° to –47°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Norphenylephrine Hydrochloride RS
  3-(2-Amino-1-hydroxyethyl)phenol hydrochloride.
  $C_8H_{11}NO_2 \cdot HCl$ 189.64
  USP Phenylephrine Hydrochloride RS

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USP Phenylephrine Related Compound C RS
1-(3-Hydroxyphenyl)-2-(methylamino)ethan-1-one hydrochloride.
$C_9H_{11}NO_2 \cdot HCl$ 201.65
USP Phenylephrine Related Compound D RS
(*R*)-3-{2-[Benzyl(methyl)amino]-1-hydroxyethyl}phenol.
$C_{16}H_{19}NO_2$ 257.33
USP Phenylephrine Related Compound E RS
2-[Benzyl(methyl)amino]-1-(3-hydroxyphenyl)ethan-1-one hydrochloride.
$C_{16}H_{17}NO_2 \cdot HCl$ 291.77

Phenylephrine Hydrochloride Injection

DEFINITION

Phenylephrine Hydrochloride Injection is a sterile solution of Phenylephrine Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 115.0% of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the phenylephrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

Add the following:

▲• **B.** The UV absorption spectrum of the phenylephrine peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**

▲Solution A: Phosphoric acid and water (1:1000)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
2.5	98	2
6	65	35
6.1	98	2
9	98	2

Standard solution: 0.1 mg/mL of USP Phenylephrine Hydrochloride RS in water
Sample solution: Nominally 0.1 mg/mL of phenylephrine hydrochloride in water from a volume of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 273 nm. For *Identification B*, use a diode array detector in the range of 245–400 nm.
Column: 4.6-mm × 15-cm; 2.6-µm packing L1
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenylephrine from the *Sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)▲ (USP 1-Aug-2019)

Acceptance criteria: 90.0%–115.0%

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, and **Sample solution:** Prepare as directed in the *Assay*.
System suitability solution: 0.1 mg/mL of USP Phenylephrine Hydrochloride RS and 0.005 mg/mL of USP Phenylephrine Related Compound F RS in water
Sensitivity solution: 0.1 µg/mL of USP Phenylephrine Hydrochloride RS in water
Standard solution: 0.0002 mg/mL of USP Phenylephrine Hydrochloride RS in water
Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.
Detector: UV 215 nm
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—The relative retention times for phenylephrine related compound F and phenylephrine are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between phenylephrine and phenylephrine related compound F, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

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r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylephrine	1.0	1.0	—
Phenylephrone (phenylephrine related compound C)[a]	1.2	2.8	0.7
Phenylephrine-citrate adduct[b]	2.9	1.0	0.4
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.3

[a] 1-(3-Hydroxyphenyl)-2-(methylamino)ethan-1-one hydrochloride.
[b] 2-Hydroxy-2-(2-{[(*R*)-2-hydroxy-2-(3-hydroxyphenyl)ethyl](methyl)amino}-2-oxoethyl)succinic acid. ▲(USP 1-Aug-2019)

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 25.0 USP Endotoxin Units/mg of phenylephrine hydrochloride.
- **pH** ⟨791⟩: 3.0–6.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. ▲Store at controlled room temperature.▲ (USP 1-Aug-2019)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Phenylephrine Hydrochloride RS
  ▲USP Phenylephrine Related Compound F RS
  (*R*)-2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,8-diol hydrochloride monohydrate.
  $C_{10}H_{13}NO_2 \cdot HCl \cdot H_2O$ 233.69▲ (USP 1-Aug-2019)

Phenylephrine Hydrochloride Nasal Jelly

» Phenylephrine Hydrochloride Nasal Jelly contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_9H_{13}NO_2 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Phenylephrine Hydrochloride RS
Identification—Dissolve a suitable quantity in water to obtain a solution having a concentration of about 60 µg per mL, and centrifuge, if necessary: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Phenylephrine Hydrochloride RS, concomitantly measured.
Minimum fill ⟨755⟩: meets the requirements.

Assay—

Mobile phase—Prepare a mixture of methanol and water (1:1) containing 1.1 g of sodium 1-octanesulfonate per liter, adjust with phosphoric acid to a pH of 3.0, filter, and degas. Make adjustments to the methanol and water ratio, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Dilution solvent—Prepare a mixture of methanol and water (1:1), and adjust with phosphoric acid to a pH of 3.0.

Standard preparation—Dissolve an accurately weighed quantity of USP Phenylephrine Hydrochloride RS in *Dilution solvent* to obtain a Stock standard solution having a known concentration of about 2 mg per mL. Dilute an accurately measured volume of this solution with *Dilution solvent* to obtain the *Standard preparation* having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately weighed amount of Nasal Jelly, equivalent to about 10 mg of phenylephrine hydrochloride, to a 100-mL volumetric flask. Dilute with *Dilution solvent* to volume, and mix.

Resolution solution—Transfer 5.0 mL of Stock standard solution to a 100-mL volumetric flask, add 10 mg of USP Epinephrine Bitartrate RS, dilute with *Dilution solvent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution:* the resolution, *R,* is not less than 1.5, and the tailing factor for the phenylephrine peak is not more than 2.0. Chromatograph replicate injections of the *Standard preparation:* the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_2 \cdot HCl$ in the portion of Nasal Jelly taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Hydrochloride Nasal Solution

DEFINITION

Phenylephrine Hydrochloride Nasal Solution contains NLT 90.0% and NMT 115.0% of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$).

USP Monographs

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IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: 1.1 g/L of sodium 1-octane sulfonate in a mixture of methanol and water (50:50). Adjust with phosphoric acid to a pH of 3.0.

Diluent: Methanol and water (50:50). Adjust with phosphoric acid to a pH of 3.0.

System suitability solution: 0.1 mg/mL of USP Phenylephrine Hydrochloride RS and 0.1 mg/mL of USP Epinephrine Bitartrate RS in *Diluent*

Standard stock solution: 2 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent* from the *Standard stock solution*

Sample solution: Nominally 0.1 mg/mL of phenylephrine hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between phenylephrine and epinephrine, *System suitability solution*

Tailing factor: NMT 2.0 for the phenylephrine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

▲USP Epinephrine Bitartrate RS▲ (USP 1-Aug-2019)

USP Phenylephrine Hydrochloride RS

Phenylephrine Hydrochloride Ophthalmic Solution

DEFINITION

Phenylephrine Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Phenylephrine Hydrochloride. It contains NLT 90.0% and NMT 115.0% of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$). It may contain a suitable antimicrobial agent and buffer and may contain suitable antioxidants.

IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: 1.1 g/L of sodium 1-octane sulfonate in a mixture of methanol and water (50:50). Adjust with phosphoric acid to a pH of 3.0.

Diluent: Methanol and water (50:50). Adjust with phosphoric acid to a pH of 3.0.

System suitability solution: 0.1 mg/mL of USP Phenylephrine Hydrochloride RS and 0.1 mg/mL of USP Epinephrine Bitartrate RS in *Diluent*

Standard stock solution: 2 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent* from the *Standard stock solution*

Sample solution: Nominally 0.1 mg/mL of phenylephrine hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between phenylephrine and epinephrine, *System suitability solution*

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Tailing factor: NMT 2.0 for the phenylephrine peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements.
- **pH** ⟨791⟩**:** 4.0–7.5 for buffered Ophthalmic Solution and 3.0–4.5 for unbuffered Ophthalmic Solution

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers of NMT 15-mL size.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Epinephrine Bitartrate RS▲ (USP 1-Aug-2019)
USP Phenylephrine Hydrochloride RS

Phenylephrine Hydrochloride Tablets

DEFINITION
Phenylephrine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$).

IDENTIFICATION
- **A.** The UV absorption spectra of the phenylephrine peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.
- **B.** The retention time of the phenylephrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

It is suggested to use plastic vials for analysis.
Buffer: 3.45 g/L of monobasic ammonium phosphate in water. Adjust with 10% phosphoric acid or 10% ammonium hydroxide solution to a pH of 4.5 ± 0.10, if necessary.
Solution A: Dilute 10 mL of glacial acetic acid with water to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Diluent: Methanol and *Solution A* (30:70)
Standard solution: 0.1 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of phenylephrine hydrochloride prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, add 50% of the final volume of *Solution A*, and stir vigorously for NLT 30 min. Add 30% of the final volume of methanol and stir for NLT an additional 90 min. To ensure that particles do not collect above the solvent level, periodically rinse the particulate into the solution with *Solution A*. Allow the resulting solution to cool to room temperature and dilute with *Solution A* to volume. Pass a portion through a suitable filter of 0.45-µm pore size. Discard the first 2–3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm. For *Identification A*, use a diode-array detector in the range of 200–350 nm.
Column: 4.6-mm × 10-cm; 5-µm packing L9
Flow rate: 2.0 mL/min
Injection volume: 25 µL
Run time: NLT 1.75 times the retention time of phenylephrine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.5–3.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenylephrine from the *Sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

It is suggested to use plastic vials for analysis.
Medium: Simulated gastric fluid without pepsin; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Buffer, Mobile phase, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: (*L*/900) mg/mL of USP Phenylephrine Hydrochloride RS in *Medium*, where *L* is the label claim of phenylephrine hydrochloride in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 10–20-µm pore size.
Chromatographic system: Proceed as directed in the *Assay*, except for the following.
Injection volume: 100 µL
Run time: NLT 1.5 times the retention time of phenylephrine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

Official Monographs

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r_U = peak response of phenylephrine from the *Sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Trifluoroacetic acid and water (1:1000)
Solution B: Trifluoroacetic acid and acetonitrile (1:1000)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	96.5	3.5
3	96.5	3.5
44	62.0	38.0
45	96.5	3.5
50	96.5	3.5

Diluent: Phosphoric acid and water (0.5: 1000)
System suitability solution: 0.0002 mg/mL of USP Phenylephrine Related Compound F RS, 0.0002 mg/mL of USP Phenylephrine Related Compound G RS, and 0.2 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
Standard solution: 0.002 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
Sensitivity solution: 0.0002 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent* from the *Standard solution*
Sample solution: Nominally 0.2 mg/mL of phenylephrine hydrochloride prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, add about 50% of the final volume of *Diluent*, and shake for NLT 1 h. Dilute with *Diluent* to volume. Pass a portion through a suitable filter of 0.45-µm pore size. Discard the first 2 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 35.0°
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.0 between phenylephrine related compound G and phenylephrine, *System suitability solution*
Relative standard deviation: NMT 6.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor of each individual degradation product (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks below 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isoquinoline 4,6-diol analog[a]	0.67	0.70	1.0
Phenylephrine related compound F[b]	0.88	1.0	0.3
Phenylephrine related compound G[c]	0.94	1.0	0.3
Phenylephrine	1.00	1.0	—
Phenylephrone (Phenylephrine related compound C)[d]	1.29	1.8	0.5
3-Hydroxybenzaldehyde	3.00	3.1	0.3
Phenylephrine isoquinolinone analog[e]	4.19	8.0	0.3
Any unspecified degradation product	—	1.0	0.3
Total degradation products	—	—	2.4

[a] 2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,6-diol.
[b] 2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,8-diol.
[c] (*R*)-*N*-(2-Hydroxy-2-(3-hydroxyphenyl)ethyl)-*N*-methylglycine.
[d] 1-(3-Hydroxyphenyl)-2-(methylamino)ethan-1-one.
[e] 1-(3-Hydroxybenzoyl)-2-methylisoquinolin-6(2*H*)-one.

(Postponed indefinitely)

ADDITIONAL REQUIREMENTS

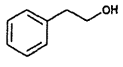
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at 20°–25°.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Phenylephrine Hydrochloride RS
USP Phenylephrine Related Compound F RS
2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,8-diol.
$C_{10}H_{13}NO_2$ 179.22
(Postponed indefinitely)

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USP Phenylephrine Related Compound G RS
(*R*)-*N*-(2-Hydroxy-2-(3-hydroxyphenyl)ethyl)-*N*-methylglycine.
$C_{11}H_{15}NO_4$ 225.24
(Postponed indefinitely)

Phenylethyl Alcohol



$C_8H_{10}O$ 122.16
Benzeneethanol;
Phenethyl alcohol [60-12-8].

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩**
  Sample: 10 mL
  Analysis: Evaporate the *Sample* in a suitable crucible, and ignite to constant weight.
  Acceptance criteria: NMT 0.005%

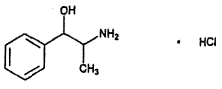
SPECIFIC TESTS

- **SPECIFIC GRAVITY ⟨841⟩:** 1.017–1.020
- **REFRACTIVE INDEX ⟨831⟩:** 1.531–1.534 at 20°
- **CHLORINATED COMPOUNDS**
  Sample: 2 drops
  Analysis: Wind a 1.5- × 5-cm strip of 20-mesh copper gauze around the end of a copper wire. Heat the gauze in the nonluminous flame of a Bunsen burner until it glows without coloring the flame green. Permit the gauze to cool, and heat several times until a good coat of oxide has formed. Apply the *Sample* to the cooled gauze with a medicine dropper, ignite, and permit it to burn freely in the air. Again cool the gauze, add a second *Sample*, and burn as before. Continue this process until a total of 6 drops has been added and ignited, and then hold the gauze in the outer edge of the Bunsen flame, adjusted to a height of about 4 cm.
  Acceptance criteria: No transient green color or other color is imparted to the flame.
- **ALDEHYDE**
  Sample: 5 mL
  Analysis: Shake the *Sample* with 5 mL of 1 N sodium hydroxide, and allow to stand for 1 h.
  Acceptance criteria: No yellow color appears in the organic (top) layer.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool, dry place.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Phenylethyl Alcohol RS

Phenylpropanolamine Hydrochloride



$C_9H_{13}NO \cdot HCl$ 187.67
Benzenemethanol, α-(1-aminoethyl)-, hydrochloride, (*R**,*S**)-, (±);
(±)-Norephedrine hydrochloride [154-41-6].

DEFINITION

Phenylpropanolamine Hydrochloride contains NLT 98.0% and NMT 101.0% of phenylpropanolamine hydrochloride ($C_9H_{13}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Solution: 500 µg/mL
  Medium: Water
  Analytical wavelength: 256 nm
  Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
- **C. MELTING RANGE OR TEMPERATURE ⟨741⟩**
  Sample: Dissolve 1 g in 10 mL of water, add 10 mL of saturated sodium carbonate solution, and mix. Separate the precipitate by vacuum filtration, using a sintered-glass filter of medium pore size. Wash with three 5-mL portions of ice-cold water. Dry the crystals at 80° for 1 h.
  Acceptance criteria: The *Sample* melts between 101° and 104°.

ASSAY

- **PROCEDURE**
  Sample: 500 mg
  Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.77 mg of phenylpropanolamine hydrochloride ($C_9H_{13}NO \cdot HCl$).
  Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **LIMIT OF CATHINONE HYDROCHLORIDE**
  Diluent: Dilute hydrochloric acid (1 in 120)
  Standard solution: 100 µg/mL of USP Cathinone Hydrochloride RS in *Diluent*
  Sample solution: 100 mg/mL of Phenylpropanolamine Hydrochloride in *Diluent*
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum absorbance at about 285 nm
  Cell: 1 cm
  Blank: *Diluent*
  Analysis: Concomitantly determine the absorbances of the *Sample solution* and the *Standard solution*, using the *Blank*.

USP Monographs

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Acceptance criteria: The absorbance of the *Sample solution* is NMT than that of the *Standard solution* (NMT 0.10%).

• **LIMIT OF AMPHETAMINE HYDROCHLORIDE**

Mobile phase: Acetonitrile, phosphoric acid, triethylamine, and water (50:8:5:950)

System suitability solution: 5 µg/mL each of USP Phenylpropanolamine Hydrochloride RS and USP Dextroamphetamine Sulfate RS in water

Sample stock solution: 250 mg/mL of Phenylpropanolamine Hydrochloride in water. Sonicate if necessary.

Sample solution: 100 mg/mL of Phenylpropanolamine Hydrochloride in water from *Sample stock solution*

Amphetamine standard stock solution: 2.5 µg/mL of USP Dextroamphetamine Sulfate RS in water

Standard solution: 1 µg/mL of USP Dextroamphetamine Sulfate RS and 100 mg/mL of Phenylpropanolamine Hydrochloride in water from *Amphetamine standard stock solution* and *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 206 nm

Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L1

Flow rate: 1 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phenylpropanolamine and amphetamine are 1.0 and 2.1, respectively.]

Suitability requirements

Resolution: NLT 15 between phenylpropanolamine and amphetamine, *System suitability solution*

Column efficiency: NLT 10,000 theoretical plates, *System suitability solution*

Relative standard deviation: NMT 3.0% for amphetamine, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Record the chromatograms, and measure the responses at the locus of the amphetamine peak.

Calculate the percentage of amphetamine hydrochloride in the portion of Phenylpropanolamine Hydrochloride taken:

$$\text{Result} = [r_U/(r_S - r_U)] \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 0.2$$

r_U = amphetamine peak response from the *Sample solution*

r_S = amphetamine peak response from the *Standard solution*

C_S = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (µg/mL)

C_U = concentration of Phenylpropanolamine Hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amphetamine hydrochloride, 171.67

M_{r2} = molecular weight of amphetamine sulfate, 368.49

Acceptance criteria: NMT 0.001%

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩: 191°–196°

• **pH** ⟨791⟩

Sample solution: A solution (3 in 100)

Acceptance criteria: 4.2–5.5

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **LABELING:** Label it to indicate that it is for veterinary use only.

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

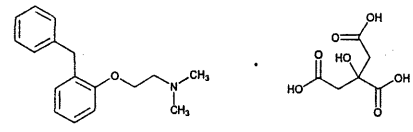
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cathinone Hydrochloride RS
α-Aminopropiophenone hydrochloride.
$C_9H_{11}NO \cdot HCl$ 185.65

USP Dextroamphetamine Sulfate RS

USP Phenylpropanolamine Hydrochloride RS

Phenyltoloxamine Citrate



$C_{17}H_{21}NO \cdot C_6H_8O_7$ 447.48

N,N-Dimethyl-2-(α-phenyl-*o*-tolyloxy)ethylamine, citrate (1:1) salt.

2-(2-Dimethylaminoethoxy)diphenylmethane, citrate (1:1) salt

Phenyltoloxamine dihydrogen citrate [1176-08-5].

» Phenyltoloxamine Citrate contains not less than 99.0 percent and not more than 101.0 percent of $C_{17}H_{21}NO \cdot C_6H_8O_7$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at room temperature.

USP Reference standards ⟨11⟩—

USP Phenyltoloxamine Citrate RS

USP Phenyltoloxamine Related Compound A RS
2-(2-Benzylphenoxy)ethylmethylamine hydrochloride.
$C_{16}H_{19}NO \cdot HCl$ 277.79

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Melting range, *Class 1a* ⟨741⟩: between 137° and 143°.

pH ⟨791⟩: between 3.2 and 4.2, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it in vacuum at 80° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—

Resolution solution—In a separatory funnel dissolve about 10 mg each of USP Phenyltoloxamine Citrate RS and USP Phenyltoloxamine Related Compound A RS, accurately weighed, in 50 mL of water. Add 5 mL of ammonium hydroxide, and extract with three 10-mL portions of methylene chloride. Combine the extracts, dry the solution over anhydrous sodium sulfate, and gently evaporate to dryness. Dissolve the residue in 20 mL of methylene chloride.

Test solution—In a separatory funnel dissolve about 400 mg of Phenyltoloxamine Citrate, accurately weighed, in 50 mL of water. Proceed as directed for *Resolution solution*, beginning with "Add 5 mL of ammonium hydroxide."

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Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a split injection system, a flame-ionization detector, and a 0.32-mm × 25-m column coated with a 0.45-µm film of phase G27. The carrier gas is helium, flowing at a rate of about 29 cm per second, with a split flow rate of about 25 mL per minute. The column temperature is programmed as follows. Initially the temperature of the column is equilibrated at 190° for 3 minutes, then the temperature is increased at a rate of 4° per minute to 240°, and maintained at 240° for 8 minutes. The injection port and the detector temperatures are maintained at 280°. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between phenyltoloxamine and phenyltoloxamine related compound A is not less than 1.5.

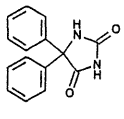
Procedure—Inject a volume (about 1 µL) of the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Phenyltoloxamine Citrate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response of each impurity; and r_s is the sum of the responses of all the peaks, excluding the solvent peaks: not more than 0.2% of phenyltoloxamine related compound A; not more than 0.1% of any other individual impurity; and not more than 1.0% of total impurities is found.

Assay—Dissolve about 0.5 g of Phenyltoloxamine Citrate, accurately weighed, in 80 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 44.75 mg of $C_{17}H_{21}NO \cdot C_6H_8O_7$.

Phenytoin



$C_{15}H_{12}N_2O_2$ 252.27

2,4-Imidazolidinedione, 5,5-diphenyl-; 5,5-Diphenylhydantoin [57-41-0].

DEFINITION

Phenytoin contains NLT 98.0% and NMT 102.0% of phenytoin ($C_{15}H_{12}N_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Solution A: Prepare a 0.05 M monobasic potassium phosphate solution and adjust with phosphoric acid to a pH of 2.5.

Solution B: Methanol and acetonitrile (60:40)

Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
23	60	40
38	42	58
45	30	70
50	30	70
51	60	40
55	60	40

Diluent: *Solution B* and water (1:1)

Standard solution: 0.2 mg/mL of USP Phenytoin RS in *Diluent.* Dissolve with the aid of sonication if necessary.

Sample solution: 0.2 mg/mL of Phenytoin in *Diluent.* Dissolve with the aid of sonication if necessary.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenytoin ($C_{15}H_{12}N_2O_2$) in the portion of Phenytoin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay.*

Standard solution: 1 µg/mL of USP Phenytoin RS, 5 µg/mL of USP Phenytoin Related Compound A RS, 9 µg/mL of USP Phenytoin Related Compound B RS, and 1 µg/mL of USP Benzophenone RS in *Diluent*

Sample solution: 1 mg/mL of Phenytoin in *Diluent*

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times are given in *Table 2.*]
Suitability requirements
Signal-to-noise ratio: NLT 10
Relative standard deviation: NMT 5.0% for the phenytoin peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified impurity in the portion of Phenytoin taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of specified impurity from the *Sample solution*
r_S = peak area of corresponding impurity from the *Standard solution*
C_S = concentration of corresponding impurity in the *Standard solution* (mg/mL)
C_U = concentration of Phenytoin in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Phenytoin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area for each unspecified impurity
r_S = peak area of phenytoin from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenytoin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenytoin related compound A	0.14	0.5
Phenytoin related compound B	0.53	0.9
Phenytoin	1.0	—
Benzophenone	2.11	0.1
Benzil	2.23	—
Any individual unspecified impurity	—	0.10
Total impurities[a]	—	0.9

[a] Excluding benzophenone.

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 4 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩
  USP Benzophenone RS
  Diphenylmethanone.
  $C_{13}H_{10}O$ 182.22
  USP Phenytoin RS
  USP Phenytoin Related Compound A RS
  2,2-Diphenylglycine.
  $C_{14}H_{13}NO_2$ 227.26
  USP Phenytoin Related Compound B RS
  2,2-Diphenyl-2-ureidoacetic acid.
  $C_{15}H_{14}N_2O_3$ 270.28

Add the following:

▲Phenytoin Compounded Topical Gel

DEFINITION

Phenytoin Compounded Topical Gel contains NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$).

Prepare Phenytoin Compounded Topical Gel 10 mg/g to 50 mg/g as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

For Phenytoin Compounded Topical Gel containing 10 mg/g of phenytoin:

Phenytoin	1 g
Polyethylene Glycol 300	10 g
PCCA Spira-Wash Gel,[a] a sufficient quantity to make	100 g

[a] PCCA, Houston, TX.

For Phenytoin Compounded Topical Gel containing 50 mg/g of phenytoin:

Phenytoin	5 g
Polyethylene Glycol 300	10 g
PCCA Spira-Wash Gel,[a] a sufficient quantity to make	100 g

[a] PCCA, Houston, TX.

In an appropriately sized electronic mortar and pestle container, add *Phenytoin*, *Polyethylene Glycol 300*, and sufficient *PCCA Spira-Wash Gel* to bring to final weight. Mix the mixture with an electronic mortar and pestle for 3 min at a speed of about 1450 rpm. Process through an ointment mill once at the middle setting and once at the finest setting to reduce the particle size of the active ingredient and reduce air content of the preparation. Return the mixture to the electronic mortar and pestle and mix again for 1 min at a speed of about 1130 rpm. Package in a light-resistant calibrated dispenser.

ASSAY

- **Procedure**
  Solution A: 0.1% trifluoroacetic acid in acetonitrile
  Solution B: 0.1% trifluoroacetic acid in water
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	3	97
1.5	55	45
1.6	3	97
2.0	3	97

Standard solution: 0.05 mg/mL of USP Phenytoin RS in methanol
Sample solution
For Topical Gel 10 mg/g: Transfer 0.5 g of Topical Gel into a 50-mL centrifuge tube, add 19.5 mL of methanol, and vortex for 1 min. Sonicate for 2 min and vortex for 1 min. Centrifuge the mixture for 10 min at 6000 rpm.

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Transfer 2 mL of the supernatant to a 10-mL volumetric flask and dilute with methanol to volume. Centrifuge for 10 min at 14,000 rpm.

For Topical Gel 50 mg/g: Transfer 0.5 g of Topical Gel into a 50-mL centrifuge tube, add 19.5 mL of methanol, and vortex for 1 min. Sonicate for 2 min and vortex for 1 min. Centrifuge the mixture for 10 min at 6000 rpm. Transfer 0.4 mL of the supernatant to a 10-mL volumetric flask and dilute with methanol to volume. Centrifuge for 10 min at 14,000 rpm.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Temperatures
Autosampler: 8°
Column: 65°
Flow rate: 1 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for phenytoin is about 1.3 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) in the portion of Topical Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenytoin from the *Sample solution*
r_S = peak response of phenytoin from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 7.2–8.2
- **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩: 500–3500 mPa · s

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in a tight, light-resistant calibrated dispenser. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 180 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator.
- **LABELING:** Label it to indicate that it is for external use only and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Phenytoin RS▲ (USP 1-May-2020)

Phenytoin Oral Suspension

DEFINITION
Phenytoin Oral Suspension is Phenytoin suspended in a suitable medium. It contains NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Shake a volume of Oral Suspension equivalent to 100 mg of phenytoin with 50 mL of a mixture of ether and chloroform (1 in 2) in a separator, evaporate the extract to dryness, and dry under vacuum at 105° for 4 h. Weigh 2–4 mg of the residue and 200 mg of potassium bromide in a mortar. Pestle, mix, and grind well, and prepare the potassium bromide pellet.
Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: Prepare a 0.05 M monobasic potassium phosphate solution, and adjust with phosphoric acid to a pH of 2.5.
Solution B: Methanol and acetonitrile (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
23	60	40
38	42	58
45	30	70
50	30	70
51	60	40
55	60	40

Diluent: *Solution B* and water (1:1)
Standard solution: 0.2 mg/mL of USP Phenytoin RS in *Diluent*. Dissolve with the aid of sonication, if necessary.
Sample solution: Nominally 0.2 mg/mL of phenytoin prepared as follows. Weigh and transfer a suitable volume of Oral Suspension containing the equivalent of 20 mg of phenytoin to a 100-mL volumetric flask. Add 20 mL of methanol, and dissolve. Dilute with *Diluent* to volume. Dissolve with the aid of sonication, if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Buffer 1: Dissolve 36.3 g of tris(hydroxymethyl) aminomethane and 60 g of sodium lauryl sulfate in 6 L of water, adjust with hydrochloric acid to a pH of 7.5, and degas.
Medium: *Buffer 1*; 900 mL
Apparatus 2: 35 rpm
Time: 60 min
Buffer 2: 2.76 g/L of monobasic sodium phosphate in water
Mobile phase: Methanol, acetonitrile, and *Buffer 2* (27:23:50). Adjust with phosphoric acid to a pH of 3.0.
Standard solution: 0.14 mg/mL of USP Phenytoin RS prepared as follows. Transfer a suitable amount of USP Phenytoin RS to a suitable volumetric flask. Dissolve in 3% of the flask volume of methanol. Dilute with *Medium* to volume.
Sample solution: Shake the sample suspension well (100 shakes). Determine the density, *d* (g/mL), of Oral Suspension using appropriate means. Using a 5-mL syringe, collect approximately 5 mL of Oral Suspension, and record the weight. With the paddles lowered, gently empty the contents of each syringe into the bottom of each vessel containing *Medium*. Start rotating the paddles. Reweigh each syringe, and determine the weight (g) of Oral Suspension delivered into each vessel. At the end of 60 min, remove 4 mL from each vessel, and pass through a nylon filter of 0.45-µm pore size, presaturated with *Medium*. [Note—Dilute with *Medium* if necessary to a concentration that is similar to the *Standard solution*.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for phenytoin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (d/W) \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor (necessary only if the *Sample solution* requires dilution)
d = density of Oral Suspension (g/mL)
W = weight of Oral Suspension delivered (g)
L = label claim of Oral Suspension (mg/mL)

Tolerances: NLT 80% (*Q*) of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩
For single-unit containers
Acceptance criteria: Meets the requirements

• **Deliverable Volume** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL of USP Phenytoin RS, 9 µg/mL of USP Phenytoin Related Compound A RS, and 9 µg/mL of USP Phenytoin Related Compound B RS in *Diluent*
Sample solution: Nominally 1 mg/mL of phenytoin prepared as follows. Weigh and transfer a suitable volume of Oral Suspension to an appropriate volumetric flask. Add methanol to about 20% of the final flask volume and dissolve. Dilute with *Diluent* to volume. Dissolve with the aid of sonication, if necessary.
System suitability
Sample: *Standard solution*
Suitability requirements
Signal-to-noise ratio: NLT 10
Relative standard deviation: NMT 5.0% for the phenytoin peak
Analysis
Samples: *Standard solution* and *Sample solution*
[Note—The relative retention times are given in *Table 2*.]
Calculate the percentage of phenytoin related compound A and phenytoin related compound B in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each specified impurity from the *Sample solution*
r_S = peak area of each specified impurity from the *Standard solution*
C_S = concentration of each specified impurity in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area for each unspecified impurity
r_S = peak area of phenytoin from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity less than 0.05%.

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Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenytoin related compound A	0.14	0.9
Phenytoin related compound B	0.53	0.9
Phenytoin	1.0	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	0.9

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature. Protect from freezing and light.
- **LABELING:** The label bears a statement that the patient must use an accurately calibrated measuring device with multiple-dose containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Phenytoin RS
  USP Phenytoin Related Compound A RS
  2,2-Diphenylglycine.
  $C_{14}H_{13}NO_2$ 227.26
  USP Phenytoin Related Compound B RS
  2,2-Diphenyl-2-ureidoacetic acid.
  $C_{15}H_{14}N_2O_3$ 270.28

Phenytoin Chewable Tablets

DEFINITION

Phenytoin Chewable Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Triethylamine and water (1:99)
Mobile phase: Methanol, acetonitrile, water, *Solution A*, and acetic acid (270:230:500:5:1)
Standard solution: 0.5 mg/mL of USP Phenytoin RS in *Mobile phase*
Sample solution: Nominally 0.5 mg/mL of phenytoin from NLT 20 finely powdered Chewable Tablets in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: NLT 2 times the retention time of the phenytoin peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Dissolve 6 g of tromethamine in 600 mL of water. Dilute with water to 1 L, and adjust with phosphoric acid to a pH of 9.0. Dissolve 10 g of sodium lauryl sulfate in 1 L of the prepared buffer; 900 mL.
Apparatus 2: 100 rpm
Time: 120 min
Solution A, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 3.0 mg/mL of USP Phenytoin RS in methanol
Standard solution: 0.012 mg/mL of USP Phenytoin RS prepared as follows. Transfer a suitable volume of the *Standard stock solution* to a suitable volumetric flask, and dilute with *Medium* to volume to obtain an intermediate concentration of 0.06 mg/mL of USP Phenytoin RS. Transfer a suitable volume of the resulting solution to a suitable volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Withdraw a portion of the solution under test and filter, discarding the first 3 mL of the filtrate. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
Solution B: Acetonitrile and methanol (40:60)
Mobile phase: See *Table 1*.

USP Monographs

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
23	60	40
38	42	58
45	30	70
50	30	70
51	60	40
55	60	40

Diluent: *Solution B* and water (50:50)
Standard solution: 1 µg/mL of USP Phenytoin RS, 5 µg/mL of USP Phenytoin Related Compound A RS, and 9 µg/mL of USP Phenytoin Related Compound B RS in *Diluent*
Sample solution: Nominally 1000 µg/mL of phenytoin prepared as follows. Finely powder NLT 20 Chewable Tablets and transfer a portion of the powder to an appropriate volumetric flask. Add *Diluent* to about 50% of the final flask volume, and shake the mixture mechanically for 15 min or until dissolved. Dilute with *Diluent* to volume, centrifuge, and use the supernatant. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0% for the phenytoin peak
Signal-to-noise ratio: NLT 10 for the phenytoin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenytoin related compound A and phenytoin related compound B in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenytoin related compound A or phenytoin related compound B from the *Sample solution*
r_S = peak response of phenytoin related compound A or phenytoin related compound B from the *Standard solution*
C_S = concentration of USP Phenytoin Related Compound A RS or USP Phenytoin Related Compound B RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of phenytoin in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of phenytoin from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of phenytoin in the *Sample solution* (µg/mL)

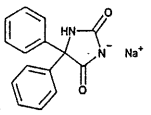
Acceptance criteria: See *Table 2*. Disregard any degradation product less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenytoin related compound A	0.14	0.5
Phenytoin related compound B	0.51	0.9
Phenytoin	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature. Protect from moisture.
- **LABELING:** Label the Chewable Tablets to indicate that they are to be chewed before swallowing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Phenytoin RS
  USP Phenytoin Related Compound A RS
  2,2-Diphenylglycine.
  $C_{14}H_{13}NO_2$ 227.26
  USP Phenytoin Related Compound B RS
  2,2-Diphenyl-2-ureidoacetic acid.
  $C_{15}H_{14}N_2O_3$ 270.28

Phenytoin Sodium



$C_{15}H_{11}N_2NaO_2$ 274.25
2,4-Imidazolidinedione, 5,5-diphenyl-, monosodium salt; 5,5-Diphenylhydantoin sodium salt [630-93-3].

DEFINITION
Phenytoin Sodium contains NLT 98.0% and NMT 102.0% of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

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• **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩
Solution A: Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of tetramethylammonium hydroxide TS, and add 20 mL of dehydrated alcohol.
Solution B: 158 mg/mL of ammonium carbonate in water
Sample solution: Ignite 1 g, and cool. Add 2 mL of water to the residue, and neutralize the solution with hydrochloric acid. Filter, and dilute the filtrate with water to 4 mL.
Analysis: To 0.1 mL of the *Sample solution* add 1.5 mL of *Solution A*, and cool in ice water for 30 min. A voluminous, white, crystalline precipitate is formed. Place in water at 20°, and stir for 5 min.
Acceptance criteria: The precipitate does not disappear. Add 1 mL of ammonia TS. The precipitate dissolves completely. Add 1 mL of *Solution B*. No precipitate is formed.
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 0.05 M monobasic ammonium phosphate buffer, adjusted with phosphoric acid to a pH of 2.5
Mobile phase: Acetonitrile, methanol, and *Buffer* (35:20:45)
System suitability solution: 0.1 mg/mL of USP Phenytoin RS and 0.15 mg/mL of benzoin in *Mobile phase*
Standard solution: 0.05 mg/mL of USP Phenytoin RS in *Mobile phase*
Sample solution: 0.05 mg/mL of Phenytoin Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for phenytoin and benzoin are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 1.5 between phenytoin and benzoin, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) in the portion of Phenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = concentration of Phenytoin Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of phenytoin sodium, 274.25
M_{r2} = molecular weight of phenytoin, 252.27

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.5 µg/mL of benzophenone, 1 µg/mL of USP Phenytoin RS, 9 µg/mL of USP Phenytoin Related Compound A RS, and 9 µg/mL of USP Phenytoin Related Compound B RS in *Mobile phase*
Sample solution: 1 mg/mL of Phenytoin Sodium in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for phenytoin and benzoin are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 1.5 between phenytoin and benzoin, *System suitability solution*
Relative standard deviation: NMT 5.0% for each compound, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenytoin related compound A, phenytoin related compound B, and benzophenone in the portion of Phenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenytoin related compound A, phenytoin related compound B, or benzophenone from the *Sample solution*
r_S = peak response of phenytoin related compound A, phenytoin related compound B, or benzophenone from the *Standard solution*
C_S = concentration of the corresponding analyte in the *Standard solution* (µg/mL)
C_U = concentration of Phenytoin Sodium in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Phenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of phenytoin from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (µg/mL)
C_U = concentration of Phenytoin Sodium in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of phenytoin sodium, 274.25
M_{r2} = molecular weight of phenytoin, 252.27

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%, w/w)
Phenytoin related compound A	0.5	0.5
Phenytoin related compound B	0.6	0.9
Phenytoin	1.0	—
Benzophenone	2.9	0.1

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Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%, w/w)
Any individual unspecified impurity	—	0.10
Total impurities[a]	—	0.9

[a] Excluding benzophenone.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 4 h.
  Acceptance criteria: NMT 2.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Phenytoin RS
  USP Phenytoin Related Compound A RS
  Diphenylglycine.
  $C_{14}H_{13}NO_2$ 227.26
  USP Phenytoin Related Compound B RS
  Diphenylhydantoic acid.
  $C_{15}H_{14}N_2O_3$ 270.29
  USP Phenytoin Sodium RS

Extended Phenytoin Sodium Capsules

DEFINITION

Extended Phenytoin Sodium Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)
  Sample: 300 mg of phenytoin sodium from the contents of Capsules in 50 mL of water in a separator. Add 10 mL of 3 N hydrochloric acid, and extract with three successive portions, measuring 100, 60, and 30 mL, respectively, of ether and chloroform (1:2). Evaporate the combined extracts, and dry the residue of phenytoin at 105° for 4 h.
  Acceptance criteria: The spectrum of the *Sample* corresponds to that of a similarly prepared USP Phenytoin RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 0.05 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.5.
  Mobile phase: Methanol and *Buffer* (55:45)
  Standard solution: 0.6 mg/mL of USP Phenytoin RS in *Mobile phase*. [NOTE—Dissolve the required quantity of phenytoin in a small amount of methanol before diluting with *Mobile phase*.]
  Sample stock solution: Transfer the contents of 10 Capsules to a 250-mL volumetric flask. Add 150 mL of methanol, and sonicate for 20 min. Cool to room temperature, and dilute with methanol to volume.
  Sample solution: Nominally 0.6 mg/mL of phenytoin from the *Sample stock solution* in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 229 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 3000 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phenytoin from the *Sample solution*
r_S = peak response of phenytoin from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of phenytoin sodium, 274.25
M_{r2} = molecular weight of phenytoin, 252.27

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: Water; 900 mL
  Apparatus 1: 50 rpm
  Times: 30, 60, and 120 min
  Mobile phase: Methanol and water (70:30)
  Standard solution: Dissolve USP Phenytoin RS in methanol, and dilute with water to obtain a concentration similar to that of the *Sample solution*.
  Sample solution: Pass a portion of the solution under test through a suitable filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 229 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 3200 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (100/L)$$

r_U = peak response from the *Sample solution*

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r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of phenytoin sodium, 274.25
M_{r2} = molecular weight of phenytoin, 252.27
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances (for products labeled as 30-mg Capsules): The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 40% (Q) in 30 min, 56% (Q') in 60 min, and NLT 65% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 1*.

Table 1

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between Q – 15% and Q – 5%, is within the range Q' ± 10%, and is NLT Q'' + 5% at the stated *Times*.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between Q – 10% and Q, is within the range Q' ± 8%, and is NLT Q''; no unit is outside the range between Q – 20% and Q + 10%, no unit is outside the range Q' ± 18%, and no unit is less than Q'' – 10% at the stated *Times*.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between Q – 10% and Q, is within the range Q' ± 8%, and is NLT Q''; NMT 2 units are outside the range between Q – 20% and Q + 10%, and no unit is outside the range Q – 30% and Q + 20%; NMT 2 units are outside the range Q' ± 18%, and no unit is outside the range Q' ± 25%; NMT 2 units are less than Q'' – 10%, and no unit is less than Q'' – 20% at the stated *Times*.

Tolerances (for products labeled as 100-mg Capsules): The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 45% (Q) in 30 min, 60% (Q') in 60 min, and NLT 70% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 2*.

Table 2

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between Q – 25% and Q – 5%, is equal to Q' ± 20%, and is NLT Q'' + 5% at the stated *Times*.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between Q – 20% and Q, is within the range Q' ± 15%, and is NLT Q''; no unit is outside the range between Q – 30% and Q + 10%, no unit is outside the range Q' ± 25%, and no unit is less than Q'' – 10% at the stated *Times*.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between Q – 20% and Q, is within the range Q' ± 15%, and is NLT Q''; NMT 2 units are outside the range between Q – 30% and Q + 10%, and no unit is outside the range between Q – 40% and Q + 20%; NMT 2 units are outside the range Q' ± 25%, and no unit is outside the range Q' ± 35%; NMT 2 units are less than Q'' – 10%, and no unit is less than Q'' – 20% at the stated *Times*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Proceed as directed in *Test 1*, except use *Apparatus 1* at 75 rpm and the following *Tolerances*.

Tolerances (for products labeled as 100-mg Capsules): The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 45% (Q) in 30 min, 65% (Q') in 60 min, and NLT 70% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 3*.

Table 3

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between Q – 25% and Q – 5%, is equal to Q' ± 20%, and is NLT Q'' + 5% at the stated *Times*.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between Q – 25% and Q – 5%, is within the range of Q' – 20% and Q' + 10%, and is NLT Q''; no unit is outside the range between Q – 30% and Q + 5%, no unit is outside the range Q' – 25% and Q' + 20%, and no unit is less than Q'' –10% at the stated *Times*.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between Q – 25% and Q – 5%, is within the range of Q' – 20% and Q' + 10%, and is NLT Q''; NMT 2 units are outside the range between Q – 30% and Q + 5%; and no unit is outside the range of Q – 40% and Q + 15%; NMT 2 units are outside the range Q' – 25% and Q' + 20%, and no unit is outside the range Q' – 35% and Q' + 25%; NMT 2 units are less than Q'' – 10%; and no unit is less than Q'' – 20% at the stated *Times*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 1: 75 rpm
Times: 30, 60, and 120 min
Determine the amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved by using the method described in *Test 1*.
Tolerances (for products labeled as 200- and 300-mg Capsules): The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 30% (Q) in 30 min, 50% (Q') in 60 min, and NLT 60% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 4*.

Table 4

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between Q – 20% and Q + 5%, is equal to Q' – 20% and Q' + 25%, and is NLT Q'' + 5% at the stated *Times*.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between Q – 20% and Q, is within the range of Q' ± 20%, and is NLT Q''; no unit is outside the range between Q – 25% and Q + 10%, no unit is outside the range Q' ± 25%, and no unit is less than Q'' – 10% at the stated *Times*.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between Q – 20% and Q, is within the range of Q' ± 20%, and is NLT Q''; NMT 2 units are outside the range between Q – 25% and Q + 10%, and no unit is outside the range Q – 25% and Q + 15%; NMT 2 units are outside the range Q' ± 25%; and no unit is outside the range Q' ± 30%; NMT 2 units are less than Q'' – 10%; and no unit is less than Q'' – 20% at the stated *Times*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium, Apparatus 1, Times, and **Analysis:** Proceed as directed for *Test 1*.

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Tolerances (for products labeled as 30-mg Capsules): The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 40% (Q) in 30 min, 56% (Q') in 60 min, and NLT 65% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 5*.

Table 5

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between Q – 10% and Q, is within the range Q' – 9% and Q' + 7%, and is NLT Q'' + 5% at the stated *Times*.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between Q – 8% and Q + 2%, is within the range Q' – 9% and Q' + 7%, and is NLT Q''; no unit is outside the range between Q – 20% and Q + 10%, no unit is outside the range Q' – 19% and Q' + 17%, and no unit is less than Q'' – 10% at the stated *Times*.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between Q – 8% and Q + 2%, is within the range Q' – 9% and Q' + 7%, and is NLT Q''; NMT 2 units are outside the range between Q – 20% and Q + 10%, and no unit is outside the range Q – 30% and Q + 20%; NMT 2 units are outside the range Q' – 19% and Q' + 17%, and no unit is outside the range Q' – 26% and Q' + 24%; NMT 2 units are less than Q'' – 10%, and no unit is less than Q'' – 20% at the stated *Times*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium, Apparatus 1, Times, and **Analysis:** Proceed as directed for *Test 1*.

Tolerances (for products labeled as 100-mg Capsules): The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 45% (Q) in 30 min, 65% (Q') in 60 min, and NLT 80% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 6*.

Table 6

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between Q – 25% and Q – 5%, is between Q' + 20% and Q'– 15%, and is NLT Q'' + 5% at the stated *Times*.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between Q – 25% and Q – 5%, is within the range Q' ± 15%, and is NLT Q''; no unit is outside the range between Q – 30% and Q + 10%, no unit is outside the range Q' + 25% and Q'– 20%, and no unit is less than Q'' – 10% at the stated *Times*.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between Q – 25% and Q – 5%, is within the range Q' ± 15%, and is NLT Q''; NMT 2 units are outside the range between Q – 30% and Q + 10%, and no unit is outside the range Q – 35% and Q + 20%; NMT 2 units are outside the range between Q' + 25% and Q'– 20%, and no unit is outside the range Q' + 30% and Q'– 25%; NMT 2 units are less than Q'' – 10%, and no unit is less than Q'' – 20% at the stated *Times*.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 600 µg/mL of USP Phenytoin RS, 3 µg/mL of USP Phenytoin Related Compound A RS, and 3 µg/mL of USP Phenytoin Related Compound B RS in methanol

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for phenytoin related compound A, phenytoin related compound B, and phenytoin are 0.38, 0.45, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between phenytoin related compound B and phenytoin; NLT 1.5 between phenytoin related compound A and phenytoin related compound B

Tailing factor: NMT 2.0 for the phenytoin peak

Relative standard deviation: NMT 2.0% for phenytoin; NMT 5.0% for phenytoin related compound A or phenytoin related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each phenytoin related compound in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenytoin related compound A or phenytoin related compound B from the *Sample solution*

r_S = peak response of phenytoin related compound A or phenytoin related compound B from the *Standard solution*

C_S = concentration of the corresponding analyte in the *Standard solution* (µg/mL)

C_U = nominal concentration of phenytoin in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of phenytoin from the *Standard solution*

C_S = concentration of USP Phenytoin RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of phenytoin in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 7*.

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenytoin related compound A	0.38	0.5
Phenytoin related compound B	0.45	1.0
Phenytoin	1.0	—
Any individual, unspecified degradation product	—	0.2

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Protect from moisture. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Phenytoin RS
  USP Phenytoin Related Compound A RS
  Diphenylglycine.
  $C_{14}H_{13}NO_2$ 227.26
  USP Phenytoin Related Compound B RS
  Diphenylhydantoic acid.
  $C_{15}H_{14}N_2O_3$ 270.29

Phenytoin Sodium Injection

DEFINITION

Phenytoin Sodium Injection is a sterile solution of Phenytoin Sodium with Propylene Glycol and Alcohol in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
  Sample: Transfer an equivalent of 250 mg of phenytoin sodium from a volume of Injection to a separator containing 25 mL of water. Extract first with 50 mL of ethyl acetate and then with two additional 30-mL portions of ethyl acetate. Wash each extract with two 20-mL portions of sodium acetate solution (10 mg/mL). Evaporate the combined ethyl acetate extracts, and dry the residue of phenytoin at 105° to constant weight.
  Acceptance criteria: Residue obtained from the *Sample* meets the requirements.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Methanol and water (55:45)
  Standard solution: 0.23 mg/mL of USP Phenytoin RS in *Mobile phase*
  Sample solution: Nominally 0.25 mg/mL of phenytoin sodium from a volume of Injection equivalent to 250 mg of phenytoin sodium in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 25-cm; packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenytoin sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of phenytoin sodium, 274.25
M_{r2} = molecular weight of phenytoin, 252.27

Acceptance criteria: 95.0%–105.0%

OTHER COMPONENTS

- **ALCOHOL AND PROPYLENE GLYCOL CONTENT**
  Internal standard solution: 0.02 mL/mL of methanol and 0.04 mL/mL of ethylene glycol in water
  Standard stock solution: 0.01 mL/mL of alcohol from USP Alcohol Determination–Alcohol RS and 0.04 mL/mL of USP Propylene Glycol RS in water
  Standard solution: 0.005 mL/mL of alcohol and 0.02 mL/mL of propylene glycol prepared as follows. Pipet 5 mL each of *Standard stock solution* and *Internal standard solution* into a 10-mL volumetric flask.
  Sample solution: 0.05 mL/mL of Injection in water prepared as follows. Pipet 5 mL of Injection and 50 mL of the *Internal standard solution* into a 100-mL volumetric flask, and dilute with water to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: GC
  Detector: Flame ionization
  Column: 30-m × 0.53-mm (ID) capillary column with a 1-µm coating of G16 phase
  Temperatures
  Injection port: 240°
  Detector: 250°
  Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	4
50	15	230	5

Carrier gas: Hydrogen or helium
Flow rate: 5 mL/min
Injection volume: 0.2 µL
Injection type: Split ratio, 10:1
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between methanol and alcohol; NLT 5.0 between propylene glycol and ethylene glycol
Relative standard deviation: NMT 2.0% for each of the response ratios of alcohol to methanol and propylene glycol to ethylene glycol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the alcohol content, as a percentage, in the portion of Injection taken:

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$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alcohol to methanol from the *Sample solution*
R_S = peak response ratio of alcohol to methanol from the *Standard solution*
C_S = concentration of alcohol in the *Standard solution* (mL/mL)
C_U = nominal concentration of the Injection in the *Sample solution* (mL/mL)

Calculate the propylene glycol content, as a percentage, in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of propylene glycol to ethylene glycol from the *Sample solution*
R_S = peak response ratio of propylene glycol to ethylene glycol from the *Standard solution*
C_S = concentration of propylene glycol in the *Standard solution* (mL/mL)
C_U = nominal concentration of the Injection in the *Sample solution* (mL/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methanol[a]	0.27	—
Alcohol	0.32	9.0–11.0
Propylene glycol	0.98	37.0–43.0
Ethylene glycol[a]	1.0	—

[a] Internal standard included for peak identification purposes only.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.3 USP Endotoxin Unit/mg of phenytoin sodium.
- **pH** ⟨791⟩: 10.0–12.3
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements under small-volume injections.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, at controlled room temperature.
- **LABELING:** The label states the following: "Do not use the Injection if it is hazy or contains a precipitate."
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alcohol Determination–Alcohol RS
  USP Phenytoin RS
  USP Propylene Glycol RS

Physostigmine Salicylate

$C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$ 413.47
Pyrrolo[2,3-*b*]indol-5-ol, 1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethyl-, methylcarbamate (ester), (3*aS*-*cis*)-, mono(2-hydroxybenzoate);
Physostigmine monosalicylate [57-64-7].

DEFINITION
Physostigmine Salicylate contains NLT 97.0% and NMT 102.0% of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$), calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩
  Analysis: Use 1 g of sodium bicarbonate instead of 2 mL of 1 N sodium hydroxide.
  Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Salicylate* ⟨191⟩: Meets the requirements

ASSAY
- **PROCEDURE**
  Sample: 250 mg of Physostigmine Salicylate
  Analysis: Dissolve the *Sample* in 25 mL of chloroform, and add 25 mL of glacial acetic acid. Titrate with 0.02 N perchloric acid in dioxane VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N perchloric acid is equivalent to 8.270 mg of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$).
  Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩
  Sample: 100 mg
  Acceptance criteria: Negligible
- **SULFATE**
  Sample solution: Precipitate the salicylic acid from 10 mL of a cold, saturated solution of Physostigmine Salicylate with a slight excess of 3 N hydrochloric acid, and filter.
  Analysis: Add 5 drops of barium chloride TS to the *Sample solution*.
  Acceptance criteria: No turbidity is produced immediately.

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: –91° to –94°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry over silica gel for 24 h.
  Acceptance criteria NMT 1.0%
- **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩
  Sample solution: Dissolve 100 mg in 5 mL of sulfuric acid.
  Acceptance criteria: At the end of 5 min, the *Sample solution* has no more color than *Matching Fluid I*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Physostigmine Salicylate RS

Physostigmine Salicylate Injection

DEFINITION
Physostigmine Salicylate Injection is a sterile solution of Physostigmine Salicylate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$). It may contain an antimicrobial agent and an antioxidant.
[NOTE—Do not use the Injection if it is more than slightly discolored.]

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IDENTIFICATION

• **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩
Analysis: Use 1 g of sodium bicarbonate instead of 2 mL of 1 N sodium hydroxide.
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL,** *Salicylate* ⟨191⟩**:** Meets the requirements

ASSAY

• **PROCEDURE**
Buffer: 3.85 g/L of ammonium acetate in water. Adjust, if necessary, with glacial acetic acid or ammonium hydroxide to a pH of 6 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (50:50)
Solution A: 100 µL of USP Benzyl Alcohol RS and 1 µL of benzaldehyde in 400 mL of acetonitrile
Standard solution: 0.03 mg/mL of USP Physostigmine Salicylate RS in *Solution A*
Sample solution: Nominally 0.03 mg/mL from a suitable volume of Injection containing NLT 3 mg of physostigmine salicylate diluted with acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *Solution A* and *Standard solution*
[NOTE—If the peaks due to benzyl alcohol and benzaldehyde co-elute when *Solution A* is injected, the *Standard solution* will exhibit only two peaks instead of three. In a suitable system, benzyl alcohol and benzaldehyde elute before physostigmine.]
Suitability requirements
Resolution: NLT 2.0 between the physostigmine peak and the adjacent peak (benzyl alcohol or benzaldehyde, or the combination of these), *Standard solution*
Column efficiency: NLT 1200 theoretical plates from the analyte peak, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Physostigmine Salicylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of physostigmine salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 83.4 USP Endotoxin Units/mg of physostigmine salicylate
• **pH** ⟨791⟩**:** 3.5–5.0
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, protected from light.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzyl Alcohol RS
USP Physostigmine Salicylate RS

Delete the following:

▲Physostigmine Salicylate Ophthalmic Solution

DEFINITION

Physostigmine Salicylate Ophthalmic Solution is a sterile, aqueous solution of physostigmine salicylate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$). It may contain suitable antimicrobial agents, buffers, and stabilizers and suitable additives to increase its viscosity.

IDENTIFICATION

• **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩
Analysis: Use 1 g of sodium bicarbonate instead of 2 mL of 1 N sodium hydroxide.
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL,** *Salicylate* ⟨191⟩**:** Meets the requirements

ASSAY

• **PROCEDURE**
Buffer: 3.85 g/L of ammonium acetate in water adjusted, if necessary, with glacial acetic acid or ammonium hydroxide to a pH of 6 ± 0.1
Mobile phase: Acetonitrile and *Buffer* (50:50)
Standard solution: 0.03 mg/mL of USP Physostigmine Salicylate RS in acetonitrile
Sample solution: Nominally 0.03 mg/mL of physostigmine salicylate from a volume of Ophthalmic Solution containing NLT 3 mg of physostigmine salicylate diluted with acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1200 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Physostigmine Salicylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of physostigmine salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

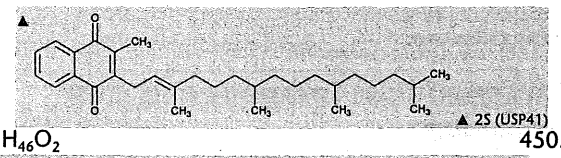
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SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 2.0–4.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Physostigmine Salicylate RS▲ (USP 1-May-2020)

Phytonadione

Change to read:



▲ 2S (USP41)

$C_{31}H_{46}O_2$ 450.70

▲2-Methyl-3-(3,7,11,15-tetramethylhexadec-2-en-1-yl) naphthalene-1,4-dione;▲ 2S (USP41)
Phylloquinone [▲81818-54-4▲ 2S (USP41)].

DEFINITION
Phytonadione is a mixture of *E*- and *Z*-isomers containing NLT 97.0% and NMT 103.0% of phytonadione isomers ($C_{31}H_{46}O_2$). It contains NMT 21.0% of the *Z*-isomer.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Analytical wavelength: 248 nm
Sample solution: 10 µg/mL in *n*-hexane
Acceptance criteria: Meets the requirements. Absorptivities do not differ by more than 3.0%.

ASSAY
- **PROCEDURE**

[NOTE—Protect solutions containing phytonadione from exposure to light.]

Mobile phase: *n*-Hexane and *n*-amyl alcohol (2000: 1.5)
Internal standard solution: 2.5 mg/mL of cholesteryl benzoate in *Mobile phase*
Standard solution: Prepare a 96-µg/mL solution of USP Phytonadione RS in *Mobile phase*. Pipet 10 mL of this solution and 7 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Prepare as directed for the *Standard solution*, using Phytonadione instead of USP Phytonadione RS.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*

[NOTE—The relative retention times for the internal standard, (*Z*)-phytonadione, and (*E*)-phytonadione are 0.7, 0.9, and 1.0, respectively.]

Suitability requirements
Resolution: NLT 1.5 between (*Z*)-phytonadione and (*E*)-phytonadione
Relative standard deviation: NMT 2.0% for the ratios of the sum of peak areas of (*Z*)-phytonadione and (*E*)-phytonadione to the peak area of the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phytonadione isomers ($C_{31}H_{46}O_2$) in the portion of Phytonadione taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio [sum of the peak areas of (*Z*)-phytonadione and (*E*)-phytonadione/peak area of the internal standard] from the *Sample solution*
R_S = internal standard ratio [sum of the peak areas of (*Z*)-phytonadione and (*E*)-phytonadione/peak area of the internal standard] from the *Standard solution*
C_S = concentration of USP Phytonadione RS in the *Standard solution* (µg/mL)
C_U = concentration of Phytonadione in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% of phytonadione isomers

OTHER COMPONENTS
- ***Z*-ISOMER CONTENT**

[NOTE—Protect solutions containing phytonadione from exposure to light.]

Mobile phase, Internal standard solution, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in the *Assay*, except calculate the percentage of *Z*-isomer in the portion of Phytonadione taken:

$$\text{Result} = [r_Z/(r_Z + r_E)] \times 100$$

r_Z = peak area of the (*Z*)-phytonadione isomer from the *Sample solution*
r_E = peak area of the (*E*)-phytonadione isomer from the *Sample solution*

Acceptance criteria: NMT 21.0%

IMPURITIES
- **LIMIT OF MENADIONE**

Sample: 20 mg of Phytonadione
Analysis: Mix the *Sample* with 0.5 mL of a mixture of 6 N ammonium hydroxide and alcohol (1:1). Add 1 drop of ethyl cyanoacetate, and shake gently.
Acceptance criteria: No purple or blue color is produced.

SPECIFIC TESTS
- **REFRACTIVE INDEX** ⟨831⟩: 1.523–1.526
- **REACTION:** A 50-mg/mL solution of Phytonadione in dehydrated alcohol is neutral to litmus.

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ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store in a cool place.▲ 25 (USP41)
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Phytonadione RS

Phytonadione Injectable Emulsion

» Phytonadione Injectable Emulsion is a sterile, aqueous dispersion of Phytonadione. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{31}H_{46}O_2$. It contains suitable solubilizing and/or dispersing agents.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Phytonadione RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 14.0 USP Endotoxin Units per mg of phytonadione.

pH ⟨791⟩: between 3.5 and 7.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

[NOTE—Use low-actinic glassware throughout this assay, and otherwise protect the solutions from exposure to light.]

Mobile phase—Prepare a suitable degassed mixture of dehydrated alcohol and water (95:5).

Standard preparation—Dissolve an accurately weighed quantity of USP Phytonadione RS in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.1 mg per mL.

Assay preparation 1 (containing 10 mg or more of phytonadione per mL)—Pipet a volume of Injectable Emulsion, equivalent to 10 mg of phytonadione, into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation 2 (containing less than 10 mg of phytonadione per mL)—Pipet a volume of Injectable Emulsion, equivalent to 1 mg of phytonadione, into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 0.7 mL per minute. Chromatograph five replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak response for the major peak. Calculate the quantity, in mg, of $C_{31}H_{46}O_2$ in each mL of the Injectable Emulsion taken by the formula:

$$D(C/V)(r_U/r_S)$$

in which D is 100 if the Injectable Emulsion contains 10 mg or more of phytonadione per mL, or 10 if the Injectable Emulsion contains less than 10 mg of phytonadione per mL; C is the concentration, in mg per mL, of USP Phytonadione RS in the *Standard preparation;* V is the volume, in mL, of Injectable Emulsion taken; and r_U and r_S are the peak responses of phytonadione obtained from the appropriate *Assay preparation* and the *Standard preparation,* respectively.

Add the following:

▲Phytonadione Compounded Oral Suspension

DEFINITION

Phytonadione Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of phytonadione ($C_{31}H_{46}O_2$).

Prepare Phytonadione Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Phytonadione Injectable Emulsion[a] equivalent to	30 mg of phytonadione
Simple Syrup,[b] a sufficient quantity to make	30 mL

[a] Phytonadione 10-mg/mL Injectable Emulsion, Hospira, Inc., Lake Forest, IL.
[b] Simple Syrup, Humco, Inc., Texarkana, TX.

Open the ampule(s) of *Phytonadione Injection Emulsion* and withdraw the contents through a 5-µm filter needle. Remove the filter needle and transfer the *Phytonadione Injection Emulsion* into a calibrated container. Add sufficient *Simple Syrup* to bring to final volume. Mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and water (95:5)

Standard solution: 0.04 mg/mL of USP Phytonadione RS in methanol

Sample solution: Transfer 1.0 mL of Oral Suspension into a 25-mL volumetric flask and add methanol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 10-cm; 2.6-µm packing L1
Temperatures
Autosampler: 4°
Column: 35°
Flow rate: 1.2 mL/min
Injection volume: 40 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for phytonadione is about 11.6 min.]
Suitability requirements
Tailing factor: NMT 2.0

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Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phytonadione ($C_{31}H_{46}O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phytonadione from the *Sample solution*

r_S = peak response of phytonadione from the *Standard solution*

C_S = concentration of USP Phytonadione RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phytonadione in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 3.2–4.2

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistance containers. Store at controlled room temperature or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or NMT 90 days after the date on which it was compounded when stored in a refrigerator
- **Labeling:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩

USP Phytonadione RS▲ (USP 1-Aug-2019)

Phytonadione Tablets

DEFINITION

Phytonadione Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phytonadione ($C_{31}H_{46}O_2$).

IDENTIFICATION

- **A. Ultraviolet Absorption**

Standard solution: 0.01 mg/mL of USP Phytonadione RS in dehydrated alcohol

Sample solution: A portion of finely powdered Tablets, equivalent to 0.01 mg/mL of phytonadione in dehydrated alcohol. Shake vigorously, and filter. Use the filtrate.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution* concomitantly measured.

- **B. HPLC Identification Test**

Analysis: Proceed as directed in the *Assay.*

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY

- **Procedure**

[Note—Use low-actinic glassware throughout the *Assay,* and otherwise protect the solutions from light.]

Mobile phase: Dehydrated alcohol and water (95:5)

Standard solution: 0.10 mg/mL of USP Phytonadione RS in dehydrated alcohol

Sample solution: 0.1 mg/mL of phytodione in dehydrated alcohol prepared as follows. Mix a portion of finely powdered Tablets (NLT 20) with dehydrated alcohol to obtain a nominal concentraion of 0.4 mg/mL of phytonadione. Shake by mechanical means for 15 min, dilute with dehydrated alcohol to 0.10 mg/mL, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution* (three replicate injections)

Suitability requirements

Column efficiency: NLT 915 theoretical plates, determined from the analyte peak

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

[Note—*Z*- and *E*-isomers coelute in this chromatographic system.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phytonadione ($C_{31}H_{46}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Phytonadione RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phytonadione in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Disintegration** ⟨701⟩

Time: 30 min

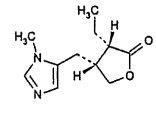
- **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **USP Reference Standards** ⟨11⟩

USP Phytonadione RS

Pilocarpine



$C_{11}H_{16}N_2O_2$ 208.26

2(3*H*)-Furanone, 3-ethyldihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-, (3*S*-*cis*)-.

Pilocarpine [92-13-7].

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» Pilocarpine contains not less than 95.0 percent and not more than 100.5 percent of pilocarpine ($C_{11}H_{16}N_2O_2$), calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, in a cold place.

USP Reference standards ⟨11⟩—
USP Pilocarpine RS
USP Pilocarpine Nitrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197F*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests ⟨197⟩, Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 20 µg per mL.
Medium: water.

Specific rotation ⟨781S⟩: between +102° and +107°.
Test solution: 20 mg per mL, in pH 6.0 phosphate buffer.

Refractive index ⟨831⟩: between 1.5170 and 1.5210 at 25°, determined in a liquid specimen. If crystals are present, first warm to about 40°.

Water Determination, *Method I* ⟨921⟩: not more than 0.5%.

Chloride—
Standard chloride solution—Transfer 165 mg of sodium chloride to a 100-mL volumetric flask, and dissolve in and dilute with water to volume. Transfer 25.0 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume. This solution contains 25 µg of chloride per mL.
Test solution—Transfer about 1.0 g of Pilocarpine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume.
Procedure—Transfer 5.0 mL of the *Test solution* to a test tube, add 0.6 mL of diluted nitric acid and 0.3 mL of silver nitrate TS: any opalescence produced is not greater than that produced by an identically treated solution containing 5.0 mL of the *Standard chloride solution* (0.25%).

Sulfate—
Standard sulfate solution—Dissolve 148 mg of anhydrous sodium sulfate in water, and dilute with water to 100 mL. Dilute 10.0 mL of this solution with water to 1000 mL. This solution contains 10 µg of sulfate per mL.
Procedure—To about 1 g of Pilocarpine in a test tube add 1 mL of 6 N hydrochloric acid and 4 mL of water, and mix. For the control, transfer 4.0 mL of *Standard sulfate solution* to a test tube, add 1 mL of 6 N hydrochloric acid, and mix. Adjust both solutions with pH indicator paper by the dropwise addition of 3 N hydrochloric acid or 6 N ammonium hydroxide, if necessary, to a pH of between 2 and 3. Add water to maintain the same volume in the control and test specimen tubes. To each tube add 1 mL of barium chloride TS, and mix: any turbidity produced in the specimen tube after 10 minutes' standing is not greater than that produced in the control (0.004%).

Limit of nitrate—
Standard preparation—Prepare a solution of USP Pilocarpine Nitrate RS to contain 43 µg per mL. This solution contains the equivalent of 10 µg of nitrate ion per mL.
Test preparation—Prepare a solution of Pilocarpine to contain 200 mg per mL.
Procedure—Transfer 0.5-mL portions of the *Test preparation* and of the *Standard preparation*, respectively, to separate test tubes, and to each tube add 1 drop of a 1 in 100 solution of sulfanilic acid in 5 N acetic acid and 1 drop of a 3 in 1000 solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 5 N acetic acid. Adjust the *Standard preparation* and the *Test preparation* with pH indicator paper by the dropwise addition of 3 N hydrochloric acid or 1 N ammonium hydroxide, if necessary to a pH of between 2 and 3. To each solution add a few granules of acid-washed, nitrate-free zinc. Heat the test tubes in a water bath at a temperature of about 32°. Allow 5 minutes for the development of a pink color: any pink color observed in the *Test preparation* is not greater than that observed in the *Standard preparation* (0.005%).

Related compounds—
Buffer solution , *Mobile phase*, *System suitability preparation*, and *Chromatographic system*—Proceed as directed in the *Assay*.
Standard solution—Prepare a solution in water of isopilocarpine nitrate to contain 1.5 µg per mL.
Test preparation—Prepare as directed for *Assay preparation* in the *Assay*.
Procedure—Separately inject equal volumes (about 40 µL) of the *Standard solution* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of isopilocarpine in the portion of Pilocarpine taken by the formula:

$$(208.26/271.27)50(C/W)(r_U/r_S)$$

in which 208.26 and 271.27 are the molecular weights of pilocarpine and isopilocarpine nitrate, respectively, C is the concentration, in µg per mL, of isopilocarpine nitrate in the *Standard solution*, W is the weight, in mg, of Pilocarpine taken, and r_U and r_S are the peak responses due to isopilocarpine in the *Test preparation* and the *Standard solution*, respectively: not more than 2% of isopilocarpine is found. Calculate the percentage of all other impurities from the chromatogram of the *Test preparation* taken by the formula:

$$(208.26/271.27)50(C/W)(r_i/r_S)$$

in which r_i is the peak response due to the impurity: no one impurity corresponding to one of the four peaks in the *System suitability preparation* exceeds 3%; no other individual impurity exceeds 0.5%. The sum total of all impurities, including isopilocarpine, is not more than 5.0%.

Assay—
Buffer solution—Transfer 13.5 mL of phosphoric acid to a 1-liter beaker containing 700 mL of water. Add 3 mL of triethylamine, and dilute with water to 1000 mL. Adjust with 20% sodium hydroxide to a pH of 3.0.
Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (98:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—Do not store this *Mobile phase* for more than 2 days.]
Standard preparation—Prepare a solution in water having an accurately known concentration of about 40 µg of USP Pilocarpine Nitrate RS per mL. [NOTE—Use this solution within 24 hours of its preparation.]
Assay preparation—Transfer an accurately weighed quantity of about 15 mg of Pilocarpine to a 500-mL volumetric flask. Dilute with water to volume, and mix. [NOTE—Use this solution within 24 hours of its preparation.]
System suitability preparation—Transfer accurately weighed quantities of about 30 mg each of pilocarpine hydrochloride

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and isopilocarpine nitrate to a 50-mL volumetric flask, and dilute with water to volume. Transfer 25 mL of this solution to a suitable flask, add 5 mL of 1 N sodium hydroxide, and reflux for 1 hour. Cool, and adjust the solution with 0.25 M phosphoric acid to a pH of 7.0. Quantitatively transfer this solution to a 50-mL volumetric flask, dilute with water to volume, and mix. Dilute the remaining original solution with water to volume, and mix. Add 1 mL each of the refluxed and unrefluxed solutions to a 10-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 12.5-cm column that contains 3-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* four peaks are observed; the resolution, *R*, between two adjacent peaks is not less than 1.2, the column efficiency determined for the pilocarpine peak is not less than 1500 theoretical plates, and the tailing factor, *T*, for the pilocarpine peak is not greater than 1.5. The relative retention times for the major peaks are about 0.67 for isopilocarpine, 0.76 for pilocarpine, 0.85 for pilocarpic acid, and 1.0 for isopilocarpic acid.

Procedure—Separately inject equal volumes (about 40 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of pilocarpine ($C_{11}H_{16}N_2O_2$) in the portion of Pilocarpine taken by the formula:

$$(208.26/271.27)500C(r_U/r_S)$$

in which 208.26 and 271.27 are the molecular weights of pilocarpine and pilocarpine nitrate, respectively, C is the concentration, in mg per mL, of USP Pilocarpine Nitrate RS in the *Standard preparation*, and r_U and r_S are the peak responses for pilocarpine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pilocarpine Ocular System

» Pilocarpine Ocular System contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of pilocarpine ($C_{11}H_{16}N_2O_2$). It is sterile.

Packaging and storage—Preserve in single-dose containers in a cold place.

USP Reference standards ⟨11⟩—
USP Pilocarpine RS
USP Pilocarpine Hydrochloride RS
USP Pilocarpine Nitrate RS

Identification—Cut around the inside margin of the Ocular System, then discard the ring encircling the Ocular System, extract the remaining portion with 0.5 mL of methanol in a small capped vial, shaking vigorously for 1 to 2 minutes. Evaporate the methanol extract on a sodium chloride plate forming a thin film: the IR absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Pilocarpine RS.

Sterility Tests ⟨71⟩: meets the requirements.

Uniformity of dosage units ⟨905⟩: meets the requirements for capsules.

Drug release pattern—Place each of the Ocular Systems in suitable porous holders made of an inert material, and suspend each from a nickel wire. To the upper end of the wire attach a tag identifying the specimen. Put each assembly into a test tube containing 27.0 mL of saline TS so that the system lies at the bottom of the tube and the identifying tag extends from the open top of the tube. Put the tubes into a horizontally reciprocating shaker in which the temperature is maintained at 37 ± 0.5°. Agitate the tubes with a horizontal amplitude of about 4 cm and a frequency of about 35 cycles per minute. At 7, 24, 48, 72, 96, and 168 hours, remove the assemblies from their tubes, and each time replace them in similar tubes containing 27.0 mL of fresh saline TS. Determine the amount of pilocarpine in solution in each tube, after adjusting the volume to 27.0 mL to make up for any evaporative losses, by measuring the UV absorbance in 1-cm cells at the wavelength of maximum absorbance at about 215 nm, with a suitable spectrophotometer, against saline TS as the blank. Concomitantly measure the absorbance of a Standard solution of USP Pilocarpine Hydrochloride RS having a known concentration of about 20 µg in each mL of saline TS. Calculate the quantity, in µg, of $C_{11}H_{16}N_2O_2$ in each solution taken by the formula:

$$(208.26/244.72)(A_U/A_S)27C$$

in which 208.26 and 244.72 are the molecular weights of pilocarpine and pilocarpine hydrochloride, respectively; A_U and A_S are the absorbances of the test solution and the Standard solution, respectively; and C is the concentration, in µg per mL, of USP Pilocarpine Hydrochloride RS in the Standard solution. Calculate the amount of pilocarpine released in 168 hours by adding the pilocarpine content of each set of tubes collected over 168 hours.

Tolerances—The amount of $C_{11}H_{16}N_2O_2$ from each Ocular System released during the total 0 to 168 hours tested conforms to *Acceptance Table 1* under *Drug Release* ⟨724⟩. The drug release range for this time period is not less than 80.0% and not more than 120.0% of the labeled release pattern.

Assay—

Buffer solution, Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Pilocarpine.*

Assay preparation—Select not fewer than 10 Ocular Systems. Cut each System into 4 pieces, transfer quantitatively to a 500-mL volumetric flask, and rinse all cutting utensils with 20 to 30 mL of methanol into the flask. Make additional rinses of the utensils with about 250 mL of *Mobile phase,* and collect all the rinses in the flasks. Allow the flasks to stand for 30 minutes, sonicate for about 15 minutes, dilute with water to volume, and mix. Transfer an aliquot of the supernatant, equivalent to 6 mg of pilocarpine to a 200-mL volumetric flask, dilute with water to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Pilocarpine.* Calculate the quantity, in mg, of pilocarpine in each Ocular System taken by the formula:

$$(208.26/271.27)(10/V)(C/N)(r_U/r_S)$$

in which 208.26 and 271.27 are the molecular weights of pilocarpine and pilocarpine nitrate, respectively; V is the volume, in mL, of the supernatant taken (see *Assay preparation*); C is the concentration, in µg per mL, of USP Pilocarpine Nitrate RS in the *Standard preparation; N* is the number of Ocular Systems taken; and r_U and r_S are the peak

USP Monographs

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responses for pilocarpine obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Pilocarpine Hydrochloride

$C_{11}H_{16}N_2O_2 \cdot HCl$ 244.72

2(3*H*)-Furanone, 3-ethyldihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-, monohydrochloride, (3*S*-*cis*)-;
Pilocarpine monohydrochloride [54-71-7].

DEFINITION

Pilocarpine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{11}H_{16}N_2O_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

Sample solution: 50 mg/mL

ASSAY

- **PROCEDURE**

Buffer: 4.4 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.5 ± 0.1.
Mobile phase: Acetonitrile, methanol, and *Buffer* (2:35:63)
Standard solution: 0.5 mg/mL of USP Pilocarpine Hydrochloride RS in water. [NOTE—Sonicate if necessary.]
System suitability solution: Transfer a known quantity of USP Pilocarpine Hydrochloride RS in a suitable volumetric flask, and add water, equivalent to 10% of the volume of the flask, to dissolve. [NOTE—Sonicate as needed.] Add 0.1 N sodium hydroxide, equivalent to 10% of the volume of the flask, quench immediately with the same volume of 0.1 N hydrochloric acid, and mix. Dilute with water to volume. [NOTE—The initial concentration of USP Pilocarpine Hydrochloride RS is 0.5 mg/mL. Isopilocarpine is formed in the *System suitability solution* preparation.]
Sample solution: 0.5 mg/mL of Pilocarpine Hydrochloride in water. [NOTE—Sonicate if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3-µm packing L11
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between isopilocarpine and pilocarpine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{11}H_{16}N_2O_2 \cdot HCl$ in the portion of Pilocarpine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Pilocarpine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
ORGANIC IMPURITIES
- **Procedure 1: Related Compounds**

Mobile phase, Standard solution, System suitability solution, and **Sample solution:** Proceed as directed in the *Assay.*
Sensitivity solution: 0.25 µg/mL of USP Pilocarpine Hydrochloride RS in water from the *Standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3-µm packing L11
Column temperature: 35°
Flow rate: 1.0 mL/min
Run time: NLT 5 times the retention time of the pilocarpine peak
Injection size: 10 µL
System suitability
Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between isopilocarpine and pilocarpine, *System suitability solution*
Signal-to-noise ratio: NLT 10 for the pilocarpine peak, *Sensitivity solution*
Relative standard deviation: NMT 2.0% for the pilocarpine peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Pilocarpine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_S = peak area of pilocarpine from the *Standard solution*
C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Pilocarpine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1.*
[NOTE—Disregard any unspecified impurity peaks less than 0.05%.]
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Isopilocarpine[a]	0.94	1.0
Pilocarpine	1.00	—
Pilocarpic acid[b]	1.15	0.5
Isopilocarpic acid[c]	1.19	0.1

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Impurity Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	0.1

[a] (3*R*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one.
[b] (2*S*,3*R*)-2-Ethyl-4-hydroxy-3-[(1-methyl-1*H*-imidazol-5-yl)methyl]butanoic acid.
[c] (2*R*,3*R*)-2-Ethyl-4-hydroxy-3-[(1-methyl-1*H*-imidazol-5-yl)methyl]butanoic acid.

• **Procedure 2: Other Alkaloids**
Sample solution: 10 mg/mL in water
Analysis: Divide the *Sample solution* into two portions. To one portion add a few drops of 6 N ammonium hydroxide, and to the other, add a few drops of potassium dichromate TS.
Acceptance criteria: No turbidity is produced in either solution.

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩**:** +88.5° to +91.5°
Sample solution: 20 mg/mL, in water
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 2 h: it loses NMT 3.0% of its weight.
• **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩
Sample solution: 50 mg/mL in sulfuric acid
Acceptance criteria: The solution has no more color than *Matching Fluid B*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pilocarpine Hydrochloride RS

Pilocarpine Hydrochloride Ophthalmic Solution

DEFINITION
Pilocarpine Hydrochloride Ophthalmic Solution is a sterile, buffered, aqueous solution of pilocarpine hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$). It may contain suitable antimicrobial agents and stabilizers, and suitable additives to increase its viscosity.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Ammonium hydroxide in isopropyl alcohol (1 in 50)
Mobile phase: *n*-Hexane and *Solution A* (700:300)
Standard solution: 1.6 mg/mL of USP Pilocarpine Hydrochloride RS in methanol
Sample solution: Nominally equivalent to 1.6 mg/mL of pilocarpine hydrochloride from a volume of Ophthalmic Solution diluted with methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 2 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for pilocarpine hydrochloride is 16 min.]
Suitability requirements
Relative standard deviation: NMT 2.0% for pilocarpine hydrochloride for three replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$) in each mL of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
• **pH** ⟨791⟩**:** 3.5–5.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pilocarpine Hydrochloride RS

Pilocarpine Hydrochloride Tablets

DEFINITION
Pilocarpine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to the major peak of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 10 N sodium hydroxide, 85% phosphoric acid, triethylamine, and water (7:6:1:500). Adjust with 10 N sodium hydroxide to a pH of 3.0.
Mobile phase: Methanol and *Solution A* (3:100)
Standard solution: 50 µg/mL of USP Pilocarpine Hydrochloride RS
System suitability solution: Transfer 10 mL of the *Standard solution* to a test tube. Add 100 µL of 2 N sodium hydroxide, mix well, and allow it to stand for 5 min. Add 100 µL of 2 N hydrochloric acid and mix well. [NOTE—This preparation contains pilocarpine, isopilocarpine, and two unidentified compounds.]
Sample stock solution: Nominally 0.1 mg/mL of pilocarpine hydrochloride in water prepared as follows.

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Place Tablets, equivalent to 50 mg of pilocarpine hydrochloride, in a 500-mL volumetric flask. Fill the flask 75% full with water. Stir for at least 30 min or more if necessary, until the Tablets are completely disintegrated and the powder is finely dispersed. Dilute with water to volume.

Sample solution: Nominally 50 µg/mL of pilocarpine hydrochloride in water from *Sample stock solution*. Pass a suitable amount of solution through a PVDF filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm. For *Identification B*, use a diode array detector in the range of 190–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for isopilocarpine, pilocarpine, and two unidentified peaks are 0.9, 1.0, 1.2, and 1.5, respectively.]
Suitability requirements
Resolution: NLT 1.2 between isopilocarpine and pilocarpine; NLT 1.2 between pilocarpine and the peak at a relative retention time of 1.2; NLT 1.2 between the peaks at relative retention times of 1.2 and 1.5, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pilocarpine hydrochloride from the *Sample solution*
r_S = peak response of pilocarpine hydrochloride from the *Standard solution*
C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pilocarpine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Buffer solution: 13.5 mL of *phosphoric acid* and 3.0 mL of triethylamine in 1000 mL of *water*. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 3.
Mobile phase: *Methanol* and *Buffer solution* (3:17)
Standard stock solution: 0.1 mg/mL of USP Pilocarpine Hydrochloride RS in *Medium*
Standard solution
For Tablets labeled to contain 7.5 mg: Transfer 15.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Medium* to volume.
For Tablets labeled to contain 5 mg: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Medium* to volume.
Sample solution: Pass the solution under test through a suitable polyethylene filter of 45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 75% (Q) of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Mobile phase, Standard solution, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Place 1 Tablet in a suitable volumetric flask, fill the flask about 75% full with *water*, and vigorously stir for NLT 30 min to ensure complete disintegration. Dilute with water to volume to obtain a final concentration of 0.05 mg/mL of pilocarpine hydrochloride. Pass the solution through a PVDF filter of 0.45-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pilocarpine hydrochloride from the *Sample solution*
r_S = peak response of pilocarpine hydrochloride from the *Standard solution*
C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pilocarpine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Procedure
Mobile phase, System suitability solution, Sample stock solution, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.5 µg/mL of USP Pilocarpine Hydrochloride RS

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Sample solution: Nominally 100 µg/mL of pilocarpine hydrochloride in water from *Sample stock solution*. Pass a suitable amount of solution through a PVDF filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 100 µL

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pilocarpine hydrochloride from the *Standard solution*
C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pilocarpine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isopilocarpine	0.9	0.79	1.0
Pilocarpine	1.0	1.0	—
Pilocarpic acid	1.2	1.0	0.5
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.2

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Pilocarpine Hydrochloride RS

Pilocarpine Nitrate

$C_{11}H_{16}N_2O_2 \cdot HNO_3$ 271.27
2(3*H*)-Furanone, 3-ethyldihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-, (3*S*-*cis*)-, mononitrate.
Pilocarpine mononitrate [148-72-1].

» Pilocarpine Nitrate contains not less than 98.5 percent and not more than 101.0 percent of $C_{11}H_{16}N_2O_2 \cdot NO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Pilocarpine Nitrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Mix a solution (1 in 10) with an equal volume of ferrous sulfate TS, and superimpose the mixture upon 5 mL of sulfuric acid contained in a test tube: the zone of contact becomes brown.

Melting range ⟨741⟩: between 171° and 176°, with decomposition, but the range between beginning and end of melting does not exceed 3°.

Specific rotation ⟨781S⟩: between +79.5° and +82.5°.
Test solution: 20 mg per mL, in water.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 2.0% of its weight.

Readily carbonizable substances ⟨271⟩—Dissolve 100 mg in 5 mL of sulfuric acid : the solution has no more color than *Matching Fluid A*.

Chloride—To 5 mL of a solution (1 in 50), acidified with nitric acid, add a few drops of silver nitrate TS: no opalescence is produced immediately.

Other alkaloids—Dissolve 200 mg in 20 mL of water, and divide the solution into two portions. To one portion add a few drops of 6 N ammonium hydroxide and to the other add a few drops of potassium dichromate TS: no turbidity is produced in either solution.

Assay—Dissolve about 600 mg of Pilocarpine Nitrate, accurately weighed, in 30 mL of glacial acetic acid, warming slightly to effect solution. Cool to room temperature, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.13 mg of $C_{11}H_{16}N_2O_2 \cdot NO_3$.

Pilocarpine Nitrate Ophthalmic Solution

» Pilocarpine Nitrate Ophthalmic Solution is a sterile, buffered, aqueous solution of Pilocarpine Nitrate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{11}H_{16}N_2O_2 \cdot HNO_3$. It may contain suitable antimicrobial agents and stabilizers, and suitable additives to increase its viscosity.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Pilocarpine Nitrate RS

Identification—
A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

B: It responds to *Identification* test *B* under *Pilocarpine Nitrate*.

Sterility Tests ⟨71⟩ : meets the requirements.

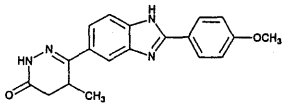
pH ⟨791⟩: between 4.0 and 5.5.

Assay—Proceed with Ophthalmic Solution as directed in the *Assay* under *Pilocarpine Hydrochloride Ophthalmic Solution*,

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except to read pilocarpine nitrate instead of pilocarpine hydrochloride throughout and to calculate the quantity, in mg, of $C_{11}H_{16}N_2O_2 \cdot HNO_3$ in each mL of the Ophthalmic Solution taken by the formula given therein.

Pimobendan



$C_{19}H_{18}N_4O_2$ 334.37

3(2*H*)-Pyridazinone, 4,5-dihydro-6-[2-(4-methoxyphenyl)-1*H*-benzimidazol-5-yl]-5-methyl-, (±)-;
(±)-4,5-Dihydro-6-[2-(*p*-methoxyphenyl)-5-benzimidazolyl]-5-methyl-3(2*H*)-pyridazinone;
6-[2-(4-Methoxyphenyl)-1*H*-benzimidazol-5-yl]-5-methyl-4,5-dihydropyridazin-3(2*H*)-one [74150-27-9].

DEFINITION

Pimobendan contains NLT 98.0% and NMT 102.0% of pimobendan ($C_{19}H_{18}N_4O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M.▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 3.0 g/L of monobasic potassium phosphate in water. Adjust with suitably diluted phosphoric acid to a pH of 2.5.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
6	80	20
20	20	80
20.1	85	15
30	85	15

Standard solution: 0.1 mg/mL of USP Pimobendan RS in methanol
Sample solution: 0.1 mg/mL of Pimobendan in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1 or 4.0-mm × 12.5-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pimobendan ($C_{19}H_{18}N_4O_2$) in the portion of Pimobendan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pimobendan RS in the *Standard solution* (mg/mL)
C_U = concentration of Pimobendan in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 5 µg/mL each of USP Pimobendan Related Compound A RS and USP Pimobendan Related Compound B RS in methanol
Standard solution: 50 µg/mL of USP Pimobendan RS in methanol
Sample solution: 5.0 mg/mL of Pimobendan in methanol
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between pimobendan related compound A and pimobendan related compound B, *System suitability solution*
Tailing factor: 0.8–1.5, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Pimobendan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pimobendan from the *Standard solution*
C_S = concentration of USP Pimobendan RS in the *Standard solution* (µg/mL)
C_U = concentration of Pimobendan in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pimobendan	1.0	—
Pimobendan related compound A	1.3	0.1
Pimobendan related compound B	1.4	0.1

USP Monographs

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Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other individual impurity	—	0.1
Total impurities	—	0.2

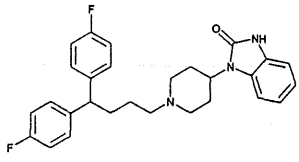
SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I*
  Sample: 0.5 g
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Pimobendan RS
  USP Pimobendan Related Compound A RS
  4-[2-(4-Methoxyphenyl)-1*H*-benzimidazol-5-yl]-3-methyl-4-oxobutanoic acid.
  $C_{19}H_{18}N_2O_4$ 338.36
  USP Pimobendan Related Compound B RS
  N-[2-Amino-4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl]-4-methoxybenzamide.
  $C_{19}H_{20}N_4O_3$ 352.39

Pimozide



$C_{28}H_{29}F_2N_3O$ 461.55
2*H*-Benzimidazol-2-one, 1-[1-[4,4-bis(4-fluorophenyl)butyl]-4-piperidinyl]-1,3-dihydro-;
1-[1-[4,4-Bis(*p*-fluorophenyl)butyl]-4-piperidyl]-2-benzimidazolinone [2062-78-4].

DEFINITION

Pimozide contains NLT 98.0% and NMT 102.0% of pimozide ($C_{28}H_{29}F_2N_3O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K.▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Solution A: 2.5 g/L of ammonium acetate and 8.5 g/L of tetrabutylammonium hydrogen sulfate in water
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	70	30
15	70	30
20	80	20
25	80	20

System suitability solution: 0.05 mg/mL of USP Pimozide RS and 0.02 mg/mL of USP Mebendazole RS in methanol
Standard solution: 1 mg/mL of USP Pimozide RS in methanol
Sample solution: 1 mg/mL of Pimozide in methanol. [NOTE—Sonication may be needed to dissolve the sample.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between pimozide and mebendazole, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pimozide ($C_{28}H_{29}F_2N_3O$) in the portion of Pimozide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pimozide from the *Sample solution*
r_S = peak response of pimozide from the *Standard solution*
C_S = concentration of USP Pimozide RS in the *Standard solution* (mg/mL)
C_U = concentration of Pimozide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**
  Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
  Standard solution: 0.05 mg/mL of USP Pimozide RS in methanol
  Sample solution: 10 mg/mL of Pimozide in methanol. [NOTE—Sonication may be needed to dissolve the sample.]
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—See *Table 2* for the relative retention times.]
  Suitability requirements
  Resolution: NLT 5.0 between pimozide and mebendazole, *System suitability solution*
  Tailing factor: NMT 2.0, *Standard solution*

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Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Pimozide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of pimozide from the *Standard solution*

C_S = concentration of USP Pimozide RS in the *Standard solution* (mg/mL)

C_U = concentration of Pimozide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pimozide amine[a]	0.1	0.5
Mebendazole[b]	0.88	—
Desfluoro pimozide[c]	0.9	0.5
o-Pimozide isomer[d]	0.95	0.5
Pimozide	1.0	—
Didehydropimozide[e]	1.1	0.5
Pimozide olefin[f]	1.2	0.5
Pimozide *N*-oxide[g]	1.3	0.5
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] 1-(Piperidin-4-yl)benzimidazolin-2-one.

[b] Included for system suitability purposes only. Not a process impurity or degradation product.

[c] 1-{1-[4-(4-Fluorophenyl)-4-phenylbutyl]piperidin-4-yl}benzimidazolin-2-one.

[d] 1-{1-[4-(2-Fluorophenyl)-4-(4-fluorophenyl)butyl]piperidin-4-yl}-1*H*-benzimidazol-2-one.

[e] 1-{1-[4,4-Bis(4-fluorophenyl)butyl]-1,2,3,6-tetrahydropyridin-4-yl} benzimidazolin-2-one.

[f] 1-{1-[4,4-Bis(4-fluorophenyl)but-3-en-1-yl]piperidin-4-yl}benzimidazolin-2-one.

[g] 1-[4,4-Bis(4-fluorophenyl)butyl]-4-(2-oxobenzimidazol-1-yl)piperidine 1-oxide.

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° to a constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩

USP Mebendazole RS

USP Pimozide RS

Pimozide Tablets

DEFINITION

Pimozide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of pimozide ($C_{28}H_{29}F_2N_3O$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

Sample: Grind an appropriate number of Tablets to prepare a 1-mg/mL solution of pimozide in dichloromethane. Shake the solution for 5 min, and pass through a suitable filter. Evaporate the filtrate to dryness under reduced pressure. Use the dried residue.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Protect all pimozide solutions from light.

Solution A: 2.5 g/L of ammonium acetate and 8.5 g/L of tetrabutylammonium hydrogen sulfate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	70	30
15	70	30
16	80	20
30	80	20

System suitability solution: 0.04 mg/mL of USP Pimozide RS and 0.02 mg/mL of USP Mebendazole RS in methanol

Standard solution: 0.4 mg/mL of USP Pimozide RS in methanol

Sample solution: Nominally 0.4 mg/mL of pimozide prepared as follows. Transfer a suitable number of powdered Tablets (NLT 20) to a suitable volumetric flask. Add about 70% of the flask volume of methanol, and mechanically shake for 30 min. Dilute with methanol to volume, and mix well with the aid of sonication for 10 min. Centrifuge, and pass a portion of the supernatant through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for mebendazole and pimozide are 0.88 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the pimozide and mebendazole peaks, *System suitability solution*

USP Monographs

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Relative standard deviation: NMT 2.0% for pimozide, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pimozide ($C_{28}H_{29}F_2N_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pimozide from the *Sample solution*

r_S = peak response of pimozide from the *Standard solution*

C_S = concentration of USP Pimozide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pimozide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 5 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.5.

Mobile phase: Acetonitrile and *Buffer* (40:60)

Standard solution: (*L*/900) mg/mL of USP Pimozide RS in *Mobile phase*, where *L* is the label claim of pimozide in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 25-cm; 5-µm packing L1

Flow rate: 0.8 mL/min

Injection volume: 100 µL

Run time: 2.5 times the retention time of pimozide

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pimozide ($C_{28}H_{29}F_2N_3O$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of pimozide from the *Sample solution*

r_S = peak response of pimozide from the *Standard solution*

C_S = concentration of USP Pimozide RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of pimozide ($C_{28}H_{29}F_2N_3O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Protect all pimozide solutions from light.

Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of USP Pimozide RS in methanol

Sample solution: Nominally 2 mg/mL of pimozide prepared as follows. Transfer a suitable number of powdered Tablets (NLT 20) to a suitable volumetric flask. Add about 70% of the flask volume of methanol, and mechanically shake for 30 min. Dilute with methanol to volume, and mix well with the aid of sonication for 10 min. Centrifuge, and pass a portion of the supernatant through a suitable filter of 0.45-µm pore size.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mebendazole and pimozide are 0.88 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between pimozide and mebendazole, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*

r_S = peak response of pimozide from the *Standard solution*

C_S = concentration of USP Pimozide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pimozide in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual degradation product: NMT 1.0%

Total degradation products: NMT 2.0%

ADDITIONAL REQUIREMENTS

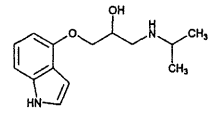
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Mebendazole RS

USP Pimozide RS

Pindolol



$C_{14}H_{20}N_2O_2$ 248.32

2-Propanol,1-(1*H*-indol-4-yloxy)-3-(1-methylethyl)amino-; 1-(Indol-4-yloxy)-3-(isopropylamino)-2-propanol [13523-86-9].

DEFINITION

Pindolol contains NLT 98.5% and NMT 101.0% of pindolol ($C_{14}H_{20}N_2O_2$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 0.05 M sodium acetate, previously adjusted with glacial acetic acid to a pH of 5.0 (350:650)

System suitability solution: 0.005 mg/mL each of USP Pindolol RS and indole in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Pindolol RS in *Mobile phase* prepared as follows. To a suitable amount of USP Pindolol RS, add *Mobile phase* to fill about 90% of the total volume, and sonicate for about 5 min to dissolve.

Standard solution: 0.1 mg/mL of USP Pindolol RS from *Standard stock solution* in *Mobile phase*

Sample stock solution: 1 mg/mL of Pindolol in *Mobile phase* prepared as follows. To a suitable amount of Pindolol, add *Mobile phase* to fill about 90% of the total volume, and sonicate for about 5 min to dissolve.

Sample solution: 0.1 mg/mL of Pindolol from *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 219 nm

Column: 4.6-mm × 15-cm; 3-µm packing L10

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for indole and pindolol are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 7 between indole and pindolol

Column efficiency: NLT 3000 theoretical plates for pindolol

Relative standard deviation: NMT 2% for pindolol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pindolol ($C_{14}H_{20}N_2O_2$) in the portion of Pindolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for pindolol from the *Sample solution*

r_S = peak response for pindolol from the *Standard solution*

C_S = concentration of USP Pindolol RS in the *Standard solution* (mg/mL)

C_U = concentration of Pindolol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Chromatographic system, and System suitability: Prepare as directed in the *Assay*. [NOTE—Decreasing the acetonitrile concentration in *Mobile phase* results in less resolution between pindolol and impurities that elute on the tail of the pindolol peak; increasing the acetonitrile concentration results in less resolution between impurities with longer retention times.]

Sample solution: Use the *Sample stock solution* in the *Assay*.

Analysis

Samples: *System suitability solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Pindolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response of each impurity from the *Sample solution*

r_S = peak area response for pindolol from the *System suitability solution*

C_S = concentration of USP Pindolol RS in the *System suitability solution* (mg/mL)

C_U = concentration of Pindolol in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Pindolol RS

Pindolol Tablets

» Pindolol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pindolol ($C_{14}H_{20}N_2O_2$).

Packaging and storage—Preserve in well-closed containers, protected from light.

USP Reference standards ⟨11⟩—

USP Pindolol RS

Identification—

A: Examine the chromatograms obtained in the test for *Chromatographic purity:* the principal spot obtained from the *Test solution* is similar in R_F value, color, and intensity to that obtained from the *Standard stock solution*.

B: The retention time exhibited by pindolol in the chromatogram of the *Assay preparation* corresponds to that of pindolol in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Pindolol RS in *Dissolution Medium* to obtain a solution having a known concentration of about 0.002*J* mg per mL, *J* being the labeled quantity, in mg, of pindolol in each Tablet.

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Mix equal volumes of this solution and of *Mobile phase* to obtain the *Standard solution*.

Resolution solution—Dissolve a quantity of nortriptyline hydrochloride in *Standard solution* to obtain a solution having a concentration of about 0.005 mg of nortriptyline hydrochloride per mL.

Test solution—Filter a portion of the solution under test. Mix equal volumes of the filtrate and of *Mobile phase* to obtain the *Test solution*.

Procedure—Proceed as directed for *Procedure* under the *Assay*. Calculate the quantity of $C_{14}H_{20}N_2O_2$ dissolved by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pindolol RS in the *Standard solution;* and r_U and r_S are the pindolol peak responses obtained from the *Test solution* and the *Standard solution*, respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{14}H_{20}N_2O_2$ is dissolved in 15 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity— [NOTE—Protect solutions and chromatographic plate (after application of solutions) from light.]

p-Dimethylaminobenzaldehyde spray—Dissolve 1 g of *p*-dimethylaminobenzaldehyde in a mixture of 50 mL of hydrochloric acid and 50 mL of alcohol, and mix. [NOTE—Store this solution in a tightly closed, light-resistant container, and discard after 4 weeks.]

Solvent mixture—Prepare a solution of methanol and glacial acetic acid (99:1).

Standard stock solution—Dissolve an accurately weighed quantity of USP Pindolol RS in *Solvent mixture* to obtain a solution containing 5.0 mg per mL.

Standard solution 1—Dilute an accurately measured volume of *Standard stock solution* quantitatively and stepwise with *Solvent mixture* to obtain a solution containing 0.025 mg per mL.

Standard solution 2—Dilute 6.0 mL of *Standard solution 1* with *Solvent mixture* to 10.0 mL, and mix.

Standard solution 3—Dilute 4.0 mL of *Standard solution 1* with *Solvent mixture* to 10.0 mL, and mix.

Standard solution 4—Dilute 2.0 mL of *Standard solution 1* with *Solvent mixture* to 10.0 mL, and mix.

Test solution— [NOTE—Prepare this solution immediately before use, and apply last.] Transfer a portion of powdered Tablets, equivalent to 50 mg of pindolol, to a 50-mL flask, add 10.0 mL of *Solvent mixture*, insert the stopper in the flask, and shake by mechanical means for 15 minutes. Centrifuge a portion of the resultant suspension, and promptly test the clear supernatant.

Procedure—Prepare a lined chromatographic chamber (see *Chromatography* ⟨621⟩) with a developing solvent consisting of a mixture of methylene chloride, methanol, and formic acid (75: 23.5: 1.5), and equilibrate for 30 minutes. Separately apply 2-µL portions of the *Standard stock solution*, each of the *Standard solutions*, and the *Test solution* to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in the chromatographic chamber, and allow the solvent front to move about two-thirds of the length of the plate. Remove the plate from the chamber, immediately spray with the *p-Dimethylaminobenzaldehyde spray*, heat the plate at 60° for 15 minutes, and promptly examine the chromatogram: no individual secondary spot observed in the chromatogram of the *Test solution* is greater in size or intensity than the principal spot observed in the chromatogram of *Standard solution 1*, corresponding to 0.5%, and the total of any such spots observed does not exceed 3.0%. [NOTE—In a valid determination, spots from all solutions must be visible.]

Assay—

Ammonium carbonate solution—Dissolve 300 mg of ammonium carbonate in 50 mL of water, and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and *Ammonium carbonate solution* (475:475:50), making adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Prepare a solution of USP Pindolol RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

Resolution solution—Dissolve a quantity of nortriptyline hydrochloride in *Standard preparation* to obtain a solution having a concentration of about 0.2 mg of nortriptyline hydrochloride per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of pindolol, to a 100-mL volumetric flask. Add 4 mL of water, and sonicate for 2 minutes, with occasional shaking to disperse the powder. Add 30 mL of *Mobile phase*, sonicate for 15 minutes, and allow to cool. Dilute with *Mobile phase* to volume, mix, and filter. Use the clear filtrate as the *Assay preparation*.

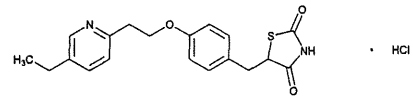
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L16. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution between pindolol and nortriptyline is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for pindolol and 1.0 for nortriptyline. Calculate the quantity, in mg, of pindolol ($C_{14}H_{20}N_2O_2$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pindolol RS in the *Standard preparation;* and r_U and r_S are the pindolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pioglitazone Hydrochloride



$C_{19}H_{20}N_2O_3S \cdot HCl$ 392.90

2,4-Thiazolidinedione, 5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy] phenyl]methyl]-, monohydrochloride, (±)-;
(±)-5-[*p*-[2-(5-Ethyl-2-pyridyl)ethoxy]benzyl]-2,4-thiazolidinedione monohydrochloride [112529-15-4].

DEFINITION

Pioglitazone Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{19}H_{20}N_2O_3S \cdot HCl$, calculated on the anhydrous basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS–GENERAL, *Chloride* ⟨191⟩:** Dissolve 25 mg of Pioglitazone Hydrochloride in 0.5 mL of nitric acid, and add 2 mL of dilute nitric acid. It meets the requirements of the test for *Chloride*.
- **C.** The retention time of the pioglitazone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)

Standard solution: Prepare a 0.5 mg/mL solution of USP Pioglitazone Hydrochloride RS in methanol, and dilute with *Mobile phase* to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride.

System suitability stock solution: 0.5 mg/mL of USP Pioglitazone Hydrochloride RS and 0.13 mg/mL of benzophenone in methanol

System suitability solution: Dilute *System suitability stock solution* with *Mobile phase* to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride and 13 µg/mL of benzophenone.

Sample solution: Prepare a 0.5 mg/mL solution of pioglitazone hydrochloride in methanol, and dilute with *Mobile phase* to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 2.5°
Flow rate: 0.7 mL/min
[NOTE—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]
Injection size: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.]
Suitability requirements
Tailing factor: NMT 1.5 for pioglitazone and benzophenone, *System suitability solution*
Resolution: NLT 15 between pioglitazone and benzophenone, *System suitability solution*
Relative standard deviation: NMT 2.0% for six replicate injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{20}N_2O_3S \cdot HCl$ in the portion of Pioglitazone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Pioglitazone Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

- **Procedure**

Mobile phase and **System suitability stock solution:** Proceed as directed in the *Assay*.

System suitability solution: Dilute the *System suitability stock solution* with *Mobile phase* to obtain a solution containing 25 µg/mL of pioglitazone hydrochloride and 6.5 µg/mL of benzophenone.

Sample solution: 0.2 mg/mL of pioglitazone hydrochloride dissolved in 20% of the final volume with methanol, then diluted with *Mobile phase* to final volume

Standard solution: 1 µg/mL of pioglitazone hydrochloride prepared by diluting the *Sample solution* with *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 2.5°
Flow rate: 0.7 mL/min
[NOTE—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]
Injection size: 40 µL
Run time: At least four times the retention time of pioglitazone

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for pioglitazone and benzophenone, *System suitability solution*
Resolution: NLT 15 between pioglitazone and benzophenone, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Pioglitazone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of pioglitazone from the *Standard solution*
D = dilution factor used to prepare the *Standard solution*, 0.005

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydroxypioglitazone[a]	0.7	0.15
Pioglitazone	1.0	—
Didehydropioglitazone[b]	1.4	0.15
N-Alkylpioglitazone[c]	3.0	0.15

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Impurity Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other individual impurity	—	0.10

[a] (±)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}-5-hydroxythiazolidine-2,4-dione.
[b] (*Z*)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzylidene}thiazolidine-2,4-dione.
[c] (±)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}-3-[2-(5-ethylpyridin-2-yl)ethyl]thiazolidine-2,4-dione.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Pioglitazone Hydrochloride RS

Pioglitazone Tablets

DEFINITION
Pioglitazone Tablets contain an amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S \cdot HCl$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$).

IDENTIFICATION
- **A.** The retention time of the pioglitazone peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B. ULTRAVIOLET ABSORPTION**
  Sample solution: Dissolve a quantity of finely powdered Tablets in 0.1 N hydrochloric acid to obtain a solution containing 25 µg/mL of pioglitazone. [NOTE—Vigorous shaking and filtration may be needed.]
  Acceptance criteria: The UV absorption spectrum exhibits a maximum between 267 and 271 nm.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)
  Standard solution: Prepare 0.5 mg/mL solution of USP Pioglitazone Hydrochloride RS in methanol, and dilute with *Mobile phase* to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride.
  System suitability stock solution: 0.5 mg/mL of USP Pioglitazone Hydrochloride RS and 0.13 mg/mL of benzophenone in methanol
  System suitability solution: Dilute the *System suitability stock solution* with *Mobile phase* to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride and 13 µg/mL of benzophenone.
  Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 23 mg of pioglitazone, to a glass-stoppered flask, and add 50 mL of methanol. Disperse the particles by sonication for about 2 min, then centrifuge. Dilute a portion of the supernatant with *Mobile phase* to obtain a solution having a nominal concentration of 45 µg/mL of pioglitazone.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 269 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 25 ± 2.5°
  Flow rate: 0.7 mL/min. [NOTE—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]
  Injection size: 20 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  [NOTE—The approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.]
  Suitability requirements
  Tailing factor: NMT 1.5 for pioglitazone and benzophenone, *System suitability solution*
  Resolution: NLT 15 between pioglitazone and benzophenone, *System suitability solution*
  Relative standard deviation: NMT 2.0% for six replicate injections, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of $C_{19}H_{20}N_2O_3S$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Hydrochloric acid and potassium chloride buffer, pH 2.0 [mix 50 mL of 0.2 N hydrochloric acid and 150 mL of potassium chloride solution (150 mg/mL), dilute with water to 1 L, and adjust with 5 N hydrochloric acid to a pH of 2.0]; 900 mL
  Apparatus 2: 75 rpm
  Time: 15 min
  Standard solution: Transfer 23 mg of USP Pioglitazone Hydrochloride RS to a 50-mL volumetric flask, dissolve in 10 mL of methanol, and dilute with *Medium* to volume. Dilute this solution with *Medium* to obtain a final concentration of about L/900, where L is the label claim (mg).
  Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
  Detector: UV
  Analytical wavelength: 269 nm
  Cell: 1 cm
  Blank: *Medium*
  Calculate the percentage of pioglitazone dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = Tablet label claim (mg)
M_{r1} = molecular weight of pioglitazone, 356.44

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M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90
V = volume of *Medium* (mL), 900

Tolerances: NLT 80% (Q) of the labeled amount of pioglitazone is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

Procedure for content uniformity

Standard solution: 26 µg/mL of USP Pioglitazone Hydrochloride RS in methanol and 0.1 N hydrochloric acid (9:1)

Sample solution: Transfer 1 Tablet to an appropriate size volumetric flask such that the final concentration does not exceed 0.3 mg of pioglitazone per mL. Add 0.1 N hydrochloric acid at a volume equivalent to 10% of the total volume and shake until the Tablet is completely disintegrated. Add methanol at a volume equivalent to 70% of the total volume and shake vigorously for 10 min. Dilute with methanol to volume, mix well, and centrifuge. Dilute a portion of the supernatant with methanol and 0.1 N hydrochloric acid (9:1) to obtain a solution having a concentration of 24 µg/mL of pioglitazone.

Spectrometric conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 269 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{19}H_{20}N_2O_3S$ in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Mobile phase and **System suitability stock solution:** Proceed as directed in the *Assay*.

Diluent: *Mobile phase* and methanol (4:1)

Standard solution: 1 µg/mL of USP Pioglitazone Hydrochloride RS in *Diluent*. [NOTE—If necessary, dissolve USP Pioglitazone Hydrochloride RS in a minimal amount of methanol and then dilute with *Diluent* to final concentration.]

System suitability solution: Dilute the *System suitability stock solution* with *Mobile phase* to obtain a solution containing 25 µg/mL of pioglitazone hydrochloride and 6.5 µg/mL of benzophenone.

Sample solution: Weigh and finely powder 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 18 mg of pioglitazone, to a 100-mL volumetric flask and add 20 mL of methanol. Disperse the particles by sonication for about 1 min, then dilute with *Mobile phase* to volume, mix well, centrifuge, and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 2.5°
Flow rate: 0.7 mL/min

[NOTE—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

Run time: At least 30 min
Injection size: 40 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Tailing factor: NMT 1.5 for pioglitazone and benzophenone, *System suitability solution*

Resolution: NLT 15 between pioglitazone and benzophenone, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.]

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of pioglitazone from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria

Individual impurities: NMT 0.2%
Total impurities: NMT 0.6%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Pioglitazone Hydrochloride RS

Pioglitazone and Glimepiride Tablets

DEFINITION

Pioglitazone and Glimepiride Tablets contain an amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S \cdot HCl$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$), and NLT 90.0% and NMT 110.0% of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$).

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION**

Sample: Transfer 1 Tablet to a suitable container, and add 0.1 N hydrochloric acid to obtain a final concentration of about 0.1 mg/mL of glimepiride. Shake until the Tablet disintegrates. Pass a 2-mL portion of the resulting suspension through a suitable filter of 0.45-µm pore size.

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Use the filtrate for the identification of pioglitazone, and use the filter for the identification of glimepiride.

Pioglitazone

Sample solution: Dilute a portion of the filtrate obtained in the *Sample* with 0.1 N hydrochloric acid to obtain a solution containing about 0.03 mg/mL of pioglitazone.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 267 and 271 nm.

Glimepiride

Sample solution: Wash the filter, as obtained in the *Sample*, with 100 mL of 0.1 N hydrochloric acid, and discard the filtrate. Wash the filter with 20 mL of methanol, and use the filtrate.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 227 and 231 nm.

• **B.** The retention times of the pioglitazone and glimepiride peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 6.9 g/L of monobasic sodium phosphate in water, adjusted with diluted phosphoric acid to a pH of 4.0

Mobile phase: Acetonitrile and *Buffer* (1:1)

Diluent: Acetonitrile and 0.1 N hydrochloric acid (9:1)

Standard stock solution: 0.66 mg/mL of USP Pioglitazone Hydrochloride RS and 0.08 mg/mL of USP Glimepiride RS in *Diluent*

Standard solution: 66 µg/mL of pioglitazone hydrochloride and 8 µg/mL of glimepiride in *Mobile phase* from the *Standard stock solution*

Resolution stock solution: Dilute 1.0 mL of ethyl benzoate with *Mobile phase* to 100 mL. Further dilute 1.0 mL of the resulting solution with *Mobile phase* to 100 mL.

System suitability solution: Transfer 5.0 mL of the *Standard stock solution* and 5.0 mL of the *Resolution stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample stock solution: Transfer 20 Tablets to a suitable container. Add 200.0 mL of *Diluent*, and shake vigorously for at least 20 min. If disintegration is not complete, sonicate until the Tablets are completely disintegrated, and then shake for an additional 10 min. Pass through a suitable filter of 0.2-µm pore size, discarding the first few mL of filtrate. Further dilute 5.0 mL of the filtrate with *Diluent* to 50.0 mL.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with the nominal concentrations listed in *Table 1*.

Table 1

Labeled Amounts of Pioglitazone and Glimepiride (mg/Tablet)	Nominal Concentrations in the *Sample solution*	
	Pioglitazone (µg/mL)	Glimepiride (µg/mL)
30 and 2	60	4
30 and 4	60	8

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 228 nm

Column: 4.6-mm × 5-cm; 3-µm packing L1

Column temperature: 25 ± 5°

Flow rate: 0.8 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the pioglitazone peak of about 2.3 min.]

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—See *Table 2* for the approximate relative retention times.]

Table 2

Name	Relative Retention Time
Pioglitazone	1.0
Ethyl benzoate	1.7
Glimepiride	2.3

Suitability requirements

Resolution: NLT 4 between pioglitazone and ethyl benzoate; NLT 3 between ethyl benzoate and glimepiride, *System suitability solution*

Relative standard deviation: NMT 1.0% for the pioglitazone peak; NMT 1.0% for the glimepiride peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of pioglitazone from the *Sample solution*

r_S = peak response of pioglitazone from the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of glimepiride from the *Sample solution*

r_S = peak response of glimepiride from the *Standard solution*

C_S = concentration of USP Glimepiride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of glimepiride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% for each of the labeled amounts of pioglitazone and glimepiride

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Pioglitazone

Medium: Hydrochloric acid buffer pH 2.0 (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 75 rpm

Time: 15 min

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Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay.*
Standard stock solution: 0.37 mg/mL of USP Pioglitazone Hydrochloride RS dissolved first in methanol using 20% of the final volume, and then diluted with *Medium* to volume
Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of pioglitazone from the *Sample solution*
r_S = peak response of pioglitazone from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (mg/mL)
L = labeled amount of pioglitazone (mg/Tablet)
V = volume of *Medium,* 900 mL
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Tolerances: NLT 80% (*Q*) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved.
Glimepiride
Medium: pH 6.8 sodium phosphate buffer containing 0.2% of sodium dodecyl sulfate (6.9 g/L of monobasic sodium phosphate, 0.896 g/L of sodium hydroxide, and 2 g/L of sodium dodecyl sulfate in water, adjusted with 1 N sodium hydroxide to a pH of 6.8); 900 mL
Apparatus 2: 75 rpm
Time: 15 min
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay.* The flow rate may be adjusted to achieve the retention time of the glimepiride peak of about 5.4 min.
Standard stock solution: 0.22 mg/mL of USP Glimepiride RS in acetonitrile
Standard solution: *L*/900 mg/mL of USP Glimepiride RS in *Medium,* where *L* is the labeled amount of glimepiride, in mg/Tablet, from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of glimepiride from the *Sample solution*
r_S = peak response of glimepiride from the *Standard solution*
C_S = concentration of USP Glimepiride RS in the *Standard solution* (mg/mL)
L = labeled amount of glimepiride (mg/Tablet)
V = volume of *Medium,* 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*
Pioglitazone
Medium: Hydrochloric acid buffer pH 2.0 (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL, deaerated
Apparatus 2: 75 rpm
Time: 30 min
Buffer: 0.02 M sodium phosphate buffer pH 2.5 (2.75 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.5)
Solution A: Acetonitrile and *Buffer* (28:72)
Solution B: Acetonitrile and *Buffer* (70:30)
Mobile phase: See *Table 3.*

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4.0	0	100
7.0	0	100

Return to original conditions and re-equilibrate the system.
Standard stock solution: 0.2 mg/mL of USP Pioglitazone Hydrochloride RS dissolved first in alcohol using 20% of the final volume, and then diluted with *Medium* to volume
Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 25-mL volumetric flask, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 225 nm for pioglitazone (0–4.0 min) and UV 230 nm for glimepiride (4.0–7.0 min)
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of pioglitazone from the *Sample solution*

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r_S = peak response of pioglitazone from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (mg/mL)
L = labeled amount of pioglitazone (mg/Tablet)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Tolerances: NLT 80% (*Q*) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved.

Glimepiride

Medium: pH 6.8 sodium phosphate buffer containing 0.2% of sodium dodecyl sulfate (6.9 g/L of monobasic sodium phosphate, 0.896 g/L of sodium hydroxide, and 2 g/L of sodium dodecyl sulfate in water, adjusted with 1 N sodium hydroxide to a pH of 6.8); 900 mL
Apparatus 2: 75 rpm
Time: 15 min
Mobile phase and **Chromatographic system:** Proceed as directed in *Dissolution Test 2, Pioglitazone.*
Standard stock solution: 0.2 mg/mL of USP Glimepiride RS in alcohol
Standard solution: *L*/900 mg/mL of USP Glimepiride RS in *Medium*, where *L* is the labeled amount of glimepiride, in mg/Tablet, from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of glimepiride from the *Sample solution*
r_S = peak response of glimepiride from the *Standard solution*
C_S = concentration of USP Glimepiride RS in the *Standard solution* (mg/mL)
L = labeled amount of glimepiride (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements for *Content Uniformity* for pioglitazone and glimepiride

IMPURITIES

• **Organic Impurities: Pioglitazone**

Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)
Diluent: Acetonitrile and 0.1 N hydrochloric acid (9:1)
Standard stock solution: 0.2 mg/mL of USP Pioglitazone Hydrochloride RS in *Diluent*
Standard solution: 2 µg/mL of USP Pioglitazone Hydrochloride RS in *Mobile phase* from the *Standard stock solution*
Resolution stock solution: Dilute 1.0 mL of ethyl benzoate to 100.0 mL with acetonitrile. Further dilute 1.0 mL of the resulting solution with acetonitrile to 100.0 mL.
System suitability solution: Transfer 2.0 mL of the *Resolution stock solution* into a 100-mL volumetric flask. Add 1.0 mL of the *Standard stock solution*, and dilute with *Mobile phase* to volume.
Sample stock solution: Transfer 10 Tablets to an appropriate volumetric flask such that the nominal glimepiride concentration is 0.4 mg/mL. Add *Diluent* to approximately 80% of the total volume. Shake vigorously for at least 20 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size, discarding the first few mL of filtrate.
Sample solution: Transfer a suitable volume of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing 240 µg/mL of pioglitazone.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 5°
Flow rate: 0.8 mL/min. [Note—The flow rate may be adjusted to achieve the retention time of the pioglitazone peak of about 7 min.]
Injection volume: 40 µL
Run time: At least 4 times the retention time of the pioglitazone peak
System suitability
Samples: *Standard solution* and *System suitability solution*
[Note—Elution order is the pioglitazone peak followed by ethyl benzoate.]
Suitability requirements
Resolution: NLT 10 between pioglitazone and ethyl benzoate, *System suitability solution*
Tailing factor: NMT 1.5 for the pioglitazone peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each pioglitazone related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of pioglitazone from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria
Any individual pioglitazone related impurity: NMT 0.2%
Total pioglitazone related impurities: NMT 0.6%
[Note—Disregard the peak due to glimepiride, which elutes at about 16.5 min.]

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Change to read:

• **ORGANIC IMPURITIES: GLIMEPIRIDE**
Buffer: 0.007 M sodium phosphate, pH 1.6 (0.97 g/L of monobasic sodium phosphate in water, adjusted with dilute phosphoric acid to a pH of 1.6)
Diluent: Acetonitrile and 0.1 N hydrochloric acid (9:1)
Solution A: Acetonitrile and *Buffer* (52:48)
Solution B: Acetonitrile and *Buffer* (70:30)
Mobile phase: See *Table* ▲*4*▲ (ERR 1-Oct-2018).

Table ▲*4*▲ (ERR 1-Oct-2018)

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
60	0	100
60.1	100	0
70	100	0

Standard stock solution: 0.2 mg/mL of USP Glimepiride RS in *Diluent*
Standard solution: 2 µg/mL of USP Glimepiride RS in *Solution A* from the *Standard stock solution*
Resolution stock solution: Dilute 1.0 mL of ethyl benzoate with acetonitrile to 100.0 mL. Further dilute 1.0 mL of the resulting solution with acetonitrile to 100.0 mL.
System suitability solution: Transfer 2 mL of the *Resolution stock solution* into a 100-mL volumetric flask, add 1.0 mL of the *Standard stock solution,* and dilute with *Solution A* to volume.
Sample stock solution: Transfer 10 Tablets to an appropriate volumetric flask such that the nominal glimepiride concentration is 0.4 mg/mL. Add *Diluent* to approximately 80% of the total volume. Shake vigorously for at least 20 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size, discarding the first few mL of filtrate.
Sample solution: Equivalent to 0.2 mg/mL glimepiride in *Solution A* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 228 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 25 ± 5°
Flow rate: 1.0 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the glimepiride peak of about 25 min.]
Injection volume: 40 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—Elution order is ethyl benzoate followed by glimepiride.]
Suitability requirements
Resolution: NLT 10 between ethyl benzoate and glimepiride, *System suitability solution*
Tailing factor: NMT 1.5 for glimepiride, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each glimepiride related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of glimepiride from the *Standard solution*
C_S = concentration of USP Glimepiride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glimepiride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table* ▲*5*▲ (ERR 1-Oct-2018))

Acceptance criteria: See *Table* ▲*5*▲ (ERR 1-Oct-2018).
[NOTE—Disregard the peaks due to inactive ingredients and to pioglitazone that elute before the glimepiride sulfonamide peak.]

Table ▲*5*▲ (ERR 1-Oct-2018)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Glimepiride sulfonamide (glimepiride related compound B)[a]	0.2	1.39	1.5
Glimepiride	1.0	1.0	—
Any other glimepiride related individual impurity	—	1.0	0.2
Total glimepiride related impurities	—	—	2.5

[a] [*p*-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl] sulfonamide.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **LABELING:** When more than one dissolution test is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Glimepiride RS
USP Pioglitazone Hydrochloride RS

Pioglitazone and Metformin Hydrochloride Tablets

DEFINITION
Pioglitazone and Metformin Hydrochloride Tablets contain an amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S \cdot HCl$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$), and NLT 95.0% and NMT 105.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩▲, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
[NOTE—The UV spectra of the major peaks of the *Sample solution* and the *Standard solution* as obtained in the *Assay* may also be used to meet the *Acceptance criteria.*]

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Pioglitazone

Sample solution: Transfer a quantity of finely powdered Tablets to a suitable container, and add water to obtain a final concentration of about 0.03 mg/mL of pioglitazone. Sonicate for about 30 s. Pass through a 5-mL portion of the resulting suspension using a suitable filter of 0.45-µm pore size, then wash the filter with 10 mL of water, and discard the filtrate. Wash the filter with 5 mL of 0.1 N hydrochloric acid, and use the filtrate.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 265 and 271 nm.

Metformin hydrochloride

Sample solution: Transfer a quantity of finely powdered Tablets to a suitable container, and add a suitable quantity of water, based on the labeled amount of metformin hydrochloride in the sample, to obtain a final concentration of about 0.4 mg/mL of metformin hydrochloride. Sonicate for about 30 s, and pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate. Dilute a portion of the filtrate with water to obtain a solution containing about 8 µg/mL of metformin hydrochloride.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 230 and 235 nm.

• **B.** The retention times of the pioglitazone and metformin peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: 7.2 g/L of sodium dodecyl sulfate in a mixture of 0.05 M monobasic ammonium phosphate and acetonitrile (1:1)

Diluent: Methanol and 0.1 N hydrochloric acid (1:1)

System suitability stock solution: 0.5 mg/mL of *p*-methoxyacetophenone and 0.4 mg/mL of butylparaben in *Diluent*

Pioglitazone standard stock solution: 0.84 mg/mL of USP Pioglitazone Hydrochloride RS in *Diluent*

Mixed standard stock solution: 2.5 mg/mL of USP Metformin Hydrochloride RS and 0.084 mg/mL of USP Pioglitazone Hydrochloride RS in 0.1 N hydrochloric acid from the *Pioglitazone standard stock solution*

System suitability solution: Transfer 10.0 mL of the *Mixed standard stock solution* and 5.0 mL of the *System suitability stock solution* to a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

Standard solution: 16.8 µg/mL of USP Pioglitazone Hydrochloride RS and 0.5 mg/mL of USP Metformin Hydrochloride RS in 0.1 N hydrochloric acid from the *Mixed standard stock solution*

Sample stock solution: Weigh and finely powder NLT 10 Tablets. Transfer an amount of powdered Tablets, equivalent to about 15 mg of pioglitazone, to a 200-mL volumetric flask. Add 120 mL of 0.1 N hydrochloric acid, shake for about 30 min, and then sonicate for about 5 min. Dilute with 0.1 N hydrochloric acid to volume, and mix well. Pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate.

Sample solution: Transfer a suitable volume of the *Sample stock solution* (see *Table 1*) to a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

Table 1

Labeled Amount of Pioglitazone and Metformin Hydrochloride (mg/Tablet)	Volume of Sample Stock Solution Used to Prepare the Sample Solution (mL)	Nominal Concentrations in the Sample Solution	
		Pioglitazone (µg/mL)	Metformin Hydrochloride (mg/mL)
15 and 500	10	15	0.5
15 and 850	5	7.5	0.425

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 255 nm for metformin and *p*-methoxyacetophenone; UV 225 nm for pioglitazone and butylparaben. If this procedure is used for *Identification A*, use a diode-array detector set at 200–400 nm.

Column: 6.0-mm × 15-cm; 5-µm packing L7

Column temperature: 25 ± 5°

Flow rate: 1 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the metformin peak of about 5 min.]

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—See *Table 2* for the approximate relative retention times.]

Table 2

Name	Relative Retention Time
Metformin	1.0
p-Methoxyacetophenone	1.2
Pioglitazone	1.8
Butylparaben	2.1

Suitability requirements

Resolution: NLT 2.5 between metformin and *p*-methoxyacetophenone; NLT 2.5 between pioglitazone and butylparaben, *System suitability solution*

Relative standard deviation: NMT 1.0% for the metformin peak; NMT 1.0% for pioglitazone peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of pioglitazone from the *Sample solution*

r_S = peak response of pioglitazone from the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

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M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metformin from the *Sample solution*
r_S = peak response of metformin from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% for each of the labeled amounts of pioglitazone and metformin hydrochloride

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: pH 2.5 McIlvaine buffer (could be prepared by adjusting 0.1 M citric acid with 0.2 M dibasic sodium phosphate to a pH of 2.5); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Diluent and **Mobile phase:** Proceed as directed in the *Assay*.
Pioglitazone standard stock solution: 0.37 mg/mL of USP Pioglitazone Hydrochloride RS in *Diluent*
Standard solution: 0.0185 mg/mL of USP Pioglitazone Hydrochloride RS from the *Pioglitazone standard stock solution* and (*L*/900) mg/mL of USP Metformin Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet, of metformin hydrochloride
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system: Proceed as directed in the *Assay*, except use an *Injection volume* of 5 µL.
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5 for the metformin peak; NMT 2.0 for the pioglitazone peak
Relative standard deviation: NMT 2.0% for the metformin peak; NMT 2.0% for the pioglitazone peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of pioglitazone from the *Sample solution*
r_S = peak response of pioglitazone from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim of pioglitazone (mg/Tablet)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of metformin hydrochloride from the *Sample solution*
r_S = peak response of metformin hydrochloride from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim of metformin hydrochloride (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved; NLT 80% (*Q*) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: pH 2.5 McIlvaine buffer (could be prepared by adjusting 0.1 M citric acid with 0.2 M dibasic sodium phosphate to a pH of 2.5); 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Solution A: 1.4 g/L of dibasic sodium phosphate anhydrous and 1.4 g/L of sodium dodecyl sulfate in water
Solution B: Phosphoric acid and water (50:50)
Mobile phase: Acetonitrile and *Solution A* (34:66). Adjust with *Solution B* to a pH of 7.1.
Diluent A: Acetonitrile and *Medium* (50:50)
Diluent B: Acetonitrile and water (70:30)
Pioglitazone standard stock solution: 0.019 mg/mL of USP Pioglitazone Hydrochloride RS in *Diluent B*. Sonicate as needed to dissolve.
Metformin standard stock solution: 0.92 mg/mL of USP Metformin Hydrochloride RS in *Medium*. Sonicate as needed to dissolve.
Standard solution: 0.003 mg/mL of USP Pioglitazone Hydrochloride RS from the *Pioglitazone standard stock solution* and 0.11 mg/mL of USP Metformin Hydrochloride RS from the *Metformin standard stock solution* in *Diluent A*
Sample solution: Pass a portion of the solution under test through a suitable filter and dilute with *Diluent A* to a metformin concentration that is similar to the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 5°
Column: 40°
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–2.0 for the metformin peak; 0.8–2.0 for the pioglitazone peak
Relative standard deviation: NMT 2.0% for the metformin peak; NMT 2.5% for the pioglitazone peak
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of pioglitazone from the *Sample solution*
r_S = peak response of pioglitazone from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim of pioglitazone (mg/Tablet)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak response of metformin hydrochloride from the *Sample solution*
r_S = peak response of metformin hydrochloride from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim of metformin hydrochloride (mg/Tablet)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved; NLT 80% (Q) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, ***Content Uniformity:*** Meet the requirements for pioglitazone and metformin hydrochloride

IMPURITIES

• **ORGANIC IMPURITIES: PIOGLITAZONE**

Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)
Diluent: Methanol and 0.1 N hydrochloric acid (1:1)
Standard stock solution: 0.2 mg/mL of USP Pioglitazone Hydrochloride RS, dissolved first in methanol using 20% of the final volume, then diluted with *Mobile phase* to volume
System suitability solution: Prepare a solution containing 0.3 mg/mL of benzophenone in methanol. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of the *Standard stock solution*, and dilute with *Mobile phase* to volume. This solution contains 20 µg/mL of USP Pioglitazone Hydrochloride RS and 6 µg/mL of benzophenone.
Standard solution: 1 µg/mL of USP Pioglitazone Hydrochloride RS in *Mobile phase* from the *Standard stock solution*
Sample solution: Weigh and finely powder 10 Tablets. Transfer an amount of powdered Tablets, equivalent to about 18 mg of pioglitazone, to a 100-mL volumetric flask, and add 50 mL of *Diluent*. Shake for 30 min, and dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 5°
Flow rate: 0.8 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the pioglitazone peak of about 7 min.]
Injection volume: 40 µL
Run time: At least 4 times the retention time of the pioglitazone peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Elution order is the pioglitazone peak followed by benzophenone.]
Suitability requirements
Resolution: NLT 10 between pioglitazone and benzophenone, *System suitability solution*
Tailing factor: NMT 1.5 for the pioglitazone peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each pioglitazone related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of pioglitazone from the *Standard solution*
C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria
Any individual pioglitazone related impurity: NMT 0.2%
Total pioglitazone related impurities: NMT 0.6%
[NOTE—Disregard the peaks due to metformin and its impurities that elute before 4.5 min, corresponding to the relative retention time of the pioglitazone peak of about 0.64.]

• **ORGANIC IMPURITIES: METFORMIN**

Solution A: 1.74 g of sodium 1-pentanesulfonate and 1.15 g of monobasic ammonium phosphate in 1000 mL of water
Solution B: Acetonitrile and water (7:3)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	70	30
15.1	0	100
25	0	100
25.1	100	0
35	100	0

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System suitability solution: 5 µg/mL of USP Metformin Hydrochloride RS and 2 µg/mL of melamine in water
Standard solution: 5 µg/mL of USP Metformin Hydrochloride RS in water
Sample solution: Accurately weigh 10 Tablets, and finely powder. Transfer an amount of powdered Tablets, equivalent to about 100 mg of metformin hydrochloride, to a 100-mL volumetric flask, and add 50 mL of water. Shake for 30 min. Dilute with water to volume, and pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L62
Column temperature: 25 ± 5°
Flow rate: 1.0 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the metformin peak of about 8 min.]
Run time: 15 min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for melamine and metformin are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4 between melamine and metformin hydrochloride, *System suitability solution*
Tailing factor: NMT 1.5 for the metformin hydrochloride peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each metformin hydrochloride related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

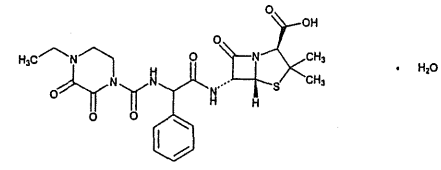
r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of metformin hydrochloride from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria
Any individual impurity: NMT 0.1%
Total impurities: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Metformin Hydrochloride RS
USP Pioglitazone Hydrochloride RS

Piperacillin



$C_{23}H_{27}N_5O_7S \cdot H_2O$ 535.57
$C_{23}H_{27}N_5O_7S$ 517.56
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl] amino] phenylacetyl]amino]-3,3-dimethyl-7-oxo-, monohydrate, [2*S*-2α,5α,6β(*S**)]];
(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid monohydrate [66258-76-2].
Anhydrous [61477-96-1].

DEFINITION

Piperacillin contains NLT 960 µg/mg and NMT 1030 µg/mg of piperacillin ($C_{23}H_{27}N_5O_7S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, water, 0.2 M monobasic sodium phosphate, and 0.4 M tetrabutylammonium hydroxide (450:447:100:3). Adjust with phosphoric acid to a pH of 5.50.
System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*
Standard solution: 0.4 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.
Sample solution: 0.4 mg/mL of Piperacillin in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*
Tailing factor: NMT 1.2 for the piperacillin peak, *System suitability solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the potency of piperacillin ($C_{23}H_{27}N_5O_7S$) in the portion of Piperacillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)

Acceptance criteria: 960–1030 µg/mg on the anhydrous basis

IMPURITIES

• **AMPICILLIN, PIPERACILLIN PENICILLOIC ACID, PIPERACILLIN RELATED COMPOUND E, AND ACETYLATED PENICILLOIC ACID OF PIPERACILLIN**

Mobile phase, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution 1: 0.08 mg/mL of USP Ampicillin RS in *Mobile phase*

Standard solution 2: 0.04 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of ampicillin in the portion of Piperacillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of ampicillin from the *Sample solution*
r_S = peak response of ampicillin from *Standard solution 1*
C_S = concentration of USP Ampicillin RS in *Standard solution 1* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of ampicillin in USP Ampicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentages of specified impurities other than ampicillin in the portion of Piperacillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F_1) \times F_2 \times 100$$

r_U = peak response of each specified impurity other than ampicillin from the *Sample solution*
r_S = peak response of piperacillin from *Standard solution 2*
C_S = concentration of USP Piperacillin RS in *Standard solution 2* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)
F_1 = relative response factor (see *Table 1*)
F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E[a]	0.24	2.4	0.2
Ampicillin	0.31	1.0	0.2
Acetylated penicilloic acid of piperacillin[b]	0.37	1.1	0.4
Piperacillin penicilloic acid[c]	0.62	0.7	1.0
Piperacillin	1.0	—	—

[a] 1-Ethyl-2,3-piperazinedione.
[b] (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[c] (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

• **PIPERACILLINYLAMPICILLIN**

Mobile phase: Methanol, water, 0.2 M monobasic sodium phosphate, and 0.4 M tetrabutylammonium hydroxide (615:282:100:3). Adjust with phosphoric acid to a pH of 5.50.

Standard solution: 0.04 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution: 0.4 mg/mL of Piperacillin in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Relative standard deviation: NMT 2%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of piperacillinylampicillin in the portion of Piperacillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F_1) \times F_2 \times 100$$

r_U = peak response of piperacillinylampicillin from the *Sample solution*
r_S = peak response of piperacillin from the *Standard solution*
C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)
F_1 = relative response factor (see *Table 2*)
F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*.

USP Monographs

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Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin	1.0	—	—
Piperacillinylampicillin[a]	2.55	0.7	2.0
Total impurities[b]	—	—	3.8

[a] (2*S*,5*R*,6*R*)-6-((*R*)-2-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[b] Total impurities is the sum of all impurities reported in the tests for *Ampicillin*, *Piperacillin Penicilloic Acid*, *Piperacillin Related Compound E, and Acetylated Penicilloic Acid of Piperacillin*, and *Piperacillinylampicillin*.

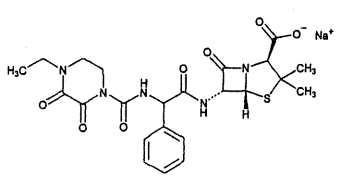
SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: 2.0%–4.0%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 40 mg/mL in methanol
  Acceptance criteria: +155° to +175°
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Piperacillin is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.07 USP Endotoxin Unit/mg of piperacillin.
- **STERILITY TESTS** ⟨71⟩: Where the label states that Piperacillin is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ampicillin RS
  USP Piperacillin RS

Piperacillin Sodium



$C_{23}H_{26}N_5NaO_7S$ 539.54

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2*S*-[2α,5α,6β(*S**)]];

Sodium (2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [59703-84-3].

DEFINITION

Piperacillin Sodium has a potency equivalent to NLT 863 µg/mg and NMT 1007 µg/mg of piperacillin ($C_{23}H_{27}N_5O_7S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the chromatogram compares qualitatively to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements

Add the following:

▲• **C.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Methanol, water, 0.2 M monobasic sodium phosphate, and 0.4 M tetrabutylammonium hydroxide (450:447:100:3). Adjust with phosphoric acid to a pH of 5.50.

System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*

Standard solution 1: 0.4 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution: 0.4 mg/mL of Piperacillin Sodium in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. ▲For *Identification C*, use a diode array detector in the range of 190–400 nm.▲ (USP 1-Dec-2019)
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution 1*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*
Tailing factor: NMT 1.2 for piperacillin, *System suitability solution*
Relative standard deviation: NMT 2% for piperacillin, *Standard solution 1*

Analysis
Samples: *Standard solution 1* and *Sample solution*
Calculate the potency, in µg/mg, of piperacillin ($C_{23}H_{27}N_5O_7S$) in the portion of Piperacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution 1*
C_S = concentration of USP Piperacillin RS in *Standard solution 1* (mg/mL)
C_U = concentration of Piperacillin Sodium in the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)

Acceptance criteria: 863–1007 µg/mg on the anhydrous basis

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IMPURITIES

• **PIPERACILLIN PENICILLOIC ACID AND ACETYLATED PENICILLOIC ACID OF PIPERACILLIN**

Mobile phase, System suitability solution, Standard solution 1, and **Sample solution:** Prepare as directed in the *Assay*.

Standard solution 2: 0.04 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution 1*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*

Tailing factor: NMT 1.2 for piperacillin, *System suitability solution*

Relative standard deviation: NMT 2% for piperacillin, *Standard solution 1*

Analysis

Samples: *Sample solution* and *Standard solution 2*

Calculate the percentages of piperacillin penicilloic acid and acetylated penicilloic acid of piperacillin in the portion of Piperacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F_1) \times F_2 \times 100$$

r_U = peak response of piperacillin penicilloic acid or acetylated penicilloic acid of piperacillin from the *Sample solution*

r_S = peak response of piperacillin from *Standard solution 2*

C_S = concentration of USP Piperacillin RS in *Standard solution 2* (mg/mL)

C_U = concentration of Piperacillin Sodium in the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

F_1 = relative response factor (see *Table 1*)

F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E[a,b]	0.24	—	—
Ampicillin	0.31	—	—
Acetylated penicilloic acid of piperacillin[c]	0.37	1.1	1.0
Piperacillin penicilloic acid[d]	0.62	0.7	3.5
Piperacillin	1.0	—	—

[a] This impurity is not to be reported.

[b] 1-Ethyl-2,3-piperazinedione.

[c] (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[d] (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 400 mg/mL

Acceptance criteria: 5.5–7.5

• **WATER DETERMINATION** ⟨921⟩, *Method I*

Test preparation: Prepare as described for hygroscopic substances.

Acceptance criteria: NMT 1.0%

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Where the label states that Piperacillin Sodium must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Piperacillin is used can be met.▲ (USP 1-Dec-2019)

Change to read:

• **STERILITY TESTS** ⟨71⟩: ▲It meets the requirements where the label states that Piperacillin Sodium is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms.▲ (USP 1-Dec-2019)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Change to read:

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. ▲Where Piperacillin Sodium must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.▲ (USP 1-Dec-2019)

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ampicillin RS

USP Piperacillin RS

Piperacillin for Injection

DEFINITION

Piperacillin for Injection contains an amount of Piperacillin Sodium equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$).

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Dissolve 250 mg in water. Add 0.5 mL of 2 N hydrochloric acid and 5 mL of ethyl acetate. Stir, and allow to stand for 10 min in ice water. Pass through a suitable sintered-glass filter, applying suction. Wash the crystals with 5 mL of water and 5 mL of ethyl acetate, then dry in an oven at 60° for 60 min.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, 0.2 M monobasic sodium phosphate, 0.4 M tetrabutylammonium hydroxide, and water (450:100:3:447). Adjust with phosphoric acid to a pH of 5.50.

System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution 1 (where it is labeled for use as a single-dose container): Equivalent to 0.4 mg/mL of piperacillin from Piperacillin for Injection constituted as directed below. Constitute Piperacillin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents using a suitable hypodermic needle and syringe, and dilute with *Mobile phase*.

Sample solution 2 (where the label states the quantity of piperacillin in a given volume of the constituted solution): Equivalent to 0.4 mg/mL of piperacillin from Piperacillin for Injection constituted as directed below. Constitute Piperacillin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Dilute an aliquot of the constituted solution with *Mobile phase*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*

Tailing factor: NMT 1.2 for the piperacillin peak, *System suitability solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$) in the container or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of piperacillin in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Sample solution 1, Sample solution 2, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*. Evaluate the *Relative standard deviation* using the *Standard solution* prepared in the *Assay*.

Standard solution: 40 µg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Analysis

Samples: *Sample solution 1* or *Sample solution 2* and *Standard solution*

Calculate the percentage of piperacillin penicilloic acid and acetylated penicilloic acid of piperacillin in the portion of Piperacillin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F_1) \times F_2 \times 100$$

r_U = peak response of piperacillin penicilloic acid or acetylated penicilloic acid of piperacillin from *Sample solution 1* or *Sample solution 2*

r_S = peak response of piperacillin from the *Standard solution*

C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of piperacillin in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

F_1 = relative response factor (see *Table 1*)

F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E[a,b]	0.24	—	—
Ampicillin[a]	0.31	—	—
Acetylated penicilloic acid of piperacillin[c]	0.37	1.1	1.0
Piperacillin penicilloic acid[d]	0.62	0.7	3.5

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin	1.0	—	—

[a] These are process impurities that are listed here for information only; they are controlled in the drug substance and are not to be reported.
[b] 1-Ethyl-2,3-piperazinedione.
[c] (2*R*,4*S*)-3-Acetyl-2-[(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[d] (2*R*,4*S*)-2-[(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

SPECIFIC TESTS
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.07 USP Endotoxin Unit/mg of piperacillin.
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩**:** 4.8–6.8, in a solution of 200 mg/mL of piperacillin
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.9%
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ampicillin RS
  USP Piperacillin RS

Piperacillin and Tazobactam for Injection

DEFINITION
Piperacillin and Tazobactam for Injection contains amounts of Piperacillin Sodium and Tazobactam Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amounts of piperacillin ($C_{23}H_{27}N_5O_7S$) and tazobactam ($C_{10}H_{12}N_4O_5S$), the labeled amounts representing proportions of piperacillin to tazobactam of 8:1. It may contain small amounts of a suitable buffer and stabilizer.

IDENTIFICATION
- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Buffer: 27.6 g/L of monobasic sodium phosphate
Solution A: 80 mL of 40% aqueous tetrabutylammonium hydroxide diluted with water to 100 mL
Mobile phase: Methanol, water, *Buffer*, and *Solution A* (510:432:50:8). Adjust with phosphoric acid to a pH of 5.5.
Standard solution: 0.1 mg/mL of USP Tazobactam RS and 1 mg/mL of USP Piperacillin RS in *Mobile phase.* Refrigerate the *Standard solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.
Sample solution: Nominally 0.125 mg/mL of tazobactam and 1 mg/mL of piperacillin from Piperacillin and Tazobactam for Injection in *Mobile phase.* Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Autosampler temperature: 5 ± 3°
System suitability
[NOTE—The relative retention times for tazobactam and piperacillin are 0.36 and 1.0, respectively.]
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for tazobactam and piperacillin
Relative standard deviation: NMT 2.0% for tazobactam and piperacillin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$) in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of piperacillin from the *Sample solution*
r_S = peak response of piperacillin from the *Standard solution*
C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of piperacillin in the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount of tazobactam ($C_{10}H_{12}N_4O_5S$) in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of tazobactam from the *Sample solution*
r_S = peak response of tazobactam from the *Standard solution*
C_S = concentration of USP Tazobactam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tazobactam in the *Sample solution* (mg/mL)
P = potency of tazobactam in USP Tazobactam RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

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IMPURITIES

• ORGANIC IMPURITIES, PROCEDURE 1

Organic Impurities, Procedure 1 is recommended when the impurity profile includes piperacillin impurities 4, 5, and 6.

Buffer: Dilute the contents of one vial of tetrabutylammonium hydrogen sulfate ion pairing reagent with water to 1 L.

Solution A: Phosphoric acid and water (1:4)

Mobile phase: Acetonitrile and *Buffer* (25:75). Adjust with *Solution A* to a pH of 3.8.

Diluent: Acetonitrile and water (25:75)

Standard stock solution 1: 60 µg/mL of USP Tazobactam Related Compound A RS in *Diluent*

Standard stock solution 2: 0.5 mg/mL of USP Tazobactam RS in *Diluent*

Standard stock solution 3: 1.0 mg/mL of USP Piperacillin RS in acetonitrile and *Diluent* (1:24). Dissolve first in acetonitrile, using about 4% of the final volume, and dilute with *Diluent* to volume.

System suitability solution: 6 µg/mL of tazobactam related compound A from *Standard stock solution 1* and 25 µg/mL of tazobactam from *Standard stock solution 2* in *Diluent*

Standard solution: 25 µg/mL of tazobactam from *Standard stock solution 2* and 0.2 mg/mL of piperacillin from *Standard stock solution 3* in *Mobile phase*. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Sample solution: Nominally 25 µg/mL of tazobactam and 0.2 mg/mL of piperacillin from Piperacillin and Tazobactam for Injection in *Mobile phase*. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3-µm packing L11

Flow rate: 1 mL/min

Injection volume: 20 µL

Autosampler temperature: 5 ± 3°

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3 between tazobactam related compound A and tazobactam, *System suitability solution*

Tailing factor: NMT 1.8 for tazobactam and piperacillin, *Standard solution*

Relative standard deviation: NMT 2% for tazobactam and piperacillin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of piperacillin from the *Standard solution*

C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of piperacillin in the *Sample solution* (mg/mL)

P = potency of USP Piperacillin RS (µg/mg)

F_1 = correction factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)[a]
Tazobactam related compound A[b]	0.12	0.75	1.0
Tazobactam	0.25	—	—
Piperacillin impurity 4[c]	0.31	1.0	1.0
Piperacillin penilloic acid[d,e]	0.36	1.0	1.0
Piperacillin penicilloic acid[d,f]	0.51	0.56	5.0
Acetylated penicilloic acid of piperacillin[g]	0.55	1.0	1.0
Piperacillin impurity 5[c]	0.62	1.0	1.0
Piperacillin impurity 6[c]	0.67	1.0	1.0
Piperacillin	1.0	—	—
Any individual unspecified impurity	—	1.0	1.0
Total impurities[h]	—	—	5.0

[a] Calculated relative to the peak area of piperacillin.
[b] (2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.
[c] Specified unidentified impurities.
[d] This compound has two epimers that usually co-elute but that may be separated as a result of minor changes in the chromatographic conditions.
[e] (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[f] (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[g] (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[h] Total impurities does not include piperacillin penicilloic acid.

• ORGANIC IMPURITIES, PROCEDURE 2

Organic Impurities, Procedure 2 is recommended when the impurity profile includes piperacillin dimer ethyl ester and piperacillin dimer thiazolamide derivative.

Solution A: 3.12 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 3.5.

Solution B: Methanol

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	85	15
10	65	35
35	55	45
60	35	65
65	90	10
75	90	10

System suitability solution 1: 10 µg/mL of USP Amoxicillin Related Compound A RS and 6 µg/mL of USP Tazobactam Related Compound A RS in *Solution A*. Refrigerate *System suitability solution 1* immediately after preparation and

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during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

System suitability solution 2: 0.2 mg/mL each of USP Piperacillin RS and USP Tazobactam RS in a mixture of methanol and *Solution A* (30:70). Prepare the solution by dissolving the compounds in methanol and diluting with *Solution A* to volume. Refrigerate *System suitability solution 2* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Sample solution: Nominally 2 mg/mL of piperacillin and 0.25 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in *Solution A*. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 30°

Autosampler: 5 ± 3°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution 1* and *System suitability solution 2*

Suitability requirements

Resolution: NLT 1.5 between tazobactam related compound A and amoxicillin related compound A, *System suitability solution 1*

Tailing factor: NMT 2.0 for the piperacillin and tazobactam peaks, *System suitability solution 2*

Relative standard deviation: NMT 10.0% for the piperacillin and tazobactam peaks, *System suitability solution 2*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the responses of all peaks from the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tazobactam related compound A[a]	0.11	0.3
Amoxicillin related compound A[b]	0.13	0.2
Piperacillin related compound E[c]	0.17	0.8
Tazobactam	0.25	—
Formyl penicillamine[d]	0.34	0.2
Ampicillin	0.45	0.2
Piperazinedionecarbonyl D-phenylglycine[e]	0.53	0.2
Acetylated penicilloic acid of piperacillin[f]	0.64	0.5
Piperacillin penicilloic acid, isomer 1[g]	0.74	0.15
Piperacillin penicilloic acid, isomer 2[h]	0.78	1.5
L-Piperacillin[i,j]	0.81	—
Piperacillin penilloic acid[k]	0.91	0.5
Piperacillin	1.0	—
Piperacillin methyl ester[i,l]	1.2	—
Piperacillin dimer ethyl ester[m]	1.3	0.2
Piperacillin dimer thiazolamide derivative[n]	1.5	0.2
Piperacillin penicillamide[o]	1.6	0.3
Piperacillin dimer[p]	1.7	0.4
Piperacillinylampicillin[q]	1.9	0.3
Any individual unspecified impurity	—	0.1
Total impurities	—	4.0

[a] (2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.

[b] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[c] 1-Ethylpiperazine-2,3-dione.

[d] 2-Formamido-3-mercapto-3-methylbutanoic acid.

[e] (*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.

[f] *N*-Acetyl piperacillin open ring; (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[g] (2*S*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[h] Piperacillin open ring; (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[i] (2*S*,5*R*,6*R*)-6-[(*S*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[j] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[k] Piperacillin penilloic analog; (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[l] (2*S*,5*R*,6*R*)-Methyl 6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

[m] 2-(((3-Acetyl-4-(ethoxycarbonyl)-5,5-dimethylthiazolidin-2-yl)methyl)amino)-2-oxo-1-phenylethyl 6-(2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

[n] (2*R*,4*S*)-2-{(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-3-((2*R*,4*S*)-2-{(*R*)-carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carbonyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

[o] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[p] (2*R*,4*S*)-2-{(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-3-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[q] Piperacillin amide dimer; (2*S*,5*R*,6*R*)-6-((*R*)-2-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

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• **ORGANIC IMPURITIES, PROCEDURE 3**

Organic Impurities, Procedure 3 is recommended when the impurity profile includes piperacillinpenicillenic acid and piperazinedionecarbonyl D-phenylglycylglycine.

Buffer: 27.6 g/L of monobasic sodium phosphate dihydrate

Solution A: 0.4 M aqueous tetrabutylammonium hydroxide

Solution B: Methanol, *Solution A*, *Buffer*, and water (275:3:100:622). Adjust with phosphoric acid to a pH of 5.5.

Solution C: Methanol, *Solution A*, *Buffer*, and water (615:3:100:282). Adjust with phosphoric acid to a pH of 5.5.

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution B (%)	Solution C (%)
0	100	0
6	100	0
55	71	29
73	10	90
85	10	90

System suitability solution: 60 µg/mL of USP Piperacillin Related Compound E RS, 0.1 mg/mL of USP Tazobactam Related Compound A RS, and 0.76 mg/mL of USP Tazobactam RS in *Solution C*

Standard solution 1: 6 mg/mL of USP Piperacillin RS in *Solution C*

Standard solution 2: 0.06 mg/mL of USP Piperacillin RS in *Solution C*

Sample solution: Nominally 5.1 mg/mL of piperacillin and 0.64 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in water. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 4°. Analyze within 10 h of preparation.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 40°

Autosampler: 4°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution 2*

Suitability requirements

Resolution: NLT 1.5 between tazobactam related compound A and piperacillin related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for piperacillin, *Standard solution 2*

Relative standard deviation: NMT 10.0% for the tazobactam peak, *System suitability solution*

Analysis

Samples: *Standard solution 2* and *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of piperacillin from *Standard solution 2*

C_S = concentration of USP Piperacillin RS in *Standard solution 2* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

F_1 = correction factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5*. Disregard peaks that are 0.05 times the response of the peak in *Standard solution 2*. Disregard peaks that elute after piperacillinylampicillin.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E[a]	0.05	2.4	0.5
Tazobactam related compound A[b]	0.06	0.52	1.0
Tazobactam	0.09	—	—
Formyl penicillamine[c]	0.12	0.31	0.2
Ampicillin	0.14	0.79	0.3
Piperazinedionecarbonyl D-phenylglycine[d]	0.30	1.0	0.5
Piperazinedionecarbonyl D-phenylglycylglycine[e]	0.36	1.0	0.2
Acetylated penicilloic acids of piperacillin[f]	0.57	1.0	0.3
Piperacillinpenicillenic acid[g]	0.60	1.0	0.2
Ampicillin hydantoin analog[h]	0.65	1.0	0.3
Piperacillin penicilloic acid, isomer 1[i]	0.71	1.0	
Piperacillin penicilloic acid, isomer 2[j, k]	0.83	1.0	3.0
Piperacillin oxalylamide[l]	0.75	1.0	0.2
L-Piperacillin[m, n]	0.80	1.0	—
	0.87	1.0	
Piperacillin penilloic acids[o, p]	0.92	1.0	1.0
Piperacillin	1.0	—	—
Piperazinedionecarbonyl D-phenylglycylampicillin[n, q]	1.26	1.0	—
Open ring piperacillinylampicillin[n, r]	1.36	1.0	—
Piperacillin penicillamide[s]	1.38	1.0	0.2
Piperacillin dimer[t]	1.41	1.0	0.5
Piperacillinylampicillin[u]	1.54	1.0	1.0

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Table 5 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	1.0	0.1

[a] 1-Ethylpiperazine-2,3-dione.
[b] (2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.
[c] 2-Formamido-3-mercapto-3-methylbutanoic acid.
[d] (*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.
[e] (*R*)-2-[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido] acetic acid.
[f] (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[g] 2-{[(*E*)-(2-[(*R*)-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)(phenyl)methyl]-5-oxooxazol-4(5*H*)-ylidene}methyl]amino}-3-mercapto-3-methylbutanoic acid.
[h] (2*S*,5*R*,6*R*)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[i] (2*S*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[j] (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[k] The limit is for the sum of the two epimers of piperacillin open ring.
[l] (2*S*,5*R*,6*R*)-6-(2-{3-[2-(1-Carboxy-*N*-ethylformamido)ethyl]ureido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[m] (2*S*,5*R*,6*R*)-6-[(*S*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[n] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.
[o] (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido] methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[p] The limit is for the sum of the two isomers of piperacillin penilloic analog.
[q] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[r] (2*S*,5*R*,6*R*)-6-{(2*R*)-2-(2-[(4*S*)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido] acetamido)-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[s] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[t] (2*R*,4*S*)-2-{(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-3-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[u] (2*S*,5*R*,6*R*)-6-((*R*)-2-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

- **ORGANIC IMPURITIES, PROCEDURE 4**

Organic Impurities, Procedure 4 is recommended when the impurity profile includes piperacillin sulfoxide and piperacillin methyl penicilloate.

Buffer: 4 g/L of monobasic sodium phosphate dihydrate

Solution A: Acetonitrile and *Buffer* (2:98) adjusted with 1 M sodium hydroxide to a pH of 6.0 ± 0.05

Solution B: Acetonitrile

Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	90	10
25	82	18
30	75	25
40	50	50
45	50	50
50	100	0
60	100	0

System suitability solution: 10 µg/mL of USP Amoxicillin Related Compound A RS and 6 µg/mL of USP Tazobactam Related Compound A RS in *Buffer*

Standard stock solution: 1 mg/mL of USP Piperacillin RS and 36 µg/mL of USP Tazobactam RS, prepared as follows. Dissolve suitable amounts of USP Piperacillin RS and USP Tazobactam RS in a small amount of acetonitrile. Dilute with *Buffer* to volume.

Standard solution: 50 µg/mL of piperacillin and 1.8 µg/mL of tazobactam from *Standard stock solution* in *Buffer*

Sample solution: Nominally 5 mg/mL of piperacillin and 0.625 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in *Buffer*. Store the *Sample solution* at 2°–8°, and use within 1 h.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 2°–8°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between tazobactam related compound A and amoxicillin related compound A, *System suitability solution*
Tailing factor: NMT 2.0 for the piperacillin and tazobactam peaks, *Standard solution*
Relative standard deviation: NMT 5.0% for the piperacillin and tazobactam peaks, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity other than tazobactam related compound A in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity other than tazobactam related compound A from the *Sample solution*
r_S = peak response of piperacillin from the *Standard solution*
C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of piperacillin in the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of tazobactam related compound A in the portion of Piperacillin and Tazobactam for Injection taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of tazobactam related compound A from the *Sample solution*
r_S = peak response of tazobactam from the *Standard solution*
C_S = concentration of USP Tazobactam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tazobactam in the *Sample solution* (mg/mL)
P = potency of tazobactam in USP Tazobactam RS (mg/mg)

Acceptance criteria: See *Table 7*.

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tazobactam related compound A[a]	0.08	1.0
Amoxicillin related compound A[b]	0.15	0.2
Piperacillin related compound E[c]	0.18	0.8
Tazobactam	0.25	—
Ampicillin	0.51	0.2
Acetylated penicilloic acid of piperacillin[d]	0.59	0.6
Piperazinedionecarbonyl D-phenylglycine[e]	0.63	0.1
Piperacillin penicilloic acid, isomer 1[f]	0.65	2.0
Piperacillin penicilloic acid, isomer 2[g]	0.74	
Ampicillin hydantoin analog, isomer 1[h]	0.78	0.2
Ampicillin hydantoin analog, isomer 2[h]	0.80	0.15
Piperacillin sulfoxide[i]	0.90	0.15
Piperacillin penilloic analog, isomer 1[j]	0.94	0.5
Piperacillin penilloic analog, isomer 2[j]	0.95	
Piperacillin methyl penicilloate[k, l]	0.98	—
Piperacillin	1.0	—
Piperacillin dimer[m]	1.2	0.3
Piperacillinylampicillin[l, n]	1.31	—

Table 7 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.10

[a] (2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.
[b] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[c] 1-Ethylpiperazine-2,3-dione.
[d] (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[e] (*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.
[f] (2*S*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[g] (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[h] (2*S*,5*R*,6*R*)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[i] (2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4-oxide.
[j] (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[k] (2*R*,4*S*)-2-{(*R*)-1-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-methoxy-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[l] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.
[m] (2*R*,4*S*)-2-{(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-3-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[n] (2*S*,5*R*,6*R*)-6-((*R*)-2-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS

- **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 0.08 USP Endotoxin Units in a portion equivalent to 1 mg of a mixture of piperacillin and tazobactam (0.89 and 0.11 mg, respectively).
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements
- **pH** ⟨791⟩
  Sample solution: Nominally 40 mg/mL of piperacillin
  Acceptance criteria: 5.0–7.0
- **Water Determination** ⟨921⟩, *Method I*: NMT 2.5%
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*. Store at controlled room temperature.
- **Labeling:** Label it to indicate its sodium content. If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP Reference Standards** ⟨11⟩
  USP Amoxicillin Related Compound A RS
  (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
  $C_8H_{12}N_2O_3S$ 216.26
  USP Piperacillin RS
  USP Piperacillin Related Compound E RS
  1-Ethylpiperazine-2,3-dione.
  $C_6H_{10}N_2O_2$ 142.16

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USP Tazobactam RS
USP Tazobactam Related Compound A RS
(2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.
$C_7H_{12}N_4O_4S$ 248.26

Piperazine



$C_4H_{10}N_2$ 86.14
Piperazine.
Piperazine [110-85-0].

» Piperazine contains not less than 98.0 percent and not more than 101.0 percent of $C_4H_{10}N_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Piperazine RS
Color of solution—Dissolve 10.0 g in water, and dilute with water to 50.0 mL: the solution has no more color than a standard solution prepared by adding 2.0 mL of ferric chloride CS to water and diluting with water to 50.0 mL, when compared in matched color-comparison tubes.

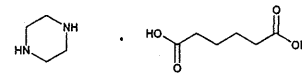
Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*.▲ (CN 1-May-2020)
B: In the test for *Chromatographic purity,* the principal spot in the chromatogram of *Test solution 2,* observed after spraying with the ninhydrin solutions, corresponds in R_F value, color, and size to that in the chromatogram of *Standard solution 1.*
Melting range ⟨741⟩: between 109° and 113°.
Water Determination, *Method I* ⟨921⟩: not more than 2.0%.

Chromatographic purity—
Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).
Standard solution 1—Prepare a solution of USP Piperazine RS in *Solvent* containing 10 mg per mL.
Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.
Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.
Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of USP Piperazine RS per mL.
Test solution 1—Prepare a solution of Piperazine in *Solvent* containing 100 mg per mL.
Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent.*
Procedure—Apply separate 5-µL portions of *Standard solution 1, Standard solution 2, Standard solution 3, Resolution solution, Test solution 1,* and *Test solution 2* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% (w/v) solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% (w/v) solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.
Assay—Weigh accurately about 150 mg of Piperazine, and dissolve in 75 mL of glacial acetic acid. Titrate potentiometrically with 0.1 N perchloric acid VS, using a silver-glass electrode system. As the endpoint is approached, warm the solution to 60° to 70°, then complete the titration. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 4.307 mg of $C_4H_{10}N_2$.

Piperazine Adipate



$C_4H_{10}N_2 \cdot C_6H_{10}O_4$ 232.28
Piperazine, compound with 1,4-butanediacarboxylic acid (1:1).
Piperazine, compound with hexanedioic acid (1:1) [142-88-1].

» Piperazine Adipate contains not less than 98.0 percent and not more than 101.0 percent of $C_4H_{10}N_2 \cdot C_6H_{10}O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Piperazine Adipate RS

Identification—

Change to read:

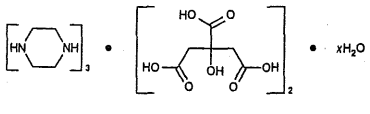
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020)
B: In the test for *Chromatographic purity,* the principal spot in the chromatogram obtained from *Test solution 2* observed after spraying with the ninhydrin solutions corresponds in R_F value, color, and size to that in the chromatogram obtained from *Standard solution 1.*
C: To 10 mL of a 1 in 20 solution of it add 5 mL of hydrochloric acid, and extract with three 10-mL portions of

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ether. Evaporate the combined ether extracts to dryness, wash the residue with water, and dry at 105°: the residue of adipic acid so obtained melts at between 150° and 154°.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).
Test solution 1—Prepare a solution of Piperazine Adipate in *Solvent* containing 100 mg per mL.
Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.
Standard solution 1—Prepare a solution of USP Piperazine Adipate RS in *Solvent* containing 10 mg per mL.
Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.
Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.
Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of Piperazine Adipate per mL.
Procedure—Separately apply 5-µL portions of *Test solution 1, Test solution 2, Standard solution 1, Standard solution 2, Standard solution 3,* and the *Resolution solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.
Assay—Dissolve about 100 mg of Piperazine Adipate, accurately weighed, in 10 mL of glacial acetic acid, heating gently if necessary. Add 60 mL of glacial acetic acid and 0.25 mL of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid until the brownish-yellow color of the solution turns green. Each mL of 0.1 N perchloric acid is equivalent to 11.61 mg of $C_4H_{10}N_2 \cdot C_6H_{10}O_4$.

Piperazine Citrate



$(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7 \cdot xH_2O$ (anhydrous) 642.65

Piperazine, 2-hydroxy-1,2,3-propanetricarboxylate (3:2), hydrate.
Piperazine citrate (3:2) hydrate [41372-10-5].
Anhydrous 642.66
[144-29-6].

» Piperazine Citrate contains not less than 98.0 percent and not more than 100.5 percent of $(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Piperazine Citrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: In the test for *Chromatographic purity,* the principal spot in the chromatogram of *Test solution 2,* observed after spraying with the ninhydrin solutions, corresponds in R_F value, color, and size to that in the chromatogram of *Standard solution 1*.
C: It responds to the tests for *Citrate* ⟨191⟩.
Water Determination, *Method I* ⟨921⟩: not more than 12.0%.

Chromatographic purity—
Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).
Standard solution 1—Prepare a solution of USP Piperazine Citrate RS in *Solvent* containing 10 mg per mL.
Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.
Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.
Test solution 1—Prepare a solution of Piperazine Citrate in *Solvent* containing 100 mg per mL.
Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.
Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of Piperazine Citrate per mL.
Procedure—Apply separate 5-µL portions of *Standard solution 1, Standard solution 2, Standard solution 3, Resolution solution, Test solution 1,* and *Test solution 2* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% (w/v) solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% (w/v) solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the

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principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.
Assay—Dissolve about 200 mg of Piperazine Citrate, accurately weighed, in 100 mL of glacial acetic acid, warming slightly if necessary to effect solution. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 10.71 mg of $(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7$.

Piperazine Citrate Syrup

» Piperazine Citrate Syrup is prepared from Piperazine Citrate or from Piperazine to which an equivalent amount of Citric Acid is added. It contains an amount of piperazine citrate equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of piperazine hexahydrate ($C_4H_{10}N_2 \cdot 6H_2O$).

Packaging and storage—Preserve in tight containers.

Identification—
A: To 2 mL of Syrup add 5 mL of 3 N hydrochloric acid, then add, with stirring, 1 mL of sodium nitrite solution (1 in 2). Chill in an ice bath for 15 minutes, stirring if necessary, to induce crystallization, filter the precipitate on a sintered-glass funnel, wash with 10 mL of cold water, and dry at 105°: the *N,N'*-dinitrosopiperazine so obtained melts between 156° and 160°.
B: It responds to the tests for *Citrate* ⟨191⟩.
Assay—Determine the specific gravity of Syrup, and transfer an accurately weighed portion of the Syrup, equivalent to about 200 mg of piperazine citrate, to a 250-mL beaker. Add 10 mL of water and 75 mL of trinitrophenol TS, stir well, and allow to stand in a refrigerator for not less than 2 hours. Collect the precipitate in a tared filtering crucible, wash with five 10-mL portions of dehydrated alcohol, and dry at 105° to constant weight. *[Caution—Picrates may explode.]* The weight of the dipicrate, multiplied by 0.3568, gives the equivalent of $C_4H_{10}N_2 \cdot 6H_2O$ in the portion of Syrup taken.

Delete the following:

▲Piperazine Citrate Tablets

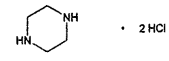
» Piperazine Citrate Tablets contain an amount of piperazine citrate equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of piperazine hexahydrate ($C_4H_{10}N_2 \cdot 6H_2O$).

Packaging and storage—Preserve in tight containers.

Identification—
A: Finely powder a number of Tablets, equivalent to about 200 mg of piperazine citrate, mix with 5 mL of 3 N hydrochloric acid, and filter. To the filtrate add, with stirring, 1 mL of sodium nitrite solution (1 in 2). Chill in an ice bath for 15 minutes, stirring if necessary, to induce crystallization, filter the precipitate on a sintered-glass funnel, wash with 10 mL of cold water, and dry at 105°: the N,N'-dinitrosopiperazine so obtained melts between 156° and 160°.
B: To a quantity of powdered Tablets, equivalent to about 500 mg of piperazine citrate, add 10 mL of water, shake, and filter: the filtrate responds to the tests for *Identification Tests—General* ⟨191⟩, *Citrate*.

Dissolution ⟨711⟩, *Procedure for a Pooled Sample—*
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of piperazine hexahydrate ($C_4H_{10}N_2 \cdot 6H_2O$) dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_4H_{10}N_2 \cdot 6H_2O$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨ 905 ⟩ : meet the requirements.
Assay—Weigh and finely powder not less than 20 Tablets. Shake an accurately weighed portion of the powder, equivalent to about 200 mg of piperazine citrate, for 1 hour with 10 mL of a mixture of 1 part of 3 N hydrochloric acid and 3 parts of water, filter, and wash the residue with two 10-mL portions of water. To the combined extract and washings add 75 mL of trinitrophenol TS, and proceed as directed in the *Assay* under *Piperazine Citrate Syrup*, beginning with "stir well." ▲(USP 1-May-2020)

Piperazine Dihydrochloride



$C_4H_{10}N_2 \cdot 2HCl \cdot xH_2O$ 159.06 (anhydrous)
Piperazine dihydrochloride hydrate [142-64-3].

» Piperazine Dihydrochloride contains not less than 98.5 percent and not more than 100.5 percent of $C_4H_{10}N_2 \cdot 2HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Piperazine Dihydrochloride RS.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲(CN 1-May-2020)—
Test specimen: previously dried at 105° for 3 hours.
B: In the test for *Chromatographic purity*, the principal spot in the chromatogram obtained from *Test solution 2*, observed after spraying with the ninhydrin solutions, corresponds in R_F value, color, and size to that in the chromatogram obtained from *Standard solution 1*.

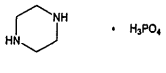
USP Monographs

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C: It meets the requirements of the test for *Chloride* ⟨191⟩.
pH ⟨791⟩**:** between 3.0 and 3.4, in a solution (1 in 20).
Water Determination, *Method I* ⟨921⟩**:** not more than 10.0%.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).
Test solution 1—Prepare a solution of Piperazine Dihydrochloride in *Solvent* containing 100 mg per mL.
Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.
Standard solution 1—Prepare a solution of USP Piperazine Dihydrochloride RS in *Solvent* containing 10 mg per mL.
Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.
Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.
Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of Piperazine Dihydrochloride per mL.
Procedure—Separately apply 5-µL portions of *Test solution 1, Test solution 2, Standard solution 1, Standard solution 2, Standard solution 3,* and the *Resolution solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plates from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.
Assay—Dissolve about 140 mg of Piperazine Dihydrochloride in 4 mL of ethylene glycol using a 150-mL beaker. Add 25 mL of glacial acetic acid containing 1.2 g of mercuric acetate, rinsing the walls of the beaker with a small amount of the glacial acetic acid. Add 0.25 mL of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 7.953 mg of $C_4H_{10}N_2 \cdot 2HCl$.

Piperazine Phosphate



$C_4H_{10}N_2 \cdot H_3PO_4 \cdot H_2O$ 202.15
Piperazine phosphate (1:1), monohydrate.
Piperazine phosphate (1:1), monohydrate [18534-18-4].
Anhydrous 184.13 [14538-56-8].

» Piperazine Phosphate contains not less than 98.5 percent and not more than 100.5 percent of $C_4H_{10}N_2 \cdot H_3PO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, and store at room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩**—**
USP Piperazine Phosphate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)
Test specimen: previously dried at 105° for 3 hours.
B: In the test for *Chromatographic purity,* the principal spot in the chromatogram obtained from *Test solution 2,* observed after spraying with the ninhydrin solutions, corresponds in R_F value, color, and size to that in the chromatogram obtained from *Standard solution 1*.
C: It meets the requirements of the test for *Phosphate* ⟨191⟩.
pH ⟨791⟩**:** between 6.0 and 6.5, in a solution (1 in 100).
Water, *Method I* ⟨921⟩**:** between 8.0% and 9.5%.

Chromatographic purity—
Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).
Test solution 1—Prepare a solution of Piperazine Phosphate in *Solvent* containing 100 mg per mL.
Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.
Standard solution 1—Prepare a solution of USP Piperazine Phosphate RS in *Solvent* containing 10 mg per mL.
Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.
Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.
Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of Piperazine Phosphate per mL.
Procedure—Separately apply 5-µL portions of *Test solution 1, Test solution 2, Standard solution 1, Standard solution 2, Standard solution 3,* and the *Resolution solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%).

USP Monographs

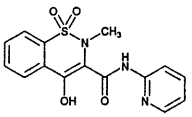
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In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.

Change to read:

Assay—Dissolve about 200 mg of Piperazine Phosphate in 4 mL of ethylene glycol using a 150-mL beaker. Add 25 mL of glacial acetic acid, rinsing the walls of the beaker with a small amount of the glacial acetic acid. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to ▲9.207 mg of $C_4H_{10}N_2 \cdot H_3PO_4$.▲ (ERR 1-Jun-2019)

Piroxicam



$C_{15}H_{13}N_3O_4S$ 331.35

2*H*-1,2-Benzothiazine-3-carboxamide, 4-hydroxy-2-methyl-*N*-2-pyridinyl-, 1,1-dioxide;
4-Hydroxy-2-methyl-*N*-2-pyridyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide [36322-90-4].

DEFINITION

Piroxicam contains NLT 97.0% and NMT 103.0% of piroxicam ($C_{15}H_{13}N_3O_4S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M.**▲ (CN 1-May-2020) Do not dry specimens.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Solution: 10 µg/mL
Medium: Hydrochloric acid in methanol (1 in 1200)
Acceptance criteria: Meets the requirements

- **C. THIN-LAYER CHROMATOGRAPHY**

Diluent: Chloroform and methanol (1:1)
Standard solution: 1 mg/mL of USP Piroxicam RS in *Diluent*
Sample solution: 1 mg/mL in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Absorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 20 µL
Developing solvent system: Toluene and glacial acetic acid (95:5)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and air-dry. Place the plate in the developing chamber, and develop as before. Remove the plate from the chamber, mark the solvent front, and air-dry. Locate the spots on the plate by viewing under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 7.72 g of anhydrous citric acid in 400 mL of water.
Solution B: Dissolve 5.35 g of dibasic sodium phosphate in 100 mL of water.
Solution C: Add *Solution B* to *Solution A*, and dilute with water to 1000 mL.
Mobile phase: Methanol and *Solution C* (450:550)
Diluent: 0.01 N methanolic hydrochloric acid
Standard stock solution: 0.25 mg/mL of USP Piroxicam RS in *Diluent*
Standard solution: 0.05 mg/mL of USP Piroxicam RS prepared as follows. Transfer 10.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add about 25 mL of *Diluent* and 10.0 mL of water, and dilute with *Diluent* to volume.
Sample stock solution: 0.5 mg/mL of Piroxicam in *Diluent*
Sample solution: Transfer 10.0 mL of *Sample stock solution* to a second 100-mL volumetric flask, add about 50 mL of *Diluent* and 20.0 mL of water, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 500 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of piroxicam ($C_{15}H_{13}N_3O_4S$) in the portion of Piroxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Piroxicam RS in the *Standard solution* (mg/mL)
C_U = concentration of Piroxicam in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%

SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Piroxicam RS

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Piroxicam Capsules

DEFINITION

Piroxicam Capsules contain NLT 92.5% and NMT 107.5% of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Diluent: Chloroform and methanol (1:1)

Standard solution: 1 mg/mL of USP Piroxicam RS in *Diluent*

Sample solution: Dissolve a portion of the contents of Capsules in *Diluent* to obtain a solution containing about 1 mg/mL. Shake by mechanical means for 10 min, and filter a portion. Use the filtrate for the analysis.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Absorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 20 µL

Developing solvent system: Toluene and glacial acetic acid (95:5)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and air-dry. Place the plate in the developing chamber, and develop as before. Remove the plate from the chamber, mark the solvent front, and air-dry. Locate the spots on the plate by viewing under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **Procedure**

Solution A: Dissolve 7.72 g of anhydrous citric acid in 400 mL of water.

Solution B: Dissolve 5.35 g of dibasic sodium phosphate in 100 mL of water.

Solution C: Add *Solution B* to *Solution A*, and dilute with water to make 1000 mL.

Mobile phase: Methanol and *Solution C* (450:550)

Diluent: 0.01 N methanolic hydrochloric acid

Standard stock solution: 0.25 mg/mL of USP Piroxicam RS in *Diluent*

Standard solution: 0.05 mg/mL of USP Piroxicam RS prepared as follows. Transfer 10.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 25 mL of *Diluent* and 10.0 mL of water, and dilute with *Diluent* to volume.

Sample stock solution: Transfer, as completely as possible, the contents of NLT 20 Capsules to a suitable tared container, and determine the average weight per capsule. Mix the combined contents, and transfer an amount nominally equivalent to about 50 mg of piroxicam to a 100-mL volumetric flask. Add about 70 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of this mixture to obtain a clear solution.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add about 50 mL of *Diluent* and 20.0 mL of water, dilute with *Diluent* to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Piroxicam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of piroxicam in the *Sample solution* (mg/mL)

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: Simulated gastric fluid TS, prepared without pepsin; 900 mL

Apparatus 1: 50 rpm

Time: 45 min

Standard stock solution: 0.5 mg/mL of USP Piroxicam RS in methanol

Standard solution: A known concentration of USP Piroxicam RS in *Medium* from *Standard stock solution*. Dilute with *Medium*, if necessary.

Sample solution: Filter a portion of the solution under test, suitably dilute with *Medium*, if necessary.

[Note—Use a suitable filter that does not adsorb piroxicam.]

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 333 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) dissolved by comparing the UV absorbance of *Sample solution* with that of *Standard solution*.

Tolerances: NLT 75% (*Q*) of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

SPECIFIC TESTS

• **Water Determination,** *Method I* ⟨921⟩: NMT 8.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards** ⟨11⟩

USP Piroxicam RS

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Piroxicam Compounded Cream

DEFINITION

Piroxicam Compounded Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$).

Prepare Piroxicam Compounded Cream as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Piroxicam	3 g
White Petrolatum	25 g
Stearyl Alcohol	15 g
Propylparaben	0.06 g
Methylparaben	0.15 g
Propylene Glycol	12.0 g
Sodium Lauryl Sulfate	1 g
Sodium Hydroxide, 1 N	2.5 mL
Purified Water, a sufficient quantity to make	100 g

In an appropriate container (final weight tared), mix the *White Petrolatum* and *Stearyl Alcohol* together, and heat to 80 ± 5° to form a clear oil phase. In a separate container, mix the *Propylparaben, Methylparaben, Propylene Glycol, Sodium Lauryl Sulfate*, and about 30 mL of *Purified Water* together, and heat to 80 ± 5° to form a clear aqueous phase. Add the aqueous phase to the oil phase with continuous stirring, and allow it to cool to 50° to form an emulsion. In a mortar, triturate the *Piroxicam* with the *Sodium Hydroxide* to form a suspension. Using additional *Purified Water* to rinse the mortar, add the piroxicam suspension to the previously prepared emulsion, transferring the suspension stepwise and quantitatively to the emulsion. Add sufficient *Purified Water* with stirring to bring to final weight. Package, and label.

ASSAY

- **PROCEDURE**

Solution A: 2.7 g/L of citric acid and 5.4 g/L of dibasic sodium phosphate in *Purified Water*. Pass through a filter of 0.45-µm pore size.

Mobile phase: Methanol and *Solution A* (50:50). Filter, and degas.

Diluent: 0.01 N methanolic hydrochloric acid prepared by diluting 0.9 mL of hydrochloric acid with methanol to a final volume of 1 L

Standard solution: Dissolve an accurately weighed quantity of USP Piroxicam RS in 2 mL of chloroform, and dilute with *Diluent* to obtain a solution with a nominal concentration of about 50 µg/mL of piroxicam.

Sample solution: Add 340 mg of Cream to 4 mL of chloroform and 150 mL of *Diluent*. Shake the mixture on a wrist action shaker for 15 min, and dilute with *Diluent* to 200 mL. Pass the solution through a filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Blank: Add 340 mg of *Purified Water* to 4 mL of chloroform and 150 mL of *Diluent*. Shake the mixture on a wrist action shaker for 15 min, and dilute with *Diluent* to 200 mL. Pass the solution through a filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Columns

Guard: 4.6-mm × 2-cm; packing L1

Analytical: 4.6-mm × 30-cm; 10-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for piroxicam is about 7 min.]

Suitability requirements

Relative standard deviation: NMT 2.8% for replicate injections

Analysis

Samples: *Standard solution, Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Piroxicam RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of piroxicam in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in a tight, light-resistant, plastic resealable container. Store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **LABELING:** Label it to state the *Beyond-Use Date.*
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Piroxicam RS

Piroxicam Compounded Oral Suspension

DEFINITION

Piroxicam Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$).

Prepare Piroxicam Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩.

Piroxicam powder	1 g
Ora-Blend,[a] a sufficient quantity to make	100 mL

[a] Perrigo, Minneapolis, MN.

Pour the weighed *Piroxicam powder* into a suitable mortar. Wet the powder with a small amount of *Ora-Blend*, and triturate to make a smooth paste. Add the *Ora-Blend* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container. Add sufficient *Ora-Blend* to bring the preparation to final volume. Shake to mix well.

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ASSAY

• **PROCEDURE**

Mobile phase: Mix 500 mL of methanol and 500 mL of 0.1 M sodium acetate, and adjust with phosphoric acid to a pH of 4.0. Add 10 mL of acetonitrile, filter, and degas.

Standard solution: 0.2 mg/mL of piroxicam prepared from USP Piroxicam RS in methanol

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 1.0 mL of the Oral Suspension into a 50-mL volumetric flask, dilute with methanol to volume, and mix well to dissolve. Pass through a PVDF filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 361 nm

Column: 3.9-mm × 15-cm; 4-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for piroxicam is about 4.0 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of piroxicam from the *Sample solution*

r_S = peak response of piroxicam from the *Standard solution*

C_S = concentration of piroxicam in the *Standard solution* (mg/mL)

C_U = nominal concentration of piroxicam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.7–4.7

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

• **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date.*

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Piroxicam RS

Plantago Seed

DEFINITION

Plantago Seed is the cleaned, dried, ripe seed of *Plantago psyllium* auct. or of *Plantago indica* L. (*Plantago arenaria* Waldst. & Kit.), known in commerce as Spanish or French psyllium; or of *Plantago ovata* Forssk., known in commerce as blond psyllium, or Indian plantago seed (Fam. Plantaginaceae).

CONTAMINANTS

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS

• **BOTANICAL CHARACTERISTICS**

Macroscopic

Unground *Plantago psyllium* auct. seed: Ovate to ovate-elongate, concavo-convex; mostly from 1.3–2.7 mm in length, rarely up to 3 mm, and from 600 µm to 1.1 mm in width. It is light brown to moderate brown, darker along the margin, and very glossy; the convex dorsal surface exhibiting a lighter colored longitudinal area extending nearly the length of the seed and representing the embryo lying beneath the seed coat, and showing a sometimes indistinct transverse groove nearer the broader end. The concave ventral surface has a deep cavity, in the center of the base of which is an oval, yellowish white hilum.

Unground *Plantago indica* seed: Ovate-oblong to elliptical, concavo-convex; from 1.6–3 mm in length and from 1–1.5 mm in width. Externally it is dark reddish brown to moderate yellowish brown, occasionally somewhat glossy, often dull, rough, and reticulate; the convex dorsal surface having a longitudinal lighter-colored area extending lengthwise along the center and beneath the seed coat, and a median transverse groove, dent, or fissure. The ventral surface has a deep concavity, the edges somewhat flattened and frequently forming a sharp indented angle with the base of the cavity, the latter showing a light-colored oval hilum.

Unground *Plantago ovata* seed: Broadly elliptical to ovate, boat-shaped, from 2–3.5 mm in length and from 1–1.5 mm in width. It is pale brown to moderate brown with a dull surface, the convex surface having a small, elongated, glossy brown spot. The concave surface has a deep cavity in the center of the base of which occurs a hilum covered with a thin membrane.

Odor and taste: All varieties of *Plantago* seed are nearly odorless.

Microscopic: *Plantago* seed is reniform in median transverse sections. Its seed coat has a colorless epidermis of mucilaginous cells whose radial and outer walls break down to form layers of mucilage when brought into contact with water, and a reddish brown to yellow pigment layer in the seeds of *Plantago indica* and *Plantago psyllium*, a broad endosperm with thick-walled outer palisade cells, and irregular inner endosperm cells; and a straight embryo extending lengthwise through the center. The endosperm and embryo cells contain fixed oil and aleurone grains, the latter being rounded, oval, pyriform, or irregularly shaped, from 2–8 µm in diameter.

• **WATER ABSORPTION**

Sample: 1 g of Plantago Seed

Analysis: Place the *Sample* in a 25-mL graduated cylinder, add water to the 20-mL mark, and shake the cylinder at intervals during 24 h. Allow the seeds to settle for 12 h, and note the total volume occupied by the swollen seeds.

Acceptance criteria: Seeds occupy the following volumes: *Plantago psyllium*, NLT 14 mL; *Plantago ovata*, NLT 10 mL; and *Plantago indica*, NLT 8 mL.

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*: NMT 0.50%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Total Ash*: NMT 4.0%

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• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and secure against insect attack.

Plasma Protein Fraction

» Plasma Protein Fraction conforms to the regulations of the federal Food and Drug Administration concerning biologics (640.90 to 640.96) (see *Biologics* ⟨1041⟩). It is a sterile preparation of serum albumin and globulin obtained by fractionating material (source blood, plasma, or serum) from healthy human donors, the source material being tested for the absence of hepatitis B surface antigen. It is made by a process that yields a product having protein components of approved composition and sedimentation coefficient content. Not less than 83 percent of its total protein is albumin and not more than 17 percent of its total protein consists of alpha and beta globulins. Not more than 1 percent of its total protein has the electrophoretic properties of gamma globulin. It is a solution containing, in each 100 mL, 5 g of protein, and it contains not less than 94 percent and not more than 106 percent of the labeled amount. It contains no added antimicrobial agent, but it contains sodium acetyltryptophanate with or without sodium caprylate as a stabilizing agent. It has a sodium content of not less than 130 mEq per L and not more than 160 mEq per L and a potassium content of not more than 2 mEq per L. It has a pH between 6.7 and 7.3, measured in a solution diluted to contain 1 percent of protein with 0.15 M sodium chloride. It meets the requirements of the test for heat stability.

Packaging and storage—Preserve at the temperature indicated on the label.

Expiration date—The minimum expiration date is not later than 5 years after issue from manufacturer's cold storage (5°, 1 year) if labeling recommends storage between 2° and 10°; not later than 3 years after issue from manufacturer's cold storage (5°, 1 year) if labeling recommends storage at temperatures not higher than 30°.

Labeling—Label it to state that it is not to be used if it is turbid and that it is to be used within 4 hours after the container is entered. Label it also to state the osmotic equivalent in terms of plasma and the sodium content.

Podophyllum

DEFINITION

Podophyllum consists of the dried rhizomes and roots of *Podophyllum peltatum* L. (Fam. Berberidaceae). It yields NLT 5.0% of podophyllum resin.

ASSAY

• **PROCEDURE**

Sample: 10 g of Podophyllum, in fine powder

Analysis: Place the *Sample* in a 125-mL conical flask, add 35 mL of alcohol, and reflux on a steam bath for 3 h. Transfer the mixture to a small percolator, and percolate slowly with warm alcohol until the percolate measures 95 mL. Cool, add sufficient alcohol to the percolate to measure 100.0 mL, and mix. Transfer 10.0 mL of this percolate to a separator, and add 10 mL each of chloroform and dilute hydrochloric acid (7:500). Shake the mixture, allow it to separate, draw off the alcohol–chloroform layer into a second separator, and wash the acid layer with three 15-mL portions of a mixture of chloroform and alcohol (2:1), adding the washings to the second separator. Add 10 mL of dilute hydrochloric acid (7:500) to the combined extract and washings, again shake the mixture, allow it to separate, and draw off the alcohol–chloroform layer into a tared vessel. Wash the acid layer three times with 15-mL portions of the alcohol–chloroform mixture, adding the washings to the tared vessel. Evaporate the combined extracts on a steam bath to approximately 1 mL, add 5 mL of dehydrated alcohol, again evaporate to dryness, and dry the residue at 80° for 4 h. The weight of this residue is the weight of resin in 1 g of the Podophyllum taken.

Acceptance criteria: NLT 5.0% of podophyllum resin

CONTAMINANTS

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis,Acid-Insoluble Ash*: NMT 2.0%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis,Foreign Organic Matter*: NMT 2.0%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS

• **BOTANICAL CHARACTERISTICS**

Macroscopic: Consists of nearly cylindrical rhizomes, jointed, compressed or flattened somewhat on upper and lower surfaces, and sometimes branched. It occurs as pieces of rhizome up to 20 cm in length, with internodes 2–9 mm in diameter, some of the nodes being somewhat thickened. The rhizome is dusky red to light yellowish brown, longitudinally wrinkled or nearly smooth, with irregular, somewhat V-shaped scars of scale leaves; some of the nodes are annulate, the upper portion having large, circular, depressed stem-scars and buds or stem-bases. On the lower portion there are numerous root-scars or roots, the latter 2–7 cm in length and 2 mm in thickness. The fracture is short and weak, the fractured surface being yellowish orange to pale yellow or grayish white.

Microscopic: The rhizome shows an outer portion consisting of a brown epidermis, often necrosed, and 1–3 layers of brown to olive-brown suberized cells; a cortex 20 cells in width, consisting chiefly of nearly isodiametric cells, the cells containing single or compound starch grains and resin masses and, in scattered cells of the nodes, rosette aggregates of calcium oxalate; a circle of 16–34 open collateral vascular bundles, separated by rather wide medullary rays, each bundle containing a few lignified vessels, a more or less distinct cambium, and a rather large phloem. The pith is large, the cells being more or less rounded and containing starch grains and reddish-brown

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resin masses. The roots show an epidermal layer of brownish suberized cells and a single row of hypodermal cells; a broad cortex of thin-walled nearly isodiametric cells; a distinct endodermis of tangentially elongated cells having uniformly thickened walls; and a 4- to 7-rayed vascular bundle.

Powdered Podophyllum: It is pale brown to weak yellow and has a slight odor. It contains numerous starch grains, simple or 2- to 6-compound, the individual grains being spheroidal, plano- to angular-convex, or polygonal, up to 20 µm in diameter; occasional rosette aggregates of calcium oxalate, up to 80 µm in diameter; vessels with simple pits or reticulate thickenings; fragments of starch- and resin-bearing parenchyma and reddish-brown to yellow cork cells.

Indian podophyllum: *Podophyllum peltatum* is differentiated from *Podophyllum hexandrum* Royle (Indian podophyllum) by the reaction described in the test for *Distinction from resin of Indian podophyllum* in Podophyllum Resin. [NOTE—Distinction from resin of Indian podophyllum.]

Sample: 400 mg

Analysis: Add the *Sample* to 3 mL of 60% alcohol, then add 0.5 mL of 1 N potassium hydroxide, shake the mixture gently, and allow to stand for 2 h.

Acceptance criteria: The solution does not gelatinize.

Podophyllum Resin

» Podophyllum Resin is the powdered mixture of resins extracted from Podophyllum by percolation with Alcohol and subsequent precipitation from the concentrated percolate upon addition to acidified water. It contains not less than 40.0 percent and not more than 50.0 percent of hexane-insoluble matter.
[**CAUTION**—Podophyllum Resin is highly irritating to the eye and to mucous membranes in general.]

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—

A: It is soluble in 1 N potassium hydroxide or in 1 N sodium hydroxide, forming a yellow liquid, which gradually becomes darker on standing and from which the resin is precipitated by hydrochloric acid.

B: A hot solution of it deposits most of its solids on cooling, and if the cooled liquid is filtered, the filtrate turns brown upon the addition of a few drops of ferric chloride TS.

Residue on ignition ⟨281⟩: not more than 1.5%.

Distinction from resin of Indian podophyllum—Add 400 mg to 3 mL of 60 percent alcohol, then add 0.5 mL of 1 N potassium hydroxide, shake the mixture gently, and allow to stand for 2 hours: it does not gelatinize.

Hexane-insoluble matter—Transfer about 1 g of Podophyllum Resin, accurately weighed, to a glass-stoppered, 100-mL conical flask, add 30.0 mL of chloroform, insert the stopper tightly, and shake for 30 minutes, using a mechanical wrist-action shaker, or equivalent. Filter with suction through a medium- or fine-porosity, sintered-glass filter, into a small filter flask. Wash the conical flask and the filter with two 5-mL portions of chloroform, adding the washings to the filtrate. Transfer the filtrate with the aid of chloroform to a 50-mL volumetric flask, add chloroform to volume, and mix. Pipet 20 mL of the resulting solution into a 250-mL conical flask containing 160 mL of solvent hexane. Gently swirl, allow to stand for 10 minutes, and transfer the resulting precipitate to a tared, fine-porosity, sintered-glass filter, wash the flask and the precipitate with two 20-mL portions of solvent hexane, dry the precipitate at 70° for 1 hour, and weigh the *Hexane-insoluble matter* so obtained. Multiply by 2.5 to find the amount present in the quantity of Podophyllum Resin taken.

Podophyllum Resin Topical Solution

» Podophyllum Resin Topical Solution is a solution in Alcohol consisting of Podophyllum Resin and an alcoholic extract of Benzoin. It contains, in each 100 mL, not less than 10 g and not more than 13 g of hexane-insoluble matter.
[**CAUTION**—Podophyllum Resin Topical Solution is highly irritating to the eye and to mucous membranes in general.]

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—

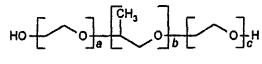
A: A 1 in 5 solution in chloroform is levorotatory.

B: The precipitate obtained as directed in the test for *Hexane-insoluble matter* responds to *Identification* test *A* under *Podophyllum Resin*.

Alcohol Determination ⟨611⟩: between 69.0% and 72.0% of C_2H_5OH.

Hexane-insoluble matter—Using a 10.0-mL quantity of the Topical Solution, proceed as directed for *Hexane-insoluble matter* under *Podophyllum Resin*. Multiply the weight of hexane-insoluble matter found by 2.5 to find the amount present in the 10.0 mL taken.

Poloxalene



Oxirane, methyl-, polymer with oxirane.
Polyethylene-polypropylene glycol [9003-11-6].

» Poloxalene is a synthetic block copolymer of ethylene oxide and propylene oxide. It contains not less than 98.0 percent and not more than 103.0 percent of poloxalene.

Packaging and storage—Preserve in tight containers, protected from light. Store in a cool place.

USP Reference standards ⟨11⟩—
USP Poloxalene RS

Labeling—Label it to indicate that it is for veterinary use only.

Identification—

Color reagent—Dissolve 12.7 g of ammonium thiocyanate and 2.0 g of cobalt nitrate in 100 mL of water.

Procedure—Add 10 mL of ethylene chloride to 0.5 g of Poloxalene, and shake for 1 minute. Add 1 mL of *Color*

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reagent, and shake for 1 minute: a blue color is produced in the lower layer.

Average molecular weight—

Phthalic anhydride-pyridine solution—Prepare as directed in the test for *Average molecular weight* under *Poloxamer*.

Procedure—Using about 12 g of Poloxalene, accurately weighed, proceed as directed for *Procedure* in the test for *Average molecular weight* under *Poloxamer:* the average molecular weight is between 2850 and 3150.

pH ⟨791⟩: between 5.0 and 7.5, in a solution (1 in 40).

Water Determination, *Method Ia* ⟨921⟩: not more than 0.4%.

Hydroxyl value ⟨401⟩—

Esterification reagent—Dissolve 166 g of phthalic anhydride and 28 g of imidazole in 1000 mL of pyridine, and allow to stand for 2 hours before using. Store in a light-resistant bottle.

Procedure—Transfer a quantity of Poloxalene, determined by dividing 420 by the expected hydroxyl value and accurately weighed, to a glass-stoppered, 250-mL conical flask, and add 25.0 mL of *Esterification reagent*. Transfer 25.0 mL of *Esterification reagent* to a second glass-stoppered, 250-mL conical flask to provide the reagent blank. Add glass beads to the flasks, swirl to dissolve the Poloxalene, and fit both flasks with glass-jointed reflux condensers previously rinsed with 10 mL of pyridine, and heat on a steam bath for 15 minutes. Add 10 mL of pyridine through each condenser, insert the stoppers, and cool under running water for 1 minute. After removing the condensers, add 10 mL of water and 1 mL of phenolphthalein TS, and titrate with 0.5 N sodium hydroxide VS to a light pink endpoint that persists for at least 15 seconds. Calculate the hydroxyl value by the formula:

$$(56.11N/W)(B - U)$$

in which N is the normality of the 0.5 N sodium hydroxide titrant; W is the weight, in g, of the Poloxalene taken; B and U are the volumes, in mL, of 0.5 N sodium hydroxide consumed by the reagent blank and the solution of Poloxalene, respectively. [NOTE—If B minus U is greater than 10 mL, repeat the test using a smaller sample.] The hydroxyl value is between 36.0 and 40.0.

Cloud point—Add 10 g of Poloxalene to 190.0 mL of water in a 250-mL beaker. Add a magnetic stirring bar, place on a stirrer and hot plate, and stir until dissolution is complete. Place a probe from an electronic thermometer with an accuracy of 0.2° in the solution within 3 mm of the stirring bar. Continue stirring at a rate that minimizes the formation of bubbles. Adjust the hot plate so that the temperature of the solution increases at a rate of about 1° per minute. Continue to view the solution, and record the temperature when the probe can no longer be seen. This occurs between 42.5° and 46.5°.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Poloxalene RS in ethylene dichloride to obtain a solution having a known concentration of about 0.1575 mg per mL.

Assay preparation—Transfer about 105 mg of Poloxalene, accurately weighed, to a 100-mL volumetric flask, add about 85 mL of ethylene dichloride, and swirl to dissolve. Dilute with ethylene dichloride to volume, and mix. Transfer 15.0 mL of this solution to a second 100-mL volumetric flask, dilute with ethylene dichloride to volume, and mix.

Procedure—Transfer 10.0 mL of the *Standard preparation*, the *Assay preparation*, and ethylene dichloride (to serve as a blank) to glass-stoppered tubes, add 4.0 mL of the *Color reagent* specified in the *Identification* test, shake vigorously for 3 minutes, and then centrifuge for 5 minutes. Carefully remove the lower ethylene dichloride layers from the three tubes, and using the ethylene dichloride layer from the tube containing the blank to zero the spectrophotometer, determine the UV absorbance at 630 nm of the solutions from the *Standard preparation* and the *Assay preparation*. Calculate the percentage of poloxalene in the portion of Poloxalene taken by the formula:

$$100(A_U/A_S)(C_S/C_U)$$

in which A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively; and C_S and C_U are the concentrations, in mg per mL, of the *Standard preparation* and the *Assay preparation*, respectively.

Polycarbophil

Polycarbophil.
Polycarbophil [9003-97-8].

» Polycarbophil is polyacrylic acid cross-linked with divinyl glycol.

Packaging and storage—Preserve in tight containers.

Identification—

A: A dispersion (1 in 100) is orange with thymol blue TS and yellow with cresol red TS.

B: Adjust a dispersion (1 in 100) with 1 M sodium hydroxide to a pH of about 7.5. A very viscous gel is produced.

pH ⟨791⟩—To 1.0 g add 100 mL of water, and shake by mechanical means for 1 hour: the pH of the mixture is not more than 4.0.

Loss on drying ⟨731⟩—Dry it in vacuum at 45° for 4 hours: it loses not more than 1.5% of its weight.

Residue on ignition ⟨281⟩: not more than 4.0%.

Absorbing power—Transfer about 50 mg, accurately weighed, to an accurately tared 50-mL centrifuge tube fitted with a tight closure. Add 35 mL of sodium bicarbonate solution (1.5 in 100), and shake manually, venting as necessary to release liberated carbon dioxide. Shake and vent at least 3 times. Close the tube tightly, and shake vigorously by mechanical means for 60 minutes. Centrifuge at 2000 rpm for 1 hour. By means of a 50-mL syringe fitted with a 13-gauge needle, draw off the supernatant taking care that the solid material is not disturbed. Repeat the process of the addition of 35 mL of sodium bicarbonate solution, of the shaking, and of the withdrawing of supernatant. Accurately weigh the tube with its contents, and calculate the weight of the absorbed solution by subtracting the weight of Polycarbophil taken and the tare weight of the tube. The absorbed weight is not less than 62 g per g of Polycarbophil on the dried basis.

Limit of acrylic acid—

pH 3.0 phosphate buffer—Prepare 0.01 M monobasic potassium phosphate, and adjust with phosphoric acid to a pH of 3.0 ± 0.5.

Mobile phase—Prepare a degassed solution consisting of *pH 3.0 phosphate buffer* and methanol (8:2).

Standard solution—Dissolve an accurately weighed quantity of acrylic acid in water to obtain a solution containing 1.0 mg per mL. Dilute quantitatively, and stepwise if necessary, with a 2.5% alum solution to obtain a solution having a known concentration of about 30 µg per mL.

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Test solution—Transfer about 0.1 g of Polycarbophil into a vial. Add 20 mL of a 2.5% alum solution and mix. Heat at 50° for 20 minutes, then shake for 1 hour, centrifuge, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution*, and record peak responses as directed for *Procedure:* the relative standard deviation for replicate injections of the *Standard solution* is not more than 5.0%, and the tailing factor is not more than 2.5.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the response for the acrylic acid peak. Calculate the percentage of acrylic acid in the portion of Polycarbophil taken by the formula:

$$(0.002C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of acrylic acid, W is the weight, in g, of Polycarbophil taken, and r_U and r_S are the acrylic acid peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.3% acrylic acid is found.

Limit of ethyl acetate—

Internal standard solution—Dissolve an accurately weighed quantity of methyl ethyl ketone in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.075 mg per mL.

Standard solution—Dissolve 0.225 mg, accurately weighed, of ethyl acetate into a 10-mL volumetric flask. Add 2.0 mL of the *Internal standard solution*, dilute with methanol to volume, and mix.

Test solution—Transfer about 50 mg of Polycarbophil into a 10-mL volumetric flask. Add 2.0 mL of the *Internal standard solution*, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 10-ft. × 2-mm column that contains 1% liquid phase G25 on support S12. The column is maintained at about 160°, and the injection port and detector block are maintained at about 250°. The carrier gas is dry helium flowing at a rate of about 30 mL per minute. Chromatograph the *Standard solution*, and record the chromatogram as directed for *Procedure:* the relative retention times are about 1.3 for ethyl acetate, and 1.0 for methyl ethyl ketone, and the relative standard deviation for replicate injections of the ethyl acetate peak is not more than 2%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of ethyl acetate in the portion of Polycarbophil taken by the formula:

$$(0.001C/W)(R_U/R_S)$$

in which C is the concentration of ethyl acetate, in µg per mL, W is the weight of Polycarbophil, in g, and R_U and R_S are the ratios of the responses of the ethyl acetate peak to the methyl ethyl ketone peak obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.45% is found.

Polyethylene Glycol 3350

Poly(oxy-1,2-ethanediyl), α-hydro-ω-hydroxy-; 1,2-Ethanediol, homopolymer [25322-68-3].

DEFINITION

Polyethylene Glycol 3350 is an addition polymer of ethylene oxide and water, represented by the formula $H(OCH_2CH_2)_nOH$, in which n represents the average number of oxyethylene groups. The apparent weight-average molecular weight is 3015–3685 g/mol (Da). It contains NLT 97.0% and NMT 103.0% of polyethylene glycol 3350, calculated on the anhydrous basis. It may contain a suitable antioxidant.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A** or **197F.**▲ (CN 1-May-2020) Use a thin film of test specimen, melted if necessary, in the range from 4000 to 650 cm^{-1}, when the measurement is performed by using⟨197F⟩
- **B. CHROMATOGRAPHIC IDENTITY**

Analysis: Proceed as directed in the *Assay*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

- **PROCEDURE**

Mobile phase: 50 µg/mL of sodium azide in water

Standard solution: 20 mg/mL of USP Polyethylene Glycol 3350 RS in *Mobile phase*

Sample solution: 20 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Differential refractive index

Columns

Guard: 6-mm × 4-cm; 6-µm packing L25

Analytical: 7.8-mm × 30-cm; 6-µm packing L25

Temperatures

Detector: 35 ± 1°

Column: 35 ± 1°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for polyethylene glycol 3350 is about 8.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of polyethylene glycol 3350 $[H(OCH_2CH_2)_nOH]$ in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Polyethylene Glycol 3350 RS in the *Standard solution* (mg/mL)

C_U = concentration of Polyethylene Glycol 3350 in the *Sample solution* (mg/mL)

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Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩
Sample: 2 g
Analysis: Proceed as directed, moistening the residue with 2 mL of sulfuric acid.
Acceptance criteria: NMT 0.1%

• **Limit of Ethylene Oxide and Dioxane**
Analysis: Proceed as directed in *Ethylene Oxide and Dioxane* ⟨228⟩, *Method II*.
Acceptance criteria
Ethylene oxide: NMT 1 µg/g
Dioxane: NMT 10 µg/g

• **Limit of Ethylene Glycol and Diethylene Glycol**
Mobile phase: 50 µg/mL of sodium azide in water
Eluant: Water
Standard stock solution: 10 mg/g of USP Diethylene Glycol RS and 10 mg/g of USP Ethylene Glycol RS in *Eluant*
Standard solution: Transfer 0.1 g of *Standard stock solution* to a 100-mL volumetric flask. Dilute with water to volume. The *Standard solution* contains 0.01 mg/mL of USP Diethylene Glycol RS and 0.01 mg/mL of USP Ethylene Glycol RS.
Sample solution: 10 mg/mL of Polyethylene Glycol 3350 in *Eluant*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractive index
Column: 7.8-mm × 30-cm; 7-µm packing L89, 125-Å pore size
Temperatures
Detector: 35 ± 1°
Column: 35 ± 1°
Flow rate: 0.5 mL/min
Injection volume: 100 µL
Run time: 30 min
System suitability
Sample: *Standard solution*
[Note—The relative retention times for diethylene glycol and ethylene glycol are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 0.9 between diethylene glycol and ethylene glycol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of diethylene glycol (or ethylene glycol) in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diethylene glycol (or ethylene glycol) from the *Sample solution*
r_S = peak response of diethylene glycol (or ethylene glycol) from the *Standard solution*
C_S = concentration of USP Diethylene Glycol RS (or USP Ethylene Glycol RS) in the *Standard solution* (mg/mL)
C_U = concentration of Polyethylene Glycol 3350 in the *Sample solution* (mg/mL)

Acceptance criteria
Ethylene glycol: NMT 0.062%
Sum of diethylene glycol and ethylene glycol: NMT 0.2%

• **Limit of Formaldehyde and Acetaldehyde**
Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time[a] (min)	Solution A (%)	Solution B (%)
0	50	50
11	0	100

[a] The equilibration time is 5 min.

[Note—Use amber containers and amber autosampler vials.]
2,4-DNPH solution: Transfer 250 mg of 2,4-dinitrophenylhydrazine (2,4-DNPH) to a 50-mL volumetric flask, add 20.0 mL of acetonitrile, and swirl to mix. Add 3.0 mL of hydrochloric acid to the flask, and swirl to mix. Sonicate until all solids are dissolved, and dilute with acetonitrile to volume.
Formaldehyde-2,4-DNPH solution: 100 µg/mL of aldehyde equivalent in acetonitrile[1]
Acetaldehyde-2,4-DNPH solution: 1000 µg/mL of aldehyde equivalent in acetonitrile[2]
Formaldehyde stock solution: Transfer 500 µL of *Formaldehyde-2,4-DNPH solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume. The formaldehyde concentration is 5.0 µg/mL.
Acetaldehyde stock solution: Transfer 500 µL of *Acetaldehyde-2,4-DNPH solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume. The acetaldehyde concentration is 50.0 µg/mL.
Standard solution: Transfer 1.5 mL of *Formaldehyde stock solution* and 1.2 mL of *Acetaldehyde stock solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume, and mix well. The concentrations of formaldehyde and acetaldehyde are 0.75 and 6.0 µg/mL, respectively.
Sample solution: Transfer 0.5 g of Polyethylene Glycol 3350 to a 10-mL volumetric flask. Add 1.0 mL of acetonitrile to the flask, and swirl to dissolve the sample. Add 2.0 mL of *2,4-DNPH solution* to the flask, and swirl to mix. Allow the solution to react for 15 min, then dilute with acetonitrile to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 360 nm
Column: 3.0-mm × 15-cm; 3.5-µm packing L7, 80-Å pore size
Column temperature: 30 ± 1°
Flow rate: 0.65 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for formaldehyde and acetaldehyde are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between formaldehyde and acetaldehyde
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the content of formaldehyde (or acetaldehyde), in µg/g, in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U)$$

[1] Available from Sigma-Aldrich Corporation, or equivalent.
[2] Available from Sigma-Aldrich Corporation, or equivalent.

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r_U = peak response of formaldehyde (or acetaldehyde) from the *Sample solution*
r_S = peak response of formaldehyde (or acetaldehyde) from the *Standard solution*
C_S = concentration of formaldehyde (or acetaldehyde) in the *Standard solution* (µg/mL)
C_U = concentration of Polyethylene Glycol 3350 in the *Sample solution* (g/mL)

Acceptance criteria
Formaldehyde: NMT 15 µg/g
Sum of formaldehyde and acetaldehyde: NMT 200 µg/g

SPECIFIC TESTS

• **APPARENT WEIGHT-AVERAGE MOLECULAR WEIGHT AND POLYDISPERSITY**
Mobile phase: Water
Standard solutions: Prepare 1.0 mg/mL each of five polyethylene glycol standards with molecular weights of 1000, 2000, 3000, 4000, and 6000 g/mol (Da) in *Mobile phase* separately in five individual flasks. Pass a portion of each solution through a 0.45-µm polytetrafluoroethylene (PTFE) syringe filter.[3] Discard the first 2 mL, and transfer the solution for analysis.
Sample solution: 1.0 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*. Pass a portion of the solution through a 0.45-µm PTFE syringe filter.[3] Discard the first 2 mL, and transfer the solution for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractive index
Column: 7.8-mm × 30-cm; 6-µm packing L37
Temperatures
Detector: 35 ± 1°
Column: 35 ± 1°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
Run time: 18 min
Analysis
Samples: *Standard solutions* and *Sample solution*
Separately inject equal volumes of the *Standard solutions* and the *Sample solution* into the chromatograph, record the chromatograms, and determine the elution peak maxima and the corresponding retention times for the five polyethylene glycol standards.
Calibration curve: Plot the retention times on the *x*-axis against the log M_P (M_P: peak molecular weights) on the *y*-axis for the peaks from the polyethylene glycol standard to generate a calibration curve using suitable gel permeation chromatography or size exclusion chromatography (GPC/SEC) software.
Calculations: Compute the data using the same GPC/SEC software, and determine the number- and weight-average molecular weights, M_N and M_W, in g/mol (Da), respectively, for the chromatogram of the *Sample solution*. Calculate the polydispersity for Polyethylene Glycol 3350:

$$\text{Result} = M_W/M_N$$

M_W = weight-average molecular weight from the *Sample solution* (g/mol)
M_N = number-average molecular weight from the *Sample solution* (g/mol)

[3] Millipore® Millex® LCR HPLC syringe filters with hydrophilic PTFE membrane is suitable, or any other equivalent filter.

Acceptance criteria: The value of apparent weight-average molecular weight is 3015–3685 g/mol. Polydispersity is 90%–110% of the value stated on the label or within the range indicated on the label.

• **HYDROXYL VALUE**
Phthalic anhydride solution: Place 49.0 g of phthalic anhydride into an amber bottle, and dissolve in 300 mL of pyridine from a freshly opened bottle or pyridine that has been freshly distilled over phthalic anhydride. Shake vigorously until completely dissolved. Add 7 g of imidazole, swirl carefully to dissolve, and allow to stand for 16 h before using.
Sample solution: Carefully introduce 25.0 mL of *Phthalic anhydride solution* into a dry, heat-resistant pressure bottle. Add 12.0 g of Polyethylene Glycol 3350. Add 25 mL of pyridine, from a freshly opened bottle or pyridine that has been freshly distilled over phthalic anhydride. Swirl to dissolve, insert the stopper in the bottle, and wrap it securely in a cloth bag.
Blank: 25.0 mL of *Phthalic anhydride solution* plus any additional pyridine added to the bottle
Analysis: Immerse the bottle in a water bath maintained at a temperature between 96° and 100°, to the same depth as that of the mixture in the bottle. Remove the bottles from the bath after 5 min and, without unwrapping, swirl for 30 s to homogenize. Heat in the water bath for 60 min, then remove from the bath, and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove from the bag, add 10 mL of water, and swirl thoroughly. Wait 2 min, add 0.5 mL of a solution of phenolphthalein in pyridine (1 in 100), and titrate with 0.5 N sodium hydroxide VS to the first pink color that persists for 15 s. Perform a blank determination. Calculate the hydroxyl value:

$$\text{Result} = [M_r \times (V_B - V_S) \times N]/W$$

M_r = molecular weight of potassium hydroxide, 56.11
V_B = volume of 0.5 N sodium hydroxide consumed in the blank test (mL)
V_S = volume of 0.5 N sodium hydroxide consumed in the actual test (mL)
N = exact normality of the sodium hydroxide solution
W = weight of Polyethylene Glycol 3350 taken for the test (g)

Acceptance criteria: 30–38

• **ACIDITY AND ALKALINITY**
Sample solution: Dissolve 5.0 g of Polyethylene Glycol 3350 in 100 mL of carbon dioxide-free water.
Analysis: Add 0.3 mL of a saturated solution of potassium chloride into the *Sample solution*. The test solution should be maintained at 25 ± 2° during the measurement. Measure the pH following *pH* ⟨791⟩.
Acceptance criteria: 4.5–7.5

• **WATER DETERMINATION** ⟨921⟩, *Method I*
Sample: 2.0 g
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from direct sunlight. Avoid exposure to excessive heat.
• **LABELING:** Label it to indicate its polydispersity (M_W/M_N) or its polydispersity range. Label it to indicate the name and amount of any added antioxidant.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diethylene Glycol RS
USP Ethylene Glycol RS
USP Polyethylene Glycol 3350 RS

USP Monographs

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Polyethylene Glycol 3350 and Electrolytes for Oral Solution

» Polyethylene Glycol 3350 and Electrolytes for Oral Solution is a mixture of Polyethylene Glycol 3350, Sodium Bicarbonate, Sodium Chloride, Sodium Sulfate (anhydrous), and Potassium Chloride. When constituted as directed in the labeling it contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of polyethylene glycol 3350, potassium (K^+), sodium (Na^+), bicarbonate (HCO_3^-), chloride (Cl^-), and sulfate ($SO_4^=$), the labeled amounts per L being 10 mmol (10 mEq) of potassium, 125 mmol (125 mEq) of sodium, 20 mmol (20 mEq) of bicarbonate, 35 mmol (35 mEq) of chloride, and 40 mmol (80 mEq) of sulfate.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Polyethylene Glycol 3350 RS
Completeness of solution ⟨641⟩: meets the requirements.

Identification—
A: The IR absorption spectrum of a mineral oil dispersion of it in a calcium fluoride cell exhibits maxima only at the same wavelengths as that of a similar preparation of USP Polyethylene Glycol 3350 RS.
B: A solution (1 in 20) responds to the tests for *Sodium* ⟨191⟩, *Potassium* ⟨191⟩, *Bicarbonate* ⟨191⟩, *Sulfate* ⟨191⟩, and *Chloride* ⟨191⟩.
pH ⟨791⟩: between 7.5 and 9.5, in the solution prepared as directed in the labeling.
Uniformity of dosage units ⟨905⟩: meets the requirements.
Osmolality and Osmolarity ⟨785⟩, ***Osmolality***: between 235 and 304 mOsmol, in the solution prepared as directed in the labeling.

Assay for potassium and sodium—
Mobile phase—Dilute 0.5 mL of nitric acid with water to obtain 4000 mL of solution. Degas, and place the solution in a suitable plastic container. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Dissolve a suitable quantity of ammonium bromide in water to obtain a solution having a concentration of about 2 mg per mL.
Standard preparation—To a 100-mL volumetric flask transfer about 90 mg of potassium chloride, previously dried at 105° for 2 hours and accurately weighed, and about 880 mg of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, add 10.0 mL of *Internal standard solution,* dilute with water to volume, and mix. Pass this solution through a filter having a 0.5-µm or finer porosity, and store the filtrate in a suitable plastic container. This *Standard preparation* contains about 9 µg (0.00012 mEq) of potassium chloride and about 88 µg (0.0015 mEq) of sodium chloride per mL.
Assay preparation—Constitute the contents of a container of Polyethylene Glycol 3350 and Electrolytes for Oral Solution with an accurately measured volume of water, as specified in the labeling. Transfer 6.0 mL of this stock solution, equivalent to about 0.06 mEq of potassium, to a 500-mL volumetric flask, add 10 mL of *Internal standard solution,* dilute with water to volume, and mix. This solution contains about 0.00012 mEq of potassium and 0.0015 mEq of sodium per mL. [NOTE—Reserve the remaining portion of the stock solution for the *Assay for bicarbonate,* and reserve the remaining portion of the *Assay preparation* for the *Assay for chloride and sulfate* and the *Assay for polyethylene glycol 3350.*]
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a conductivity detector, a 4-mm × 5-cm guard column containing packing L17, and a 4-mm × 30-cm analytical column maintained at 35 ± 1° containing packing L17. The flow rate is about 0.9 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure:* the relative retention times are about 0.6 for sodium, 0.8 for ammonium, and 1.0 for potassium; the resolution, *R,* between the sodium and ammonium peaks is not less than 1.1, and between the ammonium and potassium peaks is not less than 0.9. [NOTE—Maintain column backpressure at less than 1000 pounds per square inch. Backpressure may be reduced by changing the in-line filters and frits in the columns. Column efficiency may be improved by backflushing the analytical column with 30 mL of 0.1 N nitric acid or by injecting four successive 100-µL portions of 0.1 N nitric acid into the chromatograph.]
Procedure— [NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the mEq of potassium per L of constituted Oral Solution taken by the formula:

$$(500/74.55)(C/6)(R_U/R_S)$$

in which 74.55 is the molecular weight of potassium chloride; C is the concentration, in µg per mL, of potassium chloride in the *Standard preparation;* and R_U and R_S are the peak response ratios of potassium to ammonium obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the mEq of sodium per L of constituted Oral Solution taken by the formula:

$$(500/58.44)(C/6)(R_U/R_S)$$

in which 58.44 is the molecular weight of sodium chloride; C is the concentration, in µg per mL, of sodium chloride in the *Standard preparation;* and R_U and R_S are the peak response ratios of sodium to ammonium obtained from the *Assay preparation* and the *Standard preparation,* respectively.
Assay for bicarbonate—Transfer 400.0 mL of the stock solution remaining from the *Assay for potassium and sodium,* equivalent to about 672 mg of sodium bicarbonate (8 mEq), to a suitable container, add methyl red TS, and titrate with 1 N sulfuric acid VS. Calculate the mEq of bicarbonate (HCO_3^-) per L of constituted Oral Solution taken by the formula:

$$2.5V_A$$

in which V_A is the volume, in mL, of 1 N sulfuric acid consumed.

Assay for chloride and sulfate—
Mobile phase—Transfer 34 g of boric acid, 8.6 g of lithium hydroxide, 23.5 mL of gluconic acid solution (1:1), and 125 mL of glycerin to a 1000-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Add 15 mL of this buffer solution to 865 mL of water, mix, and degas. Add 120 mL of acetonitrile, mix, and degas. [NOTE—Protect the *Mobile phase* from air to prevent absorption of carbon dioxide.] Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Increasing the proportion of buffer solution decreases the retention times of the analytes.

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Internal standard solution—Dissolve a suitable quantity of ammonium bromide in water to obtain a solution having a concentration of about 2.2 mg per mL.

Standard preparation—To a 100-mL volumetric flask transfer about 246 mg of sodium chloride (4.2 mEq), previously dried at 105° for 2 hours and accurately weighed, and about 682 mg of anhydrous sodium sulfate (9.6 mEq), previously dried at 105° for 2 hours and accurately weighed, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with water to volume, and mix. Filter this solution through a 0.5-µm or finer porosity filter, and store the filtrate in a suitable glass container. This *Standard preparation* contains about 24.6 µg of sodium chloride (0.00042 mEq of chloride) and about 68.2 µg of sodium sulfate (0.00096 mEq of sulfate) per mL.

Assay preparation—Use the *Assay preparation* prepared as directed in the *Assay for potassium and sodium*. This solution contains about 0.042 mEq of chloride and 0.096 mEq of sulfate per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a conductivity detector, a 4-mm × 5-cm guard column containing packing L23, and a 4-mm × 30-cm analytical column maintained at 35 ± 1° containing packing L23. The flow rate is about 0.9 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure:* the relative retention times are about 0.25 for chloride, 0.4 for bromide, and 1.0 for sulfate, the resolution, *R*, between the chloride and bromide peaks is not less than 1.5 and between the bromide and sulfate peaks is not less than 4.5. [NOTE—Maintain column backpressure at less than 1000 pounds per square inch. Backpressure may be reduced by changing the in-line filters and frits in the columns. Column efficiency may be improved by backflushing the analytical column with 50 mL of the buffer solution used to prepare the *Mobile phase*.]

Procedure— [NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the mEq of chloride per L of constituted Oral Solution taken by the formula:

$$(500/58.44)(C/6)(R_U/R_S)$$

in which 58.44 is the molecular weight of sodium chloride, *C* is the concentration, in µg per mL, of sodium chloride in the *Standard preparation*, and R_U and R_S are the peak response ratios of chloride to bromide obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the mEq of sulfate per L of constituted Oral Solution taken by the formula:

$$(500/71.02)(C/6)(R_U/R_S)$$

in which 71.02 is one-half of the molecular weight of sodium sulfate, *C* is the concentration, in µg per mL, of anhydrous sodium sulfate in the *Standard preparation*, and R_U and R_S are the peak response ratios of sulfate to bromide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for polyethylene glycol 3350—

Salt solution—Prepare a solution in water containing 0.35 mg of sodium chloride, 0.18 mg of potassium chloride, 0.40 mg of sodium bicarbonate, 1.37 mg of anhydrous sodium sulfate, and 0.88 mg of ammonium bromide per mL.

Mobile phase—Dilute 40.0 mL of *Salt solution* with water to 1000 mL. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 360 mg of USP Polyethylene Glycol 3350 RS, accurately weighed, to a 500-mL volumetric flask, add 20.0 mL of *Salt solution* and about 250 mL of water, dissolve by swirling, dilute with water to volume, and mix. This *Standard preparation* contains about 0.72 mg of polyethylene glycol 3350 per mL.

Assay preparation—Use the *Assay preparation*, prepared as directed in the *Assay for potassium and sodium*. This solution contains about 0.72 mg of polyethylene glycol 3350 per mL.

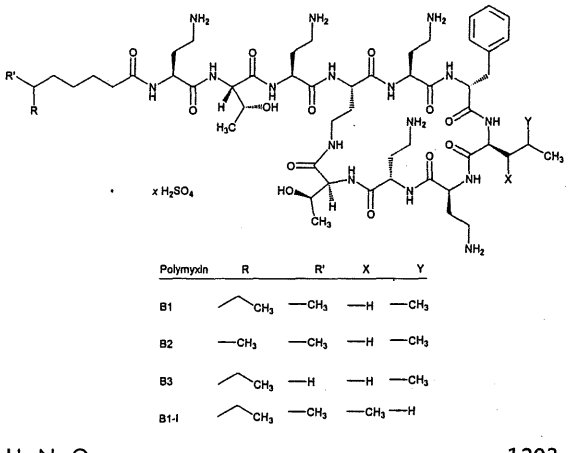
Chromatographic system (see *Chromatography* ⟨621⟩)—[NOTE—Use peak heights where peak responses are indicated.] The liquid chromatograph is equipped with a refractive index detector maintained at 34 ± 0.5°, a 7.8-mm × 4.5-cm guard column containing packing L25, and a 7.8-mm × 30-cm analytical column containing packing L25 and maintained at ambient temperature. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%. [NOTE—Maintain column backpressure at less than 1000 pounds per square inch. Backpressure may be reduced by cleaning the frits in the guard column or by replacing the guard column. Baseline drift may be reduced by maintaining strict control of ambient temperature, by insulating the lines, the *Mobile phase* reservoir, and the columns, and by increasing the time of equilibration.]

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the content, in g, of polyethylene glycol 3350 per L of constituted Oral Solution taken by the formula:

$$500(C/6)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of polyethylene glycol 3350 in the *Standard preparation*, and r_U and r_S are the polyethylene glycol 3350 peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Polymyxin B Sulfate



$C_{56}H_{98}N_{16}O_{13}$ 1203.48

Polymyxin B1:

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N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-({(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methyloctanamide [4135-11-9].

$C_{55}H_{96}N_{16}O_{13}$ 1189.45

Polymyxin B2:

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-({(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methylheptanamide [34503-87-2].

$C_{55}H_{96}N_{16}O_{13}$ 1189.45

Polymyxin B3:

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-({(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl] octanamide [71140-58-4].

$C_{56}H_{98}N_{16}O_{13}$ 1203.48

Polymyxin B1-I:

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-({(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-[(*S*)-sec-butyl]-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methyloctanamide.

Polymyxin B, sulfate;
Polymyxin B sulfate [1405-20-5].
Polymyxin B [1404-26-8].

DEFINITION

Polymyxin B Sulfate is the sulfate salt of a kind of polymyxin, a substance produced by the growth of *Bacillus polymyxa* (Prazmowski) Migula (Fam. Bacillaceae), or a mixture of two or more such salts. It has a potency of NLT 6000 Polymyxin B units/mg, calculated on the dried basis.

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Composition of Polymyxins*.
- **B.**

Solution A: 10 mg/mL of cupric sulfate
Sample solution: Dissolve 2 mg in 5 mL of water.
Analysis: To the *Sample solution* add 5 mL of 2.5 N sodium hydroxide, and mix. Add 5 drops of *Solution A*, shaking after the addition of each drop.
Acceptance criteria: A reddish-violet color is produced.

- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sulfate*: A 50-mg/mL solution of Polymyxin B Sulfate meets the requirements.

ASSAY

- **PROCEDURE**

Analysis: Proceed with Polymyxin B Sulfate as directed for *Antibiotics—Microbial Assays* ⟨81⟩.
Acceptance criteria: NLT 6000 Polymyxin B units/mg on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩

Analysis: Proceed as directed in the chapter, moistening the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.
Acceptance criteria: NMT 5.0% where used for prescription compounding

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the test for *Composition of Polymyxins*.
Analysis
Samples: *Standard solution* (the *Standard solution* is used to determine the disregard limit) and *Sample solution*
Calculate the percentage of each impurity in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the responses of all peaks from the *Sample solution*

Acceptance criteria: Disregard any peak less than 0.007 times the peak response of the polymyxin B1 peak from the *Standard solution*.
Any individual impurity: NMT 3.0%
Total impurities: NMT 17.0%

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 5 mg/mL
Acceptance criteria: 5.0–7.5

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry 100 mg in a capillary-stoppered bottle under vacuum at 60° for 3 h.
Acceptance criteria: NMT 7.0%

- **STERILITY TESTS** ⟨71⟩: Meets the requirements where the label states that Polymyxin B Sulfate is sterile
- **PYROGEN TEST** ⟨151⟩

Sample solution: Nominally 20×10^3 Polymyxin B units/mL in pyrogen-free saline TS
Analysis: Proceed as directed in the chapter using a test dose of 1.0 mL/kg.
Acceptance criteria: Meets the requirements where intended for injectable dosage forms or where the label states that Polymyxin B Sulfate must be subjected to further processing during the preparation of injectable dosage forms

- **COMPOSITION OF POLYMYXINS**

Buffer: 4.5 g/L of sodium sulfate anhydrous. The pH is adjusted to 2.3 with dilute phosphoric acid (10% w/w) prior to final dilution.
Mobile phase: Acetonitrile and *Buffer* (20:80)
Diluent: Acetonitrile and water (20:80)
Standard solution: 0.5 mg/mL of USP Polymyxin B Sulfate RS in *Diluent*
Sample solution: 0.5 mg/mL of Polymyxin B Sulfate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1. [NOTE—A 4.6-mm × 25-cm; 5-µm packing L1 column was also found to be suitable.]
Column temperature: 30°
Flow rate: 1 mL/min

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Injection volume: 20 µL
Run time: 1.4 times the retention time of polymyxin B1
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between polymyxin B2 and polymyxin B3
Relative standard deviation: NMT 2.0% for the polymyxin B1 peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of polymyxin B1 in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of polymyxin B1 from the *Sample solution*
r_S = peak response of polymyxin B1 from the *Standard solution*
C_S = concentration of USP Polymyxin B Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Polymyxin B Sulfate in the *Sample solution* corrected for loss on drying (mg/mL)
P = potency of polymyxin B1 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B2 in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of polymyxin B2 from the *Sample solution*
r_S = peak response of polymyxin B2 from the *Standard solution*
C_S = concentration of USP Polymyxin B Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Polymyxin B Sulfate in the *Sample solution* corrected for loss on drying (mg/mL)
P = potency of polymyxin B2 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B3 in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of polymyxin B3 from the *Sample solution*
r_S = peak response of polymyxin B3 from the *Standard solution*
C_S = concentration of USP Polymyxin B Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Polymyxin B Sulfate in the *Sample solution* corrected for loss on drying (mg/mL)
P = potency of polymyxin B3 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B1-I in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of polymyxin B1-I from the *Sample solution*
r_S = peak response of polymyxin B1-I from the *Standard solution*
C_S = concentration of USP Polymyxin B Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Polymyxin B Sulfate in the *Sample solution* corrected for loss on drying (mg/mL)
P = potency of polymyxin B1-I in USP Polymyxin B Sulfate RS (mg/mg)

Acceptance criteria: See *Table 1*. Report the components on the dried basis.

Table 1

Name	Relative Retention Time	Acceptance Criteria, (%)
Polymyxin B2[a]	0.5	—
Polymyxin B3	0.6	NMT 6.0
Polymyxin B1-I	0.8	NMT 15.0
Polymyxin B1[a]	1.0	—
Sum of polymyxins B1, B2, B3, and B1-I	—	NLT 80.0

[a] These components are not reported individually. They are only reported in the sum of polymyxins.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Where packaged for prescription compounding, the label states the number of Polymyxin B units in the container and per mg, that it is not intended for manufacturing use, that it is not sterile, and that its potency cannot be assured for longer than 60 days after opening. Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Polymyxin B Sulfate RS

Polymyxin B for Injection

» Polymyxin B for Injection contains an amount of Polymyxin B Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of polymyxin B.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, protected from light.
Labeling—Label it to indicate that where it is administered intramuscularly and/or intrathecally, it is to be given only to patients hospitalized so as to provide constant supervision by a physician.
USP Reference standards ⟨11⟩—
USP Polymyxin B Sulfate RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

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Pyrogen—It meets the requirements of the *Pyrogen Test* ⟨151⟩, the test dose being 1.0 mL per kg of a solution in pyrogen-free saline TS containing 20,000 Polymyxin B Units per mL.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Residue on ignition ⟨281⟩: not more than 5.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.
Other requirements—It meets the requirements for *pH* and *Loss on drying* under *Polymyxin B Sulfate*. It also meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Polymyxin B for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer B.6* to obtain a solution containing a convenient number of Polymyxin B Units per mL.

Assay preparation 2 (where the label states the quantity of polymyxin B in a given volume of constituted solution)—Constitute 1 container of Polymyxin B for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer B.6* to obtain a solution containing a convenient number of Polymyxin B Units per mL.

Procedure—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Polymyxin B Sulfate and Bacitracin Zinc Topical Aerosol

» Polymyxin B Sulfate and Bacitracin Zinc Topical Aerosol contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of polymyxin B and bacitracin.

Packaging and storage—Preserve in pressurized containers, and avoid exposure to excessive heat.
Identification—Collect in a suitable container the expelled contents of 1 Aerosol container, shake with a volume of 0.1 N hydrochloric acid sufficient to obtain a solution containing about 500 USP Bacitracin Units per mL, centrifuge, and use the clear supernatant as the test solution. Proceed as directed under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩. The specified result is observed.
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Collect aseptically in a suitable container half the contents expelled from 5 containers, dissolve in 500 mL of *Fluid A* containing 0.25 g of sodium thioglycollate and adjusted with sodium hydroxide to a pH of 6.6 ± 0.6, pass through a membrane filter as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined* under *Sterility Tests* ⟨71⟩, except to place the filter on the surface of Soybean–Casein Digest Agar Medium in a Petri dish, incubate for 7 days at 30° to 35°, and count the number of colonies on the filter. Similarly prepare a second specimen, except to incubate at 20° to 25°. Not more than 20 colonies are observed from the two specimens. It meets also the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* under *Microbial Enumeration Tests* ⟨61⟩ and *Tests for Specified Microorganisms* ⟨62⟩.
Water Determination, *Method I* ⟨921⟩—Store 1 container of Topical Aerosol in a freezer for not less than 2 hours, open the container, and transfer 10.0 mL of the freshly mixed specimen to a titration vessel containing 20 mL of a mixture of toluene and methanol (7:3) instead of methanol. In titrating the specimen, determine the endpoint at a temperature of 10° or higher: not more than 0.5% of water is found.
Other requirements—It meets the requirements for *Pressure Test, Minimum Fill,* and *Leakage Test* under *Topical Aerosols* ⟨603⟩.
Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, expelling the entire contents of 1 container of Topical Aerosol, according to the directions in the labeling, into a 2000-mL conical flask held in a horizontal position. Add 500.0 mL of 0.01 N hydrochloric acid, and shake to dissolve. Immediately dilute an accurately measured volume of this acidic solution quantitatively and stepwise with *Buffer B.6* to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.
Assay for bacitracin—Proceed as directed for bacitracin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of the acidic solution obtained in the *Assay for polymyxin B* immediately diluted quantitatively and stepwise with *Buffer B.1* to yield a *Test Dilution* having a bacitracin concentration assumed to be equal to the median dose level of the Standard. [NOTE—Add additional hydrochloric acid to each test dilution of the Standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution*.]

Polymyxin B Sulfate and Bacitracin Zinc Topical Powder

» Polymyxin B Sulfate and Bacitracin Zinc Topical Powder contains not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of polymyxin B and bacitracin.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
USP Polymyxin B Sulfate RS
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Collect aseptically in a suitable container about 1 g from not less than 5 containers, dissolve in 500 mL of *Fluid A*, filter through a membrane filter as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined* under *Sterility Tests* ⟨71⟩, except to place the filter on the surface of Soybean–Casein Digest Agar Medium in a Petri dish, incubate for 7 days at 30° to 35°, and count the number of colonies on the filter. Similarly prepare a second specimen, except to incubate at 20° to 25°. Not more than 20 colonies are observed from the two specimens. It meets also the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* under

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Microbial Enumeration Tests ⟨61⟩ and *Tests for specified microorganisms* ⟨62⟩.
Water Determination, *Method I* ⟨921⟩: not more than 7.0%.
Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Topical Powder, equivalent to about 5000 USP Polymyxin B Units, shaken with 20 mL of water in a suitable volumetric flask. Dilute with *Buffer B.6* to volume, and mix. Dilute an accurately measured volume of the solution so obtained quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.
Assay for bacitracin—Proceed as directed for bacitracin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Topical Powder, equivalent to about 800 USP Bacitracin Units, added to a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Dilute this solution quantitatively with *Buffer B.1* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. In preparing each test dilution of the Standard, add additional hydrochloric acid to each to obtain the same concentration of hydrochloric acid as in the *Test Dilution.*

Polymyxin B Sulfate and Hydrocortisone Otic Solution

» Polymyxin B Sulfate and Hydrocortisone Otic Solution is a sterile solution containing not less than 90.0 percent and not more than 130.0 percent of the labeled amount of polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$). It may contain one or more suitable buffers and preservatives.
[NOTE—Where Polymyxin B Sulfate and Hydrocortisone Otic Solution is prescribed without reference to the quantity of polymyxin B or hydrocortisone contained therein, a product containing 10,000 Polymyxin B Units and 5 mg of hydrocortisone per mL shall be dispensed.]

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Polymyxin B Sulfate RS
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 3.0 and 5.0.
Assay for polymyxin—Proceed with Otic Solution as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Otic Solution diluted quantitatively with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone—
Mobile phase—Prepare a suitable solution of about 500 volumes of methanol, 500 volumes of water, and 1 volume of glacial acetic acid, such that the retention time of hydrocortisone is between 6 and 10 minutes.
Standard preparation—Dissolve a suitable quantity of USP Hydrocortisone RS, accurately weighed, in a mixture of methanol and water (1:1) to obtain a solution having a known concentration of about 0.15 mg per mL.
Assay preparation—Transfer an accurately measured volume of Otic Solution, equivalent to about 15 mg of hydrocortisone, to a 100-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph five replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters such that the peak obtained from the *Standard preparation* is about 0.6 full-scale. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Solution taken by the formula:

$$(100C/V)(H_U/H_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation,* V is the volume, in mL, of the portion of Otic Solution taken, and H_U and H_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Polymyxin B Sulfate and Trimethoprim Ophthalmic Solution

» Polymyxin B Sulfate and Trimethoprim Ophthalmic Solution is a sterile, isotonic, aqueous solution of Polymyxin B Sulfate and Trimethoprim Sulfate or of Polymyxin B Sulfate and Trimethoprim that has been solubilized with Sulfuric Acid. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of polymyxin B and the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of trimethoprim ($C_{14}H_{18}N_4O_3$). It contains one or more preservatives.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.
Labeling—Label it to indicate that it is to be stored at 15° to 25°, protected from light.
USP Reference standards ⟨11⟩—
USP Polymyxin B Sulfate RS
USP Trimethoprim RS

Identification—
A: It meets the requirements for polymyxin B under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.
B: The retention time of the trimethoprim peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for trimethoprim.*

USP Monographs

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Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 4.0 and 6.2.

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution, diluted quantitatively and stepwise with *Buffer B.6*, to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Assay for trimethoprim—

Diluent—Prepare a mixture of 0.01 N hydrochloric acid and acetonitrile (870:130).

Mobile phase—Dissolve 1.65 g of ethanesulfonic acid in 1000 mL of a mixture of water and acetonitrile (870:130). Adjust with 10 N sodium hydroxide or 0.1 N hydrochloric acid to a pH of 3.5. Pass this solution through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Trimethoprim RS in *Diluent* to obtain a solution having a known concentration of about 0.04 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 1 mg of trimethoprim, to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5, when calculated at 10% height of the peak; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of trimethoprim ($C_{14}H_{18}N_4O_3$) in each mL of the Ophthalmic Solution taken by the formula:

$$25(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Trimethoprim RS in the *Standard preparation; V* is the volume, in mL, of Ophthalmic Solution taken to prepare the *Assay preparation;* and r_U and r_S are the trimethoprim peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Polyvinyl Alcohol



$(C_2H_4O)_n$

Ethenol, homopolymer;

Vinyl alcohol polymer [9002-89-5].

DEFINITION

Polyvinyl Alcohol is a water-soluble synthetic resin, represented by the formula $(C_2H_4O)_n$, in which the average value of n lies between 500 and 5000. It is prepared by 85%–89% hydrolysis of polyvinyl acetate. The apparent viscosity, in mPa · s, at 20°, of a 4% (w/w) aqueous solution is NLT 85.0% and NMT 115.0% of that stated on the label.

Change to read:

IDENTIFICATION

- **A. Infrared Absorption** ⟨197K⟩
- **B.** It meets the requirements in the test *Viscosity—Capillary Methods* ⟨911⟩, *Viscosity—Rotational Methods* ⟨912⟩, ▲or▲ (ERR 1-Jul-2019) *Viscosity—Rolling Ball Method* ⟨913⟩.
- **C.**

Sample solution: Dissolve 0.5 g of Polyvinyl Alcohol in 10 mL of water, with heat if necessary, and let the solution cool to room temperature.

Analysis 1: Transfer 5 mL of the *Sample solution* to a test tube, add 1 drop of iodine TS, mix, and allow to stand.

Acceptance criteria 1: A dark red to blue color is produced.

Analysis 2: Transfer 2 mL of the remaining *Sample solution* to a test tube, add 10 ml of Alcohol, and mix.

Acceptance criteria 2: A white, turbid, or flocculent precipitate is formed.

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 1.0%
- **Water-Insoluble Substances**

Analysis: Wash the tared 100-mesh screen used in the test for *Viscosity* immediately after with two 25-mL portions of water, and dry at 110° for 1 h.

Acceptance criteria: NMT 6.4 mg of water-insoluble substances are found (corresponding to NMT 0.1%).

- **Limit of Methanol (Methyl Alcohol) and Methyl Acetate**

Standard solution: In a 100-mL screw-cap bottle, prepare a 100-mL solution containing 0.24 µL/mL of USP Methyl Alcohol RS and 0.24 µL/mL of USP Methyl Acetate RS. Add 30 µL of USP Acetone RS to the screw-cap bottle. Close the bottle tightly with the screw cap, and heat it in a water bath, stirring continuously. Remove the bottle from the water bath, and allow it to cool to room temperature.

Sample solution: Transfer a quantity of Polyvinyl Alcohol, equivalent to 2.0 g on the dried basis, to a 100-mL screw-cap bottle, and add a magnetic stirrer. Add 98 mL of water and 30 µL of USP Acetone RS. Close the bottle tightly with the screw cap, and heat it in a water bath, stirring continuously. Once the solution becomes clear, remove the bottle from the water bath, and allow it to cool to room temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: GC

Detector: Flame ionization

Column: 3.2-mm × 2-m glass, phase S3

Temperatures

Injection port: 160°

Column: 160°

Detector: 160°

Carrier gas: Nitrogen

Flow rate: 30 mL/min

Injection volume: 0.4 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of methanol and methyl acetate in the portion of Polyvinyl Alcohol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/W) \times V \times D \times F \times 100$$

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R_U = peak response ratio of the methanol or methyl acetate peak relative to the acetone peak from the *Sample solution*
R_S = peak response ratio of the methanol or methyl acetate peak relative to the acetone peak from the *Standard solution*
C_S = concentration of methanol (methyl alcohol) or methyl acetate in the *Standard solution* (µL/mL)
W = weight of Polyvinyl Alcohol on the dried basis taken to prepare the *Sample solution* (g)
V = volume of the *Sample solution*, 100 mL
D = density of methanol (methyl alcohol) or methyl acetate, 0.79 and 0.93 g/mL, respectively
F = conversion factor, 10^{-3} mL/µL

Acceptance criteria
Methanol (methyl alcohol): NMT 1.0%
Methyl acetate: NMT 1.0%

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 110° to constant weight.
Acceptance criteria: NMT 5.0%
• **Viscosity—Capillary Methods** ⟨911⟩, **Viscosity—Rotational Methods** ⟨912⟩, or **Viscosity—Rolling Ball Method** ⟨913⟩
Sample: After performing *Loss on Drying*, weigh undried Polyvinyl Alcohol, equivalent to 6.00 g on the dried basis.
Analysis: Over a period of seconds, transfer the *Sample* with continuous slow stirring to 140 mL of water contained in a suitable tared flask. When the specimen is well wetted, increase the rate of stirring, avoiding mixing in excess air. Heat the mixture to 90°, and maintain the temperature at 90° for about 5 min. Discontinue heating, and continue stirring for 1 h. Add water to make the mixture weigh 150 g. Resume stirring to obtain a homogeneous solution. Filter the solution through a tared 100-mesh screen into a 250-mL conical flask, cool the filtrate to 15°, mix, and proceed as directed in the chapter. Determine its viscosity at 20 ± 0.1°, using an appropriate viscometer.
Acceptance criteria: 85.0%–115.0% of the labeled value.
• **pH** ⟨791⟩
Sample solution: 40 mg/mL
Acceptance criteria: 5.0–8.0
• **Degree of Hydrolysis**
Sample: 1 g of Polyvinyl Alcohol, previously dried at 110° to constant weight
Analysis: Transfer the *Sample* to a wide-mouth, 250-mL conical flask fitted by means of a suitable glass joint to a reflux condenser. Add 35 mL of dilute methanol (3 in 5), and mix gently to ensure complete wetting of the solid. Add 3 drops of phenolphthalein TS, and add 0.2 N hydrochloric acid or 0.2 N sodium hydroxide if necessary, to neutralize. Add 25.0 mL of 0.2 N sodium hydroxide VS, and reflux gently on a hot plate for 1 h. Wash the condenser with 10 mL of water, collecting the washings in the flask, cool, and titrate with 0.2 N hydrochloric acid VS. Concomitantly perform a blank determination in the same manner, using the same quantity of 0.2 N sodium hydroxide VS.
Calculation of saponification value: Calculate the saponification value:

$$\text{Result} = [(V_B - V_S) \times N \times M_r]/W$$

V_B = volume of 0.2 N hydrochloric acid VS consumed in the titration of the blank (mL)
V_S = volume of 0.2 N hydrochloric acid VS consumed in the titration of the *Sample solution* (mL)
N = actual normality of hydrochloric acid VS
M_r = molecular weight of potassium hydroxide, 56.11
W = weight of the portion of Polyvinyl Alcohol taken (g)

Calculation of degree of hydrolysis: Calculate the degree of hydrolysis, expressed as a percentage of hydrolysis of polyvinyl acetate:

$$\text{Result} = 100 - [7.84 \times S/(100 - 0.075 \times S)]$$

S = saponification value of the Polyvinyl Alcohol

Acceptance criteria: 85%–89%
• **Acid Value**
Sample: 10.0 g
Analysis: Add 200 mL of water to a borosilicate round-bottom flask attached to a reflux condenser. Heat the water on a water bath with constant stirring. Add the *Sample* to the water, and continue heating for 30 min with continuous stirring. Remove the flask from the water bath, and continue stirring until room temperature is reached. Quantitatively transfer this solution to a 250-mL volumetric flask, dilute with water to volume, and mix. Add 0.5 mL of phenolphthalein TS to 50 mL of this solution, and titrate with 0.05 N potassium hydroxide VS until the pink color persists for 15 s.
Calculate the acid value:

$$\text{Result} = D \times M_r \times [(N \times V)/W]$$

D = dilution factor
M_r = molecular weight of potassium hydroxide, 56.11
N = normality of potassium hydroxide VS used
V = volume of 0.05 N potassium hydroxide used (mL)
W = weight of the *Sample* (g)

Acceptance criteria: NMT 3.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers, and store at room temperature in a dry place.
• **Labeling:** Label it to indicate the viscosity, giving the viscosity measurement parameters, the concentration of the solution, and the type of equipment used.
• **USP Reference Standards** ⟨11⟩
USP Acetone RS
USP Methyl Acetate RS
USP Methyl Alcohol RS
USP Polyvinyl Alcohol RS

Sulfurated Potash

Thiosulfuric acid, dipotassium salt, mixture with potassium sulfide (K_2S_x).
Dipotassium thiosulfate mixture with potassium sulfide (K_2S_x) [39365-88-3].

» Sulfurated Potash is a mixture composed chiefly of potassium polysulfides and potassium thiosulfate. It contains not less than 12.8 percent of sulfur (S) in combination as sulfide.

Packaging and storage—Preserve in tight containers. Containers from which it is to be taken for immediate use in compounding prescriptions contain not more than 120 g.

USP Monographs

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Identification—

A: To a 1 in 10 solution add an excess of 6 N acetic acid: hydrogen sulfide is evolved, and sulfur is precipitated.

B: Filter the mixture from *Identification* test *A*, and add to the filtrate an excess of sodium bitartrate TS: an abundant, white, crystalline precipitate is formed within 15 minutes.

Assay for sulfides—Transfer 10 to 15 pieces of Sulfurated Potash to a mortar, and reduce to a fine powder. Transfer about 1 g of the powder, accurately weighed, to a 250-mL beaker, and dissolve in 50 mL of water. Filter, if necessary, and wash or dilute with water to 75 mL. Add, with constant stirring, 50 mL of cupric sulfate solution (1 in 20), and allow the mixture to stand, with occasional stirring, for 10 minutes. Filter through a retentive filter, and wash the precipitate with 200 mL of 0.25 N hydrochloric acid, taking care to avoid breaking up the cake. (If the filtrate is not blue in color, discard the assay specimen, and start over, using a larger volume of cupric sulfate solution.) Ignite the precipitate in a tared dish at 1000° for 1 hour, cool in a desiccator, and weigh: the weight of the cupric oxide so obtained, multiplied by 0.4030, represents the weight of S in the specimen under assay.

Potassium Acetate



$C_2H_3KO_2$ 98.14

Acetic acid, potassium salt;
Potassium acetate [127-08-2].

DEFINITION

Potassium Acetate contains NLT 99.0% and NMT 100.5% of $C_2H_3KO_2$, calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Acetate* ⟨191⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Sample: 200 mg of Potassium Acetate, previously dried
  Analysis: Dissolve the *Sample* in 25 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 9.814 mg of potassium acetate ($C_2H_3KO_2$).
  Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES

- **LIMIT OF SODIUM**
  Solution A: 100 mg/mL of potassium chloride in water
  Standard stock solution: Transfer 127.1 mg of sodium chloride, previously dried at 105° for 2 h into a 500-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.
  Standard solutions: Transfer 2.0, 5.0, and 10.0 mL of the *Standard stock solution* into separate 100-mL volumetric flasks, add 10.0 mL of *Solution A* to each flask, and dilute with water to volume. These *Standard solutions* contain 0.2, 0.5, and 1.0 µg/mL of sodium, respectively.
  [NOTE—Concentrations of sodium in the *Standard solutions* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]
  Sample solution: Transfer about 0.2 g of Potassium Acetate into a 100-mL volumetric flask containing about 50 mL of water, and swirl to dissolve. Add 10.0 mL of *Solution A*, dilute with water to volume, and mix. [NOTE—The concentration of Potassium Acetate in the *Sample solution* may be modified by using a different quantity or by further dilution to bring the absorption response within the range of responses from the *Standard solutions*.]
  Instrumental conditions
  (See *Atomic Absorption Spectroscopy* ⟨852⟩.)
  Mode: Atomic absorption spectrophotometry
  Analytical wavelength: Sodium emission line at 589 nm
  Lamp: Sodium hollow-cathode
  Flame: Oxidizing air–acetylene
  Blank solution: Transfer 10.0 mL of *Solution A* into a 100-mL volumetric flask, dilute with water to volume, and mix.
  Analysis
  Samples: *Standard solutions*, *Sample solution*, and *Blank solution*
  Determine the absorbances of the *Standard solutions* and the *Sample solution*, using the *Blank solution* to zero the instrument. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of sodium, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of sodium in the *Sample solution*.
  Calculate the percentage of sodium in the portion of Potassium Acetate taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

C	= concentration of the *Sample solution*, determined from the graph (µg/mL)
W	= weight of Potassium Acetate taken to prepare the *Sample solution* (g)
V	= final volume of the *Sample solution*, taking into account any dilution necessary (mL)
F	= conversion factor (1,000,000 µg/g)

Acceptance criteria: NMT 0.03%

SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: 50 mg/mL
  Acceptance criteria: 7.5–8.5
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 150° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Acetate Injection

» Potassium Acetate Injection is a sterile solution of Potassium Acetate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_2H_3KO_2$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.
Labeling—The label states the potassium acetate content in terms of weight and of milliequivalents in a given volume.

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Label the Injection to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

Identification—It responds to the tests for *Potassium* ⟨191⟩ and for *Acetate* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 8.80 USP Endotoxin Units per mEq.

pH ⟨791⟩: between 5.5 and 8.0, when diluted with water to 1.0% of potassium acetate.

Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Potassium stock solution—Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 hours, in water. Transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 µg of potassium (equivalent to 19.07 µg of potassium chloride) per mL.

Standard preparations—To separate 100-mL volumetric flasks transfer 10.0, 15.0, and 20.0 mL, respectively, of *Potassium stock solution*. To each flask add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix. The *Standard preparations* contain, respectively, 1.0, 1.5, and 2.0 µg of potassium per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 2 g of potassium acetate, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the solution to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the potassium emission line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a potassium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbance of the *Standard preparation* versus concentration, in µg per mL, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in µg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of $C_2H_3KO_2$ in the portion of Injection taken by the formula:

$$500C(2.510)$$

in which C is the concentration, in µg per mL, of potassium in the *Assay preparation*, and 2.510 is the ratio of the molecular weight of potassium acetate to the atomic weight of potassium.

Potassium Bicarbonate

$KHCO_3$ 100.12

Carbonic acid, monopotassium salt; Monopotassium carbonate [298-14-6].

DEFINITION

Potassium Bicarbonate contains NLT 99.5% and NMT 101.5% of $KHCO_3$, calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Bicarbonate* ⟨191⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Sample: 4 g
  Analysis: Dissolve the *Sample* in 100 mL of water, add methyl red TS, and titrate with 1 N hydrochloric acid VS. Add the acid slowly, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the pink color no longer fades after boiling. Each mL of 1 N hydrochloric acid is equivalent to 100.1 mg of $KHCO_3$.
  Acceptance criteria: 99.5%–101.5% on the dried basis

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample over silica gel for 4 h: it loses NMT 0.3% of its weight.
- **NORMAL CARBONATE**
  Sample: Grind 3.0 g of Potassium Bicarbonate with 25 mL of alcohol and 5 mL of water in a porcelain mortar.
  Barium chloride solution: 12.216 mg/mL of barium chloride in alcohol and water (7:3), prepared by dissolving the barium chloride in water, then diluting with alcohol to final volume
  Analysis: Add 3 drops of phenolphthalein TS to the *Sample*, and titrate slowly with *Barium chloride solution* until the suspension becomes colorless. Continue the grinding for 2 min, and if the color turns pink, continue the titration with *Barium chloride solution* to a colorless endpoint. Repeat the grinding for 2 min and the addition of *Barium chloride solution*, if necessary, until the suspension is colorless after 2 min of grinding. Each mL of the barium chloride solution is equivalent to 6.911 mg of K_2CO_3.
  Acceptance criteria: NMT 2.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Potassium Bicarbonate Effervescent Tablets for Oral Solution

» Potassium Bicarbonate Effervescent Tablets for Oral Solution contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of K.

Packaging and storage—Preserve in tight containers, protected from excessive heat.

Labeling—The label states the potassium content in terms of weight and in terms of milliequivalents. Where the Tablets are packaged in individual pouches, the label instructs the user not to open until the time of use.

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Identification—One Tablet dissolves in 100 mL of water with effervescence. The collected gas responds to the test for *Bicarbonate* ⟨191⟩, and the resulting solution responds to the test for *Potassium* ⟨191⟩.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Standard stock solution and *Standard solutions*—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution.*
Assay preparation—Transfer 10 Tablets to a 2000-mL volumetric flask, dissolve in 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Filter, and quantitatively dilute an accurately measured volume of the filtrate with water to obtain a solution containing 30 µg of potassium per mL. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.
Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation* instead of *Sample solution.* Calculate the quantity, in mg, of K in each Tablet taken by the formula:

$$L(C/D)$$

in which L is the labeled quantity, in mg, of potassium in each Tablet, C is the concentration, in µg per mL, of potassium in the *Assay preparation*, and D is the concentration, in µg per mL, of potassium in the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution.

Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution

» Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of K and Cl.

Packaging and storage—Preserve in tight containers, protected from excessive heat.
Labeling—The label states the potassium and chloride contents in terms of weight and in terms of milliequivalents. Where packaged in individual pouches, the label instructs the user not to open until the time of use.
Identification—A 3-g portion dissolves in 100 mL of water with effervescence. The collected gas so obtained responds to the test for *Bicarbonate* ⟨191⟩, and the resulting solution responds to the tests for *Potassium* ⟨191⟩ and for *Chloride* ⟨191⟩.

Minimum fill ⟨755⟩—
FOR SOLID PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Uniformity of dosage units ⟨905⟩—
FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Assay for potassium—
Standard stock solution and *Standard solutions*—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution.*
Assay preparation—Weigh and mix the contents of not less than 20 containers of Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution. Transfer an accurately weighed portion of the powder, equivalent to about 6 g of potassium, to a 1000-mL volumetric flask, dissolve in about 200 mL of water, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a second 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.
Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation* instead of *Sample solution.* Calculate the quantity, in mg, of K in the portion of Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution taken by the formula:

$$400C$$

in which C is the concentration, in µg per mL, of potassium in the *Assay preparation.*
Assay for chloride—Weigh and mix the contents of not less than 20 containers of Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution. Transfer a portion of the powder, equivalent to about 900 mg of chloride, to a 2000-mL volumetric flask. Add about 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Transfer 25.0 mL of this solution to a 250-mL conical flask, add 50.0 mL of 0.1 N silver nitrate VS and 15 mL of nitric acid, and boil, with constant swirling, until the solution is colorless. Cool to room temperature, add water to make about 150 mL, then add 5 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS to a permanent faint brown endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.

Potassium Bicarbonate and Potassium Chloride Effervescent Tablets for Oral Solution

» Potassium Bicarbonate and Potassium Chloride Effervescent Tablets for Oral Solution contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of K and Cl.

Packaging and storage—Preserve in tight containers, protected from excessive heat.
Labeling—The label states the potassium and chloride contents in terms of weight and in terms of milliequivalents. Where the Tablets are packaged in individual pouches, the label instructs the user not to open until the time of use.
Identification—One Tablet dissolves in 100 mL of water with effervescence. The collected gas responds to the test for *Bicarbonate* ⟨191⟩, and the resulting solution responds to the tests for *Potassium* ⟨191⟩ and for *Chloride* ⟨191⟩.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay for potassium—
Standard stock solution and *Standard solutions*—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution.*
Assay preparation—Transfer 10 Tablets to a 2000-mL volumetric flask, dissolve in 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Filter, and quantitatively dilute an accurately measured volume of the filtrate with water to obtain a solution containing 30 µg of potassium per mL. Transfer 5.0 mL of the

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resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation* instead of *Sample solution*. Calculate the quantity, in mg, of K in each Tablet taken by the formula:

$$L(C/D)$$

in which L is the labeled quantity, in mg, of potassium in each Tablet, C is the concentration, in µg per mL, of potassium in the *Assay preparation*, and D is the concentration, in µg per mL, of potassium in the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution.

Assay for chloride—Transfer a number of Tablets, equivalent to about 900 mg of chloride, to a 2000-mL volumetric flask. Add about 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Transfer 25.0 mL of this solution to a 250-mL conical flask, add 50.0 mL of 0.1 N silver nitrate VS and 15 mL of nitric acid, and boil, with constant swirling, until the supernatant is colorless. Cool to room temperature, add sufficient water to make a volume of about 150 mL, add 5 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS to a permanent faint brown endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Calculate the quantity, in mg, of chloride (Cl) in each Tablet by dividing the total amount of chloride in the Tablets taken by the number of Tablets taken.

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Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution

DEFINITION

Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution contain NLT 90.0% and NMT 110.0% of the labeled amounts of potassium bicarbonate ($KHCO_3$), sodium bicarbonate ($NaHCO_3$), and anhydrous citric acid ($C_6H_8O_7$).

IDENTIFICATION

• **A. Identification Tests—General,** *Bicarbonate* ⟨191⟩, *Potassium* ⟨191⟩, *Sodium* ⟨191⟩, and *Citrate* ⟨191⟩

Sample: One Tablet for Oral Solution

Analysis 1: Dissolve the sample in 100 mL of water, and collect the gas that evolves.

Acceptance criteria 1: The *Sample* effervesces when dissolved.

Analysis 2: Proceed as directed in *Bicarbonate* ⟨191⟩ on the gas collected from *Analysis 1*.

Acceptance criteria 2: Meets the requirements

Analysis 3: Proceed as directed in *Potassium* ⟨191⟩ and *Sodium* ⟨191⟩ on the resulting solution from *Analysis 1*.

Acceptance criteria 3: Meets the requirements

Analysis 4: Proceed as directed in *Citrate* ⟨191⟩ on 3–5 drops of the resulting solution from *Analysis 1* and 20 mL of the mixture of pyridine and acetic anhydride.

Acceptance criteria 4: Meets the requirements

ASSAY

• **Potassium Bicarbonate and Sodium Bicarbonate**

Potassium chloride stock solution: 7.5 mg/mL of potassium chloride, previously dried at 125° for 30 min, in water

Sodium chloride stock solution: 7 mg/mL of sodium chloride, previously dried at 125° for 30 min, in water

Diluent: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Before dilution to final volume, add a suitable nonionic surfactant.

Standard stock solution: 0.75 mg/mL of potassium chloride and 0.7 mg/mL of sodium chloride in water from *Potassium chloride stock solution* and *Sodium chloride stock solution*, respectively

Standard solution: 0.0375 mg/mL of potassium chloride and 0.035 mg/mL of sodium chloride in *Diluent* from the *Standard stock solution*

Sample stock solution A: Nominally 3 mg/mL of potassium bicarbonate prepared as follows. Finely powder NLT 20 Tablets for Oral Solution. Tablets for Oral Solution and powder are hygroscopic. After removal from the container, grind the Tablets for Oral Solution promptly in an atmosphere of low relative humidity, and weigh the powder promptly. Transfer a portion of the powder, equivalent to about 3000 mg of potassium bicarbonate, to a 1000-mL volumetric flask. Dissolve in 500 mL of water, swirl until effervescence ceases, and dilute with water to volume.

Sample stock solution B: Nominally 1 mg/mL of potassium bicarbonate, based on the labeled quantity, from *Sample stock solution A* in water

Sample stock solution C: Nominally 1 mg/mL of sodium bicarbonate, based on the labeled quantity, from *Sample stock solution A* in water

Sample solution A: Nominally 0.05 mg/mL of potassium bicarbonate from *Sample stock solution B* in *Diluent*

Sample solution B: Nominally 0.05 mg/mL of sodium bicarbonate from *Sample stock solution C* in *Diluent*

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: The maximum at about 766 nm

Sodium: The maximum at about 589 nm

Blank: *Diluent*

Analysis

Samples: *Standard solution*, *Sample solution A*, *Sample solution B*, and *Blank*

Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution*, *Sample solution A*, and *Sample solution B*.

Calculate the percentage of the labeled amount of potassium bicarbonate ($KHCO_3$) in the portion of Tablets for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = photometer reading of potassium from *Sample solution A*

r_S = photometer reading of potassium from the *Standard solution*

C_S = concentration of potassium chloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of potassium bicarbonate in *Sample solution A* (mg/mL)

M_{r1} = molecular weight of potassium bicarbonate, 100.12

M_{r2} = molecular weight of potassium chloride, 74.55

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Calculate the percentage of the labeled amount of sodium bicarbonate ($NaHCO_3$) in the portion of Tablets for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = photometer reading of sodium from *Sample solution B*
r_S = photometer reading of sodium from the *Standard solution*
C_S = concentration of sodium chloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium bicarbonate in *Sample solution B* (mg/mL)
M_{r1} = molecular weight of sodium bicarbonate, 84.01
M_{r2} = molecular weight of sodium chloride, 58.44

Acceptance criteria: 90.0%–110.0% of the labeled amounts of potassium bicarbonate ($KHCO_3$) and sodium bicarbonate ($NaHCO_3$)

• **ANHYDROUS CITRIC ACID**

Mobile phase, Standard preparation 1, and **Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution: Nominally 20 µg/mL of citrate prepared as follows. Transfer the equivalent of 40 mg of anhydrous citric acid from *Sample stock solution A* in the *Assay* for *Potassium Bicarbonate and Sodium Bicarbonate* into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate).*

Analysis:

Samples: *Standard preparation 1* and *Sample solution*

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure.*

Calculate the percentage of the labeled amount of anhydrous citric acid ($C_6H_8O_7$) in the portion of Tablets for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of citrate from the *Sample solution*
r_S = peak response of citrate from *Standard preparation 1*
C_S = concentration of citrate in *Standard preparation 1* (µg/mL)
C_U = nominal concentration of citrate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of anhydrous citric acid, 192.12
M_{r2} = molecular weight of citrate, 189.10

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

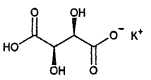
• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩**:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label Tablets for Oral Solution to state the sodium content. The label states also that the tablets are to be dissolved in water before being taken.

Potassium Bitartrate



$C_4H_5KO_6$ 188.18

Butanedioic acid 2,3-dihydroxy-, [*R*-(*R**,*R**)]-, monopotassium salt;

Potassium hydrogen tartrate [868-14-4].

DEFINITION

Potassium Bitartrate, dried at 105° for 3 h, contains NLT 99.0% and NMT 101.0% of $C_4H_5KO_6$.

IDENTIFICATION

• **A.** Ignite it: it leaves a residue that imparts a reddish purple color to a nonluminous flame.

• **B.** A saturated solution of it yields a yellowish orange precipitate with sodium cobaltinitrite TS.

• **C. IDENTIFICATION TESTS—GENERAL,** *Tartrate* ⟨191⟩**:** Meets the requirements

ASSAY

• **PROCEDURE**

Sample: Dry 6 g of Potassium Bitartrate at 105° for 3 h. Allow to cool, and weigh.

Analysis: Dissolve the *Sample* in 100 mL of boiling water, add a few drops of phenolphthalein TS, and titrate with 1 N sodium hydroxide VS to a pink endpoint. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 1 N sodium hydroxide is equivalent to 188.2 mg of $C_4H_5KO_6$.

Acceptance criteria: 99.0%–101.0% on the previously dried basis

IMPURITIES

• **INSOLUBLE MATTER**

Sample: 500 mg

Analysis: Mix the *Sample* with 3 mL of 6 N ammonium hydroxide.

Acceptance criteria: No undissolved residue remains.

• **LIMIT OF AMMONIA**

Sodium hypochlorite solution: Use a commercially available solution that contains 4.0%–6.0% of sodium hypochlorite.

Oxidizing solution: [NOTE—Prepare on the day of use.] Alkaline sodium citrate TS and *Sodium hypochlorite solution* (4:1)

Diluted sodium nitroferricyanide solution: Sodium nitroferricyanide TS and water (1:10)

Standard stock solution: Dry 300 mg of ammonium chloride over silica gel for 4 h, and use it to prepare a 0.3 mg/mL solution in water. This solution contains 100 µg/mL of ammonia.

Standard solution: 0.25 µg/mL of ammonia in water, from *Standard stock solution*

Sample solution: 2.5 mg/mL of Potassium Bitartrate in water. Heat gently to facilitate the dissolution.

Analysis

[NOTE—Carefully follow the order of addition stated below.]

Separately transfer 6.0 mL each of the *Standard solution* and the *Sample solution* to two color-comparison tubes. To each tube add 0.4 mL of phenol TS, 0.4 mL of *Diluted sodium nitroferricyanide solution,* and 1.0 mL of *Oxidizing solution.* Dilute with water to 10 mL, mix, and allow to stand for 1 h.

Acceptance criteria: The color of the *Sample solution* is not darker than the color of the *Standard solution* (NMT 0.01%).

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Bromide

KBr 119.00
Potassium bromide.
Potassium bromide [7758-02-3].

» Potassium Bromide contains not less than 98.0 percent and not more than 100.5 percent of KBr, calculated on the dried basis. It contains no added substances.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.
Appearance of solution: clear and colorless.
Test solution—Dissolve 10.0 g in carbon dioxide-free water, and dilute with the same solvent to 100 mL.

Identification—
A: A solution containing 4.5 mg of potassium bromide responds to the test for *Bromide* ⟨191⟩.
B: Responds to the test for *Potassium* ⟨191⟩.
Acidity or alkalinity—To 10 mL of the solution prepared for the test for *Appearance of solution,* add 0.1 mL of bromothymol blue TS: not more than 0.5 mL of 0.01 N hydrochloric acid or 0.01 N sodium hydroxide is required to change the color of this solution.
Loss on drying ⟨731⟩—Dry it at 100° to 105° for 3 hours: it loses not more than 1.0% of its weight.

Bromates—
Starch–mercuric iodide solution—Triturate 1.0 g of soluble starch with 5 mL of water and pour the mixture into 100 mL of boiling water, containing 10 mg of mercuric iodide.
Procedure—To 10 mL of the solution prepared for the test for *Appearance of solution* add 1 mL of *Starch–mercuric iodide solution,* 0.1 mL of a 100 g per L solution of potassium iodide, and 0.25 mL of 0.5 M sulfuric acid. Allow to stand protected from light for 5 minutes: no blue or violet color develops.
Limit of chlorine: not more than 0.6%.
Nitric acid solution and *Ferric ammonium sulfate solution*—Proceed as directed in the *Assay.*
Procedure—Dissolve 1.000 g of Potassium Bromide in 20 mL of *Nitric acid solution* in a conical flask, add and mix 5 mL of 30 percent hydrogen peroxide, and heat in a water bath until the solution is colorless. Rinse the sides of the flask with a small quantity of water, and heat in a water bath for 15 minutes. Allow to cool, dilute with water to 50 mL, and add 5.0 mL of silver nitrate VS and 1 mL of dibutyl phthalate. Mix, and back titrate the excess silver nitrate with ammonium thiocyanate VS (see *Titrimetry* ⟨541⟩), using 5 mL of *Ferric ammonium sulfate solution* as the indicator. Perform a blank titration. Not more than 1.7 mL of silver nitrate VS is used.
Iodides—To 5 mL of the solution prepared for the test for *Appearance of solution* add 0.15 mL of a 10.5 g per 100 mL ferric chloride solution, and 2 mL of dichloromethane. Shake, and allow to separate. The lower layer is colorless.
Sulfates ⟨221⟩—A 2.0-g portion shows no more sulfate than corresponds to 0.2 mL of 0.020 N sulfuric acid (0.01%).
Limit of iron: not more than 20 ppm.
Citric acid solution—Prepare a 200-mg citric acid per mL solution.
Iron standard solution—Transfer 0.863 g of ferric ammonium sulfate to a 500-mL volumetric flask, and dissolve in 25 mL of dilute sulfuric acid. Dilute with water to volume. Transfer 1.0 mL of the resulting solution to a 10-mL volumetric flask, and dilute with water to volume. Transfer 2.5 mL of this resulting solution to a 50-mL volumetric flask, and dilute with water to volume. [NOTE—Prepare immediately before use.]
Test solution—Transfer 5 mL of the solution prepared for the test for *Appearance of solution* to a 10-mL volumetric flask, and dilute with water to volume.
Procedure—To 10 mL each of the *Iron standard solution* and the *Test solution* add 2.0 mL of the *Citric acid solution* and 0.1 mL of thioglycolic acid. Make alkaline to litmus with ammonia water, and dilute with water to 20 mL. After 5 minutes, any pink color in the *Test solution* is not more intense than that in the *Iron standard solution.*
Magnesium and alkaline-earth metals—To 200 mL of water add 0.1 g of hydroxylamine hydrochloride, 10 mL of pH 10.0 ammonia–ammonium chloride buffer (prepared by dissolving 5.4 g of ammonium chloride in 20 mL of water, adding 20 mL of ammonium hydroxide and diluting to 100 mL), 1 mL of 0.1 M zinc sulfate, and about 0.2 g of eriochrome black T trituration. Heat to about 40°. Titrate this solution (see *Titrimetry* ⟨541⟩) with 0.01 M edetate disodium VS until the violet color changes to deep blue. To this solution add 10.0 g of Potassium Bromide dissolved in 100 mL of water. If the color changes to violet, titrate the solution with 0.01 M edetate disodium VS to a deep blue endpoint. The volume of 0.01 M edetate disodium consumed in the second titration does not exceed 5.0 mL (0.02%, calculated as Ca).

Assay—
Nitric acid solution—Dilute 14 mL of nitric acid with water to 100 mL.
Ferric ammonium sulfate solution—Transfer 10 g of ferric ammonium sulfate to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.
Procedure—Dissolve 2.000 g of Potassium Bromide in water, and dilute with water to 100.0 mL. To 10.0 mL of the solution add 50 mL of water, 5 mL of *Nitric acid solution,* 25.0 mL of silver nitrate VS, and 2 mL of dibutyl phthalate. Mix, and back titrate the excess silver nitrate with ammonium thiocyanate VS (see *Titrimetry* ⟨541⟩), using 2 mL of *Ferric ammonium sulfate solution* as the indicator, shaking vigorously towards the endpoint. Each mL of 0.1 M silver nitrate is equivalent to 11.90 mg of KBr. Calculate the percent content of Potassium Bromide, corrected for the chloride content, by the formula:

$$a - 3.357b$$

in which a is the percent content of KBr and KCl obtained, calculated as KBr; and b is the percent content of chlorides.

Potassium Bromide Compounded Oral Solution, Veterinary

DEFINITION
Potassium Bromide Compounded Oral Solution, Veterinary contains NLT 225 mg and NMT 275 mg of potassium bromide (KBr) per mL, equivalent to NLT 151 mg and NMT 185 mg of bromide (Br^-) per mL.
Prepare Potassium Bromide Compounded Oral Solution, Veterinary as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Potassium Bromide	25 g
Purified Water	60 mL

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Corn Syrup, *FCC*, a sufficient quantity to make	100 mL

Dissolve the *Potassium Bromide* in *Purified Water*. Add *Corn Syrup, FCC*, to bring to final volume with mixing.

ASSAY

• **PROCEDURE**

TCA solution: 20% (w/v) trichloroacetic acid in water

Gold chloride solution: 5 mg/mL of gold chloride in water

Standard stock solution: Dissolve USP Sodium Bromide RS in water to obtain a solution with a nominal concentration of 20 mg/mL of bromide.

Standard solutions: Prepare four solutions of known concentrations of about 2.0, 1.0, 0.5, and 0.25 mg/mL of bromide by diluting the *Standard stock solution* with water.

Sample solution: Dilute Oral Solution, Veterinary quantitatively with water (1:99).

Blank: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Analytical wavelength: 440 nm

System suitability

Samples: *Standard solutions* and *Blank*

Suitability requirements

Correlation coefficient: NLT 0.99, linear regression of the *Standard solutions*

Analysis

Samples: *Sample solution* and *Blank*

To 750-µL aliquots of each *Sample* add 500 µL of *TCA solution* and 250 µL of *Gold chloride solution*. Mix on a vortex mixer, and immediately read the absorbance of each *Sample*.

Calculate the concentration, in mg/mL, of bromide (Br^-) in the portion of Oral Solution, Veterinary taken:

$$\text{Result} = C \times D$$

C = concentration of the *Sample solution* (mg/mL) obtained from the standard curve

D = dilution factor of the *Sample solution*, 100

Acceptance criteria: 151–185 mg/mL of bromide (Br^-)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in a tight container, and store in a refrigerator.

• **BEYOND-USE DATE:** NMT 180 days after the date on which it was compounded when stored in a refrigerator

• **LABELING:** Label it to state the *Beyond-Use Date*. Label it to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Bromide RS

Potassium Carbonate

K_2CO_3 (anhydrous) 138.21

$K_2CO_3 \cdot 1½H_2O$ 165.23

Carbonic acid, dipotassium salt; Dipotassium carbonate [584-08-7].

DEFINITION

Potassium Carbonate contains NLT 99.5% and NMT 100.5% of K_2CO_3, calculated on the dried basis.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩**:** Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Carbonate* ⟨191⟩**:** Meets the requirements

ASSAY

• **PROCEDURE**

Sample: Dried potassium carbonate obtained in the test for *Loss on Drying*

Analysis: Transfer the *Sample* to a flask with the aid of 150 mL of water, and add 4 drops of methyl orange TS. Titrate with 1 N hydrochloric acid VS. Each mL of 1 N hydrochloric acid is equivalent to 69.11 mg of K_2CO_3.

Acceptance criteria: 99.5%–100.5% on the dried basis

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 180° for 4 h: it loses NMT 0.5% of its weight.

• **INSOLUBLE SUBSTANCES**

Sample solution: 1 g in 20 mL of water

Acceptance criteria: The solution is complete, clear, and colorless.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Potassium Chloride

KCl 74.55

Potassium chloride [7447-40-7].

DEFINITION

Potassium Chloride contains NLT 99.0% and NMT 100.5% of KCl, calculated on the dried basis.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample: 200 mg

Analysis: Dissolve the *Sample* in 10 mL of water. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 7.455 mg of KCl.

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES

• **ALUMINUM** ⟨206⟩ (where it is labeled as intended for use in hemodialysis)**:** Proceed as directed, using 2.0 g of Potassium Chloride to prepare the *Test preparation*.

Acceptance criteria: NMT 1 ppm

• **SODIUM**

Sample solution: 50 mg/mL

Acceptance criteria: *Sample solution* tested on a platinum wire does not impart a pronounced yellow color to a nonluminous flame.

• **IODIDE**

Standard stock solution: 1.64 mg/mL of potassium iodide in water

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Standard solution: Dilute 1.0 mL of *Standard stock solution* with water to 25 mL. Dilute 2.0 mL of this solution with water to 8 mL. Add 1 mL each of chloroform and diluted hydrochloric acid, then add 2 drops of a chloramine T solution (0.1 in 100), and shake gently.
Sample solution: Dissolve 2 g of Potassium Chloride in 8 mL of water. Add 1 mL each of chloroform and diluted hydrochloric acid, then add 2 drops of a chloramine T solution (0.1 in 100), and shake gently.
Acceptance criteria: The violet color of the chloroform layer is not darker than that of a concomitantly prepared *Standard solution* (NMT 0.005%).

• **BROMIDE**
Standard stock solution: 1.28 mg/mL of sodium bromide in water
Standard solution: Dilute 2.0 mL of *Standard stock solution* with water to 8 mL. Add 1 mL each of chloroform and diluted hydrochloric acid, then add 5 drops of a chloramine T solution (1 in 100), and shake gently.
Sample solution: Dissolve 2 g of Potassium Chloride in 8 mL of water. Add 1 mL each of chloroform and diluted hydrochloric acid, then add 5 drops of a chloramine T solution (1 in 100), and shake gently.
Acceptance criteria: The brown color of the chloroform layer is not darker than that of a concomitantly prepared *Standard solution* (NMT 0.1%).

• **CALCIUM AND MAGNESIUM**
Sample solution: 10 mg/mL in water
Analysis: To 20 mL of *Sample solution* add 2 mL each of 6 N ammonium hydroxide, ammonium oxalate TS, and dibasic sodium phosphate TS.
Acceptance criteria: No turbidity is produced within 5 min.

SPECIFIC TESTS

• **ACIDITY OR ALKALINITY:** To a solution of 5.0 g in 50 mL of carbon dioxide-free water add 3 drops of phenolphthalein TS: no pink color is produced. Then add 0.30 mL of 0.020 N sodium hydroxide: a pink color is produced.
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Where Potassium Chloride is intended for use in hemodialysis, it is so labeled.

Potassium Chloride Extended-Release Capsules

DEFINITION

Potassium Chloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of potassium chloride (KCl).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Potassium*
Sample solution: A portion of the filtrate, obtained as directed for *Sample stock solution* in the *Assay*
Acceptance criteria: Meet the requirements
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
Sample solution: A portion of the filtrate, obtained as directed for *Sample stock solution* in the *Assay*
Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**
Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.
Standard solutions: To separate 100-mL volumetric flasks transfer 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. To each flask add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume. The *Standard solutions* contain, respectively, 1.0, 1.5, and 2.0 µg/mL of potassium.
Sample stock solution: Place NLT 20 Capsules in a suitable container with 400 mL of water, heat to boiling, and boil for 20 min. Allow to cool, transfer the solution to a 1000-mL volumetric flask, and dilute with water to volume. Filter, discarding the first 20 mL of the filtrate. Transfer a measured volume of the subsequent filtrate, equivalent to 60 mg of potassium chloride, to a 1000-mL volumetric flask, and dilute with water to volume. [NOTE—Retain a portion of the filtrate for use in the *Identification* tests.]
Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Potassium emission line at 766.5 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbance of the *Standard solutions* versus the concentration of potassium, in µg/mL, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration of potassium in the *Sample solution* (µg/mL).
Calculate the percentage of the labeled amount of potassium chloride (KCl) in each Capsule taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = concentration of potassium in the *Sample solution* as determined in this test (µg/mL)
C_U = concentration of potassium chloride in the *Sample solution* (µg/mL)
M_r = molecular weight of potassium chloride, 74.55
A_r = atomic weight of potassium, 39.10

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
▲Test 1▲ (RB 1-Sep-2018)
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 2 h
Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.
Standard solutions: To separate 100-mL volumetric flasks transfer 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. To each flask add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume. The *Standard solutions*

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contain, respectively, 1.0, 1.5, and 2.0 µg/mL of potassium.

Sample stock solution: Filter the solution under test, and dilute quantitatively with *Medium* to obtain a solution containing 60 µg/mL of potassium chloride.

Sample solution: Add 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Potassium emission line at 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air–acetylene

Blank: Water

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbance of the *Standard solutions* versus the concentration of potassium, in µg/mL, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration of potassium in the *Sample solution* (µg/mL).

Calculate the percentage of the labeled amount of potassium chloride (KCl) dissolved:

$$\text{Result} = [C \times D \times (V/L)] \times (M_r/A_r) \times 100$$

C = concentration of potassium in the *Sample solution* as determined in this test (µg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
L = labeled amount of potassium chloride (µg/Capsule)
M_r = molecular weight of potassium chloride, 74.55
A_r = atomic weight of potassium, 39.10

Tolerances: NMT 35% (Q) of the labeled amount of potassium chloride (KCl) is dissolved in 2 h. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 1* instead of to the table shown in *Dissolution* ⟨711⟩.

Table 1

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range $Q \pm 30\%$.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 30\%$ and $Q + 35\%$, and no unit is outside the range $Q \pm 40\%$.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 30\%$ and $Q + 35\%$, and NMT 2 units are outside the range $Q \pm 40\%$.

▲**Test 2:** If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 2*.

Standard stock solution and **Standard solutions:** Prepare as directed in *Test 1*.

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 6 h

Sample stock solution: Transfer 4.0 mL of the solution under test into a 50-mL volumetric flask, dilute with water to volume, and filter.

Sample solution: Transfer 4.0 mL of the *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Blank solution: To a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Instrumental conditions: Proceed as directed in *Test 1*, except do not use the *Blank*.

System sutability

Samples: *Standard solutions*

Suitability requirements

Linearity: Correlation coefficient NLT 0.99

Relative standard deviation: NMT 5.0% from 5 replicate analyses of the 1.5-µg/mL *Standard solution*

Analysis

Samples: 1.5-µg/mL *Standard solution*, *Sample solution*, and *Blank solution*

Calculate the percentage of the labeled amount of potassium chloride (KCl) dissolved:

$$\text{Result}_i = [(A_U/A_S) \times C_S \times D \times (V/L)] \times (M_r/A_r) \times 100$$

A_U = absorbance of potassium in the *Sample solution*
A_S = absorbance of potassium in the *Standard solution*
C_S = concentration of potassium in the *Standard solution* (µg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
L = labeled amount of potassium chloride (µg/Capsule)
M_r = molecular weight of potassium chloride, 74.55
A_r = atomic weight of potassium, 39.10

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%) 750 mg/Capsule
1	1	25–45
2	2	45–65
3	4	70–90
4	6	NLT 85

The percentages of the labeled amount of potassium chloride (KCl), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Sep-2018)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at a temperature not exceeding 30°.

Add the following:

▲• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (RB 1-Sep-2018)

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Potassium Chloride for Injection Concentrate

» Potassium Chloride for Injection Concentrate is a sterile solution of Potassium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of KCl.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.
Labeling—The label states the potassium chloride content in terms of weight and of milliequivalents in a given volume. Label the Concentrate to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration. Immediately following the name, the label bears the boxed warning:

Concentrate Must be Diluted Before Use

This warning is not required when the liquid preparation is in a *Pharmacy bulk package* and the label thereon states prominently "Pharmacy Bulk Package—Not for direct infusion."

The cap of the container and the overseal of the cap must be black, and both bear the words: "Must Be Diluted" in readily legible type, in a color that stands out from its background OR the overseal may be of a clear plastic material through which the black cap is visible and the printing is readily legible.

When the nature of the container-closure system prevents compliance, the design shall follow the intent of this requirement as closely as possible, the black color being used beneath the words "Must be Diluted," which are so placed that words are readily visible as the contents of the container are being removed. Ampuls shall be identified by a black band or a series of black bands above the constriction. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

Identification—It responds to the tests for *Potassium* ⟨191⟩ and for *Chloride* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩: It contains not more than 8.80 USP Endotoxin Units per mEq.
pH ⟨791⟩: between 4.0 and 8.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Potassium stock solution and *Standard preparations*—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution*.

Assay preparation—Transfer an accurately measured volume of Concentrate, equivalent to about 600 mg of potassium chloride, to a 500-mL volumetric flask, dilute with water to volume, and mix. Proceed as directed for *Assay preparation* in the *Assay* under *Potassium Chloride Oral Solution*, beginning with "Transfer 5.0 mL of the solution to a 100-mL volumetric flask."

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Potassium Chloride Oral Solution*. Calculate the quantity, in mg, of KCl in the portion of Concentrate taken by the formula:

$$200C(1.907)$$

in which the terms are as defined therein.

Potassium Chloride Oral Solution

DEFINITION
Potassium Chloride Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of potassium chloride (KCl). It may contain alcohol.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩
Sample solution: Carefully evaporate 5 mL to dryness, and ignite the residue at dull-red heat to remove all organic matter. Cool, dissolve the residue in 10 mL of water, and filter.
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: Carefully evaporate 5 mL to dryness, and ignite the residue at dull-red heat to remove all organic matter. Cool, dissolve the residue in 10 mL of water, and filter.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.
Standard solutions: To separate 100-mL volumetric flasks transfer 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. To each flask add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume. The *Standard solutions* contain, respectively, 1.0, 1.5, and 2.0 µg/mL of potassium.
Sample stock solution: Transfer a volume of Oral Solution, equivalent to 600 mg of potassium chloride, to a 500-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of the solution to a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Potassium emission line at 766.5 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbance of the *Standard solutions* versus the concentration of potassium, in µg/mL, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration of potassium in the *Sample solution* (µg/mL).
Calculate the percentage of the labeled amount of potassium chloride (KCl) in the portion of Oral Solution taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = concentration of potassium in the *Sample solution* as determined in this test (µg/mL)

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C_U = nominal concentration of potassium chloride in the *Sample solution* (µg/mL)
M_r = molecular weight of potassium chloride, 74.55
A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩ (if present)**:** NLT 90.0% and NMT 115.0% of the labeled amount, the labeled amount being NMT 7.5% of C_2H_5OH, acetone being used as the internal standard

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Chloride for Oral Solution

» Potassium Chloride for Oral Solution is a dry mixture of Potassium Chloride and one or more suitable colors, diluents, and flavors. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of KCl.

Packaging and storage—Preserve in tight containers. **Labeling**—The label states the Potassium Chloride (KCl) content in terms of weight and in terms of milliequivalents. **Identification**—Ignite about 200 mg at a temperature not above 600°, in order to remove all organic matter, cool, dissolve the residue in 10 mL of water, and filter: the filtrate responds to the tests for *Potassium* ⟨191⟩ and for *Chloride* ⟨191⟩.

Minimum fill ⟨755⟩—

FOR SOLID PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Uniformity of dosage units ⟨905⟩—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Assay—

Standard stock solution and *Standard solutions*—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution.*

Assay preparation 1 (where it is packaged in unit-dose containers)—Weigh and mix the contents of not less than 20 containers of Potassium Chloride for Oral Solution. Transfer an accurately weighed portion of the powder, equivalent to about 1.5 g of potassium chloride, to a 500-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Transfer 5.0 mL of the solution to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Assay preparation 2 (where it is packaged in multiple-unit containers)—Transfer an accurately weighed portion of Potassium Chloride for Oral Solution, equivalent to about 1.5 g of potassium chloride, to a 500-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Proceed as directed for *Assay preparation 1*, beginning with "Transfer 5.0 mL of the solution."

Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation 1* or *Assay preparation 2* instead of *Sample solution.* Calculate the quantity of KCl, in mg, in the portion of Potassium Chloride for Oral Solution taken by the formula:

$$500C(1.907)$$

in which C is as defined therein.

Potassium Chloride Extended-Release Tablets

DEFINITION

Potassium Chloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of potassium chloride (KCl).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Potassium*

Sample solution: A portion of the filtrate, obtained as directed for the designated *Sample stock solution* in the *Assay*

Acceptance criteria: Meet the requirements

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*

Sample solution: A portion of the filtrate, obtained as directed for the designated *Sample stock solution* in the *Assay*

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

[NOTE—If necessary, first score nonsugar-coated Tablets. Retain a portion of the filtrate of either *Sample stock solution 1* or *Sample stock solution 2* for use in *Identification A* and *B.*]

Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.

Standard solutions: To separate 100-mL volumetric flasks transfer 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution.* To each flask add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, and dilute with water to volume. The *Standard solutions* contain 1.0, 1.5, and 2.0 µg/mL of potassium, respectively.

Sample preparation 1

Sample stock solution 1: Nominally 0.06 mg/mL of potassium chloride prepared as follows. Place NLT 20 Tablets in a suitable container with 400 mL of water, heat to boiling, and boil for 20 min. Allow to cool, transfer the solution to a 1000-mL volumetric flask, and dilute with water to volume. Filter and discard the first 20 mL of the filtrate. Transfer a measured volume of the subsequent filtrate, equivalent to 60 mg of potassium chloride, to a 1000-mL volumetric flask, and dilute with water to volume.

Sample solution 1: Nominally 3 µg/mL of potassium chloride prepared as follows. Transfer 5.0 mL of *Sample stock solution 1* to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Sample preparation 2 (for formulations containing crystals coated with hydrophobic polymers)

Sample stock solution 2: Nominally 0.06 mg/mL of potassium chloride prepared as follows. Place NLT 20 Tablets in a 2000-mL volumetric flask. Add 1200 mL of a mixture of acetonitrile and water (1:1), and shake by mechanical means, or stir using a magnetic bar for 90 min.

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Dilute with the mixture of acetonitrile and water (1:1) to volume. Allow to stand for 90 min. Pass through a filter of 0.2-µm pore size. Transfer a measured volume of the filtrate, and quantitatively dilute with water to obtain a solution with a concentration of 0.06 mg/mL. [NOTE—Alternatively, *Sample stock solution 2* can be prepared by the following procedure. Nominally 0.15 mg/mL of potassium chloride from NLT 20 finely powdered Tablets, prepared as follows. Transfer an appropriate amount of the powder, equivalent to about 5–6 Tablets, to a suitable volumetric flask, add 10% of the final flask volume of acetone, and sonicate for 45 min with intermittent shaking. Add 80% of the final flask volume of water and sonicate for 45 min with intermittent shaking. Cool to room temperature and dilute with water to volume. Centrifuge a portion of the solution at 5000 rpm for 10 min. Transfer an appropriate amount of the supernatant to a 100-mL volumetric flask and dilute with water to volume to obtain a solution with a concentration of 0.15 mg/mL.]

Sample solution 2: Nominally 3 µg/mL of potassium chloride prepared as follows. Transfer an appropriate amount of *Sample stock solution 2* to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Potassium emission line at 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air–acetylene

Blank: Water

Analysis

Samples: *Standard solutions*, *Sample solution 1* or *Sample solution 2*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration of potassium, in µg/mL, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration of potassium in the *Sample solution* (µg/mL).

Calculate the percentage of the labeled amount of potassium chloride (KCl) in each Tablet taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = concentration of potassium in the *Sample solution* as determined in this test (µg/mL)

C_U = nominal concentration of potassium chloride in the *Sample solution* (µg/mL)

M_r = molecular weight of potassium chloride, 74.55

A_r = atomic weight of potassium, 39.10

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

▲Test 1▲ (RB 1-Sep-2018)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 2 h

Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.

Standard solutions: To separate 100-mL volumetric flasks transfer 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. To each flask add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, and dilute with water to volume. The *Standard solutions* contain, respectively, 1.0, 1.5, and 2.0 µg/mL of potassium.

Sample stock solution: Filter the solution under test, and dilute with *Medium* to obtain a solution containing nominally 60 µg/mL of potassium chloride.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Potassium emission line at 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air–acetylene

Blank: Water

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration of potassium, in µg/mL, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration of potassium in the *Sample solution* (µg/mL).

Calculate the percentage of the labeled amount of potassium chloride (KCl) dissolved:

$$\text{Result} = [C \times D \times (V/L)] \times (M_r/A_r) \times 100$$

C = concentration of potassium in the *Sample solution* as determined in this test (µg/mL)

D = dilution factor of the *Sample solution*

V = volume of *Medium*, 900 mL

L = labeled amount of potassium chloride (µg/Tablet)

M_r = molecular weight of potassium chloride, 74.55

A_r = atomic weight of potassium, 39.10

Tolerances: NMT 35% (Q) of the labeled amount of potassium chloride (KCl) is dissolved in 2 h. The requirements are met if the quantities dissolved from the Tablets tested conform to *Table 1* instead of the table shown in *Dissolution* ⟨711⟩.

Table 1

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range $Q \pm 30\%$.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 30\%$ and $Q + 35\%$, and no unit is outside the range $Q \pm 40\%$.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 30\%$ and $Q + 35\%$, and NMT 2 units are outside the range $Q \pm 40\%$.

▲Test 2: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 2*.

Standard stock solution and **Standard solutions:** Prepare as directed in *Test 1*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 1, 2, 4, and 8 h

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Sample stock solution: Transfer 4.0 mL of the solution under test into either a 50-mL volumetric flask (for 750-mg Tablet) or a 100-mL volumetric flask (for 1500-mg Tablet), dilute with water to volume, and filter.
Sample solution: Transfer 4.0 mL of the *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, and dilute with water to volume.
Blank solution: To a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, and dilute with water to volume.
Instrumental conditions: Proceed as directed in *Test 1*, except do not use the *Blank*.
System sutability
Samples: *Standard solutions*
Suitability requirements
Linearity: Correlation coefficient NLT 0.99
Relative standard deviation: NMT 5.0% from 5 replicate analyses of the 1.5-µg/mL *Standard solution*
Analysis
Samples: 1.5-µg/mL *Standard solution*, *Sample solution*, and *Blank solution*
Calculate the percentage of the labeled amount of potassium chloride (KCl) dissolved:

$$\text{Result}_i = [(A_U/A_S) \times C_S \times D \times (V/L)] \times (M_r/A_r) \times 100$$

A_U = absorbance of potassium in the *Sample solution*
A_S = absorbance of potassium in the *Standard solution*
C_S = concentration of potassium in the *Standard solution* (µg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
L = labeled amount of potassium chloride (µg/Tablet)
M_r = molecular weight of potassium chloride, 74.55
A_r = atomic weight of potassium, 39.10

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)	
		750 mg/Tablet	1500 mg/Tablet
1	1	10–30	5–25
2	2	30–50	25–45
3	4	60–80	55–75
4	8	NLT 80	NLT 85

The percentages of the labeled amount of potassium chloride (KCl), dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Sep-2018)

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature not exceeding 30°.

Change to read:

- **LABELING:** The label states with which *Sample preparation* in the *Assay* the product complies only if *Sample preparation 1* is not used. ▲When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (RB 1-Sep-2018)

Potassium Chloride in Dextrose Injection

DEFINITION

Potassium Chloride in Dextrose Injection is a sterile solution of Potassium Chloride and Dextrose in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amount of potassium chloride (KCl) and NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.

IDENTIFICATION

- **A.**
Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water
Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A copious red precipitate of cuprous oxide is formed.
- **B.** The sample imparts a violet color to a nonluminous flame. Since the presence of small quantities of sodium masks the color, screen out the yellow color produced by the sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements

ASSAY

- **DEXTROSE**
Sample solution: Nominally 20–50 mg/mL of dextrose from Injection prepared as follows. Transfer a volume of Injection, containing 2–5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide and dilute with water to volume.
Analysis
Sample: *Sample solution*
Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).
Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%
- **POTASSIUM CHLORIDE**
Sample solution: Transfer a volume of Injection, equivalent to 75–150 mg of potassium chloride, to a conical flask. Add water, if necessary, to bring the volume to 10 mL, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

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Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Indicator: Eosin Y TS
Analysis
Sample: *Sample solution*
Titrate, with shaking, with *Titrant* to a pink endpoint.
Calculate the percentage of the labeled amount of potassium chloride (KCl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 74.55 mg/mEq
W = nominal amount of potassium chloride in the *Sample solution* (mg)

Acceptance criteria: 95.0%–110.0%

IMPURITIES

• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**
Sample solution: Nominally 2.0 mg/mL of dextrose from Injection in water
Instrumental conditions
Mode: UV
Analytical wavelength: 284 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Sample solution* and *Blank*
Determine the absorbance of the *Sample solution*.
Acceptance criteria: NMT 0.25

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
• **pH** ⟨791⟩
Sample solution: Nominally 5% of dextrose from a portion of Injection in water
Acceptance criteria: 3.5–6.5
• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably Type I or Type II.
• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL. The content of potassium, in mEq, is prominently displayed on the label.

Potassium Chloride in Dextrose and Sodium Chloride Injection

DEFINITION

Potassium Chloride in Dextrose and Sodium Chloride Injection is a sterile solution of Potassium Chloride, Dextrose, and Sodium Chloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amounts of potassium (K) and chloride (Cl) and NLT 95.0% and NMT 105.0% of the labeled amounts of dextrose ($C_6H_{12}O_6 \cdot H_2O$) and sodium (Na). It contains no antimicrobial agents.

IDENTIFICATION

• **A.** The sample imparts an intense yellow color to a nonluminous flame.
• **B.**
Analysis: To 2 mL of Injection, add 5 mL of sodium cobaltinitrite TS.
Acceptance criteria: A yellow precipitate is formed immediately. If necessary, centrifuge the solution and examine the precipitate (presence of potassium).
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements
• **D.**
Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water
Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

ASSAY

• **CHLORIDE**
Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride, to a suitable conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Titrate, with shaking, with *Titrant* to a pink endpoint. Each mL of *Titrant* is equivalent to 3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of chloride (Cl).
Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 95.0%–110.0%
• **DEXTROSE**
Sample solution: Nominally 20–50 mg/mL of dextrose from Injection prepared as follows. Transfer a volume of Injection, containing 2–5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide and dilute with water to volume.
Analysis
Sample: *Sample solution*
Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).
Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)

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α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

• **POTASSIUM AND SODIUM**

Internal standard solution: 1.04 mg/mL of lithium nitrate in water prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (equivalent to 1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (equivalent to 1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Standard stock solution: 0.0391*J* mg/mL of potassium (K) from the *Potassium stock solution* and 0.02299*J′* mg/mL of sodium (Na) from the *Sodium stock solution* prepared as follows. Transfer 0.1*J* mL of the *Potassium stock solution* and 0.1*J′* mL of the *Sodium stock solution* to a 100-mL volumetric flask, where *J* and *J′* are the labeled amounts in (mEq/L) of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Sample solution: Transfer 5.0 mL of Injection to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Instrumental conditions
Mode: Flame photometer
Analytical wavelengths
Potassium: 766 nm
Sodium: 589 nm
Lithium: 671 nm
Blank: *Internal standard solution*

Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and the *Sample solution*.
Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*
R_S = emission reading ratio of potassium to lithium from the *Standard solution*
C_S = concentration of potassium (K) in the *Standard stock solution* (mg/mL)
C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[NOTE—Each mg of potassium is equivalent to 0.02558 mEq of potassium.]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*
R_S = emission reading ratio of sodium to lithium from the *Standard solution*
C_S = concentration of sodium (Na) in the *Standard stock solution* (mg/mL)
C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium is equivalent to 0.04350 mEq of sodium.]

Acceptance criteria
Potassium: 95.0%–110.0%
Sodium: 95.0%–105.0%

IMPURITIES

• **5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2.0 mg/mL of dextrose from Injection in water
Instrumental conditions
Analytical wavelength: 284 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Sample solution* and *Blank*
Acceptance criteria: NMT 0.25

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
• **PH** ⟨791⟩: 3.5–6.5
• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
• **LABELING:** The label states the potassium, sodium, and chloride contents in terms of mEq in a given volume. The label also states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution

» Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of K and Cl.

Packaging and storage—Preserve in tight containers, protected from excessive heat.
Labeling—The label states the potassium and chloride contents in terms of weight and in terms of milliequivalents. Where Tablets are packaged in individual pouches, the label instructs the user not to open until the time of use.
Identification—One Tablet dissolves in 100 mL of water with effervescence. The collected gas responds to the test for

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Bicarbonate ⟨191⟩, and the resulting solution responds to the tests for *Potassium* ⟨191⟩, for *Chloride* ⟨191⟩, and for *Citrate* ⟨191⟩.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation*.

Assay for potassium—

Standard stock solution and *Standard solutions*—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution*.

Assay preparation—Transfer 10 Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution to a 2000-mL volumetric flask, dissolve in 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Filter, and quantitatively dilute an accurately measured volume of the filtrate with water to obtain a solution containing 30 µg of potassium per mL. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation* instead of *Sample solution*. Calculate the quantity, in mg, of potassium (K) in each Tablet taken by the formula:

$$L(C/D)$$

in which L is the labeled quantity, in mg, of potassium in each Tablet, C is the concentration, in µg per mL, of potassium in the *Assay preparation*, and D is the concentration, in µg per mL, of potassium in the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution.

Assay for chloride—Transfer a number of Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution, equivalent to about 900 mg of chloride, to a 2000-mL volumetric flask. Add about 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Transfer 25.0 mL of this solution to a 250-mL conical flask, add 50.0 mL of 0.1 N silver nitrate VS and 15 mL of nitric acid, and boil, with constant swirling, until the supernatant is colorless. Cool to room temperature, add sufficient water to make a volume of about 150 mL, add 5 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS to a permanent faint brown endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Calculate the quantity, in mg, of chloride (Cl) in each Tablet by dividing the total amount of chloride in the Tablets taken by the number of Tablets taken.

Potassium Chloride in Lactated Ringer's and Dextrose Injection

DEFINITION

Potassium Chloride in Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, and Sodium Lactate in Water for Injection. It contains, in each 100 mL, NLT 285.0 mg and NMT 315.0 mg of sodium [as sodium chloride (NaCl) and anhydrous sodium lactate ($C_3H_5NaO_3$)], NLT 4.90 mg and NMT 6.00 mg of calcium (Ca) [equivalent to NLT 18.0 mg and NMT 22.0 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)], and NLT 231.0 mg and NMT 261.0 mg of lactate ($C_3H_5O_3$) [equivalent to NLT 290.0 mg and NMT 330.0 mg of anhydrous sodium lactate ($C_3H_5NaO_3$)]. It contains NLT 95.0% and NMT 105.0% of the labeled amount of potassium chloride (KCl), NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$), and NLT 90.0% and NMT 110.0% of the labeled amount of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)]. It contains no antimicrobial agents.

IDENTIFICATION

- **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water

Analysis: Add a few drops of *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Calcium* and *Chloride*: Meets the requirements of test *A* under *Calcium* and test *A* under *Chloride*
- **C. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **D. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **E.** The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.

ASSAY

- **CALCIUM**

[NOTE—Concentrations of the *Standard solution* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Lanthanum chloride solution: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium (Ca) prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of diluted hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 1.5, and 2.0 mL of *Calcium stock solution*, respectively. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 30.0 mL of Injection to a 100-mL volumetric flask containing 5.0 mL of *Lanthanum chloride solution*. Dilute with water to volume.

Blank: Transfer 5.0 mL of *Lanthanum chloride solution* to a 100-mL volumetric flask and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene

USP Monographs

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Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (*C*), in mg/mL, of calcium in the *Sample solution*.
Calculate the quantity (mg) of calcium (Ca) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution*, as determined from the graph (mg/mL)
D = dilution factor of the *Sample solution*, 3.3
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 4.90–6.00 mg of calcium (Ca) in each 100 mL

• **CHLORIDE**
Sample solution: Transfer 10 mL of Injection into a conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Titrate, with shaking, with *Titrant* to a pink endpoint.
Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = volume of *Titrant* consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

• **DEXTROSE**
Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.
Analysis
Sample: *Sample solution*
Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).
Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

• **LACTATE**
Mobile phase: Add 1 mL of formic acid and 1 mL of dicyclohexylamine per L of water.
System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water
Standard solution: 3 mg/mL of USP Sodium Lactate RS in water
Sample solution: Use the undiluted Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between acetate and lactate, *System suitability solution*
Tailing factor: NMT 2.0 for lactate, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity (mg) of lactate ($C_3H_5O_3$) in each 100 mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V$$

r_U = peak response of lactate from the *Sample solution*
r_S = peak response of lactate from the *Standard solution*
C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of lactate, 89.07
M_{r2} = molecular weight of anhydrous sodium lactate, 112.06
V = volume of the Injection, 100 mL

Acceptance criteria: 231.0–261.0 mg of lactate ($C_3H_5O_3$) in each 100 mL

• **POTASSIUM**
Solution A: Suitable nonionic wetting agent (1 in 500)
Standard stock solution A: 100 µg/mL of potassium in water prepared as follows. Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.
Standard stock solution B: 10.93 mg/mL of sodium chloride in water
Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium prepared as follows. Transfer 10 mL of *Standard stock solution B* to each of four 100-mL volumetric flasks containing 10.0 mL of *Solution A*. To each flask add, respectively, 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution A*, and dilute with water to volume.
Sample solution: Nominally 0.015 mg/mL of potassium prepared as follows. Transfer a suitable portion of Injection into an appropriate volumetric flask. Add 10% of the final flask volume of *Solution A*. Dilute with water to volume.
Blank: Transfer 10 mL of *Standard stock solution B* to a 100-mL volumetric flask containing 10.0 mL of *Solution A*. Dilute with water to volume.
Instrumental conditions
Mode: Atomic emission spectrophotometry
Emission wavelength: 766 nm
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*

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Set a suitable flame photometer for maximum transmittance at a wavelength of 766 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium. From the graph so obtained, read the percentage transmittance of the *Sample solution*.

Calculate the percentage of the labeled amount of potassium chloride (KCl) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = concentration of potassium in the *Sample solution*, as determined from the graph (mg/mL)
C_U = nominal concentration of potassium in the *Sample solution* (mg/mL)
M_r = molecular weight of potassium chloride, 74.55
A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

- **SODIUM**

Solution A: Suitable nonionic wetting agent (1 in 500)

Standard stock solution: 100 µg/mL of sodium in water prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium prepared as follows. Transfer 10 mL of *Solution A* to each of four 100-mL volumetric flasks. To each flask add, respectively, 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, and dilute with water to volume.

Sample solution: Transfer 5 mL of Injection to a 1-L volumetric flask containing 100 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 589 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set a suitable flame photometer for maximum transmittance at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium. From the graph so obtained, read the percentage transmittance of the *Sample solution*.

Calculate the quantity (mg) of sodium (Na) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of sodium in the *Sample solution*, as determined from the graph (mg/mL)
D = dilution factor of the *Sample solution*, 200
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 285.0–315.0 mg of sodium (Na) in each 100 mL

IMPURITIES

- **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from Injection in water

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Determine the absorbance of the *Sample solution* with a suitable spectrophotometer.

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.5 USP Endotoxin Units/mL.
- **pH** ⟨791⟩: 3.5–6.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label includes also the warning: "Not for use in the treatment of lactic acidosis."
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Lactate RS

Potassium Chloride in Sodium Chloride Injection

DEFINITION

Potassium Chloride in Sodium Chloride Injection is a sterile solution of Potassium Chloride and Sodium Chloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amounts of potassium (K) and chloride (Cl) and NLT 95.0% and NMT 105.0% of the labeled amount of sodium (Na). It contains no antimicrobial agents.

IDENTIFICATION

- **A. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **B.**

Analysis: To 2 mL of Injection add 5 mL of sodium cobaltinitrite TS.

Acceptance criteria: A yellow precipitate is formed immediately. If necessary, centrifuge the solution and examine the precipitate (presence of potassium).

- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements

ASSAY

- **POTASSIUM AND SODIUM**

Internal standard solution: 1.04 mg/mL of lithium nitrate in water prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (equivalent to 1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride,

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previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (equivalent to 1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Standard stock solution: 0.0391*J* mg/mL of potassium (K) from the *Potassium stock solution* and 0.02299*J′* mg/mL of sodium (Na) from the *Sodium stock solution* prepared as follows. Transfer 0.1*J* mL of *Potassium stock solution* and 0.1*J′* mL of *Sodium stock solution* to a 100-mL volumetric flask, where *J* and *J′* are the labeled amounts (in mEq/L) of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Sample solution: Transfer 5.0 mL of Injection to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: 766 nm

Sodium: 589 nm

Lithium: 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and the *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*

R_S = emission reading ratio of potassium to lithium from the *Standard solution*

C_S = concentration of potassium (K) in the *Standard solution* (mg/mL)

C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[NOTE—Each mg of potassium is equivalent to 0.02558 mEq of potassium.]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*

R_S = emission reading ratio of sodium to lithium from the *Standard solution*

C_S = concentration of sodium (Na) in the *Standard solution* (mg/mL)

C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium is equivalent to 0.04350 mEq of sodium.]

Acceptance criteria

Potassium: 95.0%–110.0%

Sodium: 95.0%–105.0%

- **CHLORIDE**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride, to a suitable conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 35.45 mg/mEq

W = nominal amount of chloride in the *Sample solution* (mg)

[NOTE—Each mg of chloride is equivalent to 0.0282 mEq of chloride (Cl^-).]

Acceptance criteria: 95.0%–110.0%

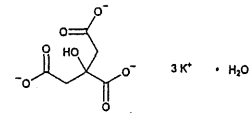
SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
- **pH** ⟨791⟩: 3.5–6.5
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass, or of a suitable plastic.
- **LABELING:** The label states the potassium, sodium, and chloride contents in terms of mEq in a given volume. The label also states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Potassium Citrate



$C_6H_5K_3O_7 \cdot H_2O$ 324.41

$C_6H_5K_3O_7$ 306.40

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, tripotassium salt, monohydrate;

Tripotassium citrate monohydrate [6100-05-6].

Anhydrous [866-84-2].

DEFINITION

Potassium Citrate contains NLT 99.0% and NMT 100.5% of potassium citrate ($C_6H_5K_3O_7$), calculated on the dried basis.

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IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩ and *Citrate* ⟨191⟩**:** A 100-mg/mL solution meets the requirements.

ASSAY

• **PROCEDURE**

Sample: 200 mg of Potassium Citrate
Blank: 25 mL of glacial acetic acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 25 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with the *Titrant* to a green endpoint. Perform a *Blank* determination.
Calculate the percentage of potassium citrate ($C_6H_5K_3O_7$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 102.1 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES

• **TARTRATE**

Sample: 1 g of Potassium Citrate
Analysis: Dissolve the *Sample* in 1.5 mL of water in a test tube, add 1 mL of 6 N acetic acid, and scratch the walls of the test tube with a glass rod.
Acceptance criteria: No crystalline precipitate is formed.

SPECIFIC TESTS

• **ALKALINITY**

Sample solution: Dissolve 1 g of Potassium Citrate in 20 mL of water.
Analysis: To the *Sample solution* add 0.20 mL of 0.10 N sulfuric acid, and add 1 drop of phenolphthalein TS.
Acceptance criteria: The *Sample solution* is alkaline to litmus. No pink color is produced after the addition of phenolphthalein TS.

• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 180° for 4 h: it loses 3.0%–6.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Citrate Extended-Release Tablets

DEFINITION

Potassium Citrate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of potassium citrate as monohydrate ($C_6H_5K_3O_7 \cdot H_2O$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Potassium*

Sample solution: Powder 5 Tablets, mix with 20 mL of water, and filter.
Acceptance criteria: The filtrate meets the requirements.

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Citrate*

Sample: A portion of powdered Tablets containing about 50 mg of potassium citrate
Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Buffer: 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.2.
Mobile phase: *Buffer*
Standard solution: 0.4 mg/mL of USP Citric Acid RS in *Mobile phase*
Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 2000 mg of potassium citrate monohydrate, to a 250-mL volumetric flask, and add 150 mL of hot water (60°–70°). Sonicate for 20 min with occasional shaking. Allow to cool to room temperature, dilute with water to volume, and mix.
Sample solution: Pass a portion of the *Sample stock solution* through a suitable filter of 0.45-µm pore size, discarding the first 5 mL of filtrate. Transfer 4 mL of the filtrate to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Reserve the remaining filtrate for use in the *Content of Potassium* test.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 55°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = citric acid peak area from the *Sample solution*
r_S = citric acid peak area from the *Standard solution*
C_S = concentration of USP Citric Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of potassium citrate monohydrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of potassium citrate monohydrate, 324.41
M_{r2} = molecular weight of citric acid ($C_6H_8O_7$), 192.13

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF POTASSIUM**

Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.
Standard solutions: Transfer 10.0, 15.0, and 20.0 mL, respectively, to separate 100-mL volumetric flasks of the *Standard stock solution*. To each flask, add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume. The *Standard solutions* contain 1.0, 1.5, and 2.0 µg/mL of potassium, respectively.

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Sample stock solution: Dilute the clear filtrate, reserved from the *Assay*, with water to obtain a solution containing about 160 µg/mL of potassium citrate monohydrate.
Sample solution: Transfer 3.0 mL of the *Sample stock solution* to a 100-mL volumetric flask.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Potassium emission line at 766.5 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbance of the *Standard solutions* versus the concentration of potassium, in µg/mL, and draw the straight line best fitting the three plotted points. From the graph obtained, determine the concentration of potassium in the *Sample solution* (µg/mL).
Calculate the percentage of potassium (K) in the portion of Tablets taken:

$$\text{Result} = C \times 100/C_U$$

C = concentration of potassium in the *Sample solution* as determined in this test (µg/mL)
C_U = concentration of potassium citrate anhydrous ($C_6H_5K_3O_7$) in the *Sample solution* calculated from the *Assay* value of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) (µg/mL)

Acceptance criteria: 36.4%–40.2%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 0.5, 1, and 3 h; without *Medium* replacement
[NOTE—Withdraw the same volume at each time point.]
Standard stock solution and **Standard solutions:** Prepare as directed in the *Content of Potassium* test.
Sample solution: Filter the solution under test and dilute quantitatively with *Medium* to obtain a solution containing about 60 µg of potassium citrate per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid. Dilute with water to volume, and mix.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Potassium emission line at 766.5 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Determine the concentration, in µg/mL, of potassium in the *Sample solution* at each time point.
Calculate the percentage of the labeled amount of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) dissolved at each time point: At 0.5 h:

$$\text{Result}_1 = C_1 \times V \times R \times F \times 100/L$$

At 1 h:

$$\text{Result}_2 = [C_2 \times (V - V_S) + C_1 \times V_S] \times R \times F \times 100/L$$

At 3 h:

$$\text{Result}_3 = \{C_3 \times [V - 2 \times V_S] + (C_1 + C_2) \times V_S\} \times R \times F \times 100/L$$

C = as C_1, C_2, C_3, concentration of potassium in the *Sample solution* at each time point (µg/mL)
V = volume of *Medium*, 900 mL
R = ratio of the molecular weight of potassium citrate monohydrate to 3 times the atomic weight of potassium, 2.765
F = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)
V_S = volume of sample withdrawn at each time point (mL)

Tolerances: The percentages of the labeled amount of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) dissolved from the Tablets are NMT 45% (Q) in 30 min, NMT 60% (Q') in 1 h, and NLT 80% (Q'') in 3 h. The requirements are met if the quantities dissolved from the Tablets tested conform to *Table 1* instead of the table shown under *Dissolution* ⟨711⟩.

Table 1

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between Q ± 10% and Q' ± 10%, and is NLT Q'' + 5% at the stated times.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between Q ± 10% and Q' ± 10% and is NLT Q''; no unit is outside the range between Q ± 15% and Q' ± 15%, and no unit is less than Q'' – 5% at the stated times.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between Q ± 10% and Q' ± 10% and is NLT Q''; NMT 1 unit is outside the range between Q ± 15%, NMT 1 unit is outside the range between Q' ± 15%, and NMT 1 unit is less than Q'' – 5% at the stated times.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 0.5, 1, 4, and 6 h. Replace the volume withdrawn with the equal volume of *Medium* preheated to 37 ± 0.5°.
Buffer: 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.2.
Mobile phase: *Buffer*
Standard solution: Prepare a solution of USP Citric Acid RS in *Medium* as directed in *Table 2*.

Table 2

Tablet Strength (mg, as potassium citrate monohydrate)	Concentration of Citric Acid (mg/mL)
540	0.35
1080	0.70
1620	1.05

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 5 mL of filtrate.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 55°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the concentration, in mg/mL, of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) in the sample withdrawn from the vessel at each time point:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = citric acid peak area from the *Sample solution*
r_S = citric acid peak area from the *Standard solution*
C_S = concentration of USP Citric Acid RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$), 324.41
M_{r2} = molecular weight of citric acid ($C_6H_8O_7$), 192.13

Calculate the percentage of the labeled amount of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) dissolved at each time point: At 0.5 h:

$$\text{Result}_1 = C_1 \times V \times 100/L$$

At 1 h:

$$\text{Result}_2 = (C_2 \times V + C_1 \times V_S) \times 100/L$$

At 4 h:

$$\text{Result}_3 = [C_3 \times V + (C_1 + C_2) \times V_S] \times 100/L$$

At 6 h:

$$\text{Result}_4 = [C_4 \times V + (C_1 + C_2 + C_3) \times V_S] \times 100/L$$

C = as C_1, C_2, C_3, C_4, concentration of potassium citrate monohydrate in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of sample withdrawn at each time point (mL)

Tolerances: The percentages of the labeled amount of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) dissolved at the times specified in *Table 3* conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 3

Time (h)	Amount Dissolved (%)
0.5	25–50
1	40–65
4	NLT 70
6	NLT 80

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Deaerated water; 900 mL
Apparatus 2: 50 rpm
[NOTE—Employ sinker if necessary to ensure that the Tablets do not float.]
Times: 0.5, 1, and 5 h; without *Medium* replacement
[NOTE—Withdraw the same volume at each time point. Pass a portion of the solution through a 0.45-µm membrane filter, discarding the first 2 mL of the filtrate.]
Diluent: Transfer 5 g of sodium chloride to a 100-mL volumetric flask, add 25 mL of water and 25 mL of concentrated hydrochloric acid, shake until dissolved, cool to room temperature, and dilute with water to volume.
Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.
Standard solutions: Transfer 5.0, 7.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. Add 4.0 mL of *Diluent* to each flask, dilute with water to volume, and mix well. The *Standard solutions* contain 0.5, 0.7, 1.0, 1.5, and 2.0 µg/mL of potassium, respectively.
Sample stock solution: Filter the solution under test, and dilute quantitatively with water as stated in *Table 4*.

Table 4

Time (h)	5 mEq Tablet Dilution	10 mEq Tablet Dilution	15 mEq Tablet Dilution
0.5	6.0 mL into 25 mL	6.0 mL into 50 mL	4.0 mL into 50 mL
1	5.0 mL into 25 mL	5.0 mL into 50 mL	3.0 mL into 50 mL
5	7.0 mL into 50 mL	7.0 mL into 100 mL	2.0 mL into 50 mL

Sample solution: Transfer 5.0 mL of respective *Sample stock solution* into 100-mL volumetric flasks. Add 4.0 mL of *Diluent*, dilute with water to volume, and mix well.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Potassium emission line at 766.5 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Blank: Dilute *Diluent* with water (4:96)
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Determine the concentration, in µg/mL, of potassium in the *Sample solution* at each time point.
Calculate the percentage of the labeled amount of potassium dissolved at each time point: At 0.5 h:

$$\text{Result}_1 = C_1 \times D_1 \times V \times 100/(L \times A)$$

At 1 h:

$$\text{Result}_2 = [C_2 \times D_2 \times (V - V_S) + C_1 \times D_1 \times V_S] \times 100/(L \times A)$$

At 5 h:

$$\text{Result}_3 = \{C_3 \times D_3 \times [V - 2 \times V_S] + (C_1 \times D_1 + C_2 \times D_2) \times V_S\} \times D \times 100/(L \times A)$$

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C = as C_1, C_2, C_3, concentration of potassium in the *Sample solution* at each time point (mg/mL)
D = as D_1, D_2, D_3, dilution factor of the *Sample solution* at each point
V = volume of *Medium*, 900 mL
L = label claim (mEq/Tablet)
A = atomic weight of potassium, 39.1
V_S = volume of sample withdrawn at each time point (mL)

Tolerances: The percentages of the labeled amount of potassium dissolved at the times specified in *Table 5* conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 5

Time (h)	Amount Dissolved (%)
0.5	30–50
1	45–65
5	NLT 85

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **Labeling:** The label states the amount of potassium citrate as monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) in mEq and in g/Tablet. The label indicates the *Dissolution* test with which the product complies.
• **USP Reference Standards** ⟨11⟩
USP Citric Acid RS

Potassium Citrate and Citric Acid Oral Solution

DEFINITION
Potassium Citrate and Citric Acid Oral Solution is a solution of Potassium Citrate and Citric Acid in a suitable aqueous medium. In each 100 mL, it contains NLT 7.55 g and NMT 8.35 g of potassium (K), and NLT 12.18 g and NMT 13.46 g of citrate ($C_6H_5O_7$), equivalent to NLT 20.9 g and NMT 23.1 g of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$). It also contains NLT 6.34 g and NMT 7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$).
[Note—The potassium ion content of Oral Solution is approximately 2 mEq/mL.]

IDENTIFICATION
• **A.**
Sample solution: A dilution of Oral Solution (1 in 40)
Analysis: To 2 mL of the *Sample solution* add 5 mL of sodium cobaltinitrite TS.
Acceptance criteria: A yellow precipitate is formed immediately (presence of potassium).
• **B.**
Sample solution: Oral Solution and hydrochloric acid (1:1)
Analysis: To 2 mL of the *Sample solution* add 10 mL of cobalt–uranyl acetate TS, and stir with a glass rod.
Acceptance criteria: No precipitate or turbidity forms after 15 min, and the *Sample solution* remains clear (absence of sodium).
• **C. Identification Tests—General,** *Citrate* ⟨191⟩
Sample solution: 3–5 drops of Oral Solution and 20 mL of the mixture of pyridine and acetic anhydride
Acceptance criteria: Meets the requirements

ASSAY
• **Potassium**
Sodium stock solution: 58.44 mg/mL of sodium chloride prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.
Potassium stock solution: 74.56 mg/mL of potassium chloride prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.
Diluent: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, then add water to volume. *Diluent* contains 15 mEq of lithium per 1000 mL.
Standard stock solution: 5.844 mg/mL of sodium chloride and 7.456 mg/mL of potassium chloride prepared as follows. Transfer 50 mL each of *Potassium stock solution* and *Sodium stock solution* to a 500-mL volumetric flask, and dilute with water to volume. Each mL of the *Standard stock solution* contains 0.1 mEq of sodium and 0.1 mEq of potassium.
Standard solution: 29.22 µg/mL of sodium chloride and 37.28 µg/mL of potassium chloride prepared as follows. Transfer 50 µL of the *Standard stock solution* to a 10-mL volumetric flask, and dilute with *Diluent* to volume.
Sample stock solution: Transfer a volume of Oral Solution, equivalent to about 2 g of potassium citrate monohydrate, to a 200-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 50 µL of the *Sample stock solution* to a 10-mL volumetric flask, and dilute with *Diluent* to volume.
Instrumental conditions
Mode: Flame photometer
Analytical wavelength: Wavelength of maximum emission at about 766 nm
Blank: *Diluent*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and the *Sample solution*.
Calculate the quantity, in g, of potassium (K) in each 100 mL of Oral Solution taken:

$$\text{Result} = [(r_U/r_S) \times (C_S \times F) \times (A_r/M_r)] \times D_1 \times D_2 \times 100$$

r_U = photometer reading of the *Sample solution*
r_S = photometer reading of the *Standard solution*
C_S = concentration of potassium chloride in the *Standard solution* (µg/mL)
F = conversion factor, 1 g per 10^6 µg
A_r = atomic weight of potassium, 39.10
M_r = molecular weight of potassium chloride, 74.55
D_1 = dilution factor of the *Sample stock solution*, 200 mL/V
V = volume of Oral Solution taken to prepare the *Sample stock solution* (mL)
D_2 = dilution factor used to prepare the *Sample solution*, 10 mL per 0.05 mL

Acceptance criteria: In each 100 mL, 7.55–8.35 g of potassium (K)

USP Monographs

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• **CITRATE**

Mobile phase, Standard preparation 1, and **Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution: Transfer 15 mL of Oral Solution to a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate).*

Analysis

Samples: *Standard preparation 1* and *Sample solution*

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure.*

Calculate the quantity, in g, of citrate ($C_6H_5O_7$) in each 100 mL of the Oral Solution taken:

$$\text{Result} = \{[(r_U/r_S) \times (C_S/F) \times D] - A[M_{r1}/M_{r2}]\} \times 100$$

r_U = peak response of citrate from the *Sample solution*

r_S = peak response of citrate from *Standard preparation 1*

C_S = concentration of citrate in *Standard preparation 1* (µg/mL)

F = conversion factor, 10^6 µg/g

D = dilution factor of the *Sample solution*

A = quantity of citric acid monohydrate in the Oral Solution determined in the *Assay* for *Citric Acid* (g/mL)

M_{r1} = molecular weight of citrate, 189.10

M_{r2} = molecular weight of citric acid monohydrate, 210.14

Acceptance criteria: In each 100 mL, 12.18–13.46 g of citrate ($C_6H_5O_7$), equivalent to NLT 20.9 g and NMT 23.1 g of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) and NLT 6.34 g and NMT 7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)

• **CITRIC ACID**

Sample solution: 15 mL of Oral Solution, diluted with water to 250 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.02 N sodium hydroxide VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Transfer 5 mL of the *Sample solution* to a suitable flask. Add 25 mL of water and 5 drops of phenophthalein TS. Titrate with *Titrant* to a pink endpoint. Record the buret reading, and calculate the volume of *Titrant* consumed. Each mL of *Titrant* is equivalent to 1.401 mg of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$).

Acceptance criteria: 6.34–7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in each 100 mL of Oral Solution

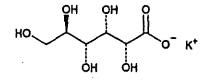
SPECIFIC TESTS

• **pH** ⟨791⟩**:** 4.9–5.4

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Gluconate



$C_6H_{11}KO_7$ 234.25

$C_6H_{11}KO_7 \cdot H_2O$ 252.26

D-Gluconic acid, monopotassium salt;
Monopotassium D-gluconate.
Anhydrous [299-27-4].
Monohydrate [35398-15-3].

DEFINITION

Potassium Gluconate is anhydrous or contains one molecule of water of hydration. It contains NLT 97.0% and NMT 103.0% of anhydrous potassium gluconate ($C_6H_{11}KO_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197M**▲ (CN 1-May-2020)

• **B.** Potassium Gluconate imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

• **C. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Potassium Gluconate

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent.* Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F to that of the *Standard solution.*

ASSAY

• **PROCEDURE**

Standard stock solution: Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1000-mL volumetric flask, add sufficient water to dissolve, and dilute with water to volume. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume. This solution contains 10 µg/mL of potassium (equivalent to 19.07 µg/mL of potassium chloride).

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Standard solutions: Transfer 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. Add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid to each flask. Dilute with water to volume and mix. The *Standard solutions* contain 1.0, 1.5, and 2.0 µg/mL of potassium, respectively.
Sample stock solution: 0.18 mg/mL of Potassium Gluconate in water. Filter the solution.
Sample solution: Transfer 5.0 mL of the filtrate from the *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid, and dilute with water to volume.
Blank: Water
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 766.5 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Analysis
Samples: *Standard solutions* and *Sample solution*
Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus their concentrations, in µg/mL, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C_K, in µg/mL, of potassium in the *Sample solution*.
Calculate the percentage of potassium gluconate ($C_6H_{11}KO_7$) in the portion of Potassium Gluconate taken:

$$\text{Result} = (C_K/C_U) \times (M_r/A_r) \times 100$$

C_K = determined concentration of potassium in the *Sample solution* (µg/mL)
C_U = concentration of Potassium Gluconate in the *Sample solution* (µg/mL)
M_r = molecular weight of potassium gluconate, 234.25
A_r = atomic weight of potassium, 39.10

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
• **REDUCING SUBSTANCES**
Sample: 1.0 g of Potassium Gluconate
Blank: 10 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: Iodine
Back-titrant: Sodium thiosulfate
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the blank determination.
Calculate the percentage of reducing substances (as dextrose) in the portion of the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N_A \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)
V_S = *Back-titrant* volume consumed by the *Sample* (mL)
N_A = actual *Back-titrant* normality (mEq/mL)
F = equivalency factor, 27 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 105° for 4 h.
Acceptance criteria
Anhydrous: NMT 3.0%
Monohydrate: 6.0%–7.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Label it to indicate whether it is the anhydrous or the monohydrate form.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Potassium Gluconate RS

Potassium Gluconate Oral Solution

DEFINITION
Potassium Gluconate Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$).

IDENTIFICATION
• **A.** Oral Solution imparts a violet color to a nonluminous flame. The presence of small quantities of sodium can mask the color unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
• **B.**
Analysis: Evaporate 5 mL on a steam bath to dryness.
Acceptance criteria: A mineral oil dispersion of the residue exhibits an IR absorption maximum in the spectral region of 6.2–6.25 µm (carboxylic acid salt).

ASSAY
• **PROCEDURE**
Standard stock solution: Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1000-mL volumetric flask, add sufficient water to dissolve, and dilute with water to volume. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume. This solution contains 10 µg/mL of potassium (equivalent to 19.07 µg/mL of potassium chloride).
Standard solutions: Transfer 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. To each flask add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid. Dilute with water to volume, and mix. The *Standard solutions* contain 1.0, 1.5, and 2.0 µg/mL of potassium, respectively.
Sample solution: Transfer an accurately measured volume of Oral Solution, equivalent to 1.8 g of potassium gluconate, to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of the solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

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Blank: Water
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 766.5 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Analysis
Samples: *Standard solutions* and *Sample solution*
Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus their concentrations, in µg/mL, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C_K, in µg/mL, of potassium in the *Sample solution*.
Calculate the percentage of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$) in the portion of Oral Solution taken:

$$\text{Result} = (C_K/C_U) \times (M_r/A_r) \times 100$$

C_K = determined concentration of potassium in the *Sample solution*, (µg/mL)
C_U = nominal concentration of potassium gluconate in the *Sample solution* (µg/mL)
M_r = molecular weight of potassium gluconate, 234.25
A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
• **ALCOHOL DETERMINATION** ⟨611⟩, *Method II—Distillation Method*: 4.5%–5.5% of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Potassium Gluconate Tablets

DEFINITION
Potassium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020): The IR absorption spectrum of potassium gluconate extracted from finely powdered Tablets exhibits maxima only at the same wavelengths as those of a similar preparation of USP Potassium Gluconate RS.

• **B.**
Sample solution: Triturate a portion of powdered Tablets with a few milliliters of water, and filter.
Acceptance criteria: The *Sample solution* imparts a violet color to a nonluminous flame; the presence of small quantities of sodium masks the color, unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks the emission at 589 nm (sodium); it is transparent to the emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

ASSAY
• **PROCEDURE**
Standard stock solution: 19.07 µg/mL of potassium chloride in water (equivalent to 10 µg/mL of potassium), prepared from potassium chloride previously dried at 105° for 2 h
Standard solutions: 1.0, 1.5, and 2.0 µg/mL of potassium from suitably diluted *Standard stock solution*, in a solution containing 4 mg/mL of sodium chloride and 1 mL of hydrochloric acid per 100 mL
Sample stock solution: Filtered water solution containing 0.18 mg/mL of potassium gluconate from NLT 20 finely powdered Tablets
Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid, and dilute with water to volume.
Blank: Water
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 766.5 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Analysis
Samples: *Standard solutions* and *Sample solution*
Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus their concentrations, in µg/mL, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of potassium in the *Sample solution*.
Calculate the percentage of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$) in the portion of Tablets taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = determined concentration of potassium in the *Sample solution* (µg/mL)
C_U = nominal concentration of potassium gluconate in the *Sample solution* (µg/mL)
M_r = molecular weight of potassium gluconate, 234.25
A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 100 rpm
Time: 45 min
Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary
Analysis: Proceed as directed in the *Assay*.
Calculate the percentage of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$) dissolved:

$$\text{Result} = (C \times D \times V/L) \times (M_r/A_r) \times 100$$

C = determined concentration of potassium in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M_r = molecular weight of potassium gluconate, 234.25
A_r = atomic weight of potassium, 39.10

USP Monographs

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Tolerances: NLT 75% (*Q*) of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Potassium Gluconate RS

Potassium Gluconate and Potassium Chloride Oral Solution

» Potassium Gluconate and Potassium Chloride Oral Solution is a solution of Potassium Gluconate and Potassium Chloride in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of potassium (K) and chloride (Cl).

Packaging and storage—Preserve in tight containers.
Labeling—Label it to state the potassium and chloride contents in terms of milliequivalents of each in a given volume of Oral Solution.

Identification—

A: To 2 mL of a dilution of Oral Solution (1 in 40) add 5 mL of sodium cobaltinitrite TS: a yellow precipitate is formed immediately (*presence of potassium*).

B: Evaporate 5 mL on a steam bath to dryness: a mineral oil dispersion of the residue so obtained exhibits an IR absorption maximum in the spectral region between 6.2 and 6.25 µm (*carboxylic acid salt*).

C: It responds to the tests for *Chloride* ⟨191⟩.

Assay for potassium—

Potassium stock solution—Dissolve in water 0.9535 g of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 1000 µg of potassium per mL.

Standard preparations—To separate 200-mL volumetric flasks transfer 19.0 mL and 25.0 mL, respectively, of *Potassium stock solution*, dilute with water to volume, and mix. The *Standard preparations* contain 95.0 µg and 125.0 µg of potassium per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 782 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of the resulting solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a potassium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of potassium. From the graph so obtained, determine the concentration, *C*, in µg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of potassium in each mL of the Oral Solution taken by the formula:

$$(50/7)(C/V)$$

in which *V* is the volume, in mL, of Oral Solution taken. Each mg of potassium is equivalent to 0.02558 mEq.

Assay for chloride—

Ionic strength adjusting solution—Use 5 M sodium nitrate.

Procedure—Transfer an accurately measured volume of Oral Solution, equivalent to about 100 mg (2.8 mEq) of chloride, to a suitable beaker. Add 2.0 mL of *Ionic strength adjusting solution* and water to make about 100 mL, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-sulfide specific ion-selective electrode and a double-junction reference electrode containing potassium nitrate solution (1 in 10). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of Cl.

Potassium Gluconate and Potassium Chloride for Oral Solution

» Potassium Gluconate and Potassium Chloride for Oral Solution is a dry mixture of Potassium Gluconate and Potassium Chloride and one or more suitable colors, diluents, and flavors. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of potassium (K) and chloride (Cl).

Packaging and storage—Preserve in tight containers.
Labeling—Label it to state the potassium and chloride contents in terms of milliequivalents. Where packaged in unit-dose pouches, the label instructs the user not to open until the time of use.

Identification—

A: Ignite about 200 mg at a temperature not above 600°, in order to remove all organic matter, cool, dissolve the residue in 10 mL of water, and filter: the filtrate responds to the tests for *Potassium* ⟨191⟩ and for *Chloride* ⟨191⟩.

B: A mineral oil dispersion of it exhibits an IR absorption maximum in the spectral region between 6.2 and 6.25 µm (*carboxylic acid salt*).

Minimum fill ⟨755⟩: meets the requirements.

Assay for potassium—

Potassium stock solution—Dissolve in water 0.9535 g of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 1000 µg of potassium per mL.

Standard preparations—To separate 200-mL volumetric flasks transfer 19.0 mL and 25.0 mL, respectively, of the *Potassium stock solution*, dilute with water to volume, and mix. The *Standard preparations* contain 95.0 µg and 125.0 µg of potassium per mL, respectively.

Assay preparation 1 (where it is packaged in unit-dose containers)—Weigh and mix the contents of not less than 20 containers of Potassium Gluconate and Potassium Chloride for Oral Solution. Transfer an accurately weighed portion of the powder, equivalent to about 782 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this stock solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Assay preparation 2 (where it is packaged in multiple-unit containers)—Transfer an accurately weighed portion of

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Potassium Gluconate and Potassium Chloride for Oral Solution, equivalent to about 780 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Transfer 7.0 mL of this stock solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a potassium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of potassium. From the graph so obtained, determine the concentration, *C*, in µg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of potassium in the portion of Potassium Gluconate and Potassium Chloride for Oral Solution taken by the formula:

$$50C/7.$$

Each mg of potassium is equivalent to 0.02558 mEq.

Assay for chloride—

Ionic strength adjusting solution—Use 5 M sodium nitrate.

Assay preparation 1 (where it is packaged in unit-dose containers)—Weigh and mix the contents of not less than 20 containers of Potassium Gluconate and Potassium Chloride for Oral Solution. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg (2.8 mEq) of chloride, to a suitable beaker.

Assay preparation 2 (where it is packaged in multiple-unit containers)—Transfer an accurately weighed portion of Potassium Gluconate and Potassium Chloride for Oral Solution, equivalent to about 100 mg (2.8 mEq) of chloride, to a suitable beaker.

Procedure—Add 2.0 mL of *Ionic strength adjusting solution* to *Assay preparation 1* or *Assay preparation 2*, add water to make about 100 mL, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-sulfide specific ion-selective electrode and a double-junction reference electrode containing potassium nitrate solution (1 in 10). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of Cl.

Potassium Gluconate and Potassium Citrate Oral Solution

» Potassium Gluconate and Potassium Citrate Oral Solution is a solution of Potassium Gluconate and Potassium Citrate in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of potassium (K).

Packaging and storage—Preserve in tight containers.
Labeling—Label it to state the potassium content in terms of milliequivalents in a given volume of Oral Solution.

Identification—

A: To 2 mL of a dilution of Oral Solution (1 in 40) add 5 mL of sodium cobaltinitrite TS: a yellow precipitate is formed immediately (*presence of potassium*).

B: It responds to the test for *Citrate* ⟨191⟩, 3 to 5 drops of Oral Solution and 20 mL of the mixture of pyridine and acetic anhydride being used.

Assay for potassium—

Potassium stock solution—Dissolve in water 0.9535 g of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 1000 µg of potassium per mL.

Standard preparations—To separate 200-mL volumetric flasks transfer 19.0 mL and 25.0 mL, respectively, of *Potassium stock solution*, dilute with water to volume, and mix. The *Standard preparations* contain 95.0 µg and 125.0 µg of potassium per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 782 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a potassium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of potassium. From the graph so obtained, determine the concentration, *C*, in µg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of potassium in each mL of the Oral Solution taken by the formula:

$$(50/7)(C/V)$$

in which *V* is the volume, in mL, of Oral Solution taken. Each mg of potassium is equivalent to 0.02558 mEq.

Potassium Gluconate, Potassium Citrate, and Ammonium Chloride Oral Solution

» Potassium Gluconate, Potassium Citrate, and Ammonium Chloride Oral Solution is a solution of Potassium Gluconate, Potassium Citrate, and Ammonium Chloride in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of potassium (K) and chloride (Cl).

Packaging and storage—Preserve in tight containers.
Labeling—Label it to state the potassium and chloride contents in terms of milliequivalents of each in a given volume of Oral Solution.

Identification—

A: To 2 mL of a dilution of Oral Solution (1 in 40) add 5 mL of sodium cobaltinitrite TS: a yellow precipitate is formed immediately (*presence of potassium*).

B: It responds to the test for *Citrate* ⟨191⟩, 3 to 5 drops of Oral Solution and 20 mL of the mixture of pyridine and acetic anhydride being used.

C: It responds to the tests for *Ammonium* ⟨191⟩ and for *Chloride* ⟨191⟩.

USP Monographs

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Assay for potassium—

Potassium stock solution—Dissolve in water 0.9535 g of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 1000 µg of potassium per mL.

Standard preparations—To separate 200-mL volumetric flasks transfer 19.0 mL and 25.0 mL, respectively, of the *Potassium stock solution*, dilute with water to volume, and mix. The *Standard preparations* contain 95.0 µg and 125.0 µg of potassium per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 782 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a potassium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of potassium. From the graph so obtained, determine the concentration, C, in µg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of potassium in each mL of the Oral Solution taken by the formula:

$$(50/7)(C/V)$$

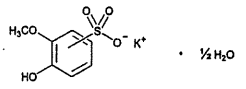
in which V is the volume, in mL, of Oral Solution taken. Each mg of potassium is equivalent to 0.02558 mEq.

Assay for chloride—

Ionic strength adjusting solution—Use 5 M sodium nitrate.

Procedure—Transfer an accurately measured volume of Oral Solution, equivalent to about 100 mg (2.8 mEq) of chloride, to a suitable beaker. Add 2.0 mL of *Ionic strength adjusting solution* and water to make about 100 mL, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-sulfide specific ion-selective electrode and a double-junction reference electrode containing potassium nitrate solution (1 in 10). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of Cl.

Potassium Guaiacolsulfonate



$C_7H_7KO_5S \cdot ½H_2O$ 251.30

Benzenesulfonic acid, hydroxymethoxy-, monopotassium salt, hemihydrate;

Potassium hydroxymethoxybenzenesulfonate hemihydrate [78247-49-1].

Anhydrous 242.30

DEFINITION

Potassium Guaiacolsulfonate contains NLT 98.0% and NMT 102.0% of potassium guaiacolsulfonate ($C_7H_7KO_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020): 7 µm–13 µm, previously dried at 105° for 18 h

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Solution: 50 µg/mL, prepared as directed in the *Assay*

Acceptance criteria: Meets the requirements

- **C. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩

Sample: A 1 in 10 solution

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Sample stock solution: Transfer about 250 mg of Potassium Guaiacolsulfonate to a 500-mL volumetric flask. Dissolve in 400 mL of water, dilute with water to volume, and mix.

Sample solution: Dilute 10.0 mL of *Sample stock solution* with pH 7.0 phosphate buffer to 100.0 mL, and mix.

Standard solution: About 50 µg/mL of USP Potassium Guaiacolsulfonate RS in the same diluent used for the *Sample solution*

Instrumental conditions

Analytical wavelength: Maximum absorbance at about 279 nm

Cell: 1 cm

Blank: A 1 in 10 mixture of water and pH 7.0 phosphate buffer

Analysis

Samples: *Sample solution* and *Standard solution*

Concomitantly determine the absorbances of the *Sample solution* and *Standard solution*, and calculate the percentage of potassium guaiacolsulfonate ($C_7H_7KO_5S$) in the portion of Potassium Guaiacolsulfonate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Potassium Guaiacolsulfonate RS in the *Standard solution* (µg/mL)

C_U = concentration of Potassium Guaiacolsulfonate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **SELENIUM** ⟨291⟩: 0.003%
- **SULFATE**

Analysis: To 10 mL of a solution (1 in 20) add 5 drops of barium chloride TS, and acidify with hydrochloric acid.

Acceptance criteria: No turbidity is produced in 1 min.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: 3.0%–6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Potassium Guaiacolsulfonate RS

USP Monographs

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Potassium Iodide

KI 166.00
Potassium iodide [7681-11-0].

DEFINITION
Potassium Iodide contains NLT 99.0% and NMT 101.5% of KI, calculated on the dried basis.

IDENTIFICATION
- **A. Identification Tests—General,** *Potassium* ⟨191⟩**:** Meets the requirements
- **B. Identification Tests—General,** *Iodide* ⟨191⟩**:** Meets the requirements

ASSAY
- **Procedure**

Sample solution: Dissolve 500 mg of Potassium Iodide in 10 mL of water.

Analysis: Add 35 mL of hydrochloric acid to the *Sample solution,* and titrate with 0.05 M potassium iodate VS until the dark brown solution that is produced becomes pale brown. Add 2–3 drops of amaranth TS, and continue the titration slowly until the red color just changes to yellow. Each mL of 0.05 M potassium iodate is equivalent to 16.60 mg of KI.

Acceptance criteria: 99.0%–101.5% on the dried basis

IMPURITIES
- **Iodate**

Iodate solution: Dilute 1 mL of potassium iodate solution (1 in 2500) with water to 100 mL.

Standard solution: Dissolve 100 mg of Potassium Iodide in ammonia- and carbon dioxide-free water, and add 1 mL of *Iodate solution* to obtain 10 mL of solution. Transfer to a color-comparison tube, add 1 mL of starch TS and 0.25 mL of 1.0 N sulfuric acid, and mix.

Sample solution: Dissolve 1.1 g in sufficient ammonia- and carbon dioxide-free water to obtain 10 mL of solution. Transfer to a color-comparison tube, add 1 mL of starch TS and 0.25 mL of 1.0 N sulfuric acid, and mix.

Acceptance criteria: Any color produced in the *Sample solution* does not exceed that produced in the *Standard solution* (NMT 4 µg/g).

- **Limit of Nitrate, Nitrite, and Ammonia**

Sample solution: Dissolve 1 g in 5 mL of water.

Analysis: To the *Sample solution* contained in a test tube of 40-mL capacity add 5 mL of 1 N sodium hydroxide and 200 mg of aluminum wire. Insert a pledget of purified cotton in the upper portion of the test tube, and place a piece of moistened red litmus paper over the mouth of the tube. Heat the test tube and its contents in a steam bath for 15 min.

Acceptance criteria: No blue coloration of the paper is discernible.

- **Thiosulfate and Barium**

Sample solution: Dissolve 0.5 g in 10 mL of ammonia- and carbon dioxide-free water.

Analysis: Add 2 drops of 2 N sulfuric acid.

Acceptance criteria: No turbidity develops within 1 min.

SPECIFIC TESTS
- **Alkalinity**

Sample solution: Dissolve 1.0 g of Potassium Iodide in 10 mL of water.

Analysis: Add 0.1 mL of 0.1 N sulfuric acid and 1 drop of phenolphthalein TS to the *Sample solution.*

Acceptance criteria: No color is produced.

- **Loss on Drying** ⟨731⟩**:** Dry a sample at 105° for 4 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.

Potassium Iodide Oral Solution

DEFINITION
Potassium Iodide Oral Solution contains NLT 94.0% and NMT 106.0% of the labeled amount of potassium iodide (KI). [Note—If Potassium Iodide Oral Solution is not to be used within a short time, add 0.5 mg of sodium thiosulfate for each g of KI. Products that have data to demonstrate acceptable stability without the addition of thiosulfate are exempt from this requirement. Crystals of potassium iodide may form in Potassium Iodide Oral Solution under normal conditions of storage, especially if refrigerated.]

IDENTIFICATION
- **A. Identification Tests—General,** *Potassium* ⟨191⟩and *Iodide* ⟨191⟩**:** Meets the requirements

ASSAY
- **Procedure**

Sample solution: 50 mg/mL of potassium iodide from Oral Solution in water

Analysis: Transfer 10.0 mL of the *Sample solution* to a 150-mL beaker, and add 40 mL of water, 25 mL of alcohol, and 1.0 mL of 1 N nitric acid. Titrate with 0.1 N silver nitrate VS, using a silver indicator electrode and an appropriate reference electrode. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N silver nitrate is equivalent to 16.60 mg of KI.

Acceptance criteria: 94.0%–106.0%

PERFORMANCE TESTS
- **Uniformity of Dosage Units** ⟨905⟩

For Oral Solution packaged in single-unit containers: Meets the requirements

- **Deliverable Volume** ⟨698⟩

For Oral Solution packaged in multiple-unit containers: Meets the requirements

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Potassium Iodide Tablets

DEFINITION
Potassium Iodide Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of potassium iodide (KI) for Tablets of 300 mg or more, and NLT 92.5% and NMT 107.5% for Tablets of less than 300 mg.

IDENTIFICATION
- **A. Identification Tests—General,** *Potassium* ⟨191⟩and *Iodide* ⟨191⟩**:** A filtered solution of powdered Tablets meets the requirements.

ASSAY
- **Procedure**

Sample solution: Transfer an equivalent to 1.2 g of potassium iodide, from finely powdered Tablets (NLT 20), to a 250-mL volumetric flask. Add 100 mL of water, shake for 20 min, and dilute with water to volume. Filter through paper, discarding the first 20 mL of the filtrate.

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Analysis: Transfer 100.0 mL of the filtrate, 25 mL of alcohol, and 1.0 mL of 1 N nitric acid to a 200-mL beaker. Titrate with 0.1 N silver nitrate VS, using a silver indicator electrode and an appropriate reference electrode. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N silver nitrate is equivalent to 16.60 mg of KI.

Acceptance criteria: 94.0%–106.0% of the labeled amount of potassium iodide (KI) for Tablets of 300 mg or more, and 92.5%–107.5% for Tablets of less than 300 mg

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

For Uncoated Tablets

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Detector: UV 227 nm

Standard solution: Potassium iodide in *Medium*

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution.*

Tolerances: NLT 75% (*Q*) of the labeled amount of potassium iodide (KI) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Iodide Delayed-Release Tablets

DEFINITION

Potassium Iodide Delayed-Release Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of potassium iodide (KI) for Tablets of 300 mg or more, and NLT 92.5% and NMT 107.5% for Tablets of less than 300 mg.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩ and *Iodide* ⟨191⟩: A filtered solution of powdered Tablets meets the requirements.

ASSAY

• **PROCEDURE**

Sample solution: Transfer an equivalent to 1.2 g of potassium iodide, from finely powdered Tablets (NLT 20), to a 250-mL volumetric flask. Add 100 mL of water, shake for 20 min, and dilute with water to volume. Filter through paper, discarding the first 20 mL of the filtrate. Transfer 100.0 mL of the filtrate, 25 mL of alcohol, and 1.0 mL of 1 N nitric acid to a 200-mL beaker.

Analysis: Transfer 100.0 mL of the filtrate, 25 mL of alcohol, and 1.0 mL of 1 N nitric acid to a 200-mL beaker. Titrate with 0.1 N silver nitrate VS, using a silver indicator electrode and an appropriate reference electrode. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N silver nitrate is equivalent to 16.60 mg of KI.

Acceptance criteria: 94.0%–106.0% of the labeled amount of potassium iodide (KI) for Tablets of 300 mg or more, and 92.5%–107.5% for Tablets of less than 300 mg

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Analysis: Proceed as directed for *Delayed-Release (Enteric-Coated) Tablets.*

Acceptance criteria: The Tablets do not disintegrate after 1 h of agitation in simulated gastric fluid TS, but they disintegrate within 90 min in simulated intestinal fluid TS.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Nitrate

KNO_3 101.10

Potassium nitrate [7757-79-1].

DEFINITION

Potassium Nitrate contains NLT 99.0% and NMT 100.5% of KNO_3.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Nitrate* ⟨191⟩: Meets the requirements

ASSAY

• **PROCEDURE**

[NOTE—Use water that is free of carbon dioxide and ammonia.]

Cation-exchange column: Transfer strongly acidic styrene-divinylbenzene cation-exchange resin (16- to 50-mesh) to a 2-cm diameter chromatographic column to a depth of about 20 cm.

Sample solution: 4 mg/mL of Potassium Nitrate in water

Analysis: Pass 100 mL of *Sample solution* through the *Cation-exchange column* at a rate of 5 mL/min, and collect the eluate in a 500-mL conical flask. Wash the resin in the column with water at a rate of 10 mL/min, collecting the eluate in the conical flask. Add 0.15 mL of phenolphthalein TS to the flask, and after 5 min titrate with 0.1 N sodium hydroxide VS to a pink endpoint. Continue collecting the wash from the column, and continue titrating, if necessary, until a 50-mL increment of eluate requires no further addition of sodium hydroxide. Each mL of 0.1 N sodium hydroxide is equivalent to 10.11 mg of KNO_3.

Acceptance criteria: 99.0%–100.5%

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩: A 500-mg portion shows no more chloride than corresponds to 0.21 mL of 0.020 N hydrochloric acid (0.03%).

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample solution: 100 mg of Potassium Nitrate in 10 mL of water. Add 15 mL of 6 N hydrochloric acid, and evaporate to dryness on a steam bath. To the residue add 7 mL of 6 N hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 35 mL of water and, if necessary, neutralize with hydrochloric acid using a litmus paper indicator. Filter, if necessary, to obtain a clear *Sample solution.*

Acceptance criteria: The *Sample solution* shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.1%).

• **ARSENIC,** *Method I* ⟨211⟩: NMT 3 ppm

• **LEAD** ⟨251⟩

Sample solution: 500 mg in 20 mL of water

Acceptance criteria: NMT 10 ppm

• **IRON** ⟨241⟩: NMT 10 ppm

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• **LIMIT OF SODIUM**

Standard stock solution: [NOTE—Sodium chloride is previously dried at 105° for 2 h.] 2.542 µg/mL of sodium chloride in water. This solution contains 1.0 µg/mL of sodium.

Sample stock solution: 2 mg/mL of Potassium Nitrate. [NOTE—The concentration of potassium nitrate in this solution may be modified by using a different quantity or by further dilution to bring the absorption response within the working range of the atomic absorption spectrometer.]

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Sodium emission line of 589 nm

Lamp: Sodium hollow-cathode

Flame: Oxidizing

Blank: Water

Analysis: Transfer 5.0 mL of the *Sample stock solution* to each of three 25-mL volumetric flasks. To these flasks, respectively, add 0.0, 5.0, and 10.0 mL of the *Standard stock solution*, dilute with water to volume, and mix. These flasks contain 0.0, 0.20, and 0.40 µg of added sodium/mL, respectively. [NOTE—Concentrations of sodium in these solutions may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Determine the absorbances of these solutions. Plot the absorbances of the three solutions versus concentration, in µg/mL of added sodium, draw the straight line best fitting the plotted points, and extrapolate the line until it intercepts the concentration axis. From the graph determine the concentration, C, in µg/mL of sodium, of the solution containing 0.0 mL of the *Standard stock solution*.

Calculate the percentage of sodium in the portion of Potassium Nitrate taken by multiplying C by 0.25.

Acceptance criteria: NMT 0.1%

• **LIMIT OF NITRITE**

Solution A: 1 mg/mL of sulfanilic acid

Solution B: 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride. [NOTE—When stored in a low-actinic glass bottle, this solution may be used for 1 week.]

Standard stock solution: 15 µg/mL of sodium nitrite (10 µg/mL of nitrite)

Standard solutions: Transfer 1.0 and 2.0 mL of *Standard stock solution* to separate 50-mL beakers, and add 19 and 18 mL of water to the respective beakers.

Sample solution: Transfer 4.0 g of Potassium Nitrate to a 50-mL beaker, add 20 mL of water, and swirl to dissolve.

Analysis: To the beakers containing the *Standard solutions* and the *Sample solution* add 5.0 mL of *Solution A* and 5.0 mL of diluted hydrochloric acid, and allow to stand for 3 min. Add 5.0 mL of *Solution B* to each beaker, mix, and allow to stand for 15 min. Determine the absorbances of the solutions at 550 nm.

Acceptance criteria: The absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution* containing 20 µg of nitrite (5 µg/g).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Nitrate Solution

» Potassium Nitrate Solution contains not less than 98.0 percent and not more than 102.0 percent of the labeled amount of KNO_3.

Packaging and storage—Preserve in tight containers.

Identification—It responds to the tests for *Potassium* ⟨191⟩ and for *Nitrate* ⟨191⟩.

Chloride ⟨221⟩—An accurately measured portion of Solution, equivalent to 500 mg of potassium nitrate, shows no more chloride than corresponds to 0.21 mL of 0.020 N hydrochloric acid (0.03%, based on the potassium nitrate content of the Solution).

Sulfate ⟨221⟩—Dilute an accurately measured portion of Solution, equivalent to 100 mg of potassium nitrate, with water to obtain 10 mL of solution, add 15 mL of 6 N hydrochloric acid, and evaporate to dryness on a steam bath. To the residue so obtained add 7 mL of 6 N hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue so obtained in about 35 mL of water and, if necessary, neutralize with hydrochloric acid using litmus paper indicator. Filter, if necessary, to obtain a clear test solution. This test solution shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.1%, based on the potassium nitrate content of the Solution).

Arsenic, *Method I* ⟨211⟩: 3 ppm, based on the potassium nitrate content of the Solution, an accurately measured portion of Solution, equivalent to 1.0 g of potassium nitrate, being tested.

Lead—Dilute an accurately measured portion of Solution, equivalent to 500 mg of potassium nitrate, with water to obtain 20 mL of test solution. This test solution contains not more than 5 µg of lead (corresponding to not more than 0.001% of Pb, based on the potassium nitrate content of the Solution) when tested as directed under *Lead* ⟨251⟩.

Iron ⟨241⟩: not more than 0.001%, based on the potassium nitrate content of the Solution, an accurately measured portion of Solution, equivalent to 1.0 g of potassium nitrate, being tested.

Limit of sodium—

Stock test solution—Transfer an accurately measured portion of Solution, equivalent to 1.0 g of potassium nitrate, to a 500-mL volumetric flask, dilute with water to volume, and mix. [NOTE—The concentration of potassium nitrate in this solution may be modified by using a different quantity or by further dilution to bring the absorption response within the working range of the atomic absorption spectrophotometer.]

Stock standard solution—Proceed as directed in the *Limit of sodium* test under *Potassium Nitrate*.

Procedure—Proceed as directed in the *Limit of sodium* test under *Potassium Nitrate*. Calculate the percentage of sodium in the portion of Solution taken by multiplying C by 0.25: the limit is 0.1%, based on the potassium nitrate content of the Solution.

Limit of nitrite—

Sulfanilic acid solution, N-(1-Naphthyl)ethylenediamine dihydrochloride solution, and *Standard solutions*—Proceed as directed in the *Limit of nitrite* test under *Potassium Nitrate*.

Test solution—Transfer an accurately measured portion of the Solution, equivalent to 4.0 g of potassium nitrate, to a 50-mL beaker, add sufficient water to obtain 20 mL of solution, and mix.

Procedure—Proceed as directed in the *Limit of nitrite* test under *Potassium Nitrate*. The absorbance of the solution from the *Test solution* does not exceed that of the solution from the

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Standard solution containing 20 µg of nitrite (5 µg per g, based on the potassium nitrate content of the Solution).

Assay— [NOTE—Use water that is carbon dioxide- and ammonia-free.]

Cation-exchange column—Transfer strongly acidic styrene-divinylbenzene cation-exchange resin (16- to 50-mesh) to a 2-cm diameter chromatographic column to a depth of about 20 cm.

Procedure—Transfer an accurately measured portion of Solution, equivalent to about 400 mg of potassium nitrate, to a beaker and add sufficient water to obtain 100 mL of solution. Proceed as directed in the *Assay* under *Potassium Nitrate* beginning with "Pass this solution through . . . ." Each mL of 0.1 N sodium hydroxide is equivalent to 10.11 mg of KNO_3.

Potassium Perchlorate

» Potassium Perchlorate contains not less than 99.0 percent and not more than 100.5 percent of $KClO_4$, calculated on the dried basis. [**CAUTION**—Great care should be taken in handling Potassium Perchlorate in solution or in the dry state, as explosions may occur if it is brought into contact with organic or other readily oxidizable substances.]

Packaging and storage—Preserve in well-closed containers.

Identification—

A: Ignite a small portion of a solution (1 in 10) on a platinum wire in a nonluminous flame: a transient violet color is imparted to the flame.

B: Add a few drops of methylene blue solution (1 in 1000) to the solution (1 in 10): a violet-colored precipitate is formed.

pH ⟨791⟩: between 5.0 and 6.5, in a 0.1 M solution.

Loss on drying ⟨731⟩—Dry it over silica gel for 12 hours: it loses not more than 0.5% of its weight.

Insoluble substances—Dissolve 20 g in 150 mL of warm water, pass through a tared medium-porosity filtering crucible, and wash with three 50-mL portions of warm water. Dry the residue at 105° for 3 hours: the weight of the residue does not exceed 1 mg (0.005%).

Chloride ⟨221⟩—A 5.0-g portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.003%).

Limit of sodium—Ignite a small portion of a solution (1 in 10) on a platinum wire in a nonluminous flame: no pronounced yellow color is imparted to the flame.

Assay—

Mobile phase—Transfer 16.6 g of phthalic acid to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 1000-mL flask, dilute with water to volume, and mix. Adjust with about 450 mg of lithium hydroxide to a pH of 4.5, filter, and degas.

Standard preparation—Transfer about 50 mg of potassium perchlorate, accurately weighed, to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Using about 50 mg of Potassium Perchlorate, accurately weighed, proceed as directed for the *Standard preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a conductivity detector and a 4.6-mm × 7.5-cm column that contains 6-µm packing L23. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $KClO_4$ in the portion of Potassium Perchlorate taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of potassium perchlorate in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Potassium Permanganate

$KMnO_4$ 158.03

Permanganic acid ($HMnO_4$), potassium salt; Potassium permanganate ($KMnO_4$) [7722-64-7].

DEFINITION

Potassium Permanganate contains NLT 99.0% and NMT 100.5% of potassium permanganate ($KMnO_4$), calculated on the dried basis.

[**CAUTION**—Observe great care in handling Potassium Permanganate, because dangerous explosions may occur if it is brought into contact with organic or other readily oxidizable substances, either in solution or in the dry state.]

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Permanganate* ⟨191⟩**:** A solution of it is deep violet-red when concentrated and pink when highly diluted.

ASSAY

• **PROCEDURE**

Sample: 1000 mg of Potassium Permanganate

Titrimetric system

Mode: Residual titration

Titrant: 0.03 N potassium permanganate VS

Endpoint detection: Visual

Analysis: For each mg of Potassium Permanganate taken, add 2.13 mg of sodium oxalate, previously dried at 110° to constant weight, to a 500-mL conical flask. Add 150 mL of water and 20 mL of 7 N sulfuric acid, and heat to 80°. Titrate the excess oxalic acid with *Titrant*. Calculate the percentage of potassium permanganate ($KMnO_4$) in the portion of Potassium Permanganate taken:

$$\text{Result} = [(F_1 \times W_S) - (V_S \times N \times F_2)] \times (100/W)$$

F_1 = equivalency factor, 0.4718 mg of potassium permanganate per mg of sodium oxalate

W_S = weight of sodium oxalate taken (mg)

V_S = *Titrant* volume consumed by the *Sample* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F_2 = equivalency factor, 31.61 mg of potassium permanganate per mEq

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W = *Sample* weight (mg)

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES

• **INSOLUBLE SUBSTANCES**

Sample: 2.0 g

Analysis: Dissolve the *Sample* in 150 mL of water that previously has been warmed to steam-bath temperature, and filter immediately through a tared, medium-porosity filtering crucible. Wash the filter with three 50-mL portions of the warm water, and dry the filtering crucible and the residue at 105° for 3 h.

Acceptance criteria: 0.2%; NMT 4 mg of residue is obtained.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over silica gel for 18 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Dibasic Potassium Phosphate

K_2HPO_4 174.18

Phosphoric acid, dipotassium salt;
Dipotassium hydrogen phosphate [7758-11-4].

DEFINITION

Dibasic Potassium Phosphate contains NLT 98.0% and NMT 100.5% of K_2HPO_4, calculated on the dried basis.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩

Sample solution: 1 in 20

Acceptance criteria: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Phosphate* ⟨191⟩

Sample solution: 1 in 20

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample: 5.2 g

Sample solution: Transfer the *Sample* to a 250-mL beaker. Add 50 mL of water and 40.0 mL of 1 N hydrochloric acid, and stir until dissolved.

Blank: Transfer 40.0 mL of 1 N hydrochloric acid to a 250-mL beaker. Add 50 mL of water.

Analysis: Titrate the *Blank* with 1 N sodium hydroxide VS, and record the volume of 1 N sodium hydroxide VS consumed. Titrate the excess acid in the *Sample solution* with 1 N sodium hydroxide VS to the inflection point at pH 4, and record the buret reading. Subtract this buret reading from that of the blank, and designate the volume of 1 N sodium hydroxide VS resulting from this subtraction as *A*. Continue the titration with 1 N sodium hydroxide VS to the inflection point at pH 8.8, record the buret reading, and calculate the volume (*B*) of 1 N sodium hydroxide required in the titration between the two inflection points (pH 4–8.8). Where *A* is equal to or less than *B*, each mL of the volume *A* of 1 N sodium hydroxide is equivalent to 174.2 mg of K_2HPO_4. Where *A* is greater than *B*, each mL of the volume $2B - A$ of 1 N sodium hydroxide is equivalent to 174.2 mg of K_2HPO_4.

Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES

• **INSOLUBLE SUBSTANCES**

Sample solution: Dissolve 10 g in 100 mL of hot water.

Analysis: Filter the *Sample solution* through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° for 2 h.

Acceptance criteria: The weight of the residue is NMT 20 mg (NMT 0.2%).

• **CARBONATE**

Sample: 1 g

Analysis: Add 3 mL of water and 2 mL of 3 N hydrochloric acid to the *Sample*.

Acceptance criteria: NMT a few bubbles are evolved.

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample: 1.0 g

Acceptance criteria: Shows no more chloride than corresponds to 0.40 mL of 0.020 N hydrochloric acid (NMT 0.03%)

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample: 0.20 g

Acceptance criteria: Shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (NMT 0.1%)

• **ARSENIC,** *Method I* ⟨211⟩**:** NMT 3 ppm

• **IRON**

Sample solution: Dissolve 0.33 g in 10 mL of water.

Analysis: To the *Sample solution* add 6 mL of hydroxylamine hydrochloride solution (1 in 10) and 4 mL of orthophenanthroline solution prepared by dissolving 1 g of orthophenanthroline in 1000 mL of water containing 1 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: Any red color produced within 1 h is not darker than that of a control prepared from 1 mL of *Standard Iron Solution* (see *Iron* ⟨241⟩): NMT 30 ppm.

• **SODIUM:** A solution (1 in 10) tested on a platinum wire imparts no pronounced yellow color to a nonluminous flame.

• **LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

Buffer: 294 g/L of sodium citrate dihydrate in water

Standard stock solution: 1.1052 mg/mL of USP Sodium Fluoride RS in water

Standard solution: Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer*, and dilute with water to volume. Each mL of this solution contains 100 µg of fluoride ion.

Sample: 2.0 g

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).

Standard response line: Transfer 50.0 mL of *Buffer* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

Analysis: Transfer the *Sample* to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer* and sufficient water to make 100 mL (*Sample solution*). Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard*

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response line determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*.
Calculate the percentage of fluoride ion in the portion of Dibasic Potassium Phosphate taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of fluoride ion in the *Sample solution* (µg/mL), as defined above
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.001%

- **LIMIT OF MONOBASIC OR TRIBASIC SALT**

Sample solution: Dissolve 3 g in 30 mL of water, and cool to 20°.
Analysis: Add 3 drops of thymol blue TS to the *Sample solution*.
Acceptance criteria: A blue color is produced, which is changed to yellow (with a greenish tinge) by the addition of NMT 0.4 mL of 1 N hydrochloric acid.

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 8.5–9.6, in a solution (1 in 20)
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° to constant weight: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sodium Fluoride RS

Potassium Phosphates Injection

» Potassium Phosphates Injection is a sterile solution of Monobasic Potassium Phosphate and Dibasic Potassium Phosphate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of monobasic potassium phosphate (KH_2PO_4) and dibasic potassium phosphate (K_2HPO_4). It contains no bacteriostat or other preservative.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
Labeling—The label states the potassium content in terms of milliequivalents in a given volume, and states also the elemental phosphorus content in terms of millimoles in a given volume. Label the Injection to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration, and that once opened any unused portion is to be discarded. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
Identification—It responds to the tests for *Potassium* ⟨191⟩ and for *Phosphate* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.10 USP Endotoxin Units per mg of potassium phosphates.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume Injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay for monobasic potassium phosphate—Transfer an accurately measured volume of Injection, equivalent to about 300 mg of monobasic potassium phosphate, to a 100-mL beaker, and dilute with water to about 50 mL. Place the electrodes of a suitable pH meter in the solution, and titrate with 0.1 N sodium hydroxide VS to the inflection point to a pH of about 9.1. Each mL of 0.1 N sodium hydroxide is equivalent to 13.61 mg of KH_2PO_4.
Assay for dibasic potassium phosphate—Transfer an accurately measured volume of Injection, equivalent to about 300 mg of dibasic potassium phosphate, to a 100-mL beaker, and dilute with water to about 50 mL. Place the electrodes of a suitable pH meter in the solution, and titrate with 0.1 N hydrochloric acid VS to the inflection point to a pH of about 4.2. Each mL of 0.1 N hydrochloric acid is equivalent to 17.42 mg of K_2HPO_4.

Potassium Phosphates Compounded Injection

DEFINITION

Potassium Phosphates Compounded Injection contains NLT 95.0% and NMT 105.0% of the labeled amount of monobasic potassium phosphate (KH_2PO_4) and dibasic potassium phosphate (K_2HPO_4). It contains no bacteriostat or other preservative.
Prepare Potassium Phosphates Compounded Injection containing 3 mM/mL of phosphorus and 4.4 mEq/mL of potassium as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Monobasic Potassium Phosphate (anhydrous)	22.4 g
Dibasic Potassium Phosphate (anhydrous)	23.6 g
Sterile Water for Injection, a sufficient quantity to make	100 mL

Dissolve the *Dibasic Potassium Phosphate (anhydrous)* in 50 mL of *Sterile Water for Injection*. Dissolve the *Monobasic Potassium Phosphate (anhydrous)* in the solution and then bring to final volume with *Sterile Water for Injection*. [NOTE—May need to heat to 77° to fully dissolve. If heating is required, allow to reach room temperature prior to passing through a filter.] Pass through a filter of 1.2-µm pore size to remove particulate matter and sterilize by autoclave.

ASSAY

- **PROCEDURE FOR POTASSIUM**

Mobile phase: 8 mM methanesulfonic acid
Standard solution: 0.050 mg/mL of potassium prepared with USP Dibasic Potassium Phosphate RS and water
Sample solution: Transfer 74 µL of Injection to a 250-mL volumetric flask and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4.6-mm × 25-cm; packing L97
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*

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[NOTE—The retention time for potassium is about 5.1 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of potassium from the *Sample solution*
r_S = peak response of potassium from the *Standard solution*
C_S = concentration of potassium in the *Standard solution* (mg/mL)
C_U = nominal concentration of potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

- **PROCEDURE FOR PHOSPHATE**

Mobile phase: 40 mM sodium hydroxide
Standard solution: 0.230 mg/mL of phosphate prepared from USP Dibasic Potassium Phosphate RS in water
Sample solution: Transfer 0.2 mL of Injection to a 250-mL volumetric flask and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4.6-mm × 25-cm; packing L103
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for phosphorus is about 5.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phosphate (PO_4) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phosphate from the *Sample solution*
r_S = peak response of phosphate from the *Standard solution*
C_S = concentration of phosphate in the *Standard solution* (mg/mL)
C_U = nominal concentration of phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 6.3–7.3
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1.10 USP Endotoxin Units/mg of potassium phosphates
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements

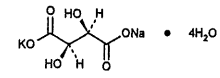
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in single-dose glass containers. Store at controlled room temperature.

Change to read:

- **BEYOND-USE DATE:** In the absence of passing a sterility ▲test and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed,▲ (CN 1-May-2020) NMT 120 days after the date on which it was compounded when stored at controlled room temperature.
- **LABELING:** Label it to state the *Beyond-Use Date*. The label states the potassium content in terms of milliequivalents in a given volume, and states also the elemental phosphorus content in terms of millimoles in a given volume. Label it not for direct injection.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Dibasic Potassium Phosphate RS

Potassium Sodium Tartrate



$C_4H_4KNaO_6 \cdot 4H_2O$ 282.22
Butanedioic acid, 2,3-dihydroxy-, [*R*-(*R**,*R**)]-, monopotassium monosodium salt, tetrahydrate.
Monopotassium monosodium tartrate tetrahydrate [6100-16-9; 6381-59-5].
Anhydrous 210.16
[304-59-6].

» Potassium Sodium Tartrate contains not less than 99.0 percent and not more than 102.0 percent of $C_4H_4KNaO_6$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

Identification—
A: Ignite it: it emits the odor of burning sugar and leaves a residue that is alkaline to litmus and that effervesces with acids.
B: To 10 mL of a solution (1 in 20) add 10 mL of 6 N acetic acid: a white, crystalline precipitate is formed within 15 minutes.
C: Responds to the tests for *Tartrate* ⟨191⟩.
Alkalinity—A solution of 1.0 g in 20 mL of water is alkaline to litmus, but after the addition of 0.20 mL of 0.10 N sulfuric acid no pink color is produced by the addition of 1 drop of phenolphthalein TS.
Water Determination, *Method I* ⟨921⟩: between 21.0% and 27.0%.

Limit of ammonia—
Sodium hypochlorite solution—Use a commercially available solution that contains between 4.0% and 6.0% of sodium hypochlorite.
Oxidizing solution— [NOTE—Prepare on the day of use.] Prepare a mixture of alkaline sodium citrate TS and *Sodium hypochlorite solution* (4:1).
Diluted sodium nitroferricyanide solution—Prepare a mixture of water and sodium nitroferricyanide TS (10:1).
Standard solution—Transfer 300 mg of ammonium chloride, previously dried over silica gel for 4 hours, to a 1-L volumetric flask, and dilute with water to volume. This solution

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contains 100 µg of ammonia per mL. Dilute this solution quantitatively, and stepwise if necessary, with water to obtain a solution having a concentration of 1.0 µg of ammonia per mL.

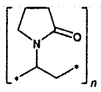
Test solution—Transfer 5.0 g of Potassium Sodium Tartrate to a 100-mL volumetric flask, and dissolve in and dilute with water to volume.

Procedure— [NOTE—Carefully follow the order of addition stated below.] Separately transfer 4.0 mL of each of the *Standard solution* and the *Test solution* to two color-comparison tubes. To each tube add 0.4 mL of phenol TS, 0.4 mL of *Diluted sodium nitroferricyanide solution*, and 1.0 mL of *Oxidizing solution*. Dilute with water to 10 mL, mix, and allow to stand for 1 hour: the color of the *Test solution* is not darker than the color of the *Standard solution* (0.002%).

Assay—Weigh accurately about 2 g of Potassium Sodium Tartrate in a tared porcelain crucible, and ignite, gently at first, until the salt is thoroughly carbonized, protecting the carbonized salt from the flame at all times. Cool the crucible, place it in a glass beaker, and break up the carbonized mass with a glass rod. Without removing the glass rod or the crucible, add 50 mL of water and 50.0 mL of 0.5 N sulfuric acid VS, cover the beaker, and boil the solution for 30 minutes. Filter, and wash with hot water until the last washing is neutral to litmus. Cool the combined filtrate and washings, add methyl red-methylene blue TS, and titrate the excess acid with 0.5 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.5 N sulfuric acid is equivalent to 52.54 mg of $C_4H_4KNaO_6$.

Povidone

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (•⬥) to specify this fact.



$(C_6H_9NO)_n$
2-Pyrrolidinone, 1-ethenyl-, homopolymer;
1-Vinyl-2-pyrrolidinone polymer;
Poly [(2-oxo-1-pyrrolidinyl)ethylene] [9003-39-8].

DEFINITION

Povidone is a chain polymer of 1-vinyl-2-pyrrolidone. It contains NLT 11.5% and NMT 12.8% of nitrogen (N: 14.01), calculated on the anhydrous basis. It has the nominal K-value of NLT 10 and NMT 120. The nominal K-value is shown on the label.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Dry at 105° for 6 h.
  ▲Acceptance criteria: Meets the requirements▲ (USP 1-May-2019)
- **•B.**
  Sample solution: 20 mg/mL of Povidone
  Analysis: To 10 mL of the *Sample solution*, add 20 mL of 1 N hydrochloric acid VS and 5 mL of potassium dichromate TS.
  Acceptance criteria: An orange-yellow precipitate is formed.⬥
- **•C.**
  Solution A: Dissolve 75 mg of cobalt nitrate and 300 mg of ammonium thiocyanate in 2 mL of water.
  Sample solution: 20 mg/mL of Povidone
  Analysis: Combine *Solution A* and 5 mL of the *Sample solution*, and render the resulting solution acidic by the addition of 3 N hydrochloric acid.
  Acceptance criteria: A pale blue precipitate is formed.⬥
- **•D.**
  Sample solution: 5 mg/mL of Povidone
  Analysis: To 5 mL of the *Sample solution*, add a few drops of iodine TS.
  Acceptance criteria: A deep red color is produced.⬥
- **E.**
  Sample solution: 50 mg/mL of Povidone in water
  Acceptance criteria: The substance dissolves.

ASSAY

Change to read:

- **▲NITROGEN DETERMINATION▲** (USP 1-May-2019)
  Sample: 0.1 g of Povidone
  Analysis: ▲Weigh the *Sample* accurately and place in a Kjeldahl flask. Add 5 g of a powdered mixture of 33 g of potassium sulfate, 1 g of cupric sulfate, and 1 g of titanium dioxide. Wash down any adhering sample from the neck of the flask with a small amount of water. Add 7 mL of sulfuric acid allowing it to flow down the inside wall of the flask. Heat the flask gradually until the solution has a clear, yellow-green color and the inside wall of the flask is free from any carbonized material and then heat for an additional 45 min. After cooling, cautiously add 20 mL of water, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 3 drops of bromocresol green-methyl red TS, and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of a solution of sodium hydroxide (2 in 5) through the funnel, rinse the funnel cautiously with 10 mL of water, immediately close the clamp attached to the rubber tube, and then start the distillation with steam to obtain 80–100 mL of the distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of water, and titrate the distillate with 0.025 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction. Each milliliter of 0.025 mol/L sulfuric acid VS equals 0.700 mg of nitrogen.▲ (USP 1-May-2019)
  Acceptance criteria: 11.5%–12.8% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **• LEAD** ⟨251⟩
  Test preparation: 1.0 g in 25 mL of water
  Acceptance criteria: NMT 10 ppm•

Change to read:

- **LIMIT OF ALDEHYDES**
  Solution A: Transfer 8.3 g of potassium pyrophosphate to a 500-mL volumetric flask and dissolve in 400 mL of water. Adjust, if necessary, with 1 N hydrochloric acid VS to a pH of 9.0, and dilute with water to volume.
  Solution B: Transfer a quantity of lyophilized aldehyde dehydrogenase, equivalent to 70 units, to a glass vial, and

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dissolve in 10.0 mL of water. [NOTE—This solution is stable for 8 h at 4°.]

Solution C: Transfer 40 mg of nicotinamide adenine dinucleotide to a glass vial, and dissolve in 10.0 mL of *Solution A*. [NOTE—This solution is stable for four weeks at 4°.]

Standard solution: Dissolve 0.140 g of acetaldehyde ammonia trimer trihydrate in water to make 200.0 mL. Dilute 1.0 mL of the solution with *Solution A* to 100.0 mL.

Sample solution: 10 mg/mL of Povidone in *Solution A*. Insert a stopper into the flask, heat at 60° for 1 h, and cool to room temperature.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 340 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and water

Pipet 0.5 mL each of the *Standard solution*, *Sample solution*, and water (used for blank test) into separate cells. Add 2.5 mL of *Solution A* and 0.2 mL of *Solution C* to each cell. Cover the cells to exclude oxygen. Mix by inversion and allow to stand for 2–3 min at 22 ± 2°. Determine the absorbances of the solutions using the water as the reference. Add 0.05 mL of *Solution B* to each cell. Cover the cells to exclude oxygen. Mix by inversion and allow to stand for 5 min at 22 ± 2°. Determine the absorbances of the solutions, using the water as the reference.

Calculate the percentage of aldehydes, expressed as acetaldehyde, in the portion of Povidone taken:

$$\text{Result} = 100 \times (C_S/C_U) \times \{[(A_{U2} - A_{U1}) - (A_{B2} - A_{B1})]/[(A_{S2} - A_{S1}) - (A_{B2} - A_{B1})]\}$$

C_S = concentration of acetaldehyde in the *Standard solution*, calculated from the weight of the acetaldehyde ammonia trimer trihydrate with the factor 0.72 (mg/mL). [NOTE—The molar mass of acetaldehyde is 44.05 g/mol, and the molar mass of acetaldehyde ammonia trimer trihydrate is 183.26 g/mol. (44.05 × 3)/183.26 = 0.72]

C_U = concentration of the *Sample solution* (mg/mL), ▲calculated on the anhydrous basis▲ (USP 1-May-2019)

A_{U2} = absorbance of the solution from the *Sample solution*, after addition of *Solution B*

A_{U1} = absorbance of the solution from the *Sample solution*, before addition of *Solution B*

A_{B2} = absorbance of the solution from the blank, after addition of *Solution B*

A_{B1} = absorbance of the solution from the blank, before addition of *Solution B*

A_{S2} = absorbance of the solution from the *Standard solution*, after addition of *Solution B*

A_{S1} = absorbance of the solution from the *Standard solution*, before addition of *Solution B*

Acceptance criteria: NMT 0.05%

Change to read:

- **LIMIT OF HYDRAZINE**

Standard solution: 9 µg/mL of salicylaldazine in toluene

Sample solution: Transfer 2.5 g to a 50-mL centrifuge tube, add 25 mL of water, and mix to dissolve. Add 500 µL of a solution (1 in 20) of salicylaldehyde in methanol. Swirl and heat in a water bath at 60° for 15 min. Allow to cool and add 2.0 mL of toluene. Insert a stopper in the tube, shake vigorously for 2 min, and centrifuge. Use the clear upper toluene layer in the centrifuge tube as the *Sample solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of dimethylsilanized chromatographic silica gel with fluorescent indicator

Application volume: 10 µL

Developing solvent system: Methanol and water (2:1)

Analytical wavelength: UV 365 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Allow the spots to dry, and develop the chromatogram with the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. ▲▲ (USP 1-May-2019) Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. ▲Locate the spots on the plate by examination under UV light.▲ (USP 1-May-2019)

Acceptance criteria: Salicylaldazine appears as a fluorescent spot having an R_F value of 0.3; and the fluorescence of any salicylaldazine spot from the *Sample solution* is not more intense than that produced by the spot from the *Standard solution* (NMT 1 ppm of hydrazine).

- **VINYLPYRROLIDINONE**

Mobile phase: Water and acetonitrile (90:10)

System suitability solution: Transfer 10 mg of vinylpyrrolidinone and 500 mg of vinyl acetate to a 100-mL volumetric flask, and dissolve in and dilute with methanol to volume. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Standard stock solution: 5 µg/mL of vinylpyrrolidinone in *Mobile phase*

Standard solution: 0.25 µg/mL of vinylpyrrolidinone diluted from the *Standard stock solution* in *Mobile phase*

Sample solution: 25 mg/mL of Povidone in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Columns

Guard: 4.0-mm × 1.0-cm; packing L1

Analytical: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between vinylpyrrolidinone and vinyl acetate, in this elution order, *System suitability solution*

Relative standard deviation: NMT 2.0% of vinylpyrrolidinone for six injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms and measure the responses for the vinylpyrrolidinone peak.

Calculate the percentage of vinylpyrrolidinone in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of vinylpyrrolidinone from the *Sample solution*

r_S = peak response of vinylpyrrolidinone from the *Standard solution*

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C_S = concentration of vinylpyrrolidinone in the *Standard solution* (mg/mL)
C_U = concentration of Povidone in the *Sample solution* (mg/mL), calculated on the anhydrous basis

Acceptance criteria: NMT 0.001%

• **2-PYRROLIDONE**
Mobile phase: Water and methanol (19:1)
Standard solution: 30 µg/mL of 2-pyrrolidinone in *Mobile phase*
Sample solution: 5 mg/mL of Povidone in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Columns
Guard: 4.0-mm × 1.0-cm; packing L1
Analytical: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
[NOTE—The retention time of 2-pyrrolidinone is about 7 min.]
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000 theoretical plates for the 2-pyrrolidinone peak
Symmetry factor: NMT 1.5 for the 2-pyrrolidinone peak
Relative standard deviation: NMT 2.0% of 2-pyrrolidinone for six injections
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms and measure the responses for the 2-pyrrolidinone peak.
Calculate the percentage of 2-pyrrolidinone in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2-pyrrolidinone from the *Sample solution*
r_S = peak response of 2-pyrrolidinone from the *Standard solution*
C_S = concentration of 2-pyrrolidinone in the *Standard solution* (mg/mL)
C_U = concentration of Povidone in the *Sample solution* (mg/mL), calculated on the anhydrous basis

Acceptance criteria: NMT 3.0%

• **PEROXIDES**
Sample solution: 40 mg/mL of Povidone in water, calculated on the anhydrous basis
Blank: To 25 mL of the *Sample solution*, add 2 mL of 13% sulfuric acid.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 405 nm
Cell: 1 cm
Analysis
Sample: *Sample solution*
To 25 mL of the *Sample solution*, add 2 mL of titanium trichloride–sulfuric acid TS, and allow to stand for 30 min. Measure the absorbance of a portion of this solution against the *Blank*.
Acceptance criteria: NMT 0.35, corresponding to NMT 400 ppm, expressed as H_2O_2

Change to read:

• **FORMIC ACID**
Mobile phase: Diluted perchloric acid (1 in 700)
Standard solution: 10 µg/mL of formic acid in water
Sample stock solution: 20 mg/mL of Povidone in water
Sample solution: Transfer a suspension of strongly acidic ion-exchange resin (use the hydrogen form of ion-exchange resin) in water to a column of about 8 mm in inside diameter to give a packing depth of about 20 mm in length. Keep the strongly acidic ion-exchange resin layer constantly immersed in water. Pour 5 mL of water and adjust the flow rate so that water drops at a rate of about 1 mL/min. When the level of the water is near the top of the strongly acidic ion-exchange resin layer, introduce 100 mL of the *Sample stock solution* into the column. Disregard the first 2 mL of the eluate, then collect 1.5 mL of the solution, and use this as the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: ▲7.9▲ (USP 1-May-2019)-mm × 30-cm; ▲10▲ (USP 1-May-2019)-µm packing L17
Column temperature: 35°
Flow rate: 1.0 mL/min
[NOTE—The retention time of formic acid is about 8 min.]
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates for the formic acid peak
Symmetry factor: 0.5–1.5 for the formic acid peak
Relative standard deviation: NMT 2.0% of formic acid for six injections
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms and measure the responses for the formic acid peak.
Calculate the percentage of formic acid in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of formic acid from the *Sample solution*
r_S = peak response of formic acid from the *Standard solution*
C_S = concentration of formic acid in the *Standard solution* (mg/mL)
C_U = concentration of Povidone in the *Sample solution* (mg/mL), calculated on the anhydrous basis

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

• **PH** ⟨791⟩
Sample solution: 50 mg/mL in water
Acceptance criteria: 3.0–5.0 for Povidone having a nominal K-value of 30 or less; 4.0–7.0 for Povidone having a nominal K-value greater than 30
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 5.0%
• **K-VALUE**
Sample solution: Weigh a quantity of undried Povidone, equivalent on the anhydrous basis, to the amount specified in *Table 1*.

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Table 1

Nominal K-value	Quantity (g)
≤18	5.00
>18 to ≤95	1.00
>95	0.10

Dissolve it in 50 mL of water in a 100-mL volumetric flask, and dilute to volume. Allow to stand for 1 h.

Analysis

Samples: *Sample solution* and water

Determine the viscosity of the *Sample solution* and the water, using a capillary-tube viscometer (see *Viscosity—Capillary Methods* ⟨911⟩), at 25 ± 0.2°.

Calculate the K-value of Povidone:

$$\text{Result} = \left[\sqrt{300c\ \log z + (c + 1.5c\ \log z)^2} + 1.5c\ \log z - c\right] / \left(0.15c + 0.003c^2\right)$$

c = weight, on the anhydrous basis, of the specimen tested in each 100.0 mL of solution (g)

z = viscosity of the *Sample solution* relative to that of water

Acceptance criteria

K-value of Povidone having a stated (nominal) K-value of NMT 15: 85.0%–115.0% of the stated values

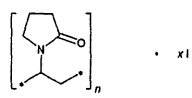
K-value of Povidone having a stated K-value or a stated K-value range with an average of more than 15: 90.0%–108.0% of the stated value or of the average of the stated range

ADDITIONAL REQUIREMENTS

- ***PACKAGING AND STORAGE:** Preserve in tight containers.◆
- **LABELING:** Label it to state, as part of the official title, the K-value or K-value range of Povidone.
- ***USP REFERENCE STANDARDS** ⟨11⟩
  USP Povidone RS

◆

Povidone–Iodine



$(C_6H_9NO)_n \cdot xI$

2-Pyrrolidinone, 1-ethenyl-, homopolymer, compound with iodine;

1-Vinyl-2-pyrrolidinone polymer, compound with iodine [25655-41-8].

DEFINITION

Povidone–Iodine is a complex of Iodine with Povidone. It contains NLT 9.0% and NMT 12.0% of available iodine (I), calculated on the dried basis.

IDENTIFICATION

- **A.**

Sample solution: 100 mg/mL

Analysis: Add 1 drop of the *Sample solution* to a mixture of 1 mL of starch TS and 9 mL of water.

Acceptance criteria: A deep blue color is produced.

- **B.**

Sample solution: 100 mg/mL

Analysis: Spread 1 mL of the *Sample solution* over an area of 20 cm × 20 cm on a glass plate, and allow to air-dry at room temperature in an atmosphere of low humidity overnight.

Acceptance criteria: A brown, dry, non-smearing film is formed, and it dissolves readily in water.

ASSAY

- **AVAILABLE IODINE**

Sample solution: Place 5 g of Povidone–Iodine in a 400-mL beaker, and add 200 mL of water. Cover the beaker, and stir by mechanical means at room temperature for NMT 1 h to dissolve as completely as possible.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Analysis: Titrate the *Sample solution* immediately with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 12.69 mg of iodine (I).

Acceptance criteria: 9.0%–12.0% of available iodine (I) on the dried basis

OTHER COMPONENTS

- **IODIDE ION**

Determination of total iodine

Sample: 500 mg of Povidone–Iodine

Titrimetric system

(See *Titrimetry* ⟨541⟩, *Residual Titrations*.)

Mode: Residual titration

Titrant: 0.1 N silver nitrate VS

Back-titrant 0.1 N ammonium thiocyanate VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 100 mL of water in a 250-mL conical flask. Add sodium bisulfite TS until the color of iodine has disappeared. Add 25.0 mL of *Titrant* and 10 mL of nitric acid.

Titrate the excess silver nitrate with *Back-titrant*, using ferric ammonium sulfate TS as the indicator. Perform a blank determination. Each mL of 0.1 N silver nitrate is equivalent to 12.69 mg of iodine (I). From the percentage of total iodine, calculated on the dried basis, subtract the percentage of available iodine (see *Assay* for available iodine), to obtain the percentage of iodide ion.

Acceptance criteria: NMT 6.6% on the dried basis

- **NITROGEN DETERMINATION,** *Method II* ⟨461⟩: 9.5%–11.5% of nitrogen (N) is found, calculated on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩

Sample: 2 g

Acceptance criteria: NMT 0.025%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Sample: 5.0 g

Analysis: Dry the *Sample* at 105° until the difference between two successive weighings at 1-h intervals is NMT 5.0 mg.

Acceptance criteria: NMT 8.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

USP Monographs

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Povidone–Iodine Topical Aerosol

» Povidone–Iodine Topical Aerosol is a solution of Povidone–Iodine under nitrogen in a pressurized container. It contains not less than 85.0 percent and not more than 120.0 percent of the labeled amount of iodine (I).

Packaging and storage—Preserve in pressurized containers, and avoid exposure to excessive heat.
Identification—Spray Topical Aerosol into a beaker or flask until about 50 mL has been collected, and allow to stand for 5 minutes to allow the entrapped propellant to escape. (Retain portions of the solution so obtained for the *pH* and *Assay* procedures.) The solution meets the requirements of the following tests.

A: Add 1 mL of a dilution containing about 0.05% of iodine to a mixture of 1 mL of starch TS and 9 mL of water: a deep blue color is produced.

B: Transfer 10 mL to a 50-mL conical flask, avoiding contact with the neck of the flask. Cover the mouth of the flask with a small disk of filter paper, and wet it with 1 drop of starch TS: no blue color appears within 60 seconds.
pH ⟨791⟩—The pH of the solution prepared for the *Identification* tests is not more than 6.0.
Other requirements—It meets the requirements for *Topical Aerosols* ⟨603⟩.
Assay—Transfer an accurately measured volume of the solution of Topical Aerosol prepared for the *Identification* tests, equivalent to about 50 mg of iodine, to a 100-mL beaker, and dilute with water to a total volume of not less than 30 mL. Titrate immediately with 0.02 N sodium thiosulfate VS, determining the endpoint potentiometrically, using a platinum-calomel electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N sodium thiosulfate is equivalent to 2.538 mg of iodine (I).

Povidone–Iodine Ointment

DEFINITION
Povidone–Iodine Ointment is an emulsion, solution, or suspension of Povidone–Iodine in a suitable water-soluble ointment base. It contains NLT 85.0% and NMT 120.0% of the labeled amount of iodine (I).

IDENTIFICATION
• **A.**
Sample solution: Alcohol dilution containing 0.05% of iodine
Analysis: Add 1 mL of the *Sample solution* to a mixture of 1 mL of starch TS and 9 mL of water.
Acceptance criteria: A deep blue color is produced.
• **B.**
Sample: 10 g
Analysis: Place the *Sample* in a 50-mL beaker, avoiding contact with the walls of the beaker. Cover the mouth of the beaker with a disk of filter paper, and wet it with 1 drop of starch TS.
Acceptance criteria: No blue color appears within 60 s.

ASSAY
• **PROCEDURE**
Sample: Nominally 50 mg of iodine from an accurately weighed quantity of Ointment
Titrimetric system
Mode: Direct titration
Titrant: 0.02 N sodium thiosulfate VS
Endpoint detection: Potentiometric
Analysis: Transfer the *Sample* to a 100-mL beaker, add water to make a total volume of NLT 30 mL, and stir until the Ointment is dissolved. Titrate immediately with *Titrant* to the endpoint, using a platinum–calomel electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N sodium thiosulfate is equivalent to 2.538 mg of iodine.
Acceptance criteria: 85.0%–120.0% of the labeled amount of iodine

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 50 mg/mL
Acceptance criteria: 1.5–6.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

Povidone–Iodine Cleansing Solution

DEFINITION
Povidone–Iodine Cleansing Solution is a solution of Povidone–Iodine with one or more suitable surface-active agents. It contains NLT 85.0% and NMT 120.0% of the labeled amount of iodine (I). It may contain a small amount of alcohol.

IDENTIFICATION
• **A.**
Sample solution 1: Dilution containing 0.05% of iodine
Analysis 1: Add 1 mL of *Sample solution 1* to a mixture of 1 mL of starch TS and 9 mL of water.
Acceptance criteria 1: A deep blue color is produced.
Sample solution 2: 10 mL
Analysis 2: Transfer *Sample solution 2* to a 50-mL conical flask, avoiding contact with the neck of the flask. Cover the mouth of the flask with a small disk of filter paper, and wet it with 1 drop of starch TS.
Acceptance criteria 2: No blue color appears within 60 s.
• **B.**
Sample solution: 2 mL
Analysis: To the *Sample solution*, in a glass-stoppered test tube, add 1 mL of peanut oil and 4 mL of water, and shake vigorously for 10 s. Allow to stand for 3 min.
Acceptance criteria: A stable emulsion is formed.

ASSAY
• **PROCEDURE**
Sample solution: Nominally 50 mg of iodine from an accurately measured volume of Solution
Titrimetric system
Mode: Direct titration
Titrant: 0.02 N sodium thiosulfate VS
Endpoint detection: Potentiometric
Analysis: Transfer the *Sample solution* to a 100-mL beaker, and add water to make a total volume of NLT 30 mL. Titrate immediately with *Titrant* to the endpoint, using a platinum–calomel electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N sodium thiosulfate is equivalent to 2.538 mg of iodine.

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Acceptance criteria: 85.0%–120.0% of the labeled amount of iodine

OTHER COMPONENTS

- **ALCOHOL DETERMINATION** ⟨611⟩ (if present): 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH)

SPECIFIC TESTS

- **pH** ⟨791⟩: 1.5–6.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Povidone–Iodine Topical Solution

DEFINITION

Povidone–Iodine Topical Solution is a solution of Povidone–Iodine. It contains NLT 85.0% and NMT 120.0% of the labeled amount of iodine (I). It may contain a small amount of alcohol.

IDENTIFICATION

- **A.**

Sample solution 1: Dilution containing 0.05% of iodine

Analysis 1: Add 1 mL of *Sample solution 1* to a mixture of 1 mL of starch TS and 9 mL of water.

Acceptance criteria 1: A deep blue color is produced.

Sample solution 2: 10 mL

Analysis 2: Transfer *Sample solution 2* to a 50-mL conical flask, avoiding contact with the neck of the flask. Cover the mouth of the flask with a small disk of filter paper, and wet it with 1 drop of starch TS.

Acceptance criteria 2: No blue color appears within 60 s.

ASSAY

- **PROCEDURE**

Sample solution: Nominally 50 mg of iodine from an accurately measured volume of Topical Solution

Titrimetric system

Mode: Direct titration

Titrant: 0.02 N sodium thiosulfate VS

Endpoint detection: Potentiometric

Analysis: Transfer the *Sample solution* to a 100-mL beaker, and add water to make a total volume of NLT 30 mL. Titrate immediately with *Titrant* to the endpoint, using a platinum–calomel electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N sodium thiosulfate is equivalent to 2.538 mg of iodine.

Acceptance criteria: 85.0%–120.0% of the labeled amount of iodine

OTHER COMPONENTS

- **ALCOHOL DETERMINATION** ⟨611⟩ (if present): 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH)

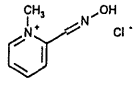
SPECIFIC TESTS

- **pH** ⟨791⟩: 1.5–6.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Pralidoxime Chloride



$C_7H_9ClN_2O$ 172.61

Pyridinium, 2-[(hydroxyimino)methyl]-1-methyl-, chloride (*E*)-;

(*E*)-2-Formyl-1-methylpyridinium chloride oxime [14018-50-9].

Unspecified isomer [51-15-0].

DEFINITION

Pralidoxime Chloride contains NLT 97.0% and NMT 102.0% of pralidoxime chloride ($C_7H_9ClN_2O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: A solution (1 in 10) meets the requirements.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Prepare a solution containing 8 mM sodium octanesulfonate and 2 mM tetraethylammonium chloride as follows. Transfer 1.87 g of sodium octanesulfonate monohydrate and 330 mg of tetraethylammonium chloride to a 1-L volumetric flask, add water to dissolve the mixture, and dilute with water to volume. Adjust with 0.01 N hydrochloric acid to a pH of 4.3.

Mobile phase: Acetonitrile and *Solution A* (17:83)

Diluent: Acetonitrile and water (17:83)

Standard stock solution: 1.25 mg/mL of USP Pralidoxime Chloride RS in water. Use sonication to dissolve, if necessary.

Standard solution: 0.125 mg/mL of USP Pralidoxime Chloride RS in *Diluent* from the *Standard stock solution*

Sample stock solution: 1.25 mg/mL of Pralidoxime Chloride in water. Use sonication to dissolve if necessary.

Sample solution: 0.125 mg/mL of Pralidoxime Chloride in *Diluent* from the *Sample stock solution*. [NOTE—This solution is stable for up to 6 h when stored at room temperature protected from light.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 3- or 3.5-µm packing L1

Flow rate: 0.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—USP Pralidoxime Chloride RS contains pralidoxime *anti*-isomer as a minor component. The relative retention times for pralidoxime *anti*-isomer and pralidoxime are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the pralidoxime *anti*-isomer and pralidoxime peaks

USP Monographs

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Tailing factor: NMT 1.5 for the pralidoxime chloride peak
Relative standard deviation: NMT 0.73% for the pralidoxime chloride peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pralidoxime chloride ($C_7H_9ClN_2O$) in the portion of Pralidoxime Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pralidoxime from the *Sample solution*
r_S = peak response of pralidoxime from the *Standard solution*
C_S = concentration of USP Pralidoxime Chloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Pralidoxime Chloride in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

OTHER COMPONENTS

• **CONTENT OF CHLORIDE**
Sample solution: Dissolve 300 mg of Pralidoxime Chloride in 150 mL of water.
Analysis: Add 20 mL of glacial acetic acid and 10 drops of (*p-tert*-octylphenoxy)nonaethoxyethanol to the *Sample solution*, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary corrections. Each milliliter of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.
Acceptance criteria: 20.2%–20.8% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
• **ORGANIC IMPURITIES**
Solution A, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard stock solution: Use the *Standard solution* prepared as directed in the *Assay*.
Standard solution: 0.125 µg/mL of USP Pralidoxime Chloride RS in *Diluent* from the *Standard stock solution*
Sensitivity solution: 0.0625 µg/mL of USP Pralidoxime Chloride RS in *Diluent* from the *Standard solution*
System suitability
Samples: *Standard stock solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between the pralidoxime *anti*-isomer and pralidoxime peaks, *Standard stock solution*
Relative standard deviation: NMT 5.0% for the pralidoxime chloride peak, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Pralidoxime Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pralidoxime from the *Standard solution*
C_S = concentration of USP Pralidoxime Chloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Pralidoxime Chloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*. The reporting level for impurities is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyridine-2-aldoxime	0.7	0.5
Pralidoxime *anti*-isomer[a]	0.9	2
Pralidoxime	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	3

[a] (*Z*)-2-[(Hydroxyimino)methyl]-1-methylpyridin-1-ium chloride.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 2.0%
• **STERILITY TESTS** ⟨71⟩: Where the label states that Pralidoxime Chloride is sterile, it meets the requirements.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Pralidoxime Chloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.10 USP Endotoxin Units/mg of pralidoxime chloride.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pralidoxime Chloride RS

Pralidoxime Chloride for Injection

DEFINITION

Pralidoxime Chloride for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of pralidoxime chloride ($C_7H_9ClN_2O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests*, *Chloride*: A solution (1 in 10) meets the requirements.
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Solution A: Prepare a solution containing 8 mM sodium octanesulfonate and 2 mM tetraethylammonium chloride

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as follows. Transfer 1.87 g of sodium octanesulfonate monohydrate and 330 mg of tetraethylammonium chloride to a 1-L volumetric flask, add water to dissolve the mixture, and dilute with water to volume. Adjust with 0.01 N hydrochloric acid to a pH of 4.3.

Mobile phase: Acetonitrile and *Solution A* (17:83)

Diluent: Acetonitrile and water (17:83)

Standard stock solution: 1.25 mg/mL of USP Pralidoxime Chloride RS in water. Use sonication to dissolve, if necessary.

Standard solution: 0.125 mg/mL of USP Pralidoxime Chloride RS in *Diluent* from the *Standard stock solution*

Sample stock solution: Nominally 1.25 mg/mL of pralidoxime chloride prepared as follows. Select a number of containers of Pralidoxime Chloride for Injection, the combined contents of which are equivalent to about 10 g of pralidoxime chloride. Dissolve the contents of each container in water and combine all of the solutions in a 1000-mL volumetric flask. Rinse each container with water and add the rinsings to the volumetric flask. Dilute with water to volume and mix. Transfer 25.0 mL of the resulting solution to a 200-mL volumetric flask and dilute with water to volume.

Sample solution: Nominally 0.125 mg/mL of pralidoxime chloride in *Diluent* from the *Sample stock solution*. [NOTE—This solution is stable for up to 6 h when stored at room temperature protected from light.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 3- or 3.5-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—USP Pralidoxime Chloride RS contains pralidoxime *anti*-isomer as a minor component. The relative retention times for pralidoxime *anti*-isomer and pralidoxime are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the pralidoxime *anti*-isomer and pralidoxime peaks
Tailing factor: NMT 1.5 for the pralidoxime chloride peak
Relative standard deviation: NMT 1.0% for the pralidoxime chloride peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pralidoxime chloride ($C_7H_9ClN_2O$) in the portion of Pralidoxime Chloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pralidoxime from the *Sample solution*
r_S = peak response of pralidoxime from the *Standard solution*
C_S = concentration of USP Pralidoxime Chloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pralidoxime chloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: Use the *Standard solution* prepared as directed in the *Assay*.
Standard solution: 0.125 µg/mL of USP Pralidoxime Chloride RS in *Diluent* from the *Standard stock solution*
System suitability
Samples: *Standard stock solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the pralidoxime *anti*-isomer and pralidoxime peaks, *Standard stock solution*
Relative standard deviation: NMT 5.0% for the pralidoxime chloride peak, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Pralidoxime Chloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pralidoxime from the *Standard solution*
C_S = concentration of USP Pralidoxime Chloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pralidoxime chloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyridine-2-aldoxime	0.7	0.5
Pralidoxime *anti*-isomer[a]	0.9	2
Pralidoxime	1.0	—
Any other individual impurity	—	0.2
Total impurities	—	3

[a] (*Z*)-2-[(Hydroxyimino)methyl]-1-methylpyridin-1-ium chloride.

SPECIFIC TESTS

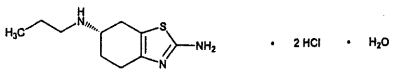
• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions*
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.10 USP Endotoxin Units/mg of pralidoxime chloride.
• **pH** ⟨791⟩: 3.5–4.5, in a solution (1 in 20)
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 2.0%
• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **OTHER REQUIREMENTS:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pralidoxime Chloride RS

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Pramipexole Dihydrochloride



$C_{10}H_{17}N_3S \cdot 2HCl \cdot H_2O$ 302.26

Benzothiazole-2,6-diamine, 4,5,6,7-tetrahydro-N^6-propyl-, dihydrochloride, monohydrate, (*S*)-;
(*S*)-2-Amino-4,5,6,7-tetrahydro-6-(propylamino)benzothiazole dihydrochloride monohydrate [191217-81-9].

DEFINITION

Pramipexole Dihydrochloride is a monohydrate and contains NLT 98.0% and NMT 102.0% of pramipexole dihydrochloride ($C_{10}H_{19}Cl_2N_3S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197A▲** (CN 1-May-2020) or ⟨197M⟩
  Wavelength range: ⟨197A⟩, 3800 cm^{-1} to 650 cm^{-1}; ⟨197M⟩, 4000 cm^{-1} to 600 cm^{-1}
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of pramipexole (*S*-enantiomer) in the *System suitability solution* in the test for *Limit of Pramipexole Related Compound D*.
- **C. Identification Tests—General ⟨191⟩, *Chemical Identification Tests, Chloride***
  Sample: 1 mg/mL of Pramipexole Dihydrochloride in water
  Acceptance criteria: Meets the requirements of test *A*

ASSAY

- **Procedure**

Solution A: Dissolve 9.1 g of potassium dihydrogen phosphate and 5.0 g of sodium 1-octanesulfonate monohydrate in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile and *Solution A* (50:50)
Diluent: Acetonitrile and *Solution A* (20:80)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
15	20	80
15.1	60	40
20	60	40

System suitability solution: 1.5 mg/mL of USP Pramipexole Dihydrochloride RS and 0.8 mg/mL of USP Pramipexole Related Compound A RS in *Diluent*
Standard solution: 1.5 mg/mL of USP Pramipexole Dihydrochloride RS in *Diluent*
Sample solution: 1.5 mg/mL of Pramipexole Dihydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 264 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40 ± 5°
Flow rate: 1.5 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for pramipexole related compound A and pramipexole are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between pramipexole related compound A and pramipexole, *System suitability solution*
Tailing factor: NMT 2.0 for pramipexole, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pramipexole dihydrochloride ($C_{10}H_{19}Cl_2N_3S$) in the portion of Pramipexole Dihydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pramipexole Dihydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Pramipexole Dihydrochloride (as monohydrate) in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of pramipexole dihydrochloride, 284.24
M_{r2} = molecular weight of pramipexole dihydrochloride monohydrate, 302.26

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.10%
- **Limit of Pramipexole Related Compound D**

Mobile phase: *n*-Hexane, dehydrated alcohol, and diethylamine (850:150:1)
System suitability stock solution: 1 mg/mL each of USP Pramipexole Dihydrochloride RS and USP Pramipexole Related Compound D RS in dehydrated alcohol
System suitability solution: 0.01 mg/mL each of USP Pramipexole Dihydrochloride RS and USP Pramipexole Related Compound D RS from the *System suitability stock solution* in *Mobile phase*
Standard stock solution: 2.0 mg/mL of USP Pramipexole Related Compound D RS in dehydrated alcohol
Standard solution: 1.5 µg/mL of USP Pramipexole Related Compound D RS in *Mobile phase* from the *Standard stock solution*
Sample solution: 0.3 mg/mL of Pramipexole Dihydrochloride prepared as follows. Transfer a suitable weighed quantity of Pramipexole Dihydrochloride into a suitable flask, dissolve in 25% of the flask volume of dehydrated alcohol, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 10-µm packing L51
Flow rate: 1.5 mL/min
Injection volume: 75 µL
System suitability
Sample: *System suitability solution*
[Note—The relative retention times for pramipexole related compound D (*R*-enantiomer) and

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pramipexole (*S*-enantiomer) are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between pramipexole related compound D and pramipexole

Tailing factor: NMT 2.4 for pramipexole

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pramipexole related compound D in the portion of Pramipexole Dihydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pramipexole related compound D from the *Sample solution*

r_S = peak response of pramipexole related compound D from the *Standard solution*

C_S = concentration of USP Pramipexole Related Compound D RS in the *Standard solution* (mg/mL)

C_U = concentration of Pramipexole Dihydrochloride (as monohydrate) in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0% of pramipexole related compound D

• **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 7.5 µg/mL of USP Pramipexole Dihydrochloride RS and 3 µg/mL of USP Pramipexole Related Compound A RS in *Diluent*

Standard solution: 1.5 µg/mL of USP Pramipexole Dihydrochloride RS in *Diluent*

Sample solution: 1.5 mg/mL of Pramipexole Dihydrochloride in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 6.0 between pramipexole related compound A and pramipexole, *System suitability solution*

Tailing factor: NMT 2.0 for pramipexole, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Pramipexole Dihydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of pramipexole from the *Standard solution*

C_S = concentration of USP Pramipexole Dihydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Pramipexole Dihydrochloride (as monohydrate) in the *Sample solution* (mg/mL)

F = relative response factor from *Table 2*

M_{r1} = molecular weight of pramipexole dihydrochloride, 284.24

M_{r2} = molecular weight of pramipexole dihydrochloride monohydrate, 302.26

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)
Pramipexole propionamide[b]	0.5	1.0	0.15
Pramipexole related compound A	0.7	1.6	0.15
Pramipexole	1.0	—	—
N-Propylpramipexole[c]	1.4	1.0	0.15
Pramipexole dimer[d]	1.7	1.0	0.15
Any unspecified individual impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] Relative response factor is relative to pramipexole dihydrochloride.

[b] (*S*)-*N*-(2-Amino-4,5,6,7-tetrahydrobenzothiazol-6-yl)propionamide.

[c] (*S*)-2,6-Di(propylamino)-4,5,6,7-tetrahydrobenzothiazole.

[d] *N* ^{6},*N* $^{6'}$-[2-Methylpentane-1,3-diyl]bis(4,5,6,7-tetrahydrobenzothiazole-2,6-diamine). This is a dimer of pramipexole (a mixture of four possible isomers).

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NLT 4.5% and NMT 7.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from moisture and light.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Pramipexole Dihydrochloride RS

[NOTE—Supplied in monohydrate form.]

USP Pramipexole Related Compound A RS

(*S*)-4,5,6,7-Tetrahydrobenzothiazole-2,6-diamine.

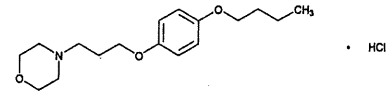
$C_7H_{11}N_3S$ 169.25

USP Pramipexole Related Compound D RS

(*R*)-2-Amino-4,5,6,7-tetrahydro-6-(propylamino) benzothiazole.

$C_{10}H_{17}N_3S$ 211.33

Pramoxine Hydrochloride



$C_{17}H_{27}NO_3 \cdot HCl$ 329.86

Morpholine, 4-[3-(4-butoxyphenoxy)propyl]-, hydrochloride; 4-[3-(*p*-Butoxyphenoxy)propyl]morpholine hydrochloride [637-58-1].

DEFINITION

Pramoxine Hydrochloride contains NLT 98.0% and NMT 102.0% of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: Dissolve 8.71 g of dibasic potassium phosphate in about 800 mL of water, adjust with dilute phosphoric acid (1 in 10) to a pH of 7.5 ± 0.1, and add water to make 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (55:45)

Standard solution: 0.5 mg/mL of USP Pramoxine Hydrochloride RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Pramoxine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 224 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$) in the portion of Pramoxine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pramoxine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Pramoxine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 170°–174°
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 1 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Pramoxine Hydrochloride RS

Pramoxine Hydrochloride Cream

DEFINITION

Pramoxine Hydrochloride Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$) in a suitable water-miscible base.

IDENTIFICATION

- **A.**

Standard: 50 mg of USP Pramoxine Hydrochloride RS

Sample: Nominally 50 mg of pramoxine hydrochloride from a quantity of Cream

Analysis: Dissolve the *Standard* and the *Sample* separately in a mixture of 25 mL of methanol and 75 mL of ether, and extract each with three 25-mL portions of a mixture of equal volumes of 3 N hydrochloric acid and water. Discard the methanol–ether solutions, render the combined extracts alkaline with 25 mL of 5 N sodium hydroxide, and extract the pramoxine with 50 mL of chloroform. Evaporate the clear chloroform extracts with the aid of a current of air to dryness.

Acceptance criteria: The UV absorption spectrum of a solution (1 in 100,000) of the residue so obtained from the *Sample*, in 0.1 N hydrochloric acid, exhibits maxima and minima at the same wavelengths as that of a similar solution of the residue similarly obtained from the *Standard*.

- **B.**

Sample: A 5-mg portion of the pramoxine obtained in *Identification* test *A*

Analysis: To the *Sample* add 1 drop of nitric acid. To the yellow solution cautiously add 5 drops of ammonium hydroxide.

Acceptance criteria: A red-brown precipitate is formed.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 3.5 g of dibasic potassium phosphate in 100 mL of water. Adjust the solution by the addition of phosphoric acid solution (1:1) to a pH of 7.5 ± 0.1.

Mobile phase: Acetonitrile, *Buffer*, and water (22:1:17)

Internal standard solution: 4 µL/mL of dibutyl phthalate in methanol

Standard stock solution: 2 mg/mL of USP Pramoxine Hydrochloride RS in methanol

Standard solution: Transfer 10 mL of the *Standard stock solution* and 5 mL of the *Internal standard solution* into a 100-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Transfer nominally 18 mg of pramoxine hydrochloride from a portion of Cream to a glass-stoppered, 250-mL conical flask. Add 15.0 mL of isopropyl alcohol and 40.0 mL of methanol, and heat on a steam bath, with swirling, to dissolve the Cream. Add 40.0 mL of methanol and 5.0 mL of the *Internal standard solution*. Cool the flask to a temperature of 10° or less to precipitate the waxes, and filter the solution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 224 nm

Columns

Guard: 4.6-mm × 3-cm; packing L1

Analytical: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

USP Monographs

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[NOTE—The relative retention times for pramoxine hydrochloride and dibutyl phthalate are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.4 between pramoxine hydrochloride and dibutyl phthalate

Relative standard deviation: NMT 2.0% in three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of pramoxine hydrochloride to the internal standard from the *Sample solution*

R_S = peak response ratio of pramoxine hydrochloride to the internal standard from the *Standard solution*

C_S = concentration of USP Pramoxine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pramoxine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Pramoxine Hydrochloride RS

Pramoxine Hydrochloride Jelly

DEFINITION

Pramoxine Hydrochloride Jelly contains NLT 94.0% and NMT 106.0% of the labeled amount of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$).

IDENTIFICATION

• **A.**

Sample solution: Place nominally 5 mg of pramoxine hydrochloride from a quantity of Jelly in a glass-stoppered conical flask. Add 25 mL of chloroform, and shake for 15 min. Filter into a small porcelain evaporating dish, and evaporate in a current of air on a steam bath.

Analysis: Add 1 drop of nitric acid to the residue. To the resulting yellow solution cautiously add 5 drops of ammonium hydroxide.

Acceptance criteria: A red-brown precipitate is formed.

ASSAY

• **PROCEDURE**

Standard solution: 150 µg/mL of USP Pramoxine Hydrochloride RS in 0.5 N sulfuric acid

Sample solution

[NOTE—If emulsions form, 2–5 mL of alcohol may be added to separate the phases.]

Transfer nominally 15 mg of pramoxine hydrochloride from a quantity of Jelly to a small beaker, and dissolve the Jelly in 0.1 N sulfuric acid, using four 5-mL portions. Warm each portion on a steam bath, and transfer to a 125-mL separator. Shake the separator vigorously after each transfer to complete the dissolution of the Jelly. To the cooled solution in the separator add 20 mL of ether, shake carefully, and proceed as directed in *Salts of Organic Nitrogenous Bases* ⟨501⟩, *Assay Preparation*, beginning with "filter the acid phase into a second 125-mL separator", except to combine the final 0.5 N sulfuric acid extracts in a 100-mL volumetric flask. Dilute with the acid to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 286 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in *Salts of Organic Nitrogenous Bases* ⟨501⟩, *Procedure*, diluting 20.0 mL each of the *Standard solution* and the *Sample solution* with 0.5 N sulfuric acid to 50.0 mL.

Calculate the percentage of the labeled amount of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$) in the portion of Jelly taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Pramoxine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pramoxine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 94.0%–106.0%

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

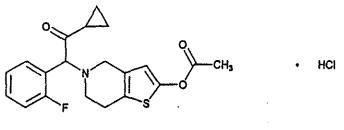
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably in collapsible tubes.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Pramoxine Hydrochloride RS

Prasugrel Hydrochloride



$C_{20}H_{20}FNO_3S \cdot HCl$ 409.90

Ethanone, 2-[2-(acetyloxy)-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl]-1-cyclopropyl-2-(2-fluorophenyl)-, hydrochloride; 5-[2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl acetate hydrochloride [389574-19-0].

USP Monographs

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DEFINITION

Prasugrel Hydrochloride contains NLT 97.0% and NMT 102.0% of prasugrel hydrochloride ($C_{20}H_{20}FNO_3S \cdot HCl$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the prasugrel peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL ⟨191⟩, *Chemical Identification Tests, Chloride*:** Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 10 mM monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.8.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Diluent: Acetonitrile and water (70:30)
Standard solution: 0.1 mg/mL of USP Prasugrel Hydrochloride RS in *Diluent*
Sample solution: 0.1 mg/mL of Prasugrel Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2.5 times the retention time of prasugrel
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0%
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of prasugrel hydrochloride ($C_{20}H_{20}FNO_3S \cdot HCl$) in the portion of Prasugrel Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of prasugrel from the *Sample solution*
r_S = peak response of prasugrel from the *Standard solution*
C_S = concentration of USP Prasugrel Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Prasugrel Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%
- **ORGANIC IMPURITIES, PROCEDURE 1**

Buffer: 10 mM monobasic potassium phosphate in water
Mobile phase: Acetonitrile, tetrahydrofuran, and *Buffer* (10:25:65)
Diluent: Acetonitrile and water (70:30)
Solution A: 150 µL of piperidine in 50 mL of acetonitrile
System suitability stock solution: 0.1 mg/mL of USP Prasugrel Hydrochloride RS in *Diluent* prepared as follows. Transfer 5 mg of USP Prasugrel Hydrochloride RS to a 50-mL volumetric flask. Add 20 mL of *Diluent* and mix to dissolve. Add 1 mL of *Solution A* and dilute with *Diluent* to volume. Heat the solution at 50° for 1 h and cool to room temperature.
System suitability solution: 0.02 mg/mL of USP Prasugrel Hydrochloride RS from the *System suitability stock solution* in *Diluent*
Standard solution: 0.015 mg/mL of USP Prasugrel Hydrochloride RS in *Diluent*
Sensitivity solution: 0.3 µg/mL of USP Prasugrel Hydrochloride RS from the *Standard solution* in *Diluent*
Sample solution: 1.5 mg/mL of Prasugrel Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Temperatures
Autosampler: 10°
Column: 40°
Flow rate: 0.9 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of prasugrel
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between desacetyl prasugrel diastereomer 1 and desacetyl prasugrel diastereomer 2 peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified process impurity in the portion of Prasugrel Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*
r_S = peak response of prasugrel from the *Standard solution*
C_S = concentration of USP Prasugrel Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Prasugrel Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desacetyl prasugrel diastereomer 1[a]	0.43	—
Desacetyl prasugrel diastereomer 2[a]	0.45	—
Desfluoro prasugrel[b]	0.9	0.20
Prasugrel	1.0	—
4-Fluoro prasugrel[c]	1.2	0.15

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Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3-Fluoro prasugrel[d]	1.3	0.30

[a] 5-[2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one. Desacetyl prasugrel diastereomer 1 and desacetyl prasugrel diastereomer 2 are a pair of diastereomers. They are used for resolution measurement only.
[b] 5-(2-Cyclopropyl-2-oxo-1-phenylethyl)-4,5,6,7-tetrahydrothieno[3,2-*c*] pyridin-2-yl acetate.
[c] 5-[2-Cyclopropyl-1-(4-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl acetate.
[d] 5-[2-Cyclopropyl-1-(3-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl acetate.

• **Organic Impurities, Procedure 2**

Buffer: 25 mM monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and water (90:10)
Solution C: 150 µL of piperidine in 50 mL of acetonitrile
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
30	0	100
37	0	100
38	100	0
45	100	0

Diluent: Acetonitrile and water (70:30)
System suitability solution: 0.1 mg/mL of USP Prasugrel Hydrochloride RS in *Diluent* prepared as follows. Transfer 5 mg of USP Prasugrel Hydrochloride RS to a 50-mL volumetric flask. Add 20 mL of *Diluent* and mix to dissolve. Add 1 mL of *Solution C* and dilute with *Diluent* to volume. Heat the solution at 50° for 1 h and cool to room temperature.
Standard solution: 0.1 mg/mL of USP Prasugrel Hydrochloride RS in *Diluent*
Sensitivity solution: 2 µg/mL of USP Prasugrel Hydrochloride RS from the *Standard solution* in *Diluent*
Sample solution: 10 mg/mL of Prasugrel Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 4-µm packing L87
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[Note—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 0.9 between the desacetyl prasugrel diastereomer 1 and desacetyl prasugrel diastereomer 2 peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified and unspecified degradation product in the portion of Prasugrel Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of prasugrel from the *Standard solution*
C_S = concentration of USP Prasugrel Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Prasugrel Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desacetyl hydroxyprasugrel[a]	0.74	1.0	0.15
Prasugrel diketone[b]	0.78	1.0	0.20
Desacetyl prasugrel diastereomer 1[c]	0.86	1.0	0.20
Desacetyl prasugrel diastereomer 2[c]	0.87	1.0	0.50
Prasugrel	1.0	—	—
Prasugrel chlorobutyryl analog[d]	1.06	0.73	0.30
Any individual unspecified degradation product	—	1.0	0.10
Total degradation products	—	—	1.0

[a] 5-[2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-7a-hydroxy-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one.
[b] 1-Cyclopropyl-2-(2-fluorophenyl)ethane-1,2-dione.
[c] 5-[2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one. Desacetyl prasugrel diastereomer 1 and desacetyl prasugrel diastereomer 2 are a pair of diastereomers.
[d] 5-[5-Chloro-1-(2-fluorophenyl)-2-oxopentyl]-4,5,6,7-tetrahydrothieno[3,2-*c*] pyridin-2-yl acetate.

SPECIFIC TESTS

• **Water Determination** ⟨921⟩, *Method I, Method Ic*: NMT 0.50%

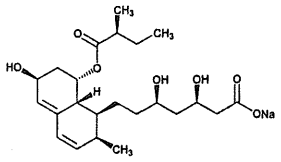
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Prasugrel Hydrochloride RS

USP Monographs

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Pravastatin Sodium



$C_{23}H_{35}NaO_7$ 446.52

1-Naphthaleneheptanoic acid, 1,2,6,7,8,8a-hexahydro-β,δ,6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy)-, monosodium salt, [1*S*-[1α(β*S**,δ*S**),2α,6α,8β(*R**),8aα]]-;
Sodium (β*R*,δ*R*,1*S*,2*S*,6*S*,8*S*,8a*R*)-1,2,6,7,8,8a-hexahydro-β,δ,6,8-tetrahydroxy-2-methyl-1-naphthaleneheptanoate, 8-[(2*S*)-2-methylbutyrate] [81131-70-6].

DEFINITION

Pravastatin Sodium contains NLT 97.5% and NMT 102.0% of pravastatin sodium ($C_{23}H_{35}NaO_7$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements ▲of test *A*▲ (CN 1-May-2018)

Add the following:

▲• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 1S (USP41)

ASSAY

Change to read:

• **PROCEDURE**

Solution A: 0.08 M phosphoric acid solution. Adjust with a 25% sodium hydroxide solution to a pH of 5.5.
Solution B: Acetonitrile and *Solution A* (20:80)
Solution C: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
7.0	90	10
10.0	62.5	37.5
17.0	62.5	37.5
17.1	100	0
20.0	100	0

System suitability solution: 0.25 mg/mL of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS and 0.001 mg/mL of USP Pravastatin Related Compound A RS in methanol
Standard solution: 0.25 mg/mL of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS in methanol
Sample solution: 0.2 mg/mL of Pravastatin Sodium in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.0-mm × 10-cm; 3-µm packing *L1*
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for pravastatin and pravastatin related compound A are about 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.2 between pravastatin and pravastatin related compound A
Relative standard deviation: NMT 2.0% for the pravastatin peak
Analysis
Samples: *Standard solution* and *Sample solution*
▲▲ 1S (USP41) Calculate the percentage of pravastatin sodium ($C_{23}H_{35}NaO_7$) in the portion of Pravastatin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of pravastatin from the *Sample solution*
r_S = peak response of pravastatin from the *Standard solution*
C_S = concentration of ▲USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS▲ 1S (USP41) in the *Standard solution* (mg/mL)
C_U = concentration of Pravastatin Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of pravastatin sodium, 446.52
M_{r2} = molecular weight of pravastatin 1,1,3,3-tetramethylbutylamine, 553.78

Acceptance criteria: 97.5%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

Delete the following:

▲• **HEAVY METALS** ⟨231⟩, *Method II*: NMT 20 ppm▲ (Official 1-Jan-2018)

Change to read:

• **ORGANIC IMPURITIES**

[NOTE—The *Standard solution* and the *Sample solution* are maintained at 15° until injected into the chromatograph.]
Diluent: Methanol and water (50:50)
Solution A: 0.08 M phosphoric acid solution. Adjust with triethylamine to a pH of 7.0.
Solution B: Acetonitrile, *Solution A*, and water (18:30:52)
Solution C: Acetonitrile, *Solution A*, and water (60:30:10)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution B (%)	Solution C (%)
0	100	0
3.0	100	0

USP Monographs

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Table 2 *(continued)*

Time (min)	Solution B (%)	Solution C (%)
26.5	0	100
26.6	100	0
30.0	100	0

System suitability solution: 0.6 mg/mL of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS and 0.001 mg/mL of USP Pravastatin Related Compound A RS in *Diluent*. [NOTE—USP Pravastatin Related Compound A RS is a sodium salt of 3α-hydroxyisocompactin acid.]
Standard solution: 1.25 µg/mL of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS in *Diluent*
Sample solution: 0.5 mg/mL of Pravastatin Sodium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1.
[NOTE—Alternatively, a 4.0-mm × 10-cm; 3-µm packing L1 can be used.]
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for pravastatin and pravastatin related compound A are about 1.0 and 1.1, respectively. ▲▲ 1S (USP41)]
Suitability requirements
Resolution: NLT 2.0 between pravastatin and pravastatin related compound A, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
▲▲ 1S (USP41) Calculate the percentage of each ▲specified or any unspecified▲ 1S (USP41) impurity in the portion of Pravastatin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pravastatin from the *Standard solution*
C_S = concentration of ▲USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS▲ 1S (USP41) in the *Standard solution* (mg/mL)
C_U = concentration of Pravastatin Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of pravastatin sodium, 446.52
M_{r2} = molecular weight of pravastatin 1,1,3,3-tetramethylbutylamine, 553.78

Acceptance criteria: See *Table 3*. The reporting level for impurities is 0.05%. ▲▲ 1S (USP41)

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3″-Hydroxypravastatin	0.33	0.2
6′-Epi-pravastatin	0.92	0.3

Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
▲Pravastatin	1.0	—▲ 1S (USP41)
▲Pravastatin related compound A▲ 1S (USP41)	1.1	0.2
Pentanoyl impurity[▲a▲ 1S (USP41)]	1.2	0.2
Pravastatin lactone	1.8	0.2
Compactin	3.1	0.2
Any ▲unspecified▲ 1S (USP41) impurity	—	▲0.10▲ 1S (USP41)
Total impurities	—	0.6

[a] (3*R*,5*R*)-3,5-Dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylpentanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoic acid.

SPECIFIC TESTS

Change to read:

• **LIMIT OF ALCOHOL** (if present)
Standard stock solution 1: Pipet 2 mL of dehydrated alcohol into a 100-mL volumetric flask, and dilute with water to volume.
Standard stock solution 2: Pipet 10 mL of *Standard stock solution 1* into a 100-mL volumetric flask, and dilute with water to volume.
Sample stock solution: Transfer 0.2 g of Pravastatin Sodium to a 20-mL volumetric flask, and dilute with water to volume.
Sample solution: Pipet 5 mL of the *Sample stock solution* into a vial fitted with a septum and a crimp cap, add 1 mL of water, seal the vial, and mix. Heat the sealed vial at 80° for 60 min.
Standard solution: Pipet 1 mL of *Standard stock solution 2* into a vial fitted with a septum and a crimp cap, and calculate the amount of alcohol (W_A) added in grams (the specific gravity of dehydrated alcohol is 0.79 g/mL). Add 5 mL of the *Sample solution* to the same vial, and seal the vial. Heat the sealed vial at 80° for 60 min.
Blank solution: Pipet 6 mL of water into a vial fitted with a septum and a crimp cap, and seal the vial. Heat the sealed vial at 80° for 60 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused silica capillary; coated with a 3-µm film of stationary phase G43
Temperatures
Transfer line: 85°
Injection port: 140°
Detector: 250°
Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	20
40	10	240	—
240	0	240	20

Carrier gas: Helium

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Flow rate: Linear velocity of 35 cm/s
Injection volume: 1 mL
Injection type: Split ratio, 1:5
System suitability
Sample: *Blank solution*
Suitability requirement: No interfering peaks are observed.
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of alcohol in the portion of Pravastatin Sodium taken:

$$\text{Result} = [r_U/(r_S - r_U)] \times (W_A/W) \times D \times 100$$ 1S (USP41)

r_U = peak response of alcohol from the *Sample solution*
r_S = peak response of alcohol from the *Standard solution*
W_A = amount of alcohol added (g)
W = weight of Pravastatin Sodium taken to prepare the *Sample solution* (g)
D = dilution factor for the *Sample solution*, 4 1S (USP41)

Acceptance criteria: NMT 3.0%
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 5 mg/mL in water
Acceptance criteria: +150° to +160° (at 20°), calculated on the anhydrous and solvent-free basis
• **PH** ⟨791⟩**:** 7.2–9.0, in a solution (1 in 20)
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 4.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store as per labeling instructions. Possible storage conditions could include the following, in the presence of stability data supporting the condition: Store under nitrogen in a cold place. Store at room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS (ERR 1-Oct-2018)
2,4,4-Trimethylpentan-2-amine (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-{[(*S*)-2-methylbutanoyl]oxy}-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoate.
$C_{23}H_{36}O_7 \cdot C_8H_{19}N$ 553.78 1S (USP41)
USP Pravastatin Sodium RS
USP Pravastatin Related Compound A RS
Sodium (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*R*,3*S*,8*S*,8a*R*)-3-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]heptanoate.
$C_{23}H_{35}NaO_7$ 446.51 1S (USP41)

Pravastatin Sodium Tablets

» Pravastatin Sodium contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$).

Packaging and storage—Preserve in tight containers. Protect from moisture and light. Store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Pravastatin Related Compound A RS
3-α-Hydroxyisocompactinor sodium (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,3*S*,8*S*,8a*R*)-3-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]heptanoate.
$C_{23}H_{35}NaO_7$ 446.51
USP Pravastatin Related Compound B RS
6′-Epi-pravastatinor sodium (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoate.
$C_{23}H_{35}NaO_7$ 446.51
USP Pravastatin Sodium RS
USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS

Identification—
A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Change to read:

B: *Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U* (CN 1-May-2020)—Finely powder a number of Tablets, and extract with water a portion equivalent to about 10 mg of pravastatin sodium. The UV absorption spectrum of a solution of pravastatin sodium in water containing about 10 µg per mL exhibits maxima at the same wavelength as that of a similar solution of USP Pravastatin Sodium RS, concomitantly measured between 220 and 340 nm.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{23}H_{35}NaO_7$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 238 nm on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS in the same *Medium.*
[NOTE—To express the concentration of the Standard solution as pravastatin sodium, use the conversion factor of (446.51/553.78), in which 446.51 and 553.78 are the molecular weights of pravastatin sodium and pravastatin 1,1,3,3-tetramethylbutylamine, respectively.]
Tolerances—Not less than 80% (*Q*) of the labeled amount of $C_{23}H_{35}NaO_7$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩**:** meet the requirements.
Related compounds— [NOTE—Maintain the *Test solution* at 15° until injected into the chromatograph. Without refrigeration, the *Test solution* should be prepared fresh.]
Diluent—Prepare a mixture of methanol and water (1:1).
Buffer pH 7.0—Prepare a 0.08 M phosphoric acid solution, adjust with triethylamine to a pH of 7.0, and mix.
Solution A—Prepare a filtered and degassed mixture of water, *Buffer pH 7.0,* and acetonitrile (52:30:18).
Solution B—Prepare a filtered and degassed mixture of acetonitrile, *Buffer pH 7.0,* and water (60:30:10).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS in *Diluent,* and dilute quantitatively, and stepwise if necessary,

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with *Diluent* to obtain a solution having a known concentration of about 1.25 µg of pravastatin 1,1,3,3-tetramethylbutylamine per mL.

System suitability solution—Dissolve accurately weighed quantities of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS and USP Pravastatin Related Compound A RS in *Diluent* to obtain a solution containing about 0.6 mg of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS and 0.001 mg of USP Pravastatin Related Compound A RS per mL. [NOTE—USP Pravastatin Related Compound A RS is a sodium salt of 3α-hydroxyisocompactin acid.]

Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of pravastatin sodium, to a 100-mL volumetric flask. Add 60 mL of the *Diluent*, sonicate for 15–20 minutes, dilute with *Diluent* to volume, and mix. Pass through a 0.45-µm nylon filter.

Chromatographic system (see *Chromatography* ⟨621⟩—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 7.5-cm column that contains 3.5-µm packing L1. Alternatively, a 4.0-mm × 10-cm column that contains 3-µm packing L1 can be used. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–3.0	100	0	isocratic
3.0–26.5	100→0	0→100	linear gradient
26.5–26.6	0→100	100→0	linear gradient
26.6–30.0	100	0	re-equilibration

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for pravastatin and 1.1 for pravastatin related compound A; and the resolution, *R,* between pravastatin and pravastatin related compound A is not less than 2.0. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 10.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, identify the impurities listed in *Table 1,* and measure the peak responses.

Table 1

Name	Relative Retention Time	Limit (%)
Oxidation impurity[1]	0.61	1
6′-Epipravastatin[2]	0.92	0.3
Pravastatin sodium	1.0	—
Pravastatin lactone	1.8	2
Any other individual impurity	—	0.2
Total impurities	—	3

[1] Sodium (3*R*,5*R*)-3,5-dihydroxy-7-((1*S*,2*S*)-6-hydroxy-2-methyl-1,2-dihydronaphthalen-1-yl)heptanoate.
[2] USP Pravastatin Related Compound B RS.

Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100\times(446.51/553.78)(C_S/C_T)(r_i/r_S)$$

in which 446.51 and 553.78 are the molecular weights of pravastatin sodium and pravastatin 1,1,3,3-tetramethylbutylamine, respectively; C_S is the concentration, in mg per mL, of pravastatin 1,1,3,3-tetramethylbutylamine in the *Standard solution;* C_T is the nominal concentration, in mg per mL, of pravastatin sodium in the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the pravastatin peak response obtained from the *Standard solution.* The reporting level for impurities is 0.1%.

Assay— [NOTE—The *Standard preparation, Assay stock preparation,* and *Assay preparation* can be stored for up to 7 days at room temperature.]

Mobile phase—Prepare a filtered and degassed mixture of methanol, water, glacial acetic acid, and triethylamine (500:500:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent 1—Transfer 16.4 g of anhydrous sodium acetate into a 2000-mL volumetric flask. Add 1600 mL of water, adjust with glacial acetic acid to a pH of 5.6, dilute with water to volume, and mix.

Diluent 2—Prepare a mixture of *Diluent 1* and methanol (80:20).

Standard preparation—Transfer an accurately weighed quantity of USP Pravastatin 1,1,3,3-Tetramethylbutylamine RS to a suitable volumetric flask, and dissolve in *Diluent 1* using sonication to obtain a solution having a known concentration of about 0.6 mg of pravastatin 1,1,3,3-tetramethylbutylamine per mL. Dilute 5.0 mL of this solution with *Diluent 2* to 25.0 mL, and mix.

Assay stock preparation—Transfer not fewer than 5 Tablets to a suitable volumetric flask with at least a (*NL*×2)-mL capacity, *N* being the number of Tablets transferred, and *L* being the label claim per Tablet, filled to at least 80% capacity with *Diluent 1*. [NOTE—It is necessary to fill the flask to 80% capacity to maintain the correct pH throughout the preparation.] Shake for at least 1 hour, and sonicate for at least 15 minutes with periodic shaking of the flask by hand, until the Tablets have completely disintegrated. Allow to cool, and dilute with *Diluent 1* to volume. Centrifuge a portion of the solution for 15 minutes at 2000 rpm, or filter.

Assay preparation—Dilute approximately 5 mL of the *Assay stock preparation* with *Diluent 2* to obtain a solution having an expected concentration of about 0.1 mg per mL, based on the label claim.

Resolution solution—Transfer about 2 mg of USP Pravastatin Related Compound B RS to a 10-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 0.1 mL of this solution and 1.0 mL of the *Standard preparation* to a small tube, and mix.

[NOTE—Pravastatin related compound B is the 6′-epipravastatin sodium.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 5-cm column than contains endcapped packing L1. Alternatively, a 3.9-mm × 7.5-cm column containing endcapped packing L1 can be used. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for pravastatin related compound B and 1.0 for pravastatin; the resolution, *R,* between the pravastatin related compound B and the pravastatin peaks is not less than 3.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak response for pravastatin. Calculate the percentage of

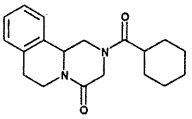
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pravastatin sodium ($C_{23}H_{35}NaO_7$) in the portion of Tablets taken by the formula:

$$100(446.51/553.78)(CVD/NL)(r_U/r_S)$$

in which 100 is the conversion factor to percentage; 446.51 and 553.78 are the molecular weights of pravastatin sodium and pravastatin 1,1,3,3-tetramethylbutylamine, respectively; *C* is the concentration, in mg per mL, of pravastatin 1,1,3,3-tetramethylbutylamine in the *Standard preparation*; *V* is the volume, in mL, of the *Assay stock preparation*; *D* is the dilution factor of the *Assay preparation*; *N* is the number of Tablets taken to prepare the *Assay stock preparation*; *L* is the label claim, in mg of pravastatin sodium per Tablet; and r_U and r_S are the pravastatin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Praziquantel



$C_{19}H_{24}N_2O_2$ 312.41

4*H*-Pyrazino[2,1-*a*]isoquinolin-4-one, 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-;

2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one [55268-74-1].

DEFINITION

Praziquantel contains NLT 98.5% and NMT 101.0% of praziquantel ($C_{19}H_{24}N_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (60:40)

Standard solution: 0.18 mg/mL of USP Praziquantel RS in *Mobile phase*

Sample solution: 0.18 mg/mL of Praziquantel in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 25-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of praziquantel ($C_{19}H_{24}N_2O_2$) in the portion of Praziquantel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Praziquantel RS in the *Standard solution* (mg/mL)

C_U = concentration of Praziquantel in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF PHOSPHATE**

Solution A: Dissolve 250 mg of cupric sulfate and 4.5 g of ammonium acetate in sufficient 2 N acetic acid to obtain 100 mL of solution.

Solution B: 30 mg/mL of ammonium molybdate

Solution C: Grind in a mortar 5 g of sodium sulfite, 94.3 g of sodium metabisulfite, and 700 mg of 4-amino-3-hydroxy-1-naphthalenesulfonic acid. Dissolve 1.5 g of this mixture in 10 mL of water, heating gently if necessary. Use this solution only when freshly prepared.

Standard stock solution: 100 µg/mL of phosphate $(PO_4)^{3-}$ prepared as follows. Dissolve 143.3 mg of dried monobasic potassium phosphate in water to make 1000 mL of solution.

Standard solution: 5 µg/mL of phosphate $(PO_4)^{3-}$ from the *Standard stock solution*

Sample solution: 10 mg/mL of Praziquantel, prepared as follows. Add 30 mL of water to 500 mg of sample, and heat to boiling. Allow to cool, and filter, collecting the filtrate in a 50-mL volumetric flask. Wash the filter with water, collect the washings in the volumetric flask, and dilute with water to volume.

Analysis: Treat 10 mL of the *Standard solution* and 10 mL of the *Sample solution* as follows. To each, add 5 mL of *Solution A*, 2 mL of *Solution B*, 1 mL of *Solution C*, and 1 mL of perchloric acid solution (3 in 100); mix; and allow to stand for 15 min.

Acceptance criteria: The *Sample solution* does not have a blue color that is darker than that of the *Standard solution* (0.05%).

- **ORGANIC IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.04 mg/mL each of USP Praziquantel Related Compound A RS, USP Praziquantel Related Compound B RS, and USP Praziquantel Related Compound C RS in *Mobile phase*

Sample solution: 20 mg/mL of Praziquantel in *Mobile phase*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of praziquantel related compound A, praziquantel related compound B, or praziquantel related compound C in the portion of Praziquantel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of praziquantel related compound A, praziquantel related compound B, or praziquantel related compound C from the *Sample solution*

r_S = peak response of praziquantel related compound A, praziquantel related compound B, or

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praziquantel related compound C from the *Standard solution*

C_S = concentration of USP Praziquantel Related Compound A RS, USP Praziquantel Related Compound B RS, or USP Praziquantel Related Compound C RS in the *Standard solution* (mg/mL)

C_U = concentration of Praziquantel in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Praziquantel related compound A[a]	0.8	0.2
Praziquantel	1.0	—
Praziquantel related compound B[b]	1.8	0.2
Praziquantel related compound C[c]	2.1	0.2

[a] 2-Benzoyl-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino [2,1-*a*]isoquinolin-4-one.
[b] 2-(Cyclohexylcarbonyl)-2,3,6,7-tetrahydro-4*H*-pyrazino [2,1-*a*]isoquinolin-4-one.
[c] 2-(*N*-Formylhexahydrohippuroyl-1,2,3,4-tetrahydroisoquinolin-1-one.

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample in a vacuum at a pressure not exceeding 5 mm of mercury at 50° over phosphorus pentoxide for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Praziquantel RS
USP Praziquantel Related Compound A RS
2-Benzoyl-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino [2,1-*a*] isoquinolin-4-one.
$C_{19}H_{18}N_2O_2$ 306.37
USP Praziquantel Related Compound B RS
2-(Cyclohexylcarbonyl)-2,3,6,7-tetrahydro-4*H*-pyrazino [2,1-*a*]isoquinolin-4-one.
$C_{19}H_{22}N_2O_2$ 310.40
USP Praziquantel Related Compound C RS
2-(*N*-Formylhexahydrohippuroyl-1,2,3,4-tetrahydroisoquinolin-1-one.
$C_{19}H_{22}N_2O_4$ 342.39

Praziquantel Tablets

DEFINITION

Praziquantel Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{19}H_{24}N_2O_2$.

IDENTIFICATION

• **Procedure**
Standard solution: 6 mg/mL of USP Praziquantel RS in methanol
Sample solution: Equivalent to 30 mg of Praziquantel from a quantity of powdered Tablets, in a centrifuge tube. Add 5 mL of methanol, agitate for 5 min, and centrifuge. Use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Ethyl acetate
Analysis
Samples: *Standard solution* and *Sample solution*
Apply separately the *Sample solution* and *Standard solution*, each as 1-cm wide bands, to a chromatographic plate. Proceed as directed in the General Chapter. Develop the chromatogram in an unsaturated chamber, using developing solvent, until the solvent front has moved 8 cm. Remove the plate from the chamber, air-dry, and examine under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal band of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **Procedure**
Mobile phase: Acetonitrile and water (3:2)
Standard solution: 0.18 mg/mL of USP Praziquantel RS in *Mobile phase*
Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 150 mg of praziquantel, to a 100-mL volumetric flask. Add 70 mL of *Mobile phase*, sonicate for 5 min, dilute with *Mobile phase* to volume, mix, and filter.
Sample solution: Transfer 3.0 mL of the filtrate to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 25-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{24}N_2O_2$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Praziquantel RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of praziquantel in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
For products for human use
Medium: 0.1 N hydrochloric acid containing 2.0 mg of sodium lauryl sulfate per mL; 900 mL
Apparatus 2: 50 rpm
Time: 60 min

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Standard solution: Prepare a solution of USP Praziquantel RS in methanol containing (L/90) mg/mL, where L is the Tablet label claim in mg. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Detector: UV 263 nm
Blank: *Medium*
Tolerances: NLT 75% (Q) of the labeled amount of $C_{19}H_{24}N_2O_2$ is dissolved.

For products for veterinary use
Medium: 0.1 N hydrochloric acid containing 2.0 mg of sodium lauryl sulfate per mL; 900 mL
Apparatus 2: 50 rpm, with peak vessels
Time: 60 min
Mobile phase: Acetonitrile and water (3:2)
Diluent: Mix 600 mL of acetonitrile with 400 mL of pH 7.4 phosphate buffer.
Standard solution: Prepare a solution containing 0.02 mg of USP Praziquantel RS per mL in methanol. Transfer 10.0 mL to a 100-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 4.0 mL to a 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, and dilute with *Diluent* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm, 10-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis:
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of praziquantel dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

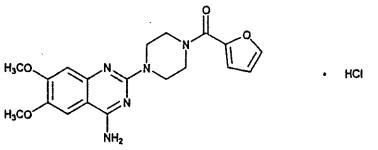
Tolerances: NLT 75% (Q) of the labeled amount of $C_{19}H_{24}N_2O_2$ is dissolved

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Praziquantel RS

Prazosin Hydrochloride



$C_{19}H_{21}N_5O_4 \cdot HCl$ 419.86
Piperazine, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)-, monohydrochloride;
1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl) piperazine monohydrochloride [19237-84-4].

DEFINITION

Prazosin Hydrochloride contains NLT 97.0% and NMT 103.0% of prazosin hydrochloride ($C_{19}H_{21}N_5O_4 \cdot HCl$), calculated on the anhydrous basis.
[Note—Care should be taken to prevent inhaling particles of Prazosin Hydrochloride and to prevent its contacting any part of the body.]

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: 1 mg/mL of Prazosin Hydrochloride in methanol prepared as follows. Dissolve 20 mg of Prazosin Hydrochloride in 20 mL of methanol with the aid of gentle heat, and evaporate to dryness. Dry the residue under vacuum at 130° for 3 h.
Analysis: Proceed as directed with the residue so obtained and a similar preparation of USP Prazosin Hydrochloride RS.
Acceptance criteria: Meets the requirements

Change to read:

• **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelengths: 246 and 329 nm
Sample solution: 7 µg/mL in methanolic 0.01 N hydrochloric acid
Acceptance criteria: Absorptivities, calculated on the anhydrous basis, do not differ by more than 4.0%.
• **C. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Diluent: Chloroform, methanol, and diethylamine (10:10:1)
Sample solution: 5 mg/mL in *Diluent*
Chromatographic system
Application volume: 10 µL
Developing solvent system: Ethyl acetate and diethylamine (190:10)
Acceptance criteria: Meets the requirements
• **D. Identification Tests—General,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

• **Procedure**
Mobile phase: Methanol, glacial acetic acid, and water (700:10:300). Add diethylamine in sufficient quantity (0.2 mL) such that the retention time of prazosin hydrochloride is 6–10 min.
Diluent: Methanol and water (700:300)
Standard stock solution: 1 mg/mL of USP Prazosin Hydrochloride RS in methanol

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Standard solution: 30 µg/mL of USP Prazosin Hydrochloride RS in *Diluent* from the *Standard stock solution*
Sample stock solution: 1 mg/mL of Prazosin Hydrochloride in methanol
Sample solution: 30 µg/mL of Prazosin Hydrochloride in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: Adjusted to obtain a retention time of 6–10 min for prazosin
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of prazosin hydrochloride ($C_{19}H_{21}N_5O_4 \cdot HCl$) in the portion of Prazosin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of prazosin from the *Sample solution*
r_S = peak response of prazosin from the *Standard solution*
C_S = concentration of USP Prazosin Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Prazosin Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% calculated on the anhydrous basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩
Sample: 1 g of Prazosin Hydrochloride
Acceptance criteria: NMT 0.4%
[Note—Save the residue for the test for *Iron*.]

• **Iron**
Standard stock solution: Dissolve 100 mg of iron wire in 10 mL of hydrochloric acid with the aid of boiling. Cool, transfer to a 1000-mL volumetric flask, and dilute with water to volume.
Standard solution: 4.0 µg/mL of iron in 0.2 N nitric acid from *Standard stock solution*
Sample solution: Dissolve the residue obtained in the test for *Residue on Ignition* in 20 mL of 2 N nitric acid. Slowly evaporate this solution to approximately 5 mL; transfer to a 25-mL volumetric flask, using 0.2 N nitric acid as a wash solvent; and dilute with 0.2 N nitric acid to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption
Analytical wavelength: 248 nm
Lamp: Iron hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis: Determine the absorbances of the *Standard solution* and the *Sample solution* against the *Blank*.
Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (0.010%).

• **Nickel**
Standard stock solution: Dissolve 100 mg of nickel in 10 mL of nitric acid with the aid of boiling. Cool, transfer to a 1000-mL volumetric flask, and dilute with water to volume.
Standard solution: 4.0 µg/mL of nickel in 0.2 N nitric acid from *Standard stock solution*
Sample solution: Prepare as directed in the test for *Iron*.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption
Analytical wavelength: 232 nm
Lamp: Nickel hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis: Determine the absorbances of the *Standard solution* and the *Sample solution* against the *Blank*.
Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (0.010%).

• **Organic Impurities**
Buffer: 3.48 g/L of sodium pentanesulfonate and 3.64 g/L of tetramethyl ammonium hydroxide. Adjust with glacial acetic acid to a pH of 5.0.
Mobile phase: Methanol and *Buffer* (500:500)
System suitability solution: 0.032 mg/mL of USP Metoclopramide Hydrochloride RS and 0.004 mg/mL of USP Prazosin Hydrochloride RS in *Mobile phase*
Standard solution: 1 µg/mL of USP Prazosin Hydrochloride RS in *Mobile phase*
Sample solution: 1 mg/mL of Prazosin Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 6 times the retention time of the prazosin peak
System suitability
Sample: *System suitability solution*
Suitability requirements
[Note—The retention times of metoclopramide and prazosin are 0.55 and 1.00, respectively.]
Resolution: NLT 8 between metoclopramide and prazosin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Prazosin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of prazosin from the *Standard solution*
C_S = concentration of USP Prazosin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Prazosin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 0.2%
Total impurities: NMT 0.5%
[Note—Disregard peaks equal to or less than 0.05%.]

SPECIFIC TESTS

• **Water Determination,** *Method I* ⟨921⟩: NMT 2.0% for the anhydrous form; 8.0%–15.0% for the polyhydrate form

Stage 6 Harmonization

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label it to indicate whether it is anhydrous or is the polyhydrate.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Metoclopramide Hydrochloride RS
  Benzamide, 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxy-, monohydrochloride, monohydrate.
  $C_{14}H_{22}ClN_3O_2 \cdot HCl \cdot H_2O$ 354.27
  USP Prazosin Hydrochloride RS

Prazosin Hydrochloride Capsules

DEFINITION

Prazosin Hydrochloride Capsules contain an amount of prazosin hydrochloride ($C_{19}H_{21}N_5O_4 \cdot HCl$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of prazosin ($C_{19}H_{21}N_5O_4$).

[**CAUTION**—Care should be taken to prevent inhaling particles of prazosin hydrochloride and to prevent its contacting any part of the body.]

IDENTIFICATION

Change to read:

- **A.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (70:1:30). Add ▲0.2 mL of diethylamine to 1 L of *Mobile phase*▲ (USP 1-Dec-2019) such that the retention time of prazosin is 6–10 min.

Solution A: To 300 mL of water add 0.85 mL of hydrochloric acid in a 1000-mL volumetric flask. Dilute with methanol to volume, and mix. Transfer 300 mL of this solution to a 500-mL volumetric flask, and dilute with methanol to volume.

Standard stock solution: 0.2 mg/mL of USP Prazosin Hydrochloride RS in *Solution A*

Standard solution: ▲0.01 mg/mL of USP Prazosin Hydrochloride RS prepared as follows.▲ (USP 1-Dec-2019) Transfer 5 mL of *Standard stock solution* to a 100-mL volumetric flask, add 45.0 mL of *Solution A* methanol

Sample stock solution: ▲Nominally 0.02 mg/mL of prazosin in *Solution A* prepared as follows.▲ (USP 1-Dec-2019) Transfer ▲a portion of▲ (USP 1-Dec-2019) the contents of NLT 20 Capsules, equivalent to about 1 mg of prazosin , to a glass-stoppered flask containing 50.0 mL of *Solution A*, and shake by mechanical means for 30 min. Place the flask in an ultrasonic bath for 30 min, cool to room temperature, and pass the contents through a suitable filter of 5-µm or finer pore size.

Sample solution: ▲Nominally 0.01 mg/mL of prazosin prepared as follows. Transfer 25.0 mL of *Sample stock solution* to a 50-mL volumetric flask, and dilute with methanol to volume.▲ (USP 1-Dec-2019)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm.▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Dec-2019)
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: ▲0.6 mL/min▲ (USP 1-Dec-2019)
Injection volume: 5 µL
▲Run time: NLT 2 times the retention time of prazosin▲ (USP 1-Dec-2019)

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prazosin ($C_{19}H_{21}N_5O_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (Mr_1/Mr_2) \times 100$$

r_U = peak response▲of prazosin▲ (USP 1-Dec-2019) from the *Sample solution*
r_S = peak response▲of prazosin▲ (USP 1-Dec-2019) from the *Standard solution*
C_S = concentration of USP Prazosin Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of prazosin in the *Sample solution* (µg/mL)
Mr_1 = molecular weight of prazosin, ▲383.41▲ (USP 1-Dec-2019)
Mr_2 = molecular weight of prazosin hydrochloride, 419.86

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid containing 3% sodium lauryl sulfate; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Standard solution: Known concentration of USP Prazosin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of prazosin ($C_{19}H_{21}N_5O_4$) dissolved, using the procedure in the *Assay*.
Tolerances: NLT 75% (*Q*) of the labeled amount of prazosin ($C_{19}H_{21}N_5O_4$) is dissolved.

Change to read:

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
  ▲▲ (USP 1-Dec-2019)

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IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A: 1.93 g of ammonium acetate in 1000 mL of water. Adjust the solution with glacial acetic acid to a pH of 5.0.

Solution B: Acetonitrile and methanol (75:25)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
15.0	85	15
66.0	45	55
75.0	45	55
76.0	85	15
85.0	85	15

Diluent: *Solution A* and *Solution B* (85:15)

Standard stock solution: 0.5 mg/mL of USP Prazosin Hydrochloride RS in *Diluent*

Standard solution: 0.5 µg/mL of USP Prazosin Hydrochloride RS from *Standard stock solution* in *Diluent*

Sensitivity solution: 0.05 µg/mL of USP Prazosin Hydrochloride RS from *Standard solution* in *Diluent*

System suitability stock solution: 0.025 mg/mL each of USP Prazosin Related Compound D RS, USP Terazosin Related Compound A RS, and USP Terazosin Related Compound C RS prepared as follows. Transfer 12.5 mg of each corresponding Reference Standard to a 500-mL volumetric flask. Add 75 mL of *Solution B* and sonicate. Dilute with *Solution A* to volume.

System suitability solution: 0.1 mg/mL of USP Prazosin Hydrochloride RS from *Standard stock solution* in *Diluent*, and 0.001 mg/mL each of USP Prazosin Related Compound D RS, USP Terazosin Related Compound A RS, and USP Terazosin Related Compound C RS in *Diluent* from *System suitability stock solution*

Sample solution: Nominally 0.1 mg/mL of prazosin hydrochloride in *Diluent* prepared as follows. Transfer a suitable portion of the contents from NLT 20 Capsules to a suitable volumetric flask that doubles the volume of *Diluent* used, add an appropriate amount of *Diluent*, and mix. Centrifuge a portion of the solution. Use the supernatant. [**CAUTION**—Do not dilute to volume.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 0.7 mL/min

Injection volume: 15 µL

System suitability

Samples: *Standard solution*, *Sensitivity solution*, and *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between prazosin and terazosin related compound C, *System suitability solution*

Tailing factor: NMT 3.0 for terazosin related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of prazosin related compound D, terazosin related compound A, or any unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each corresponding degradation product from the *Sample solution*

r_S = peak response of prazosin from the *Standard solution*

C_S = concentration of USP Prazosin Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of prazosin hydrochloride in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Prazosin related compound D	0.13	0.53	0.2
Terazosin related compound A	0.21	1.0	0.2
Prazosin	1.0	—	—
Terazosin related compound C[a]	1.1	1.0	—
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.0

[a] For resolution measurement only. Not included in the total degradation products. ▲(USP 1-Dec-2019)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. ▲Store at controlled room temperature.▲ (USP 1-Dec-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

▲USP Prazosin Hydrochloride RS

USP Prazosin Related Compound D RS

Furan-2-yl(piperazin-1-yl)methanone.

$C_9H_{12}N_2O_2$ 180.21

USP Terazosin Related Compound A RS

6,7-Dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine dihydrochloride.

$C_{14}H_{19}N_5O_2 \cdot 2HCl$ 362.26

USP Terazosin Related Compound C RS

2,2′-(Piperazine-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine) dihydrochloride.

$C_{24}H_{28}N_8O_4 \cdot 2HCl$ 565.46▲ (USP 1-Dec-2019)

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Add the following:

▲Prazosin Hydrochloride Compounded Oral Suspension

DEFINITION

Prazosin Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of prazosin ($C_{19}H_{21}N_5O_4$).

Prepare Prazosin Hydrochloride Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Prazosin Hydrochloride capsules,[a] equivalent to	30 mg of prazosin
Ora-Blend,[b] a sufficient quantity to make	30 mL

[a] Prazosin Hydrochloride 5-mg capsules, Mylan Pharmaceuticals Inc., Morgantown, WV.
[b] Perrigo, Allegan, MI.

Empty the required number of *Prazosin Hydrochloride capsules* into a suitable container. Add a small amount of *Ora-Blend* and mix well to form a smooth paste. Add a sufficient amount of *Ora-Blend* to make the contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of the *Ora-Blend*. Add sufficient *Ora-Blend* to bring to final volume. Shake well.

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Add 1.74 g of sodium 1-pentanesulfonate and 3.64 mL of a 25% tetramethylammonium ▲hydroxide▲ (ERR 1-Mar-2019) solution to 500 mL of water. Adjust with glacial acetic acid to a pH of 5.0. Add 500 mL of methanol.

Standard solution: Transfer 21.90 mg of USP Prazosin Hydrochloride RS (equivalent to 20 mg of prazosin) to a 200-mL volumetric flask and dissolve in 20 mL of methanol. Add *Mobile phase* to volume.

Sample solution: Transfer 1.0 mL of Oral Suspension into a 10-mL volumetric flask. Dissolve in about 8 mL of methanol. Add *Mobile phase* to volume. Pass through a filter of 0.22-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 15°
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for prazosin is about 10.0 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of prazosin ($C_{19}H_{21}N_5O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of prazosin from the *Sample solution*
r_S = peak response of prazosin from the *Standard solution*
C_S = concentration of USP Prazosin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prazosin hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of prazosin, 383.40
M_{r2} = molecular weight of prazosin hydrochloride, 419.86

Acceptance criteria: 90.0%–110.0%

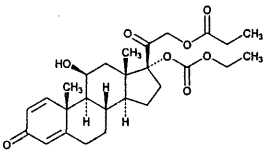
SPECIFIC TESTS

• **pH** ⟨791⟩: 4.6–5.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prazosin Hydrochloride RS▲ 2S (USP41)

Prednicarbate



$C_{27}H_{36}O_8$ 488.57

Pregna-1,4-diene-3,20-dione, 17-[(ethoxycarbonyl)oxy]-11-hydroxy-21-(1-oxopropoxy)-, (11β)-;
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 17-(ethyl carbonate) 21-propionate [73771-04-7].

DEFINITION

Prednicarbate contains NLT 97.0% and NMT 102.0% of prednicarbate ($C_{27}H_{36}O_8$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Prepare all solutions just before use.
Mobile phase: Acetonitrile and water (50:60)

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System suitability solution: 3 µg/mL each of USP Prednicarbate RS and USP Prednicarbate Related Compound A RS in *Mobile phase.*
Standard solution: 0.6 mg/mL of USP Prednicarbate RS in *Mobile phase*
Sample solution: 0.6 mg/mL of Prednicarbate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 243 nm
Column: 4-mm × 12.5-cm; packing L1
Flow rate: 0.7 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of prednicarbate
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between prednicarbate and prednicarbate related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of prednicarbate ($C_{27}H_{36}O_8$) in the portion of Prednicarbate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Prednicarbate RS in the *Standard solution* (mg/mL)
C_U = concentration of Prednicarbate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*
Sample stock solution: 0.6 mg/mL of Prednicarbate in *Mobile phase*
Sample solution: 3 µg/mL of Prednicarbate in *Mobile phase* from the *Sample stock solution*
Analysis
Samples: *Sample stock solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Prednicarbate taken:

$$\text{Result} = (r_{UA}/r_{UB}) \times D \times 100$$

r_{UA} = peak response for each individual impurity from the *Sample stock solution*
r_{UB} = peak response of prednicarbate from the *Sample solution*
D = dilution factor for the *Sample solution*

Acceptance criteria: See *Table 1.* Disregard any peak with an area less than 0.025 times the area of the main peak from the *Sample solution* (0.0125%).

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Prednicarbate	1.0	—
Prednicarbate related compound A	1.1	1.0
Any other individual impurity	—	0.5
Total impurities	—	2.0

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in alcohol
Acceptance criteria: +60° to +66°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 6 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prednicarbate RS
USP Prednicarbate Related Compound A RS
1,2-Dihydroprednicarbate.

Prednicarbate Cream

DEFINITION

Prednicarbate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of prednicarbate ($C_{27}H_{36}O_8$). It may contain a suitable preservative.

IDENTIFICATION

• **A.** The retention time of the prednicarbate peak in the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**
Solution A: 0.01 M monobasic potassium phosphate
Solution B: Acetonitrile and dehydrated alcohol (2:1)
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	67	33
5	67	33
45	40	60
50	40	60
55	20	80
70	20	80
75	67	33
85	67	33

Standard stock solution: 0.3 mg/mL of USP Prednicarbate RS in dehydrated alcohol
Standard solution: 30 µg/mL of USP Prednicarbate RS prepared as follows. Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask. Add 15 mL of

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tetrahydrofuran and 30 mL of *Solution B*, and dilute with *Solution A* to volume.

System suitability stock solution 1: 0.3 mg/mL each of USP Prednicarbate Related Compound B RS and USP Prednicarbate Related Compound C RS in dehydrated alcohol

System suitability stock solution 2: Transfer 15 mg of USP Prednicarbate Related Compound A RS to a 50-mL volumetric flask. Add 1.0 mL of *System suitability stock solution 1*, and dilute with dehydrated alcohol to volume.

System suitability solution: Transfer 10.0 mL of the *Standard solution* to a volumetric flask. Add 1.0 mL of *System suitability stock solution 2*, 1 mL of tetrahydrofuran, and 2 mL of acetonitrile. Dilute with *Solution A* to 20.0 mL.

Sample solution: Transfer the equivalent of 3.0 mg of prednicarbate from a quantity of Cream to a 100-mL volumetric flask. Add 15 mL of tetrahydrofuran, shake vigorously, and allow to stand in an ultrasonic bath until the sample has dissolved. Add 20 mL of dehydrated alcohol, and shake vigorously. Add 20 mL of acetonitrile, and shake vigorously. Immediately dilute with *Solution A* to volume, and shake vigorously. Allow to stand in an ice bath for at least 15 min. Shake vigorously, and pass through a folded paper filter. Pass the filtrate through a membrane filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 243 nm
Column: 4.0-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 60 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between prednicarbate and prednicarbate related compound A, *System suitability solution*
Tailing factor: 0.7–1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prednicarbate ($C_{27}H_{36}O_8$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Prednicarbate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prednicarbate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, System suitability stock solution 1, System suitability stock solution 2, Mobile phase, Standard stock solution, Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.03 µg/mL of USP Prednicarbate RS prepared as follows. Dilute 1.0 mL of the *Standard solution* with dehydrated alcohol to 50.0 mL. Dilute 1.0 mL of the resulting solution with *Solution A* to 20.0 mL.

System suitability
Samples: *Standard solution, System suitability solution, Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between prednicarbate and prednicarbate related compound A, *System suitability solution*
Tailing factor: 0.7–1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 3, *Sensitivity solution*

Analysis
Sample: *Sample solution*
Calculate the percentage of each related compound and unknown impurity in the portion of Cream taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each individual impurity
r_T = sum of all the peak responses

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Prednicarbate related compound B	0.57	2.0
Prednicarbate related compound C	0.64	2.0
Prednicarbate	1.0	—
Prednicarbate related compound A	1.04	—
Any individual related compound	—	0.5
Total impurities	—	5.0

PERFORMANCE TESTS

• **MINIMUM FILL:** Meets the requirements

SPECIFIC TESTS

• **CONSISTENCY:** At room temperature, a string of Cream having a length of 2 cm retains its shape on a glass plate for at least 10 min. It can be spread easily and has no visible lumps.

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic bacterial count does not exceed 10^2 cfu/g.

• **PH** ⟨791⟩

Sample solution: Add 15 mL of boiling water to 3.5 g of Cream in a 50-mL centrifuge tube, and shake vigorously until an emulsion is formed. Loosen the cap, and place in a steam bath for 5 min. Centrifuge the hot solution. After cooling to room temperature, collect the lower aqueous solution in a glass tube.

Acceptance criteria: 3.5–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prednicarbate RS
USP Prednicarbate Related Compound A RS
1,2-Dihydroprednicarbate.

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USP Prednicarbate Related Compound B RS
Prednisolone-17-ethylcarbonate.
USP Prednicarbate Related Compound C RS
Prednisolone-21-propionate.

Prednicarbate Ointment

DEFINITION

Prednicarbate Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of prednicarbate ($C_{27}H_{36}O_8$) in a suitable ointment base.

IDENTIFICATION

• **A.** The retention time of the prednicarbate peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 0.01 M monobasic potassium phosphate
Solution B: Acetonitrile and dehydrated alcohol (2:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	67	33
5	67	33
45	40	60
50	40	60
55	20	80
70	20	80
75	67	33
85	67	33

Standard stock solution: 0.3 mg/mL USP Prednicarbate RS in dehydrated alcohol
Standard solution: 30 µg/mL of USP Prednicarbate RS prepared as follows. Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask. Add 15 mL of tetrahydrofuran and 30 mL of *Solution B*, and dilute with *Solution A* to volume.
System suitability stock solution 1: 0.3 mg/mL each of USP Prednicarbate Related Compound B RS and USP Prednicarbate Related Compound C RS in dehydrated alcohol
System suitability stock solution 2: Transfer 15 mg of USP Prednicarbate Related Compound A RS to a 50-mL volumetric flask. Add 1.0 mL of *System suitability stock solution 1*, and dilute with dehydrated alcohol to volume.
System suitability solution: Transfer 10.0 mL of the *Standard solution* to a volumetric flask. Add 1.0 mL of *System suitability stock solution 2*, 1 mL of tetrahydrofuran, and 2 mL of acetonitrile. Dilute with *Solution A* to 20.0 mL.
Sample solution: Transfer the equivalent of 3.0 mg of prednicarbate from a quantity of Ointment to a 100-mL volumetric flask. Add 15 mL of tetrahydrofuran, shake vigorously, and allow to stand in an ultrasonic bath until the sample has dissolved. Add 20 mL of dehydrated alcohol, and shake vigorously. Add 20 mL of acetonitrile, and shake vigorously. Immediately dilute with *Solution A* to volume, and shake vigorously. Allow to stand in an ice bath for at least 15 min. Shake vigorously, and pass through a folded paper filter. Pass the filtrate through a membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 243 nm
Column: 4.0-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 60 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between prednicarbate and prednicarbate related compound A, *System suitability solution*
Tailing factor: 0.7–1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prednicarbate ($C_{27}H_{36}O_8$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Prednicarbate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prednicarbate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, System suitability stock solution 1, System suitability stock solution 2, Mobile phase, Standard stock solution, Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.03 µg/mL of USP Prednicarbate RS prepared as follows. Dilute 1.0 mL of the *Standard solution* with dehydrated alcohol to 50.0 mL. Dilute 1.0 mL of the resulting solution with *Solution A* to 20.0 mL.
System suitability
Samples: *Standard solution, System suitability solution, Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between prednicarbate and prednicarbate related compound A, *System suitability solution*
Tailing factor: 0.7–1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 3, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each related compound and unknown impurity in the portion of Ointment taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria: See *Table 2*.

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Table 2

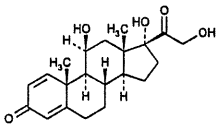
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Prednicarbate related compound B	0.57	2.0
Prednicarbate related compound C	0.64	2.0
Prednicarbate	1.0	—
Prednicarbate related compound A	1.04	—
Any individual related compound	—	0.5
Total impurities	—	5.0

PERFORMANCE TESTS
- **MINIMUM FILL:** Meets the requirements

SPECIFIC TESTS
- **CONSISTENCY:** At room temperature, a string of Ointment having a length of 2 cm retains its shape on a glass plate for at least 10 min. It can be spread easily and has no visible lumps.
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic bacterial count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Prednicarbate RS
  USP Prednicarbate Related Compound A RS
  1,2-Dihydroprednicarbate.
  USP Prednicarbate Related Compound B RS
  Prednisolone-17-ethylcarbonate.
  USP Prednicarbate Related Compound C RS
  Prednisolone-21-propionate.

Prednisolone



$C_{21}H_{28}O_5$ (anhydrous) 360.44
Pregna-1,4-diene-3,20-dione, 11,17,21-trihydroxy-, (11β)-.
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione (anhydrous) [50-24-8].
Sesquihydrate 387.48
[52438-85-4].

» Prednisolone is anhydrous or contains one and one-half molecules of water of hydration. It contains not less than 97.0 percent and not more than 102.0 percent of $C_{21}H_{28}O_5$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label it to indicate whether it is anhydrous or hydrous.
USP Reference standards ⟨11⟩—
USP Prednisolone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: methanol.
Absorptivities at 242 nm, calculated on the dried basis, do not differ by more than 2.5%. If a difference appears, dissolve portions of both the test specimen and the Reference Standard in ethyl acetate, evaporate the solutions to dryness, and repeat the test on the residues.
Specific rotation ⟨781S⟩: between +97° and +103°.
Test solution: 10 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 3 hours: anhydrous Prednisolone loses not more than 1.0%, and hydrous Prednisolone not more than 7.0%, of its weight.
Residue on ignition ⟨281⟩: negligible, from 100 mg.
Selenium ⟨291⟩: 0.003%, a 200-mg test specimen being used.

Chromatographic purity—
Solution A—Prepare a filtered and degassed mixture of water and acetonitrile (77:23).
Solution B—Prepare a filtered and degassed mixture of water and acetonitrile (60:40).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent: a mixture of water and acetonitrile (1:1).
Standard solution—Dissolve an accurately weighed quantity of USP Prednisolone RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.01 mg per mL.
System suitability solution—Dissolve an accurately weighed quantity of USP Prednisolone RS and hydrocortisone in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL and 0.06 mg per mL, respectively.
Test solution—Transfer about 25 mg of Prednisolone, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–25	100	0	isocratic
25–45	100→0	0→100	linear gradient
45–60	0	100	isocratic

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Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
60–61	0→100	100→0	linear gradient
61–100	100	0	re-equilibration

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times for prednisolone and hydrocortisone are about 1.0 and 1.06, respectively; and the height of the smallest peak is not less than 2 times the height of the valley between the prednisolone and hydrocortisone peaks. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0% for the prednisolone peak. *Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of each impurity in the portion of Prednisolone taken by the formula:

$$2500(C/W)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of USP Prednisolone RS in the *Standard solution; W* is the weight, in mg, of prednisolone used to prepare the *Test solution;* r_i is the peak response for each impurity in the *Test solution;* and r_s is the peak response obtained from the *Standard solution:* no impurity greater than 1.0% and only one peak greater than 0.5% is found; and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Prepare a solution containing a mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of betamethasone in tetrahydrofuran containing 5 mg per mL. Dilute this solution with water-saturated chloroform, and mix to obtain a solution having a final concentration of 0.5 mg per mL.

Standard preparation—Transfer about 10 mg of USP Prednisolone RS, accurately weighed, to a 100-mL volumetric flask, and dissolve in 5.0 mL of methanol. Add 20.0 mL of *Internal standard solution,* and mix. Dilute with water-saturated chloroform to 100.0 mL, and mix.

Assay preparation—Transfer about 10 mg of Prednisolone, accurately weighed, to a 100-mL volumetric flask, and dissolve in 5.0 mL of methanol. Add 20.0 mL of *Internal standard solution,* and mix. Dilute with water-saturated chloroform to 100.0 mL, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph four replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for betamethasone and 1.0 for prednisolone; the resolution, R, between prednisolone and betamethasone is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{28}O_5$ in the portion of Prednisolone taken by the formula:

$$0.1C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Prednisolone RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of prednisolone to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Delete the following:

▲Prednisolone Cream

» Prednisolone Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{28}O_5$, in a suitable cream base.

Packaging and storage—Preserve in collapsible tubes or in tight containers.

USP Reference standards ⟨ 11 ⟩ —
USP Prednisolone RS

Identification—Evaporate in a test tube to dryness about 5 mL of the dilute solution obtained in the *Assay.* Add 2 to 3 mL of warm perchloric acid, mix, and warm on a steam bath for about 2 minutes: a blood-red color develops. Add 5 mL of water: the color is not discharged.

Minimum fill ⟨ 755 ⟩ : meets the requirements.

Assay—

Sulfuric acid reagent—Prepare a solution of sulfuric acid, dehydrated alcohol, and water solution (4:3:3).

Modified phenylhydrazine-sulfuric acid TS—Dissolve 65 mg of phenylhydrazine hydrochloride in 100 mL of *Sulfuric acid reagent.*

Standard preparation—Dissolve in dehydrated alcohol a suitable quantity, accurately weighed, of USP Prednisolone RS, and dilute quantitatively and stepwise with dehydrated alcohol to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—To an accurately weighed portion of Cream, equivalent to about 20 mg of prednisolone, add 25 mL of dehydrated alcohol. Warm on a steam bath to disperse the assay specimen, then chill to congeal it. Filter through paper, previously washed with dehydrated alcohol, into a 200-mL volumetric flask. Repeat twice more, starting with "add 25 mL of dehydrated alcohol." Dilute with dehydrated alcohol to volume. (Retain about 5 mL of this dilution for the *Identification* test.)

Procedure—Pipet 2 mL of *Assay preparation* into each of two 50-mL conical flasks (identify as *Assay preparation* and *Assay preparation blank*). Pipet 2 mL of *Standard preparation* into each of two 50-mL conical flasks (identify as *Standard preparation* and *Standard preparation blank*). Pipet 2 mL of dehydrated alcohol into a 50-mL conical flask (identify as *Reagent blank*). Add 20.0 mL of *Sulfuric acid reagent* to the *Assay preparation blank* and to the *Standard preparation blank.* Add 20.0 mL of *Modified phenylhydrazine-sulfuric acid* to the *Assay preparation,* to the *Standard preparation,* and to the *Reagent blank,* respectively. Maintain the flasks in a water bath at 60° for about 45 minutes, then chill in a water-ice bath. Filter each through a fine-porosity, sintered-glass funnel, and

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identify the filtrate flasks correspondingly. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, against dehydrated alcohol. Calculate the quantity, in mg, of $C_{21}H_{28}O_5$ in the portion of Cream taken by the formula:

$$200C[(A_U - A_{UB} - A_R)/(A_S - A_{SB} - A_R)]$$

in which C is the concentration, in mg per mL, of USP Prednisolone RS in the *Standard preparation*, A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively, A_{UB} and A_{SB} are the absorbances of the solutions from the *Assay preparation blank* and the *Standard preparation blank*, respectively, and A_R is the absorbance of the *Reagent blank*. ▲ (USP 1-May-2020)

Prednisolone Oral Solution

» Prednisolone Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone ($C_{21}H_{28}O_5$). It may contain alcohol.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Prednisolone RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
pH ⟨791⟩: between 3.0 and 4.5.
Alcohol Determination, *Method II* ⟨611⟩ ***(if present)***: not less than 90.0% and not more than 115.0% of the labeled amount.

Assay—
Citrate buffer—Prepare a 0.0033 M solution of citric acid in water, adjust with 1 N sodium hydroxide to a pH of 6.2, and mix.
Diluting solution: a mixture of methanol and water (1:1).
Mobile phase—Prepare a filtered and degassed mixture of *Citrate buffer* and methanol (31:19). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve suitable quantities of prednisolone and hydrocortisone in a mixture of methanol and water (1:1) to obtain a solution containing about 100 µg per mL and 90 µg per mL, respectively.
Standard preparation—Dissolve an accurately weighed quantity of USP Prednisolone RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 0.1 mg per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 5.0 mg of prednisolone, to a 50-mL volumetric flask, dissolve in *Diluting solution*, shake by mechanical means for 15 minutes, dilute with *Diluting solution* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for hydrocortisone and 1.0 for prednisolone; the resolution, *R*, between hydrocortisone and prednisolone is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of prednisolone ($C_{21}H_{28}O_5$) in the volume of Oral Solution taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Prednisolone RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Prednisolone Tablets

» Prednisolone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{28}O_5$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Prednisolone RS
Identification—Pulverize a number of Tablets, equivalent to about 50 mg of prednisolone, and digest with 25 mL of chloroform for 15 minutes. Filter the mixture, and evaporate the filtrate on a steam bath to dryness. Wash the residue with two 10-mL portions of hot solvent hexane, decanting the supernatant each time and discarding it. Digest the residue with 25 mL of dehydrated alcohol, warming slightly, for 15 minutes. Filter the warm solution, and evaporate the filtrate to a volume of 2 to 3 mL. Add solvent hexane until the mixture just becomes turbid, chill it to effect crystallization, collect the crystals, and dry them at 60° for 1 hour: the crystals of prednisolone so obtained respond to *Identification* test *A* under *Prednisolone*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount in solution on filtered portions of the *Dissolution Medium*, suitably diluted at the wavelength of maximum absorbance at about 246 nm, with a suitable spectrophotometer in comparison with a Standard solution having a known concentration of USP Prednisolone RS. An amount of alcohol not to exceed 5% of the total volume of the Standard solution may be used to bring the prednisolone standard into solution prior to dilution with water.
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{21}H_{28}O_5$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—
Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Prednisolone*.
Test preparation—Place 1 tablet in a suitable container. Place 0.5 mL of water directly on the tablet, and allow to stand

USP Monographs

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until disintegrated (about 30 minutes). Gently agitate the container to ensure that the tablet is completely disintegrated. Add 2.0 mL of *Internal standard solution* for each mg of labeled tablet strength, and sonicate for about 10 minutes. Dilute with a quantity of water-saturated chloroform approximately four times the volume of added *Internal standard solution*. Add a few glass beads, close the container, and shake vigorously for about 30 minutes. Centrifuge, or allow to stand until a clear solution is obtained. Analyze the clear solution as directed under *Procedure*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Prednisolone*. Calculate the quantity, in mg, of $C_{21}H_{28}O_5$ in the Tablet taken by the formula:

$$(FW_S)(R_U/R_S)$$

in which F is the ratio of the volume of the *Internal standard solution*, in mL, in the *Test preparation* to the volume, in mL, of the *Internal standard solution* in the *Standard preparation*, W_S is the weight, in mg, of *USP Prednisolone RS* taken for the *Standard preparation*, and the other terms are as defined therein.

Assay—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Prednisolone*.

Assay preparation—Weigh and finely powder not less than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of prednisolone, to a 100-mL volumetric flask. Add 20.0 mL of *Internal standard solution*, and sonicate for 10 minutes. Dilute with water-saturated chloroform to volume, and shake for 30 minutes. Centrifuge this mixture, and use the clear supernatant.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Prednisolone*. Calculate the quantity, in mg, of $C_{21}H_{28}O_5$ in the portion of Tablets taken by the formula:

$$0.1C(R_U/R_S)$$

in which the terms are as defined therein.

Prednisolone Acetate

$C_{23}H_{30}O_6$ 402.48

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-, (11β)-.

11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-acetate [52-21-1].

» Prednisolone Acetate contains not less than 97.0 percent and not more than 102.0 percent of $C_{23}H_{30}O_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—

USP Prednisolone Acetate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 10 µg per mL.

Medium: methanol.

Absorptivities at 242 nm, calculated on the dried basis, do not differ by more than 2.5%.

Specific rotation ⟨781S⟩**:** between +112° and +119°.

Test solution: 10 mg per mL, in dioxane.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Chromatographic purity—

Mobile phase—Prepare a filtered and degassed mixture of isooctane, butyl chloride and methanol (49:49:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Test solution—Transfer about 10 mg of Prednisolone Acetate, accurately weighed, to a suitable container, dissolve in 10 mL of chloroform, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 6.0-mm × 4.0-cm column that contains packing L3. The flow rate is about 3 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 800 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of each impurity in the portion of Prednisolone Acetate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses for all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Prepare a solution containing a mixture of *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of beta methasone in tetrahydrofuran containing 10 mg per mL. Dilute this solution with water-saturated chloroform, and mix to obtain a solution having a final concentration of about 1 mg of betamethasone per mL.

Standard preparation—Transfer about 10 mg of USP Prednisolone Acetate RS, accurately weighed, to a 100-mL volumetric flask; add 20.0 mL of *Internal standard solution;* and dissolve, using sonication if necessary. Dilute with water-saturated chloroform to volume, and mix. Dilute 5.0 mL of the solution so obtained with water-saturated chloroform to 20.0 mL to obtain a solution having a known concentration of about 25 µg of USP Prednisolone Acetate RS per mL.

Assay preparation—Transfer about 10 mg of Prednisolone Acetate, accurately weighed, to a 100-mL volumetric flask;

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add 20.0 mL of *Internal standard solution;* and dissolve, using sonication if necessary. Dilute with water-saturated chloroform to volume, and mix. Dilute 5.0 mL of the solution so obtained with water-saturated chloroform to 20.0 mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.6 for betamethasone and 1.0 for prednisolone acetate; the resolution, *R,* between prednisolone acetate and betamethasone is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{30}O_6$ in the portion of Prednisolone Acetate taken by the formula:

$$0.4C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Prednisolone Acetate RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Prednisolone Acetate Injectable Suspension

» Prednisolone Acetate Injectable Suspension is a sterile suspension of Prednisolone Acetate in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{23}H_{30}O_6$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—
USP Prednisolone Acetate RS

Identification—Allow a volume of Injectable Suspension, equivalent to about 50 mg of prednisolone acetate, to settle. Decant and discard the supernatant. Dissolve the residue in 6 mL of alcohol. Evaporate the solution, with the aid of a current of air, to half its volume, when crystallization occurs. Chill, if necessary, to aid crystallization. Filter the crystals, and allow to dry with the aid of a current of air: the crystals so obtained respond to *Identification* test *A* under *Prednisolone Acetate.*

pH ⟨791⟩: between 5.0 and 7.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Methanol-acetonitrile solution—Prepare a solution by mixing equal volumes of methanol and acetonitrile.

Standard preparation—Dissolve an accurately weighed quantity of USP Prednisolone Acetate RS in *Methanol-acetonitrile solution,* and dilute quantitatively, and stepwise if necessary, with *Methanol-acetonitrile solution* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injectable Suspension, equivalent to about 50 mg of prednisolone acetate, to a 50-mL volumetric flask, add *Methanol-acetonitrile solution* to volume, and mix. Pipet 5 mL of this solution into a second 50-mL volumetric flask, dilute with *Methanol-acetonitrile solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak response as directed for *Procedure:* the capacity factor, k', is not less than 3.0, and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{30}O_6$ in each mL of the Injectable Suspension taken by the formula:

$$500(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Prednisolone Acetate RS in the *Standard preparation,* V is the volume, in mL, of Injectable Suspension taken, and r_U and r_S are the peak responses for prednisolone acetate obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Prednisolone Acetate Ophthalmic Suspension

» Prednisolone Acetate Ophthalmic Suspension is a sterile, aqueous suspension of prednisolone acetate containing a suitable antimicrobial preservative. It may contain suitable buffers, stabilizers, and suspending and viscosity agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{23}H_{30}O_6$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Prednisolone Acetate RS

Identification—Transfer a volume of Ophthalmic Suspension, equivalent to about 7.5 mg of Prednisolone Acetate, to a test tube, add 5 mL of chloroform, and shake. Centrifuge, and apply 20 µL of the chloroform layer and 20 µL of a Standard solution of USP Prednisolone Acetate RS in chloroform containing 1.5 mg per mL on a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a mixture of chloroform and acetone (4:1) until the solvent front has moved about three-fourths the length of the plate. Mark the solvent front, and locate the spots on the plate by examining under UV light: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

Sterility Tests ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 5.0 and 6.0.

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Assay—

Mobile phase—Prepare a suitably filtered and degassed solution of water and acetonitrile (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Prednisolone Acetate RS in a mixture of acetonitrile and water (1:1) to obtain a solution having a known concentration of about 0.1 mg per mL.

System suitability preparation—Prepare a solution of prednisolone in a mixture of acetonitrile and methanol (1:1) having a concentration of about 0.1 mg per mL. Mix equal volumes of this solution and the *Standard preparation*.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, equivalent to about 5 mg of prednisolone acetate, to a 50-mL volumetric flask. Dilute with a mixture of acetonitrile and water (1:1) to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are 0.5 for prednisolone and 1.0 for prednisolone acetate, the column efficiency is not less than 7000 theoretical plates, the tailing factor is not more than 2.0, and the resolution, *R*, between prednisolone and prednisolone acetate is not less than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{30}O_6$ in each mL of the Ophthalmic Suspension taken by the formula:

$$50(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Prednisolone Acetate RS in the *Standard preparation*, V is the volume, in mL, of Ophthalmic Suspension taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Prednisolone Compounded Oral Suspension, Veterinary

DEFINITION

Prednisolone Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$).

Prepare Prednisolone Compounded Oral Suspension, Veterinary, 20 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Prednisolone powder	2 g
Purified Water	a small amount
Syrup, NF, a sufficient quantity to make	100 mL

Pour the weighed *Prednisolone powder* into a suitable mortar. Wet the powder with a small amount of *Purified Water*, and triturate to make a smooth paste. Add the *Syrup* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar stepwise and quantitatively to a calibrated container. Add sufficient *Syrup* to bring the preparation to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (30:70). Filter, and degas.

System suitability solution: 1 mg/mL of USP Prednisolone RS and 0.06 mg/mL of USP Hydrocortisone RS in *Mobile phase*

Standard solution: 0.2 mg/mL of prednisolone prepared from USP Prednisolone RS in *Mobile phase*

Sample solution: Shake thoroughly each bottle of Oral Suspension, Veterinary. Transfer 1.0 mL of the Oral Suspension, Veterinary into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a nominal concentration of 0.2 mg/mL of prednisolone.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 246 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for prednisolone and hydrocortisone are about 1.0 and 1.06, respectively.]

Suitability requirements

Resolution: NLT 2.0 between prednisolone and hydrocortisone, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Peak-to-valley ratio: The ratio of the height of the smallest peak to the height of the valley between the prednisolone and hydrocortisone peak is NLT 2, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of prednisolone ($C_{21}H_{28}O_5$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of prednisolone from the *Sample solution*

r_S = peak response of prednisolone from the *Standard solution*

C_S = concentration of prednisolone in the *Standard solution* (mg/mL)

C_U = nominal concentration of prednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 2.6–3.6

ADDITIONAL REQUIREMENTS

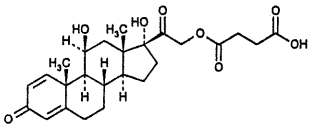
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

• **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use only.

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- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydrocortisone RS
  USP Prednisolone RS

Prednisolone Hemisuccinate



$C_{25}H_{32}O_8$ 460.52
Pregna-1,4-diene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-, (11β)-.
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(hydrogen succinate) [2920-86-7].

»Prednisolone Hemisuccinate contains not less than 98.0 percent and not more than 102.0 percent of $C_{25}H_{32}O_8$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Prednisolone Hemisuccinate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 20 µg per mL.
Medium: methanol.
Absorptivities at 243 nm, calculated on the dried basis, do not differ by more than 3.0%.
Specific rotation ⟨781S⟩: between +99° and +104°.
Test solution: 6.7 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it in vacuum at 65° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: negligible, from 100 mg.

Assay—
Standard preparation—Dissolve an accurately weighed quantity of USP Prednisolone Hemisuccinate RS in a mixture of equal volumes of alcohol and chloroform to obtain a solution having a known concentration of about 8 mg per mL.
Assay preparation—Weigh accurately about 80 mg of Prednisolone Hemisuccinate, previously dried, dissolve in a mixture of equal volumes of alcohol and chloroform to make 10.0 mL, and mix.
Procedure—Use a 20- × 20-cm chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture, which has been activated by heating at 105° for 1 hour. Using a solvent system consisting of a mixture of 5 parts of butyl alcohol, 4 parts of acetic acid, and 1 part of water, proceed as directed for *Procedure* under *Single-steroid Assay* ⟨511⟩, ending with "Centrifuge the tubes for 5 minutes," except to apply 50 µL in place of 200 µL of the solutions to the plate. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 243 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{25}H_{32}O_8$ in the portion of Prednisolone Hemisuccinate taken by the formula:

$$10C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Prednisolone Hemisuccinate RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Prednisolone Sodium Phosphate

$C_{21}H_{27}Na_2O_8P$ 484.39
Pregna-1,4-diene-3,20-dione, 11,17-dihydroxy-21-(phosphonooxy)-, disodium salt, (11β)-.
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(disodium phosphate) [125-02-0].

» Prednisolone Sodium Phosphate contains not less than 96.0 percent and not more than 102.0 percent of $C_{21}H_{27}Na_2O_8P$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Prednisolone RS
USP Prednisolone Sodium Phosphate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: The residue from the ignition of about 20 mg of it meets the requirements of the tests for *Sodium* ⟨191⟩ and for *Phosphate* ⟨191⟩.
Specific rotation ⟨781S⟩: between +95° and +102°.
Test solution: 10 mg per mL, in a mixture of pH 7.0 phosphate buffer and carbon dioxide-free water (9:1).
pH ⟨791⟩: between 7.5 and 10.5, in a solution (1 in 100).
Water Determination, *Method I* ⟨921⟩: not more than 6.5%.

Phosphate ions—
Standard phosphate solution—Dissolve 143.3 mg of dried monobasic potassium phosphate, KH_2PO_4, in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of phosphate (PO_4) in each mL.
Phosphate reagent A—Dissolve 5 g of ammonium molybdate in 1 N sulfuric acid to make 100 mL.
Phosphate reagent B—Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water, add 20 g of sodium bisulfite, mix to dissolve, and dilute with water to 100 mL.
Procedure—Dissolve about 50 mg of Prednisolone Sodium Phosphate, accurately weighed, in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25 mL, mix, and allow to stand at room temperature for 30 minutes.

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Similarly and concomitantly, prepare a standard solution, using 5.0 mL of *Standard phosphate solution* instead of the 50 mg of the substance under test. Concomitantly determine the absorbances of both solutions in 1-cm cells at 730 nm, with a suitable spectrophotometer, using water as the blank. The absorbance of the test solution is not more than that of the standard solution. The limit is 1.0% of phosphate (PO_4).

Selenium ⟨291⟩: 0.003%, a 200-mg test specimen being used.

Change to read:

Related compounds—

Buffer solution, Mobile phase, and *System suitability solution*—Proceed as directed in the *Assay.*

Standard solution—Accurately weigh a known quantity of USP Prednisolone Sodium Phosphate RS into an appropriate volumetric flask. Dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.001 mg per mL.

Test solution—Accurately weigh a known quantity of Prednisolone Sodium Phosphate into an appropriate volumetric flask. Dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* The run time is about four times the retention time of prednisolone sodium phosphate.

Procedure—Inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of the individual impurities listed in *Table 1* in the portion of Prednisolone Sodium Phosphate taken by the formula:

$$(100/F)(C_S/C_T)(r_T/r_S)$$

in which F is the relative response factor (see *Table 1* for values); C_S is the concentration, in mg per mL, of USP Prednisolone Sodium Phosphate RS in the *Standard solution;* C_T is the concentration, in mg per mL, of Prednisolone Sodium Phosphate in the *Test solution;* r_T is the peak response of each individual impurity in the *Test solution;* and r_S is the peak response of the prednisolone sodium phosphate in the *Standard solution.* The percentage limit of each individual impurity is given in *Table 1.*

Assay—

Mobile phase—Transfer 6.6 g of hexylamine and 5.32 g of monobasic ammonium phosphate into an appropriate flask. After 10 minutes, add 1850 g of water and dissolve the contents while stirring. Adjust with phosphoric acid to a pH of 6.40 ± 0.05. Add 390 g of acetonitrile, mix by stirring, and then sonicate for not more than 2 minutes.

System suitability solution—Dissolve equal, accurately weighed, quantities of USP Prednisolone RS and USP Prednisolone Sodium Phosphate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.01 mg per mL of each of the Reference Standards.

Standard preparation—Dissolve an accurately weighed quantity of USP Prednisolone RS in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Dissolve an accurately weighed quantity of the Prednisolone Sodium Phosphate in *Mobile phase* to obtain a solution having a known concentration of about 0.42 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 7.5-cm column that contains 3.5-µm packing L1. The column temperature is maintained at 35°, and the injector temperature is maintained at 6°. The flow rate is about 1.5 mL per minute, and the run time is about 22 minutes.

Table 1 (Prednisolone Sodium Phosphate)

Component	Relative Retention Time	Relative Response Factor	Limit (%)
Impurity A[1]	0.18	1	NMT 0.2
Impurity B[2]	0.29	1	NMT 1.0
D-homo A derivative[3]	0.40	1	NMT 1.0
Prednisolone (free)[4]	0.54	1.3	NMT 1.0
D-homo B derivative[5]	0.72	1	NMT 1.0
Prednisone sodium phosphate	0.77	1.0	NMT 0.5
Impurity C[6]	0.85	1	NMT 0.3
Impurity D[7]	0.94	1	NMT 0.2
▲Prednisolone Sodium Phosphate	1.00	—	—▲ (ERR 1-Apr-2019)
Impurity E[8]	2.72	1	NMT 0.5
Impurity F[9]	3.50	1	NMT 0.2
Any other individual unspecified impurity	—	1	NMT 0.1
Total of all impurities	—	—	NMT 3.0

[1] 11β,17,20-Trihydroxy-21-nor-13(17)a-homopregnane-1,4-diene-3,13a-dione 20-phosphate.
[2] 11β,17α-Dihydroxyandrosta-1,4-diene-3-one 17β-carboxylic acid.
[3] 11β,17β,20-Trihydroxy-21-nor-14(15)a-homopregnane-1,4-diene-3,16-dione 20-phosphate.
[4] 11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione.
[5] 11β,17α,20-Trihydroxy-21-nor-14(15)a-homopregnane-1,4-diene-3,16-dione 20-phosphate.
[6] 21-Oxo-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione.
[7] 11β-Hydroxyandrosta-1,4-diene-3,17-dione.
[8] 16β-Phosphoryloxyacetyl-11β,16α-dihydroxyandrosta-1,4-diene-3-one 11-phosphate.
[9] 21-Oxo-20-phosphoryloxy-11β-hydroxypregna-1,4,17-triene-3-one.

USP Monographs

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Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.52 for prednisolone and 1 for prednisolone sodium phosphate; the resolution, *R,* between prednisolone and prednisolone sodium phosphate is not less than 12; and the relative standard deviation for five replicate injections of the *Standard preparation* is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{21}H_{27}Na_2O_8P$ in the portion of the Prednisolone Sodium Phosphate taken using the formula:

$$100(C_S/C_U)(484.39/360.45)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of prednisolone in the *Standard preparation* and Prednisolone Sodium Phosphate in the *Assay preparation,* respectively; 484.39 and 360.45 are the molecular weights for prednisolone sodium phosphate and free prednisolone, respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Prednisolone Sodium Phosphate Compounded Oral Solution

DEFINITION

Prednisolone Sodium Phosphate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$). Prepare Prednisolone Sodium Phosphate Compounded Oral Solution 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Prednisolone (as Sodium Phosphate) Powder	1 g (1.34 g)
Purified Water	A small amount
Vehicle: a 1:1 mixture of Cherry Syrup[a] and Ora-Sweet[b], a sufficient quantity to make	100 mL

[a] Humco, Texarkana, TX.
[b] Perrigo Pharmaceuticals, Allegan, MI.

Pour the weighed *Prednisolone Sodium Phosphate Powder* into a suitable container. Wet the powder with a small amount of *Purified Water,* and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents, stepwise and quantitatively, to a calibrated container. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 2.0 g of 1-pentanesulfonic acid sodium in 1000 mL of water.
Solution B: Mix 980 mL of *Solution A* with 20 mL of tetrahydrofuran. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: Methanol and *Solution B* (45:55)
Standard solution: 0.4 mg/mL of prednisolone prepared from USP Prednisolone Sodium Phosphate RS in *Solution B*
Sample solution: Shake each bottle of Oral Solution thoroughly. Transfer 1.0 mL of Oral Solution to a 25-mL volumetric flask, dilute with *Solution B* to volume, and mix well.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 246 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for prednisolone is about 11.3 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prednisolone ($C_{21}H_{28}O_5$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of prednisolone from the *Sample solution*
r_S = peak response of prednisolone from the *Standard solution*
C_S = concentration of prednisolone in the *Standard solution* (mg/mL)
C_U = nominal concentration of prednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.9–4.9

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Prednisolone Sodium Phosphate RS

Prednisolone Sodium Phosphate Injection

» Prednisolone Sodium Phosphate Injection is a sterile solution of Prednisolone Sodium Phosphate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone phosphate ($C_{21}H_{29}O_8P$), present as the disodium salt.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Prednisolone RS

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Identification—

A: Dissolve 65 mg of phenylhydrazine hydrochloride in 100 mL of dilute sulfuric acid (3 in 5), add 5 mL of isopropyl alcohol, and mix. Heat 5 mL of this solution with 1 mL of *Assay preparation* (obtained as directed in the *Assay*) at 70° for 2 hours: a yellow color develops.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—

Test specimen: Place 5 mL of the *Assay preparation* obtained as directed in the *Assay*, in a glass-stoppered, 100-mL volumetric flask, mix with 5 mL of *Alkaline phosphatase solution* prepared as directed in the *Assay*, and add 50 mL of methylene chloride. Insert the stopper, and allow to stand, with occasional gentle inversion (about once every 15 minutes), for 2 hours. Filter the methylene chloride layer through a dry paper, and evaporate 25 mL of the filtrate to dryness.

Standard specimen: Prepare as directed in ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020), using USP Prednisolone RS.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.0 USP Endotoxin Units per mg of prednisolone phosphate.

pH ⟨791⟩: between 7.0 and 8.0.

Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

pH 9 Buffer with magnesium—Mix 3.1 g of boric acid and 500 mL of water in a 1-L volumetric flask, add 21 mL of 1 N sodium hydroxide and 10 mL of 0.1 M magnesium chloride, dilute with water to volume, and mix.

Alkaline phosphatase solution—Transfer 250 mg of alkaline phosphate enzyme to a 25-mL volumetric flask, dissolve by adding *pH 9 Buffer with magnesium* to volume, and mix. Prepare this solution fresh daily.

Standard preparation—Dissolve a suitable, accurately weighed quantity of USP Prednisolone RS in methylene chloride, and dilute quantitatively and stepwise with methylene chloride to obtain a solution having a known concentration of about 16 µg per mL. Pipet 100 mL of the solution into a glass-stoppered, 100-mL cylinder, and add 1.0 mL of *Alkaline phosphatase solution* and 1.0 mL of water. Allow to stand, with occasional gentle inversion, for 2 hours.

Assay preparation—Pipet a volume of Injection, equivalent to about 100 mg of prednisolone phosphate, into a separator containing 20 mL of water. Wash the solution with two 10-mL portions of methylene chloride, and discard the washings. Transfer the aqueous layer to a 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Pipet 1 mL of the *Assay preparation* into a glass-stoppered, 100-mL cylinder, add 1.0 mL of *Alkaline phosphatase solution* and about 50 mL of methylene chloride, insert the stopper, and allow to stand, with occasional gentle inversion (about once every 15 minutes), for 2 hours. Add methylene chloride to volume, mix, and allow to stand until the methylene chloride layer is clear (about 20 minutes). Concomitantly and without delay, determine the absorbances of the methylene chloride solution obtained from the *Assay preparation* and the *Standard preparation* at 241 nm, with a suitable spectrophotometer, using methylene chloride as the blank. Calculate the quantity, in mg, of $C_{21}H_{29}O_8P$ in each mL of the Injection taken by the formula:

$$6.11(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Prednisolone RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solution from the *Assay preparation* and the *Standard preparation*, respectively.

Prednisolone Sodium Phosphate Ophthalmic Solution

» Prednisolone Sodium Phosphate Ophthalmic Solution is a sterile solution of Prednisolone Sodium Phosphate in a buffered, aqueous medium. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of prednisolone phosphate ($C_{21}H_{29}O_8P$), present as the disodium salt.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Prednisolone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

Test specimen: Place 5 mL of the *Assay preparation* obtained as directed in the *Assay*, in a glass-stoppered, 100-mL volumetric flask, mix with 5 mL of *Alkaline phosphatase solution* prepared as directed in the *Assay*, and add 50 mL of methylene chloride. Insert the stopper, and allow to stand, with occasional gentle inversion (about once every 15 minutes), for 2 hours. Filter the methylene chloride layer through a dry paper, and evaporate 25 mL of the filtrate to dryness.

Standard specimen: Prepare as directed in ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020), using USP Prednisolone RS.

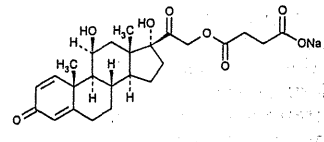
B: Dissolve 65 mg of phenylhydrazine hydrochloride in 100 mL of dilute sulfuric acid (3 in 5), add 5 mL of isopropyl alcohol, and mix. Heat 5 mL of this solution with 1 mL of *Assay preparation* (obtained as directed in the *Assay*) at 70° for 2 hours: a yellow color develops.

Sterility Tests ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 6.2 and 8.2.

Assay—Proceed with Ophthalmic Solution as directed in the *Assay* under *Prednisolone Sodium Phosphate Injection.*

Prednisolone Sodium Succinate for Injection



$C_{25}H_{31}NaO_8$ 482.50

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Pregna-1,4-diene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-, monosodium salt, (11β)-.
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(sodium succinate) [1715-33-9].

» Prednisolone Sodium Succinate for Injection is sterile prednisolone sodium succinate prepared from Prednisolone Hemisuccinate with the aid of Sodium Hydroxide or Sodium Carbonate. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone ($C_{21}H_{28}O_5$). It contains suitable buffers.

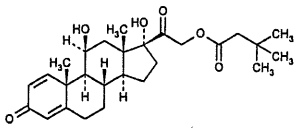
Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Prednisolone Hemisuccinate RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
Identification—Place about 50 mg in a separator, add 20 mL of water and 2 mL of 3 N hydrochloric acid, and extract with 25 mL of chloroform. Filter the extract into a suitable beaker, evaporate on a steam bath to dryness, and dry the residue at 60° for 1 hour: the residue so obtained responds to *Identification* test *A* under *Prednisolone Hemisuccinate.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.8 USP Endotoxin Units per mg of prednisolone.
pH ⟨791⟩: between 6.7 and 8.0, determined in the solution constituted as directed in the labeling.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 2.0% of its weight.
Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.
Other requirements—It meets the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Standard preparation—Dissolve about 64 mg of USP Prednisolone Hemisuccinate RS, accurately weighed, in about 100 mL of alcohol, add 5.0 mL of water, dilute with alcohol to 200.0 mL, and mix. Dilute 4.0 mL of this solution with alcohol to 100.0 mL, and mix. Pipet 20 mL of the resulting solution into a glass-stoppered, 50-mL conical flask.
Assay preparation—Transfer an accurately weighed portion of Prednisolone Sodium Succinate for Injection, equivalent to about 50 mg of prednisolone, to a 200-mL volumetric flask, dissolve in 5.0 mL of water, add alcohol to volume, and mix. Dilute 4.0 mL of this mixture with alcohol to 100.0 mL, and mix. Pipet 20 mL of the resulting solution into a glass-stoppered, 50-mL conical flask.
Procedure—Proceed as directed under *Assay for Steroids* ⟨351⟩. Calculate the quantity, in mg, of $C_{21}H_{28}O_5$ in the portion of Prednisolone Sodium Succinate for Injection taken by the formula:

$$5C(0.7827)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Prednisolone Hemisuccinate RS in the *Standard preparation*, 0.7827 is the ratio of the molecular weight of prednisolone to that of prednisolone hemisuccinate, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Prednisolone Tebutate



$C_{27}H_{38}O_6$(monohydrate) 458.59
Pregna-1,4-diene-3,20-dione, 11,17-dihydroxy-21-(3,3-dimethyl-1-oxobutyl)oxy-, (11β)-.
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(3,3-dimethylbutyrate) [7681-14-3].

» Prednisolone Tebutate contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{38}O_6$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers sealed under sterile nitrogen, in a cold place.
USP Reference standards ⟨11⟩—
USP Prednisolone Tebutate RS

Identification—
A: Dissolve a portion of it in acetone, and evaporate to dryness: the IR absorption spectrum of a mineral oil dispersion of the residue so obtained, previously dried at a pressure not exceeding 5 mm of mercury at 105° for 4 hours, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Prednisolone Tebutate RS.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: methanol.
Absorptivities at 242 nm, calculated on the dried basis, do not differ by more than 3.0%.
Specific rotation ⟨781S⟩: between +100° and +115°.
Test solution: 10 mg per mL, in chloroform.
Loss on drying ⟨731⟩—Dry it at a pressure not exceeding 5 mm of mercury at 105° for 4 hours: it loses not more than 5.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Selenium ⟨291⟩: 0.003%, a 200-mg specimen being used.

Assay—
Mobile solvent—Prepare a mixture of isooctane, tetrahydrofuran, and alcohol (89:10:1).
Standard preparation—Dissolve a suitable quantity of USP Prednisolone Tebutate RS, accurately weighed, in a mixture of tetrahydrofuran and isooctane (1:1) to obtain a solution having a known concentration of about 1 mg per mL.
Assay preparation—Weigh accurately about 50 mg of Prednisolone Tebutate, transfer to a 50-mL volumetric flask, dilute with tetrahydrofuran and isooctane (1:1) to volume, and mix.
Procedure—Inject separately 25-µL volumes of the *Standard preparation* and the *Assay preparation* into a suitable high-pressure liquid chromatograph equipped with a constant flow pump and a 30-cm × 3.9-mm column that contains packing L3, is operated at 25°, and is equipped with an UV detector capable of monitoring absorption at 254 nm. The flow rate is about 1.0 mL per minute. The retention time for Prednisolone Tebutate is about 30 minutes, but the chromatogram is run for about 45 minutes. Five replicate injections of the *Standard preparation* show a relative standard

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deviation of not more than 1.5%. Calculate the quantity, in mg, of $C_{27}H_{38}O_6$ in the portion of Prednisolone Tebutate taken by the formula:

$$50C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Prednisolone Tebutate RS in the *Standard preparation*, and R_U and R_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Prednisolone Tebutate Injectable Suspension

» Prednisolone Tebutate Injectable Suspension is a sterile suspension of Prednisolone Tebutate in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{27}H_{38}O_6$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Prednisolone Tebutate RS

Change to read:

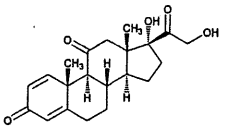
Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)—
Solution: Dilute a portion of the Injectable Suspension with methanol to obtain a solution having a concentration of about 20 µg per mL.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 8.8 USP Endotoxin Units per mg of prednisolone tebutate.
pH ⟨791⟩: between 6.0 and 8.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile solvent—Prepare a mixture of isooctane, tetrahydrofuran, and alcohol (89:10:8).
Standard preparation—Dissolve an accurately weighed quantity of USP Prednisolone Tebutate RS in a mixture of tetrahydrofuran and isooctane (1:1) to obtain a solution having a known concentration of about 1 mg per mL.
Assay preparation—Transfer to a separator an accurately measured volume, freshly mixed, of Injectable Suspension, equivalent to about 100 mg of prednisolone tebutate, and dilute with about 10 mL of water. Extract with three 25-mL portions of methylene chloride, filtering each portion through methylene chloride-washed cotton into a 100-mL volumetric flask. Add methylene chloride to volume, and mix. Pipet 10 mL of this solution into a 50-mL centrifuge tube, evaporate the methylene chloride on a steam bath just to dryness, cool, and dissolve the residue in 10.0 mL of tetrahydrofuran and isooctane (1:1). Filter through a 1-µm membrane filter.
Procedure—Introduce equal volumes, about 10 µL, of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩), operated at room temperature, by means of a suitable microsyringe or sampling valve, adjusting the sample size and other operating parameters such that the peak obtained with the *Standard preparation* is about 0.6 full-scale. Typically, the apparatus is fitted with a 3.9-mm × 30-cm column containing packing L3 and is equipped with an UV detector capable of monitoring absorption at 254 nm and a suitable recorder. In a suitable chromatogram, the coefficient of variation for five replicate injections of a single specimen is not more than 3.0%. Measure the height of the peaks, at identical retention times, obtained with the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of $C_{27}H_{38}O_6$, in the volume of Injectable Suspension taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Prednisolone Tebutate RS in the *Standard preparation*, and r_U and r_S are the peak heights obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Prednisone



$C_{21}H_{26}O_5$ 358.44
Pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-; 17,21-Dihydroxypregna-1,4-diene-3,11,20-trione [53-03-2].

DEFINITION
Prednisone contains NLT 97.0% and NMT 102.0% of prednisone ($C_{21}H_{26}O_5$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Analysis: If a difference appears, dissolve portions of both the sample and the Reference Standard in methanol, evaporate the solutions to dryness, and repeat the tests.
  Acceptance criteria: Meets the requirements
- **B.**
  Analysis 1: Dissolve 6 mg in 2 mL of sulfuric acid, and allow to stand for 5 min.
  Acceptance criteria 1: An orange color is produced.
  Analysis 2: Pour the resulting solution from *Analysis 1* into 10 mL of water.
  Acceptance criteria 2: The color changes first to yellow and then, gradually, to bluish green.

ASSAY
- **PROCEDURE**
  Mobile phase: Peroxide-free tetrahydrofuran, methanol, and water (250:62:688)
  Diluent: Methanol and water (1:1)
  Standard stock solution: 0.2 mg/mL of USP Prednisone RS in *Diluent*
  Internal standard solution: 110 µg/mL of acetanilide in *Diluent*
  Standard solution: 20 µg/mL of USP Prednisone RS and 11 µg/mL of acetanilide in *Diluent* from the *Standard stock solution* and the *Internal standard solution*, respectively. Prepare this solution fresh.
  Sample stock solution: 0.2 mg/mL of Prednisone in *Diluent*

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Sample solution: 20 µg/mL of Prednisone and 11 µg/mL of acetanilide in *Diluent* from the *Sample stock solution* and the *Internal standard solution*, respectively. Prepare this solution fresh.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention times of acetanilide and prednisone are about 6 and 8 min, respectively.]
Suitability requirements
Resolution: NLT 3 between prednisone and acetanilide
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of prednisone ($C_{21}H_{26}O_5$) in the portion of Prednisone taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of prednisone to acetanilide from the *Sample solution*
R_S = peak response ratio of prednisone to acetanilide from the *Standard solution*
C_S = concentration of USP Prednisone RS in the *Standard solution* (µg/mL)
C_U = concentration of Prednisone in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 100 mg
Acceptance criteria: Negligible

• **ORGANIC IMPURITIES**
Mobile phase: Chloroform and methanol (98:2)
Sample solution: 1.25 mg/mL of Prednisone in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 6.0-mm × 4.0-cm; packing L3
Flow rate: 1 mL/min
Injection volume: 5 µL

System suitability
Sample: *Sample solution*
Suitability requirements
Column efficiency: NLT 2500 theoretical plates
Relative standard deviation: NMT 2.0%

Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Prednisone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria
Any individual impurity: NMT 1.5%
Total impurities: NMT 2.0%

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 5 mg/mL in dioxane
Acceptance criteria: +167° to +175°

• **WATER DETERMINATION,** *Method I* ⟨921⟩
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prednisone RS

Prednisone Oral Solution

DEFINITION

Prednisone Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of prednisone ($C_{21}H_{26}O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: 50 mL of Oral Solution
Analysis: Shake the *Sample* with 25 mL of chloroform for 5 min. Filter the chloroform extract through a pledget of cotton and a layer of anhydrous sodium sulfate, and evaporate on a warm water bath with the aid of a current of air to 3 mL. Continue the evaporation to dryness at room temperature. Wash the residue with two 10-mL portions of hot solvent hexane, decanting the solvent and discarding it each time. Digest the residue with 25 mL of warm dehydrated alcohol for 15 min. Filter the mixture, and evaporate the filtrate to 3 mL. Add solvent hexane until the mixture becomes slightly cloudy, and chill in a freezer to promote the formation of crystals. Collect the crystals, and dry at 60° for 1 h.
Acceptance criteria: The crystals meet the requirements. If a difference appears, dissolve portions of both the crystals and the Reference Standard in methanol, evaporate the solutions to dryness, and repeat the tests.

ASSAY

• **PROCEDURE**
Mobile phase: Dissolve 1.36 g of monobasic sodium phosphate in 600 mL of water. To this solution add 400 mL of methanol.
Standard stock solution: 1 mg/mL of USP Prednisone RS in alcohol
Standard solution: 40 µg/mL of USP Prednisone RS in water from the *Standard stock solution*
Sample solution: Nominally 40 µg/mL of prednisone in water from a measured volume of Oral Solution

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

USP Monographs

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prednisone ($C_{21}H_{26}O_5$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Prednisone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of prednisone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS
• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩**:** 2.0%–6.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For single-unit containers
Acceptance critieria: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
• **PH** ⟨791⟩**:** 2.6–4.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prednisone RS

Prednisone Injectable Suspension

» Prednisone Injectable Suspension is a sterile suspension of Prednisone in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{26}O_5$.

Packaging and storage—Preserve in multiple-dose containers, preferably of Type I glass.
Labeling—Label it to indicate that it is for veterinary use only. Label it to indicate that it is for intramuscular administration only.
USP Reference standards ⟨11⟩—
USP Prednisone RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.
pH ⟨791⟩: between 3.0 and 7.0.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 2.27 Endotoxin Units per mg of prednisone.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of methanol and 0.05 M monobasic potassium phosphate (350:300). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Dissolve a quantity of betamethasone in methanol to obtain a solution containing about 0.4 mg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Prednisone RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.25 mg per mL. Transfer 10.0 mL of this solution to a 50-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.
Assay preparation—Transfer an accurately measured volume of well-mixed Suspension, equivalent to about 80 mg of prednisone, to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. Transfer 3.0 mL of this solution to a 50-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 1.9 for betamethasone and 1.0 for prednisone, the resolution, *R*, between the prednisone peak and the betamethasone peak is not less than 3.5, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the analyte peaks. Calculate the quantity, in mg, of prednisone in each mL of the Suspension taken by the formula:

$$5000(C/3V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Prednisone RS in the *Standard preparation*, V is the volume, in mL, of the Suspension taken to prepare the *Assay preparation*, and R_U and R_S are the ratios of the prednisone peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Prednisone Tablets

DEFINITION
Prednisone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of prednisone ($C_{21}H_{26}O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Nominally 10 mg of prednisone from pulverized Tablets
Analysis: Place the *Sample* in a 50-mL beaker, add 10 mL of water, and mix to form a slurry. Transfer the slurry to a 3-cm × 13-cm column packed with diatomaceous earth, and allow to absorb for 10 min. Elute the column with 60 mL of water-washed ether, and evaporate the eluate on a steam bath to dryness. Wash the residue with three 20-mL portions of *n*-heptane, and filter. Dry the residue at 105° for 30 min.

USP Monographs

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Acceptance criteria: The crystals meet the requirements. If a difference appears, dissolve portions of both the crystals and the Reference Standard in methanol, evaporate the solutions to dryness, and repeat the tests.

• **B.**

Analysis 1: Dissolve 6 mg of the crystals obtained in *Identification* test *A* in 2 mL of sulfuric acid, and allow to stand for 5 min.

Acceptance criteria 1: An orange color is produced.

Analysis 2: Pour the resulting solution from *Analysis 1* into 10 mL of water.

Acceptance criteria 2: The color changes first to yellow and then, gradually, to bluish green.

ASSAY

• **PROCEDURE**

Mobile phase: Peroxide-free tetrahydrofuran, methanol, and water (250:62:688). Prepare the *Mobile phase* such that, at a flow rate of 1.0 mL/min, the retention times of prednisone and acetanilide are about 8 and 6 min, respectively.

Diluent: Methanol and water (1:1)

Internal standard solution: 110 µg/mL of acetanilide in *Diluent*

Standard stock solution: 0.2 mg/mL of USP Prednisone RS in *Diluent*

Standard solution: 20 µg/mL of USP Prednisone RS and 11 µg/mL of acetanilide in *Diluent* from the *Standard stock solution* and the *Internal standard solution*, respectively. Prepare this solution fresh.

Sample stock solution: Nominally 0.2 mg/mL of prednisone prepared as follows. Transfer an amount of powder equivalent to 20 mg of prednisone from NLT 20 powdered Tablets to a suitable volumetric flask. Add 5% of the flask volume of water, and sonicate for 1 min. Add 50% of the flask volume of methanol, and sonicate again for 1 min. Dilute with water to volume.

Sample solution: Nominally 20 µg/mL of prednisone and 11 µg/mL of acetanilide in *Diluent* from the *Sample stock solution* and the *Internal standard solution*, respectively. Pass through a suitable filter of 5-µm pore size, discarding the first 20 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 25-cm; packing L1

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3 between prednisone and acetanilide

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of prednisone ($C_{21}H_{26}O_5$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of prednisone to acetanilide from the *Sample solution*

R_S = peak response ratio of prednisone to acetanilide from the *Standard solution*

C_S = concentration of USP Prednisone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of prednisone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; use 500 mL of the *Medium* for Tablets labeled to contain 10 mg of prednisone or less, and 900 mL for Tablets labeled to contain more than 10 mg of prednisone.

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Prednisone RS in *Medium*. [NOTE—An amount of alcohol not to exceed 5% of the total volume of the *Standard solution* may be used to bring the prednisone Standard into solution before dilution with *Medium*.]

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 242 nm

Tolerances: NLT 80% (*Q*) of the labeled amount of prednisone ($C_{21}H_{26}O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Mobile phase, Diluent, Internal standard solution, Standard stock solution, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*

Sample stock solution: Place 1 Tablet in a suitable volumetric flask that when the contents are diluted to volume, the resulting solution has a nominal concentration of 0.2 mg/mL of prednisone. Add 5 mL of water, swirl, sonicate for 1 min, add a volume of methanol equal to one-half the volume of the volumetric flask, and sonicate again for 1 min. Dilute with water to volume.

Sample solution: Nominally 20 µg/mL of prednisone and 11 µg/mL of acetanilide in *Diluent* from the *Sample stock solution* and the *Internal standard solution*, respectively. Pass through a suitable filter of 5-µm pore size, discarding the first 20 mL of the filtrate.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of prednisone ($C_{21}H_{26}O_5$) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of prednisone to acetanilide from the *Sample solution*

R_S = peak response ratio of prednisone to acetanilide from the *Standard solution*

C_S = concentration of USP Prednisone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of prednisone in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

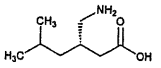
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Prednisone RS

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Pregabalin



$C_8H_{17}NO_2$ 159.23

(*S*)-3-(Aminomethyl)-5-methylhexanoic acid [148553-50-8].

DEFINITION

Pregabalin contains NLT 98.0% and NMT 102.0% of pregabalin ($C_8H_{17}NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (5:95)
Standard solution: 2.0 mg/mL of USP Pregabalin RS in *Mobile phase*
Sample solution: 2.0 mg/mL of Pregabalin in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 25°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention time of pregabalin
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pregabalin ($C_8H_{17}NO_2$) in the portion of Pregabalin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pregabalin from the *Sample solution*
r_S = peak response of pregabalin from the *Standard solution*
C_S = concentration of USP Pregabalin RS in the *Standard solution* (mg/mL)
C_U = concentration of Pregabalin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*

Standard solution: Prepare as directed in the chapter using 10.0 mL.
Sample solution: Prepare as directed in the chapter using 0.10 g of Pregabalin.
Acceptance criteria: The turbidity produced in the *Sample solution* is NMT that produced in the *Standard solution* (0.1%).

- **ORGANIC IMPURITIES**

Buffer: 0.04 M dibasic ammonium phosphate. Adjust with phosphoric acid to a pH of 6.5.
Solution A: Methanol and *Buffer* (20:80)
Solution B: Methanol and acetonitrile (10:90)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
5	98	2
16	80	20
33	80	20
34	98	2
45	98	2

Standard solution: 0.01 mg/mL of USP Mandelic Acid RS and 0.05 mg/mL each of USP Pregabalin RS and USP Pregabalin Related Compound C RS in water
Sample solution: 10.0 mg/mL of Pregabalin in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5% for mandelic acid, pregabalin, and pregabalin related compound C
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mandelic acid and pregabalin related compound C in the portion of Pregabalin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mandelic acid or pregabalin related compound C from the *Sample solution*
r_S = peak response of mandelic acid or pregabalin related compound C from the *Standard solution*
C_S = concentration of USP Mandelic Acid RS or USP Pregabalin Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Pregabalin in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Pregabalin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pregabalin from the *Standard solution*
C_S = concentration of USP Pregabalin RS in the *Standard solution* (mg/mL)

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C_U = concentration of Pregabalin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mandelic acid	0.66	—	0.10
Isobutylglutaric acid[a]	0.85	2.1	0.15
Pregabalin	1.0	1.0	—
Isobutylglutarmonoamide[b]	1.52	3.3	0.15
Pregabalin related compound C	3.95	—	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.8

[a] 3-Isobutylpentanedioic acid.
[b] 3-(2-Amino-2-oxoethyl)-5-methylhexanoic acid.

• **ENANTIOMERIC PURITY**
Buffer: Triethylamine and water (7.2: 1000). Adjust with 50% (v/v) phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (38:62)
Derivatizing reagent solution: 3 mg/mL of Marfey's reagent in acetone
Standard stock solution: 1 mg/mL of USP Pregabalin RS and 0.05 mg/mL of USP Pregabalin Related Compound A RS prepared as follows. Transfer appropriate amounts of USP Pregabalin RS and USP Pregabalin Related Compound A RS to a suitable volumetric flask. Dissolve in 50% of the final volume of water and dilute with acetone to volume.
Standard solution: Transfer 0.5 mL of the *Standard stock solution* to a 5.0-mL volumetric flask, add 0.3 mL of the *Derivatizing reagent solution* and 50 µL of 1 M sodium bicarbonate, and heat at 55° for about 1 h. Allow to cool to room temperature and dilute with water to volume.
Sample stock solution: 1 mg/mL of Pregabalin prepared as follows. Transfer an appropriate amount of Pregabalin to a suitable volumetric flask. Dissolve in 50% of the final volume of water and dilute with acetone to volume.
Sample solution: Transfer 0.5 mL of the *Sample stock solution* to a 5.0-mL volumetric flask, add 0.3 mL of the *Derivatizing reagent solution* and 50 µL of 1 M sodium bicarbonate, and heat at 55° for about 1 h. Allow to cool to room temperature and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 340 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 25°
Flow rate: 2.0 mL/min
Injection volume: 20 µL
Run time: NLT 3.8 times the retention time of pregabalin
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between pregabalin and pregabalin related compound A
Relative standard deviation: NMT 5.0% for pregabalin related compound A
Analysis
Sample: *Sample solution*
Calculate the percentage of pregabalin related compound A in the portion of Pregabalin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

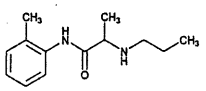
r_U = peak response of pregabalin related compound A from the *Sample solution*
r_T = sum of peak responses of pregabalin and pregabalin related compound A from the *Sample solution*

Acceptance criteria: NMT 0.15%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light and store at NMT 25°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Mandelic Acid RS
USP Pregabalin RS
USP Pregabalin Related Compound A RS
(*R*)-3-(Aminomethyl)-5-methylhexanoic acid.
$C_8H_{17}NO_2$ 159.23
USP Pregabalin Related Compound C RS
4-Isobutylpyrrolidin-2-one.
$C_8H_{15}NO$ 141.21

Prilocaine



$C_{13}H_{20}N_2O$ 220.31
Propanamide, *N*-(2-methylphenyl)-2-(propylamino)-; 2-(Propylamino)-*o*-propionotoluidide; (*RS*)-*N*-(2-Methylphenyl)-2-(propylamino)propanamide [721-50-6].

DEFINITION
Prilocaine contains NLT 98.0% and NMT 102.0% of prilocaine ($C_{13}H_{20}N_2O$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Analysis: Because of the low melting point of prilocaine, the mortar, pestle, and potassium bromide must be at ambient temperature. Record the IR spectrum using the diffuse reflectance technique.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

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ASSAY

• **PROCEDURE**

Buffer: 0.18 g/L of monobasic sodium phosphate and 2.89 g/L of dibasic sodium phosphate dihydrate in water
Mobile phase: Acetonitrile and *Buffer* (27:73)
System suitability solution: 2.5 µg/mL of USP Prilocaine RS and 3.0 µg/mL of USP Prilocaine Related Compound B RS in *Mobile phase*
Standard solution: 0.3 mg/mL of USP Prilocaine Hydrochloride RS in *Mobile phase*
Sample solution: 0.25 mg/mL of Prilocaine in *Mobile phase* [NOTE—Sonication may be needed to aid dissolution for the *Standard solution* and the *Sample solution*.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: At least 1.3 times the retention time of prilocaine
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between prilocaine and prilocaine related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of prilocaine ($C_{13}H_{20}N_2O$) in the portion of Prilocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of prilocaine from the *Sample solution*
r_S = peak response of prilocaine from the *Standard solution*
C_S = concentration of USP Prilocaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Prilocaine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of prilocaine, 220.31
M_{r2} = molecular weight of prilocaine hydrochloride, 256.77

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **LIMIT OF PRILOCAINE RELATED COMPOUND A**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1.3 µg/mL of USP Prilocaine Related Compound A RS in *Mobile phase*
Sample solution: 10 mg/mL of Prilocaine in *Mobile phase*
System suitability
Sample: *Standard solution*
Suitability requirements
Signal-to-noise ratio: NLT 10
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: Any peak corresponding to prilocaine related compound A (*o*-toluidine) in the *Sample solution* is NMT the response of the major peak in the *Standard solution* (0.01%).

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, and **System suitability solution:** Proceed as directed in the *Assay*.
Sample solution: 2.5 mg/mL of Prilocaine in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except for the run time.
Run time: At least 1.5 times the retention time of prilocaine
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for prilocaine and prilocaine related compound B are about 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 3.0 between prilocaine and prilocaine related compound B
Signal-to-noise ratio: NLT 10 for prilocaine
Analysis
Sample: *Sample solution*
Check the stability of the baseline by injecting *Mobile phase*.
Calculate the percentage of each impurity in the portion of Prilocaine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = individual peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria
Individual impurity: NMT 0.2% of any individual impurity; NMT one impurity exceeds 0.1%.
Total impurities: NMT 0.5%

SPECIFIC TESTS

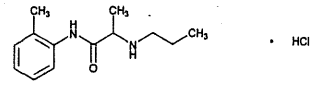
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩
Sample: 1.00 g
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store below 25°.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prilocaine RS
USP Prilocaine Hydrochloride RS
USP Prilocaine Related Compound A RS
o-Toluidine hydrochloride.
$C_7H_9N \cdot HCl$ 143.62
USP Prilocaine Related Compound B RS
(*RS*)-*N*-(4-Methylphenyl)-2-(propylamino)propanamide.
$C_{13}H_{20}N_2O$ 220.31

Prilocaine Hydrochloride



$C_{13}H_{20}N_2O \cdot HCl$ 256.77
Propanamide, *N*-(2-methylphenyl)-2-(propylamino)-, monohydrochloride;
2-(Propylamino)-*o*-propionotoluidide monohydrochloride [1786-81-8].

DEFINITION

Prilocaine Hydrochloride contains NLT 98.0% and NMT 102.0% of prilocaine hydrochloride ($C_{13}H_{20}N_2O \cdot HCl$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
  Analysis: Dissolve 100 mg in 3 mL of water, render the solution alkaline with 6 N ammonium hydroxide, and filter.
  Acceptance criteria: The filtrate meets the requirements.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 0.18 g/L of monobasic sodium phosphate and 2.89 g/L of dibasic sodium phosphate dihydrate in water
  Mobile phase: Acetonitrile and *Buffer* (26:74)
  System suitability solution: 3 µg/mL each of USP Prilocaine Hydrochloride RS and USP Prilocaine Related Compound B RS in *Mobile phase*
  Standard solution: 0.3 mg/mL of USP Prilocaine Hydrochloride RS in *Mobile phase*
  Sample solution: 0.3 mg/mL of Prilocaine Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—See *Table 1* for the relative retention times.]
  Suitability requirements
  Resolution: NLT 3.0 between prilocaine and prilocaine related compound B, *System suitability solution*
  Tailing factor: NMT 1.5, *Standard solution*
  Relative standard deviation: NMT 0.73, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of prilocaine hydrochloride ($C_{13}H_{20}N_2O \cdot HCl$) in the portion of Prilocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of prilocaine from the *Sample solution*
r_S = peak response of prilocaine from the *Standard solution*
C_S = concentration of USP Prilocaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Prilocaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**
  Buffer, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
  Standard solution 1: 3 µg/mL of USP Prilocaine Hydrochloride RS in *Mobile phase*
  Standard solution 2: 0.4 µg/mL of USP Prilocaine Related Compound A RS in *Mobile phase*
  Sample solution: 3 mg/mL of Prilocaine Hydrochloride in *Mobile phase*
  System suitability
  Samples: *System suitability solution* and *Standard solution 2*
  [NOTE—See *Table 1* for the relative retention times.]
  Suitability requirements
  Resolution: NLT 3.0 between prilocaine and prilocaine related compound B, *System suitability solution*
  Signal-to-noise ratio: NLT 10 for prilocaine related compound A, *Standard solution 2*
  Analysis
  Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
  Calculate the percentage of prilocaine related compound A in the portion of Prilocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of prilocaine related compound A from the *Sample solution*
r_S = peak response of prilocaine related compound A from *Standard solution 2*
C_S = concentration of USP Prilocaine Related Compound A RS in *Standard solution 2* (mg/mL)
C_U = concentration of Prilocaine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Prilocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of prilocaine from *Standard solution 1*
C_S = concentration of USP Prilocaine Hydrochloride RS in *Standard solution 1* (mg/mL)
C_U = concentration of Prilocaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting level for impurities is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Prilocaine related compound A	0.3	0.01
Prilocaine	1.0	—
Prilocaine related compound B	1.2	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.2

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩**
  Analysis: Dry a sample at 105° for 4 h.
  Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

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• **USP Reference Standards** ⟨11⟩
USP Prilocaine Hydrochloride RS
USP Prilocaine Related Compound A RS
o-Toluidine hydrochloride.
$C_7H_9N \cdot HCl$ 143.62
USP Prilocaine Related Compound B RS
(*RS*)-*N*-(4-Methylphenyl)-2-(propylamino)propanamide.
$C_{13}H_{20}N_2O$ 220.31

Prilocaine Hydrochloride Injection

DEFINITION
Prilocaine Hydrochloride Injection is a sterile solution of Prilocaine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of prilocaine hydrochloride ($C_{13}H_{20}N_2O \cdot HCl$).

IDENTIFICATION

Delete the following:

▲• **A. Identification—Organic Nitrogenous Bases** ⟨181⟩: Meets the requirements ▲ 2S (USP41)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ 2S (USP41)

Delete the following:

▲• **B.**
Sample: A volume of Injection equivalent to 300 mg of prilocaine hydrochloride
Analysis: Dilute the *Sample* with 5 mL of water, and add 4 mL of 6 N ammonium hydroxide. Extract with 50 mL of chloroform and filter the extract. Evaporate the filtrate on a steam bath with the aid of a current of air. Dissolve 100 mg of the prilocaine so obtained in 1 mL of alcohol, add 10 drops of cobaltous chloride TS, and shake for 2 min.
Acceptance criteria: A bright green color develops, and a precipitate is formed. ▲ 2S (USP41)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ 2S (USP41)

ASSAY

Change to read:

• **Procedure**
▲**Solution A:** Transfer 10 mL of glacial acetic acid to a 1000-mL volumetric flask containing 950 mL of water and dilute with water to volume. Adjust with ammonium hydroxide to a pH of 5.5.
Mobile phase: Acetonitrile and *Solution A* (21:79)
System suitability stock solution: 0.65 mg/mL of USP Prilocaine Related Compound A RS and 0.5 mg/mL of USP Prilocaine Related Compound B RS in *Mobile phase*
System suitability solution: 0.5 mg/mL of USP Prilocaine Hydrochloride RS, and 0.0065 mg/mL of USP Prilocaine Related Compound A RS and 0.005 mg/mL of USP Prilocaine Related Compound B RS from *System suitability stock solution*; in *Mobile phase*
Standard solution: 0.5 mg/mL of USP Prilocaine Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 0.5 mg/mL of prilocaine hydrochloride from Injection in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 3 times the retention time of prilocaine
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 6.0 between prilocaine and prilocaine related compound B; NLT 4.0 between prilocaine related compound B and prilocaine related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution* ▲ 2S (USP41)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prilocaine hydrochloride ($C_{13}H_{20}N_2O \cdot HCl$) in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Prilocaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prilocaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

Add the following:

▲• **Organic Impurities**
Solution A, Mobile phase, and **System suitability solution:** Prepare as directed in the *Assay*.
Standard solution: 0.01 mg/mL each of USP Prilocaine Hydrochloride RS and USP Prilocaine Related Compound A RS in *Mobile phase*
Sample solution: Nominally 5.0 mg/mL of prilocaine hydrochloride from Injection in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except for *Injection volume* and *Run time*.
Injection volume: 30 µL
Run time: NLT 6.5 times the retention time of prilocaine
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 6.0 between prilocaine and prilocaine related compound B; NLT 4.0 between prilocaine related compound B and prilocaine related compound A, *System suitability solution*

USP Monographs

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Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of prilocaine related compound A in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of prilocaine related compound A from the *Sample solution*

r_S = peak area of prilocaine related compound A from the *Standard solution*

C_S = concentration of USP Prilocaine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of prilocaine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of any individual unspecified degradation product from the *Sample solution*

r_S = peak area of prilocaine from the *Standard solution*

C_S = concentration of USP Prilocaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of prilocaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Prilocaine	1.0	—
Prilocaine related compound B[a]	1.7	—
Prilocaine related compound A	2.1	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	0.5

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product. ▲2S (USP41)

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.9 USP Endotoxin Units/mg of prilocaine hydrochloride
- **PH** ⟨791⟩: 6.0–7.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. ▲Store at controlled room temperature. Protect from freezing. ▲2S (USP41)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

▲▲(CN 1-May-2018)

USP Prilocaine Hydrochloride RS

▲USP Prilocaine Related Compound A RS

o-Toluidine hydrochloride.

$C_7H_9N \cdot HCl$ 143.61

USP Prilocaine Related Compound B RS

2-Propylamino-*N*-(*p*-tolyl)propanamide.

$C_{13}H_{20}N_2O$ 220.31 ▲2S (USP41)

Prilocaine and Epinephrine Injection

DEFINITION

Prilocaine and Epinephrine Injection is a sterile solution prepared from Prilocaine Hydrochloride and Epinephrine with the aid of Hydrochloric Acid in Water for Injection, or a sterile solution of Prilocaine Hydrochloride and Epinephrine Bitartrate in Water for Injection. The content of epinephrine does not exceed 0.002% (1 in 50,000). Prilocaine and Epinephrine Injection contains the equivalent of NLT 95.0% and NMT 105.0% of the labeled amount of prilocaine hydrochloride ($C_{13}H_{20}N_2O \cdot HCl$) and the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

IDENTIFICATION

- **A.**

Sample: A volume of Injection equivalent to 300 mg of prilocaine hydrochloride

Analysis: Dilute the *Sample* with 5 mL of water, and add 4 mL of 6 N ammonium hydroxide. Extract with 50 mL of chloroform, and filter the extract. Evaporate the filtrate on a steam bath with the aid of a current of air. Dissolve 100 mg of the prilocaine so obtained in 1 mL of alcohol, add 10 drops of cobaltous chloride TS, and shake for 2 min.

Acceptance criteria: A bright green color develops, and a precipitate is formed.

- **B.** The retention time of the major peak for prilocaine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Prilocaine Hydrochloride*.
- **C.** The retention time of the major peak for epinephrine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Epinephrine*.

ASSAY

- **PRILOCAINE HYDROCHLORIDE**

Solution A: 50 mL of glacial acetic acid in 930 mL of water. Adjust with 1 N sodium hydroxide to a pH of 3.40.

Mobile phase: Acetonitrile and *Solution A* (1:4). Adjust the ratio of the mobile phase components to obtain a retention time of 4–6 min for the prilocaine peak. Pass through a membrane filter of 1-µm or finer pore size, and degas.

Standard solution: 4 mg/mL of USP Prilocaine Hydrochloride RS in *Mobile phase*

System suitability stock solution: 900 µg/mL of procainamide hydrochloride in *Mobile phase*

System suitability solution: *System suitability stock solution* and *Standard solution* (1:10)

Sample solution: Nominally 4 mg/mL of prilocaine hydrochloride from Injection, in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

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Column: 3.9-mm × 30-cm; packing L1
Column temperature: 20°–25°, maintained at ±1.0° of the selected temperature
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between prilocaine and procainamide, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prilocaine hydrochloride ($C_{13}H_{20}N_2O \cdot HCl$) in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of prilocaine hydrochloride from the *Sample solution*
r_S = peak area of prilocaine hydrochloride from the *Standard solution*
C_S = concentration of USP Prilocaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prilocaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

• **EPINEPHRINE**
Solution A: Mix 50 mL of glacial acetic acid and 930 mL of water, and adjust with 1 N sodium hydroxide to a pH of 3.40. Dissolve 1.1 g of sodium 1-heptanesulfonate in this solution, and add 1.0 mL of 0.1 M disodium ethylenediaminetetraacetate.
Mobile phase: Methanol and *Solution A* (1:9). Pass through a membrane filter of 1-µm or finer pore size, and degas.
Standard solution: 1.8 µg/mL of USP Epinephrine Bitartrate RS in *Mobile phase*
Sample solution: Nominally 1 µg/mL of epinephrine from Injection in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: An electrochemical detector held at a potential of +650 mV, and a controller capable of regulating the background current
Column: 3.9-mm × 30-cm; packing L1
Column temperature: 20°–25°, maintained at ±1.0° of the selected temperature
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of epinephrine ($C_9H_{13}NO_3$) in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

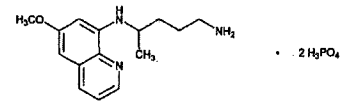
r_U = peak response of epinephrine from the *Sample solution*
r_S = peak response of epinephrine from the *Standard solution*
C_S = concentration of USP Epinephrine Bitartrate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of epinephrine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of epinephrine, 183.20
M_{r2} = molecular weight of epinephrine bitartrate, 333.29

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
• **COLOR AND CLARITY**
Standard solution: Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, and dilute with water to volume.
Sample solution: A portion of the Injection
Analysis
Samples: *Standard solution* and *Sample solution*
Visually examine the *Sample solution* in a suitable clear glass test tube against a white background: it is not pinkish, and it contains no precipitate. If any yellow color is observed in the *Sample solution*, concomitantly determine the absorbances of the *Sample solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm.
Acceptance criteria: The absorbance of the *Sample solution* does not exceed that of the *Standard solution*.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.9 USP Endotoxin Unit/mg of prilocaine hydrochloride.
• **PH** ⟨791⟩**:** 3.3–5.5
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose light-resistant containers, preferably of Type I glass.
• **LABELING:** The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Epinephrine Bitartrate RS
USP Prilocaine Hydrochloride RS

Primaquine Phosphate



$C_{15}H_{21}N_3O \cdot 2H_3PO_4$ 455.34
1,4-Pentanediamine, N^4-(6-methoxy-8-quinolinyl)-, (±)-, phosphate (1:2);
(±)-8-[(4-Amino-1-methylbutyl)amino]-6-methoxyquinoline phosphate (1:2) [63-45-6].

DEFINITION
Primaquine Phosphate contains NLT 97.0% and NMT 102.0% of primaquine phosphate ($C_{15}H_{21}N_3O \cdot 2H_3PO_4$), calculated on the dried basis.

USP Monographs

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020):** Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL ⟨191⟩, *Chemical Identification Tests, Phosphate, Pyrophosphates*:** The residue obtained by ignition meets the requirements.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Acetonitrile, tetrahydrofuran, trifluoroacetic acid, and water (9:1:0.1:90)

Standard solution: 0.4 mg/mL of USP Primaquine Phosphate RS in *Mobile phase*. [NOTE—Sonicate with intermittent shaking to dissolve, if necessary.]

System suitability stock solution: 0.4 mg/mL of USP Primaquine Related Compound A RS in *Mobile phase*

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

Sensitivity solution: 0.2 µg/mL of USP Primaquine Phosphate RS from the *Standard solution*

Sample solution: 0.4 mg/mL of Primaquine Phosphate in *Mobile phase*. [NOTE—Sonicate with intermittent shaking to dissolve, if necessary.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 75-mm; 3-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: ▲NLT▲ (USP41) 3 times the retention time of primaquine

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.5 between primaquine and primaquine related compound A, *System suitability solution*

Relative standard deviation: NMT 1.0% for primaquine, *Standard solution*

Signal-to-noise ratio: NLT 10 for the primaquine peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of primaquine phosphate ($C_{15}H_{21}N_3O \cdot 2H_3PO_4$) in the portion of Primaquine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of primaquine phosphate from the *Sample solution*

r_S = peak response of primaquine phosphate from the *Standard solution*

C_S = concentration of USP Primaquine Phosphate RS in the *Standard solution* (mg/mL)

C_U = concentration of Primaquine Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Primaquine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of primaquine phosphate from the *Sample solution*

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
▲Methoxyquinolinamine[a] ▲ (USP41)	0.24	0.20
▲Phthalhydrazide[b] ▲ (USP41)	0.29	0.60
Primaquine related compound A[c]	0.80	2.0
Primaquine	1.0	—
Secaquine[d]	1.8	0.80
Any other individual impurities	—	0.20
Total impurities	—	3.0

▲[a] 6-Methoxyquinolin-8-amine.

[b] 2,3-Dihydrophthalazine-1,4-dione.▲ (USP41)

[c] 8-[(4-Aminopentyl)amino]-6-methoxyquinoline.

[d] N^3-(6-Methoxyquinolin-8-yl)pentane-1,3-diamine.

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Primaquine Phosphate RS

USP Primaquine Related Compound A RS

8-[(4-Aminopentyl)amino]-6-methoxyquinoline.

$C_{15}H_{21}N_3O$ 259.35

Primaquine Phosphate Tablets

DEFINITION

Primaquine Phosphate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of primaquine phosphate ($C_{15}H_{21}N_3O \cdot 2H_3PO_4$).

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IDENTIFICATION

• **A.**

Sample solution: Digest the equivalent to 25 mg of primaquine phosphate from a quantity of finely powdered Tablets with 10 mL of water for 15 min, and filter.

Analysis: Dilute 0.1 mL of the *Sample solution* with 1 mL of water, and add 1 drop of gold chloride TS.

Acceptance criteria: A violet-blue color is produced at once.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, tetrahydrofuran, trifluoroacetic acid, and water (9: 1: 0.1: 90)

Standard solution: 0.4 mg/mL of USP Primaquine Phosphate RS in *Mobile phase*. [NOTE—Sonicate with intermittent shaking to dissolve, if necessary.]

System suitability stock solution: 0.4 mg/mL of USP Primaquine Related Compound A RS in *Mobile phase*

System suitability solution: Transfer 1.0 mL of *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

Sensitivity solution: 0.2 µg/mL of USP Primaquine Phosphate RS from *Standard solution*

Sample stock solution: Transfer a quantity of Tablets (NLT 20), accurately counted and weighed, to a 500-mL volumetric flask. Add 300 mL of *Mobile phase*, then sonicate and shake for 15 min. Add 150 mL of *Mobile phase*, then sonicate and shake for 15 min. Dilute with *Mobile phase* to volume, and filter.

Sample solution: 0.4 mg/mL of primaquine phosphate in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 75-mm; 3-µm packing L7

Flow rate: 1.5 mL/min

Injection size: 10 µL

Run time: Three times the retention time of primaquine

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.5 between primaquine phosphate and primaquine related compound A, *System suitability solution*

Relative standard deviation: NMT 1.0% for primaquine phosphate, *Standard solution*

Signal-to-noise ratio: NLT 10 for the primaquine peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of primaquine phosphate ($C_{15}H_{21}N_3O \cdot 2H_3PO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Primaquine Phosphate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of primaquine phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Sample solution: Pass a portion of the solution under test through an appropriate filter.

Standard solution: USP Primaquine Phosphate RS in *Medium* in a concentration similar that of the *Sample solution*

Solution A: 961 mg of sodium 1-pentanesulfonate and 1 mL of glacial acetic acid in 400 mL of water

Mobile phase: Methanol and *Solution A* (3:2)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the amount of primaquine phosphate ($C_{15}H_{21}N_3O \cdot 2H_3PO_4$) dissolved.

Tolerances: NLT 80% (*Q*) of the labeled amount of primaquine phosphate ($C_{15}H_{21}N_3O \cdot 2H_3PO_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

Procedure for content uniformity: Transfer 1 Tablet to a 50-mL volumetric flask, add about 30 mL of *Mobile phase*, then sonicate and shake for 15 min. Add *Mobile phase* up to the neck of the flask, then sonicate and shake for additional 15 min. Allow the solution to cool, and dilute with *Mobile phase* to volume. Filter a portion of the solution, and dilute 8.0 mL of this filtered solution with *Mobile phase* to 10.0 mL. Analyze the solution as directed in the *Assay*.

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = peak response of primaquine phosphate from the *Sample solution*

Acceptance criteria: See *Table 1*.

[NOTE—Disregard any impurity less than 0.05%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Specified unidentified impurity[a]	0.24	—
Specified unidentified impurity	0.29	0.60

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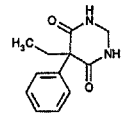
Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Primaquine related compound A[a,b]	0.80	—
Primaquine	1.0	—
Specified unidentified impurity[a]	1.8	—
Any other individual impurity	—	0.20
Total impurities	—	1.0

[a] These process impurities are listed for information only and are not quantified in the drug product.
[b] 8-[(4-Aminopentyl)amino]-6-methoxyquinoline.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Primaquine Phosphate RS
  USP Primaquine Related Compound A RS
  8-[(4-Aminopentyl)amino]-6-methoxyquinoline.
  $C_{15}H_{21}N_3O$ 259.35

Primidone



$C_{12}H_{14}N_2O_2$ 218.25
4,6(1*H*,5*H*)-Pyrimidinedione, 5-ethyldihydro-5-phenyl-;
5-Ethyldihydro-5-phenyl-4,6(1*H*,5*H*)-pyrimidinedione
[125-33-7].

DEFINITION
Primidone contains NLT 98.0% and NMT 102.0% of $C_{12}H_{14}N_2O_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020): If a difference appears, dissolve portions of both the sample and the Reference Standard in alcohol, evaporate the solutions to dryness, and repeat the tests.
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 6.8 g/L of monobasic potassium phosphate
Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (35: 0.5: 65)
Diluent: Methanol and water (35:65)
Standard stock solution: 0.5 mg/mL of USP Primidone RS in methanol
Standard solution: 0.05 mg/mL of USP Primidone RS in *Diluent*, prepared from the *Standard stock solution*
Sample stock solution: 0.5 mg/mL of Primidone in methanol
Sample solution: 0.05 mg/mL of Primidone from the *Sample stock solution* diluted with *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1.3 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{12}H_{14}N_2O_2$ in the portion of Primidone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Primidone RS in the *Standard solution* (mg/mL)
C_U = concentration of Primidone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.2%

ORGANIC IMPURITIES
- **Procedure**

Solution A: 1.36 g/L of monobasic potassium phosphate
Solution B: Methanol
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	75	25
1.0	75	25
6.0	40	60
8.0	40	60
8.5	75	25
10.0	75	25

Standard stock solution: 1000 µg/mL of USP Primidone RS in methanol
Standard solution: 1 µg/mL of USP Primidone RS in methanol, prepared from the *Standard stock solution* in methanol
System suitability solution: 1000 µg/mL of USP Primidone RS and 10 µg/mL each of USP Phenobarbital RS and USP Primidone Related Compound C RS. Prepare by diluting weighed quantities of USP Phenobarbital RS and USP Primidone Related Compound C RS with the *Standard stock solution*.
Sample solution: 1000 µg/mL of Primidone in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm

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Column: 4.6-mm × 10-cm; monolithic packing L1
Flow rate: 3.2 mL/min
Injection size: 10 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between phenobarbital and primidone related compound C, *System suitability solution*
Tailing factor: NMT 2.0 for primidone, *Standard solution*
Relative standard deviation: NMT 5.0% for primidone, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Identify the impurities using the relative retention times listed in *Impurity Table 1*. Calculate the percentage of each impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (100/F)$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of Primidone from the *Standard solution*
C_S = concentration of USP Primidone RS in the *Standard solution* (µg/mL)
C_U = concentration of Primidone in the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%
[NOTE—Disregard impurity peaks below 0.05%.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Primidone related compound A[a]	0.5	0.67	0.1
Phenobarbital	1.4	1.0	0.1
Primidone related compound C[b]	1.6	0.67	0.1
2-Cyano-2-phenylbutyramide	1.8	0.71	0.1
2-Phenylbutyric acid	2.0	0.77	0.1
Phenylpropylprimidone[c]	2.8	1.0	0.3
Any unspecified impurity	—	1.0	0.1

[a] 2-Ethyl-2-phenylmalonamide (2-ethyl-2-phenylpropanediamide).
[b] 2-Phenylbutyramide.
[c] 5-Ethyl-5-phenyl-2-(1-phenylpropyl)dihydropyrimidine-4,6(1*H*,5*H*)-dione.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Phenobarbital RS
USP Primidone RS
USP Primidone Related Compound C RS
2-Phenylbutyramide.
$C_{10}H_{13}NO$ 163.22

Delete the following:

▲Primidone Oral Suspension

DEFINITION
Primidone Oral Suspension is a suspension of Primidone in a suitable aqueous vehicle. It contains, in each 100 mL, NLT 4.5 g and NMT 5.5 g of primidone ($C_{12}H_{14}N_2O_2$).

IDENTIFICATION
• **A.** The retention time of the major peak in the *Sample solution* corresponds to that in the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Internal standard solution: 10 mg/mL of androsterone in alcohol
Standard solution: 1 mg/mL of USP Primidone RS in alcohol prepared as follows. Dissolve first in alcohol using 65% of the final volume, and boil for 1 h. Allow to cool to ambient temperature, and add the *Internal standard solution* to fill 10% of the final volume. Dilute with alcohol to volume, and filter.
Sample solution: Nominally 1 mg/mL of primidone in alcohol, from well-mixed Oral Suspension prepared as follows. Dissolve first in alcohol using 70% of the final volume, and boil for 1 h. Allow to cool to ambient temperature, and add the *Internal standard solution* to fill 10% of the final volume. Dilute with alcohol to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 4.0-mm × 120-cm; packed with 10% liquid phase G3 on support S1AB
Temperature
Detector: 310°
Injector: 310°
Column: 260°
Carrier gas: Helium
Flow rate: 40 mL/min
Injection size: 3 µL
System suitability
Sample: *Standard solution* (three replicate injections)
[NOTE—The relative retention times for primidone and androsterone are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between primidone and androsterone
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of primidone ($C_{12}H_{14}N_2O_2$), in mg, in each 100 mL of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times D \times 100$$

R_U = ratio of the peak response of primidone to the internal standard from the *Sample solution*
R_S = ratio of the peak response of primidone to the internal standard from the *Standard solution*
C_S = concentration of USP Primidone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of primidone in the *Sample solution* (mg/mL)
D = density of the Oral Suspension (g/mL)

USP Monographs

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Acceptance criteria: 4.5–5.5 g in each 100 mL

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements for oral suspension packaged in single-unit containers
- **DELIVERABLE VOLUME** ⟨698⟩: Meets the requirements for oral suspension packaged in multiple-unit containers

SPECIFIC TESTS

- **pH** ⟨791⟩: 5.5–8.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Primidone RS▲ (USP 1-May-2020)

Primidone Tablets

DEFINITION

Primidone Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of primidone ($C_{12}H_{14}N_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate
Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (35: 0.5: 65)
Diluent: Methanol and water (35:65)
Standard stock solution: 0.5 mg/mL of USP Primidone RS in methanol
Standard solution: 0.05 mg/mL of USP Primidone RS in *Diluent*, from the *Standard stock solution*
Sample stock solution: Transfer an equivalent of about 250 mg of primidone, from finely powdered Tablets (NLT 20), based on the label claim, to a 250-mL volumetric flask. Add about 125 mL of methanol, sonicate for about 15 min, and shake by mechanical means until all the solid is dispersed (usually 20 min). Allow the solution to cool to room temperature, and dilute with methanol to volume. Pass a portion of this solution through a PTFE (or equivalent) filter of 0.45-µm pore size, and discard the first 5 mL of filtrate.
Sample solution: 0.05 mg/mL of primidone in *Diluent*, from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1.3 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of primidone ($C_{12}H_{14}N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Primidone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of primidone in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Standard solution: USP Primidone RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to obtain a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 257 nm
[NOTE—Perform baseline corrections, if necessary, in determining the absorbance by extrapolating the baseline through the absorbance minima at 300 and 280 nm and beyond 257 nm.]
Tolerances: NLT 75% (*Q*) of the labeled amount of primidone ($C_{12}H_{14}N_2O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Primidone related compound A stock solution: 200 µg/mL of USP Primidone Related Compound A RS in methanol
Primidone related compound A solution: 20 µg/mL of USP Primidone Related Compound A RS in *Diluent*, from *Primidone related compound A stock solution*
Standard stock solution: 0.05 mg/mL of USP Primidone RS in methanol
System suitability solution: 1 µg/mL of USP Primidone Related Compound A RS and 2 µg/mL of USP Primidone RS in *Diluent*, from the *Primidone related compound A solution* and the *Standard stock solution*, respectively
Standard solution: 2 µg/mL of USP Primidone RS in *Diluent*, from the *Standard stock solution*
Sample solution: Transfer an equivalent of about 250 mg of primidone, from finely powdered Tablets (NLT 20), based on the label claim, to a 250-mL volumetric flask. Add about 90 mL of methanol, sonicate for about 15 min, and shake by mechanical means until all the solid is dispersed (usually 20 min). Allow the solution to cool to room temperature, and dilute with water to volume. Pass a portion of this solution through a PTFE (or equivalent) filter of 0.45-µm pore size, and discard the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
System suitability
Samples: *System suitability solution* and *Standard solution*

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[NOTE—The relative retention times for primidone related compound A and primidone are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between primidone related compound A and primidone, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Primidone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of primidone in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

[NOTE—Disregard impurity peaks that are less than 0.05%.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Primidone related compound A[a]	0.5	0.76	0.1
Primidone	1.0	—	—
Phenobarbital	1.6	1.4	0.1
Primidone related compound C[b]	1.9	0.92	0.1
2-Cyano-2-phenylbutyramide[c]	2.2	—	—
2-Phenylbutyric acid	4.1	0.91	0.1
Phenylpropylprimidone[c]	11.4	—	—
Any individual unspecified degradation product	—	1.0	0.1
Total impurities	—	—	0.5

[a] 2-Ethyl-2-phenylmalonamide.

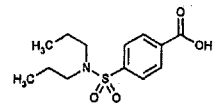
[b] 2-Phenylbutyramide.

[c] Process impurities controlled in the drug substance. Included for identification purposes only. Not reported for the drug product and not included in *Total impurities*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Tablets intended solely for veterinary use are so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Primidone RS
  USP Primidone Related Compound A RS
  2-Ethyl-2-phenylmalonamide.
  $C_{11}H_{14}N_2O_2$ 206.24

Probenecid



$C_{13}H_{19}NO_4S$ 285.36

Benzoic acid, 4-[(dipropylamino)sulfonyl]-.

p-(Dipropylsulfamoyl)benzoic acid [57-66-9].

» Probenecid contains not less than 98.0 percent and not more than 101.0 percent of $C_{13}H_{19}NO_4S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Probenecid RS

Identification—

Change to read:

A: *▲Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K▲* (CN 1-May-2020)

Change to read:

B: *▲Spectroscopic Identification Tests ⟨197⟩, Ultraviolet-Visible Spectroscopy: 197U▲* (CN 1-May-2020)

Solution: 20 µg per mL.

Medium: alcohol.

Absorptivities at 248 nm, calculated on the dried basis, do not differ by more than 3.0%.

Melting range ⟨741⟩: between 198° and 200°.

Acidity—To 2.0 g add 100 mL of water, heat on a steam bath for 30 minutes, cool, filter, and dilute with water to 100.0 mL. To 25.0 mL of this solution add 1 drop of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 0.50 mL is required to produce a pink color.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Selenium ⟨291⟩: not more than 0.003%, a 100-mg test specimen, mixed with 100 mg of magnesium oxide, being used.

Chromatographic purity—

Mobile phase , *Assay preparation*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Test preparation—Use the *Assay preparation*.

Procedure—Inject about 20 µL of the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for all of the peaks. Calculate the percentages of each peak, other than the solvent peak and the probenecid peak, in the portion of Probenecid taken by the formula:

$$100(r_i/r_T)$$

in which r_i is the response of each peak and r_T is the sum of the responses of all of the peaks, excluding that of the solvent peak: not more than 0.5% of any individual impurity and not more than 2.0% of total impurities is found.

USP Monographs

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Assay—

Sodium phosphate solution—Prepare in glacial acetic acid solution (1 in 100) a 0.05 M solution of monobasic sodium phosphate, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase—Prepare a degassed and filtered mixture (50:50) of *Sodium phosphate solution* and a 1 in 100 solution of glacial acetic acid in acetonitrile. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Probenecid RS in *Mobile phase* to obtain a solution having a known concentration of about 0.50 mg per mL.

Assay preparation—Transfer about 50 mg of Probenecid, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.3, the number of theoretical plates is not less than 3900, and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{19}NO_4S$ in the portion of Probenecid taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Probenecid RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Probenecid Tablets

» Probenecid Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{13}H_{19}NO_4S$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Probenecid RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—*Solution: Assay preparation.*

B: Finely powder a quantity of Tablets, equivalent to about 500 mg of probenecid, triturate the powder with alcohol, and filter. Evaporate the filtrate to about 20 mL, cool, acidify with hydrochloric acid until acid to litmus, remove the crystals by filtration, and recrystallize from diluted alcohol: the probenecid so obtained melts between 196° and 200°, as determined by the method for *Class Ia* under *Melting Range or Temperature* ⟨741⟩, and responds to *Identification* test *A* under *Probenecid.*

Dissolution ⟨711⟩—

Medium: simulated intestinal fluid TS, prepared without pancreatin, pH 7.5 ± 0.1; 900 mL.

Apparatus 2: 75 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{13}H_{19}NO_4S$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 244 nm on filtered portions of the solution under test, suitably diluted with 0.1 N sodium hydroxide, if necessary, in comparison with a Standard solution having a known concentration of USP Probenecid RS.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{13}H_{19}NO_4S$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 100 mg of probenecid, and transfer to a 250-mL volumetric flask. Add chloroform to volume, and mix. Filter a portion of the chloroform solution, discarding the first 20 to 25 mL of the filtrate, and pipet 5 mL of the filtrate into a 125-mL separator containing 10 mL of chloroform. Extract the chloroform layer with four 15-mL portions of sodium carbonate solution (1 in 100). Render the combined extracts distinctly acid with 5 N hydrochloric acid, and extract with four 20-mL portions of chloroform, filtering each extract through a small pledget of cotton into a 100-mL volumetric flask. Wash the cotton filter with 10 mL of chloroform, add chloroform to volume, and mix. Dissolve an accurately weighed quantity of USP Probenecid RS in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a Standard solution having a known concentration of about 20 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 257 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of $C_{13}H_{19}NO_4S$ in the portion of Tablets taken by the formula:

$$5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Probenecid RS in the Standard solution; and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

Probenecid and Colchicine Tablets

» Probenecid and Colchicine Tablets contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of colchicine ($C_{22}H_{25}NO_6$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of probenecid ($C_{13}H_{19}NO_4S$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Colchicine RS
USP Probenecid RS

Identification—

Probenecid standard solution—Prepare a solution of USP Probenecid RS in chloroform having a concentration of about 1 mg per mL.

USP Monographs

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Colchicine standard solution—Prepare a solution of USP Colchicine RS in chloroform having a concentration of about 1 mg per mL.

Probenecid test solution—Using a portion of finely powdered Tablets, prepare a filtered solution in chloroform having a concentration of about 1 mg of probenecid per mL.

Colchicine test solution—Transfer a quantity of finely powdered Tablets, equivalent to about 0.5 mg of colchicine, to a container, add 15 mL of water, mix, and filter, collecting the filtrate. Extract the filtrate with 25 mL of chloroform, and evaporate the chloroform extract to a volume of about 1 mL.

Procedure (see *Chromatography* ⟨621⟩)—Apply separately 5-µL portions of the *Probenecid test solution* and the *Probenecid standard solution*, a 7-µL portion of the *Colchicine test solution*, and a 3.5-µL portion of the *Colchicine standard solution* to a thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methanol and ammonium hydroxide (100: 1.5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, allow the solvent to evaporate, and view the plate under short-wavelength UV light: the R_F value of the principal spot in the chromatogram obtained from the *Probenecid test solution* corresponds to that obtained from the *Probenecid standard solution*. The R_F value of the principal spot in the chromatogram obtained from the *Colchicine test solution* corresponds to that obtained from the *Colchicine standard solution*.

Dissolution ⟨711⟩—

Medium: pH 6.8 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure for probenecid—Determine the amount of $C_{13}H_{19}NO_4S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 244 nm of filtered portions of the solution under test, suitably diluted with 0.1 N sodium hydroxide, if necessary, in comparison with a Standard solution having a known concentration of USP Probenecid RS.

Procedure for colchicine—Extract a filtered 200-mL portion of the solution under test with two 25-mL portions of chloroform, collecting the chloroform extracts in a suitable flask. Evaporate the combined extracts to a small volume, and transfer to a 10-mL volumetric flask. Rinse the flask with small portions of chloroform, and add the rinsings to the 10-mL volumetric flask. Dilute with chloroform to volume, and mix. Determine the amount of $C_{22}H_{25}NO_6$ dissolved from absorbances, at the wavelength of maximum absorbance at about 350 nm, of this solution, using chloroform as the blank, in comparison with a Standard solution in chloroform having a known concentration of USP Colchicine RS.

Tolerances—Not less than 80% *(Q)* of the labeled amounts of $C_{22}H_{25}NO_6$ and $C_{13}H_{19}NO_4S$ are dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay for probenecid—

Standard preparation—Dissolve an accurately weighed quantity of USP Probenecid RS on 0.1 N sodium hydroxide, and dilute quantitatively and stepwise with 0.1 N sodium hydroxide to obtain a solution having a known concentration of about 10 µg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 250 mg of probenecid, and transfer to a 250-mL volumetric flask. Add 0.1 N sodium hydroxide to volume, and mix. Filter a portion of the solution, discarding the first 20 mL of the filtrate, pipet 2 mL of the filtrate into a 200-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 244 nm, with a suitable spectrophotometer, using 0.1 N sodium hydroxide as the blank. Calculate the quantity, in mg, of $C_{13}H_{19}NO_4S$ in the portion of Tablets taken by the formula:

$$25C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Probenecid RS in the *Standard preparation*, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Assay for colchicine— [NOTE—Conduct this procedure without delay, under subdued light, using low-actinic glassware.]

Alcoholic sodium carbonate solution—Dissolve 5.0 g of anhydrous sodium carbonate in 900 mL of water, add 100 mL of isopropyl alcohol, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Colchicine RS in *Alcoholic sodium carbonate solution*, and dilute quantitatively and stepwise with *Alcoholic sodium carbonate solution* to obtain a solution having a known concentration of about 10 µg per mL.

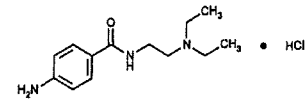
Assay preparation—Weigh and finely powder not less than 20 Probenecid and Colchicine Tablets. Weigh accurately a portion of the powder, equivalent to about 1 mg of colchicine, and transfer to a 100-mL volumetric flask. Add 75 mL of *Alcoholic sodium carbonate solution*, shake for 30 minutes, dilute with *Alcoholic sodium carbonate solution* to volume, mix, and filter, discarding the first 20 mL of the filtrate.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 350 nm, with a suitable spectrophotometer, using *Alcoholic sodium carbonate solution* as the blank. Calculate the quantity, in mg, of $C_{22}H_{25}NO_6$ in the portion of Tablets taken by the formula:

$$0.1C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Colchicine RS in the *Standard preparation*, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Procainamide Hydrochloride



$C_{13}H_{21}N_3O \cdot HCl$ 271.79

Benzamide, 4-amino-*N*-[2-(diethylamino)ethyl]-, monohydrochloride.

p-Amino-*N*-[2-(diethylamino)ethyl]benzamide monohydrochloride [614-39-1].

» Procainamide Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{21}N_3O \cdot HCl$, calculated on the dried basis.

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Packaging and storage—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Aminobenzoic Acid RS
USP Procainamide Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Standard solution—Use the *Standard solution* containing 0.2 mg of USP Procainamide Hydrochloride RS per mL, prepared as directed under *Ordinary impurities.*

Test solution—Dilute the *Test solution,* prepared as directed under *Ordinary impurities,* with methanol to obtain a solution containing about 0.2 mg of procainamide hydrochloride per mL.

Adsorbent, Eluant, and *Visualization*—Prepare as directed in *Ordinary impurities.*

Procedure—Proceed as directed for *Procedure* under *Ordinary impurities* ⟨466⟩: the R_f value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution.*

Melting range ⟨741⟩: between 165° and 169°.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.3% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Adsorbent: chromatographic silica gel.
Eluant: a mixture of chloroform, methanol, and ammonium hydroxide (70: 30: 0.7).
Visualization: 1, followed by spraying with a 1 in 2000 solution of fluorescamine in acetone and viewing with UV light at 366 nm.

Limit of free *p*-aminobenzoic acid—

Mobile phase and *Resolution solution*—Proceed as directed in the *Assay.*

Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Aminobenzoic Acid RS in *Mobile phase* to obtain a solution having a known concentration of about 0.25 µg per mL.

Test solution—Transfer 25.0 mL of the stock solution used to prepare the *Assay preparation* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.25 mg of procainamide hydrochloride per mL.

Chromatographic system—Proceed as directed for *Chromatographic system* in the *Assay.* In addition, the tailing factor for the *p*-aminobenzoic acid peak in the chromatogram obtained from the *Resolution solution* is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard solution* is not more than 3.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the *p*-aminobenzoic acid peaks. Calculate the percentage of *p*-aminobenzoic acid in the portion of Procainamide Hydrochloride taken by the formula:

$$20(C/W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Aminobenzoic Acid RS in the *Standard solution; W* is the weight, in mg, of Procainamide Hydrochloride taken to prepare the stock solution for the *Assay preparation;* and r_U and r_S are the *p*-aminobenzoic acid peak responses obtained from the *Test solution* and the *Standard solution,* respectively. The limit is 0.1%.

Assay—

Mobile phase—Prepare a suitable mixture of water, methanol, and triethylamine (140:60:1); adjust with phosphoric acid to a pH of 7.5 ± 0.1; filter; and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Procainamide Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Mobile phase* to obtain a *Standard preparation* having a known concentration of about 0.05 mg per mL.

Resolution solution—Dissolve a quantity of *p*-aminoben zoic acid in *Mobile phase* to obtain a solution containing about 0.1 mg per mL. Pipet 10 mL of this solution and 10 mL of the stock solution used to prepare the *Standard preparation* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer about 50 mg of Procainamide Hydrochloride, accurately weighed, to a 100-mL volumetric flask; dissolve in and dilute with *Mobile phase* to volume and mix. Transfer 10.0 mL of this stock solution to a second 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the *p*-aminobenzoic acid and procainamide peaks is not less than 5.0. The relative retention times are about 0.5 for *p*-aminobenzoic acid and 1.0 for procainamide hydrochloride. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{21}N_3O \cdot HCl$ in the portion of Procainamide Hydrochloride taken by the formula:

$$1000C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Procainamide Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Procainamide Hydrochloride Capsules

» Procainamide Hydrochloride Capsules contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$.

Packaging and storage—Preserve in tight containers.

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USP Reference standards ⟨11⟩—
USP Procainamide Hydrochloride RS
Identification—Capsules respond to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, 5 µL of the clear supernatant used to prepare the *Assay preparation* in the *Assay* and 5 µL of the stock solution used to prepare the *Standard preparation* in the *Assay* being applied to the plate, and a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (22:2:1) being used to develop the chromatogram.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 90 minutes.
Procedure—Determine the amount of $C_{13}H_{21}N_3O.HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 275 nm on filtered portions of the solution under test, suitably diluted with an amount of 0.01 N sodium hydroxide that is not less than twice the volume of the portion of test solution taken, in comparison with a Standard solution having a known concentration of USP Procainamide Hydrochloride RS in the same media.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$ is dissolved in 90 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Procainamide Hydrochloride.*
Assay preparation—Accurately weigh and mix the contents of 20 Capsules. Transfer an accurately weighed portion of the mixture, equivalent to about 500 mg of procainamide hydrochloride, to a 500-mL volumetric flask. Add about 350 mL of methanol to the flask, and sonicate for 10 minutes in a 40° water bath. Allow the flask to cool to room temperature, dilute with methanol to volume, and mix. Centrifuge a portion of the suspension, transfer 5.0 mL of the clear supernatant obtained to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Reserve the remainder of the clear supernatant for the *Identification* test.]
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Procainamide Hydrochloride.* Calculate the quantity, in mg, of $C_{13}H_{21}N_3O \cdot HCl$ in the portion of Capsule contents taken by the formula:

$$10{,}000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Procainamide Hydrochloride RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Procainamide Hydrochloride Injection

» Procainamide Hydrochloride Injection is a sterile solution of Procainamide Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
Labeling—Label it to indicate that the Injection is not to be used if it is darker than slightly yellow, or is discolored in any other way.
USP Reference standards ⟨11⟩—
USP Procainamide Hydrochloride RS
Identification—It responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, 5 µL of the clear supernatant being used to prepare the *Assay preparation* in the *Assay* and 5 µL of the stock solution being used to prepare the *Standard preparation* in the *Assay* being applied to the plate, and a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (22:2:1) being used to develop the chromatogram.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.35 USP Endotoxin Unit per mg of procainamide hydrochloride.
pH ⟨791⟩: between 4.0 and 6.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements under Small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Procainamide Hydrochloride.*
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of procainamide hydrochloride, to a 500-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this stock solution to a 100-mL volumetric flask, reserving the remainder of the stock solution for the *Identification* test. Dilute with *Mobile phase* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Procainamide Hydrochloride.* Calculate the quantity, in mg, of $C_{13}H_{21}N_3O \cdot HCl$ in each mL of the Injection taken by the formula:

$$10{,}000(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Procainamide Hydrochloride RS in the *Standard preparation,* V is the volume, in mL, of Injection taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Procainamide Hydrochloride Tablets

» Procainamide Hydrochloride Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Procainamide Hydrochloride RS
Identification—Tablets respond to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, 5 µL of the clear supernatant used to prepare the *Assay preparation* in the *Assay* and 5 µL of the stock solution used to prepare the *Standard preparation* in the *Assay* being applied to the plate, and a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (22:2:1) being used to develop the chromatogram.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.

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Apparatus 2: 50 rpm.
Time: 60 minutes.
Procedure—Determine the amount of $C_{13}H_{21}N_3O \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 275 nm on filtered portions of the solution under test, suitably diluted with an amount of 0.01 N sodium hydroxide that is not less than twice the volume of the portion of test solution taken, in comparison with a Standard solution having a known concentration of USP Procainamide Hydrochloride RS in the same media.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Procainamide Hydrochloride.*
Assay preparation—Weigh and finely powder not less than 20 Procainamide Hydrochloride Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of procainamide hydrochloride, to a 500-mL volumetric flask, add about 350 mL of methanol, and sonicate for 10 minutes in a 40° water bath. Allow the flask to cool to room temperature, dilute with methanol to volume, and mix. Centrifuge a portion of the suspension, transfer 5.0 mL of the clear supernatant obtained to a 100-mL volumetric flask, reserving the remainder of the clear supernatant for the *Identification* test. Dilute with *Mobile phase* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Procainamide Hydrochloride.* Calculate the quantity of $C_{13}H_{21}N_3O \cdot HCl$ in the portion of Tablets taken by the formula:

$$10{,}000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Procainamide Hydrochloride RS in the *Standard preparation,* and r_U and r_S are the procainamide peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Procainamide Hydrochloride Extended-Release Tablets

» Procainamide Hydrochloride Extended-Release Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O \cdot HCl$).

Packaging and storage—Preserve in tight containers.
Labeling—The labeling indicates the *Dissolution Test* with which the product complies.
USP Reference standards ⟨11⟩—
USP Procainamide Hydrochloride RS
Identification—It responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, 5 µL of the stock solution prepared as directed for *Assay preparation* in the *Assay* and 5 µL of the *Stock standard solution* being applied to the plate, and a solvent system consisting of a mixture of ether, methanol, and ammonium hydroxide (25:5:1) being used to develop the chromatogram.

Dissolution ⟨711⟩—
TEST 1—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 1.*
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Times: 1, 4, and 6 hours.
Procedure—Determine the amount of $C_{13}H_{21}N_3O \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 224 nm, using filtered portions of the solution under test, diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Procainamide Hydrochloride RS in the same *Medium.*
Tolerances—The percentage of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$ dissolved at the times specified conforms to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1	between 30% and 60%
4	between 60% and 90%
6	not less than 75%

TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2.*
Medium—Proceed as directed for *Method B* under *Delayed-Release Dosage Forms.*
ACID STAGE: 0.1 N hydrochloric acid; 900 mL for 1 hour.
BUFFER STAGE: 0.05 M phosphate buffer, pH 7.5; 900 mL (see *Buffer Solutions* under *Reagents, Indicators, and Solutions*) for not less than 8 hours.
Apparatus 2: 50 rpm, with sinkers.
Times: 1, 4, and 8 hours.
Procedure—Proceed as directed for *Procedure* in *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1	between 30% and 60%
4	between 60% and 90%
8	not less than 80%

TEST 3—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3.*
Medium—Proceed as directed under *Test 2.*
Apparatus 2: 50 rpm, with sinkers.
Times: 1, 3, 6, and 8 hours.
Procedure—Proceed as directed for *Procedure* in *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1	between 25% and 50%
3	between 40% and 75%
6	between 65% and 90%
8	not less than 80%

TEST 4—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4.*
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 1: 50 rpm.

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Times: 1, 2, 4, 8, and 14 hours.
Procedure—Proceed as directed for *Procedure* in *Test 1*.
Tolerances—The percentages of the labeled amount of $C_{13}H_{21}N_3O \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	not more than 30%
2	between 25% and 45%
4	between 45% and 75%
8	between 70% and 90%
14	not less than 80%

TEST 5—If the product complies with this test the labeling indicates that the product meets USP *Dissolution Test 5*.
Medium—Proceed as directed for *Method B* under *Delayed-Release Dosage Forms*.
ACID STAGE: 0.1 N hydrochloric acid; 1000 mL for 1 hour.
BUFFER STAGE: 0.05 M phosphate buffer, pH 7.5; 1000 mL (see *Buffer Solutions* under *Reagents, Indicators, and Solutions*) for not less than 8 hours.
Apparatus 2: 50 rpm, with sinkers.
Times: 1, 4, 6, and 8 hours.
Procedure—Proceed as directed for *Procedure* in *Test 1*.
Tolerances—Proceed as directed for *Tolerances* in *Test 2*.
FOR 500 MG TABLETS—

Time (hours)	Amount dissolved
1	between 30% and 45%
4	between 55% and 75%
6	not less than 65%
8	not less than 75%

FOR 750 AND 1000 MG TABLETS—

Time (hours)	Amount dissolved
1	between 30% and 50%
4	between 60% and 80%
6	between 70% and 90%
8	not less than 75%

TEST 6—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 6*.
Medium—Proceed as directed for *Test 2*.
Apparatus 2: 50 rpm.
Times: 1, 4, and 8 hours.
Procedure—Proceed as directed for *Procedure* in *Test 1*.
Tolerances—Proceed as directed for *Tolerances* in *Test 2*.
FOR 250 MG TABLETS—

Time (hours)	Amount dissolved
1	between 30% and 60%
4	between 60% and 90%
8	not less than 80%

FOR 500 MG TABLETS—

Time (hours)	Amount dissolved
1	between 30% and 50%
4	between 60% and 80%
8	not less than 85%

FOR 750 MG TABLETS—

Time (hours)	Amount dissolved
1	between 30% and 50%
4	between 60% and 80%
8	not less than 80%

TEST 8—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 8*.
Medium—Proceed as directed for *Method B* under *Delayed-Release Dosage Forms*.
ACID STAGE:0.1 N hydrochloric acid; 900 mL for 1 hour.
BUFFER STAGE:0.05 M phosphate buffer, pH 7.5; 900 mL (see *Buffer Solutions* under *Reagents, Indicators, and Solutions*) for not less than 8 hours.
Apparatus 2: 50 rpm, with sinkers.
Times: 1, 4, 6, and 8 hours.
Procedure—Proceed as directed for *Procedure* in *Test 1*.
Tolerances—Proceed as directed for *Tolerances* in *Test 2*.

Time (hours)	Amount dissolved
1	between 33% and 50%
4	between 70% and 85%
6	not less than 80%
8	not less than 85%

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase—Prepare a suitable mixture of water, methanol, and triethylamine (140:60:1), adjust with phosphoric acid to a pH of 7.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Procainamide Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL (*Stock standard solution*). Quantitatively dilute an accurately measured volume of this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL (*Standard preparation*).
Assay preparation—Transfer not fewer than 10 Tablets, accurately counted, to a 1000-mL volumetric flask, and add 100 mL of a mixture of methanol and methylene chloride (1:1). Place the flask in a 40° sonicator bath, and sonicate, with occasional shaking, until the Tablets have disintegrated completely. Add about 700 mL of methanol, and sonicate for 10 minutes, with occasional shaking. Allow to cool, dilute with methanol to volume, and mix. Filter a portion of this solution, discarding the first 10 mL of the filtrate. Quantitatively dilute an accurately measured volume of the filtrate with *Mobile phase* to obtain a solution having a concentration of about 0.5 mg of procainamide hydrochloride per mL. Use a portion of this stock solution for the *Identification* test. Quantitatively dilute another accurately measured volume of the stock solution with *Mobile phase* to obtain a solution

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having a concentration of about 0.05 mg per mL (*Assay preparation*).

Resolution solution—Prepare a solution of *p*-aminobenzoic acid in *Mobile phase* containing 0.1 mg per mL. Pipet 10 mL of this solution and 10 mL of the *Stock standard* into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

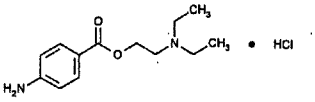
Chromatographic system (see *Chromatography*)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution:* the resolution, *R*, is not less than 2.0. Chromatograph replicate injections of the *Standard preparation:* the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for *p*-aminobenzoic acid and 1.0 for procainamide. Calculate the quantity, in mg, of procainamide hydrochloride ($C_{13}H_{21}N_3O \cdot HCl$) per Tablet taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which *L* is the labeled quantity, in mg, of procainamide hydrochloride in each Tablet; *D* is the concentration, in mg per mL, of procainamide hydrochloride in the *Assay preparation,* based on the number of Tablets taken, the labeled quantity per Tablet, and the extent of dilution; *C* is the concentration, in mg per mL, of USP Procainamide Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Procaine Hydrochloride



$C_{13}H_{20}N_2O_2 \cdot HCl$ 272.77

Benzoic acid, 4-amino-, 2-(diethylamino)ethyl ester, monohydrochloride.

2-(Diethylamino)ethyl *p*-aminobenzoate monohydrochloride [51-05-8].

» Procaine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of $C_{13}H_{20}N_2O_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Procaine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Dissolve 10 mg in 1 mL of water, add 1 drop each of hydrochloric acid and sodium nitrite solution (1 in 10), then add 1 mL of a solution prepared by dissolving 0.2 g of 2-naphthol in 10 mL of sodium hydroxide solution (1 in 10), and shake: a scarlet-red precipitate is formed.

C: It responds to the tests for *Chloride* ⟨191⟩.

Melting range ⟨741⟩: between 153° and 158°.

Bacterial Endotoxins Test ⟨85⟩—Where the label states that Procaine Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.6 USP Endotoxin Unit per mg of procaine hydrochloride.

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

Acidity—To a solution of 1.0 g in 25 mL of water add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.50 mL is required for neutralization.

Loss on drying ⟨731⟩—Dry it over silica gel for 18 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.15%.

Chromatographic purity—

Solvent—Prepare a mixture of methanol and trichloroethane (7:3).

Standard preparations—Prepare a solution of USP Procaine Hydrochloride RS in *Solvent* containing 1.6 mg per mL. Dilute quantitatively with *Solvent* to obtain Standard preparations having the following compositions:

Standard preparation	Dilution	Concentration (mg RS per mL)	Percentage (%, for comparison with test specimen)
A	2.5 in 10	0.4	0.5
B	2.0 in 10	0.32	0.4
C	1.0 in 10	0.16	0.2
D	0.5 in 10	0.08	0.1

Test preparation—Transfer 1.6 g of Procaine Hydrochloride, accurately weighed, to a suitable capped container, add 20 mL of *Solvent*, close the container, and sonicate for 2 minutes. Use this solution as the *Test preparation.*

Procedure—Apply separately 10 µL of the *Test preparation* and 10 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, prewashed with methanol and allowed to dry. Use a double-trough chromatographic chamber. Fill one trough with ammonium hydroxide, and allow the chamber to equilibrate for 1 hour. Position the plate in the other trough, and develop the chromatogram in a solvent system consisting of a mixture of methylene chloride and methanol (95:6) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations:* no secondary spot is more intense than the principal spot obtained from *Standard* (0.5%), and the sum of

USP Monographs

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the intensities of all secondary spots obtained from the *Test preparation* does not exceed 1.0%.

Assay—Transfer about 0.5 g of Procaine Hydrochloride, accurately weighed, to a beaker, add 100 mL of cold water, 5 mL of hydrochloric acid, and 100 mg of potassium bromide, and stir until dissolved. Proceed as directed under *Nitrite Titration* ⟨451⟩, beginning with "cool to about 15°." Perform a blank determination, and make any necessary correction. Each mL of 0.1 M sodium nitrite is equivalent to 27.28 mg of $C_{13}H_{20}N_2O_2$.HCl.

Procaine Hydrochloride Injection

» Procaine Hydrochloride Injection is a sterile solution of Procaine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{13}H_{20}N_2O_2 \cdot HCl$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass. The Injection may be packaged in 100-mL multiple-dose containers.

USP Reference standards ⟨11⟩—
USP Procaine Hydrochloride RS

Identification—Evaporate a portion of Injection, equivalent to about 20 mg of procaine hydrochloride, on a steam bath just to dryness, and dry over silica gel for 18 hours: the residue responds to *Identification* tests *A* and *B* under *Procaine Hydrochloride.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.6 USP Endotoxin Unit per mg of procaine hydrochloride.

pH ⟨791⟩: between 3.0 and 5.5.

Particulate Matter in Injections ⟨788⟩: meets the requirements under Small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Standard preparation—Transfer to a 125-mL separator about 50 mg, accurately weighed, of USP Procaine Hydrochloride RS, and dilute with water to 20 mL.

Assay preparation—Transfer to a 125-mL separator an accurately measured volume of Injection, equivalent to about 50 mg of procaine hydrochloride, and dilute with water to 20 mL.

Procedure—To the *Standard preparation* and also to the *Assay preparation* add 5 mL of 6 N ammonium hydroxide, then treat each as follows. Extract with five 25-mL portions of chloroform, and filter the combined extracts through about 1 g of anhydrous sodium sulfate supported on a pledget of glass wool. Receive the filtrate in a 200-mL volumetric flask, add chloroform to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, add chloroform to volume, and mix. Concomitantly determine the absorbances of both solutions at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of $C_{13}H_{20}N_2O_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(W/V)(A_U/A_S)$$

in which W is the weight, in mg, of USP Procaine Hydrochloride RS used, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Procaine Hydrochloride and Epinephrine Injection

» Procaine Hydrochloride and Epinephrine Injection is a sterile solution of Procaine Hydrochloride and Epinephrine Hydrochloride in Water for Injection. The content of epinephrine does not exceed 0.002 percent (1 in 50,000). It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

Packaging and storage—Preserve in single-dose or in multiple-dose, light-resistant containers, preferably of Type I or Type II glass.

Labeling—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards ⟨11⟩—
USP Epinephrine Bitartrate RS
USP Procaine Hydrochloride RS

Color and clarity—Using the Injection as the *Test solution,* proceed as directed for *Color and clarity* under *Epinephrine Injection.*

Identification—Evaporate a portion of Injection, equivalent to about 20 mg of procaine hydrochloride, on a steam bath just to dryness, and dry over silica gel for 18 hours, protected from light: the residue responds to *Identification* tests *A* and *B* under *Procaine Hydrochloride.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.6 USP Endotoxin Unit per mg of procaine hydrochloride.

pH ⟨791⟩: between 3.0 and 5.5.

Content of epinephrine—

Alkaline ascorbate reagent—Mix 100 mL of alcohol, 80 mL of sodium hydroxide solution (1 in 5), and 8 mL of ascorbic acid solution (1 in 50). Prepare fresh on the day of use.

Standard preparation—Place about 18 mg of USP Epinephrine Bitartrate RS, accurately weighed, in a 100-mL volumetric flask, add sodium bisulfite solution (1 in 1000) to volume, and mix. Dilute this solution quantitatively with water to obtain a solution having a known concentration of about 10 µg of epinephrine per mL.

Procedure—Pipet a volume of Injection, equivalent to about 10 µg of epinephrine, into a 50-mL beaker. Into another 50-mL beaker pipet 1 mL of *Standard preparation.* Treat the contents of each beaker as follows. Add 10 mL of dilute hydrochloric acid (1 in 120), and heat gently to reduce the volume of solution to about 5 mL. Allow to cool to room temperature, then add 5 mL of sodium acetate solution (1 in 5), followed by 0.5 mL of potassium ferricyanide solution (1 in 400), and mix. At 2 minutes, accurately timed, after the last addition, add 20 mL of *Alkaline ascorbate reagent,* transfer the contents to a corresponding 50-mL volumetric flask with the aid of water, add water to volume, and mix. At 15 to 20 minutes after the addition of the *Alkaline ascorbate reagent,* determine the

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fluorescences of each solution and of a reagent blank, with a suitable fluorometer, with an excitation wavelength setting of 420 nm and a fluorescence wavelength setting of 520 nm. Calculate the quantity, in µg, of $C_9H_{13}NO_3$ in each mL of Injection taken by the formula:

$$(C/V)[(I_U - B)/(I_S - B)]$$

in which C is the concentration of epinephrine, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and I_U, I_S, and B are the fluorescence readings of the solutions from the Injection, the *Standard preparation*, and the reagent blank, respectively.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Standard preparation—Transfer to a 125-mL separator about 50 mg, accurately weighed, of USP Procaine Hydrochloride RS, and dilute with water to 20 mL.

Assay preparation—Transfer to a 125-mL separator an accurately measured volume of Injection, equivalent to about 50 mg of procaine hydrochloride, and dilute with water to 20 mL.

Procedure—To the *Standard preparation* and also to the *Assay preparation* add 5 mL of 6 N ammonium hydroxide, then treat each as follows. Extract with five 25-mL portions of chloroform, and filter the combined extracts through about 1 g of anhydrous sodium sulfate supported on a pledget of glass wool. Receive the filtrate in a 200-mL volumetric flask, add chloroform to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, add chloroform to volume, and mix. Concomitantly determine the absorbances of both solutions at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of $C_{13}H_{20}N_2O_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(W/V)(A_U/A_S)$$

in which W is the weight, in mg, of USP Procaine Hydrochloride RS used, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solutions obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Delete the following:

▲Procaine and Tetracaine Hydrochlorides and Levonordefrin Injection

» Procaine and Tetracaine Hydrochlorides and Levonordefrin Injection is a sterile solution of Procaine Hydrochloride, Tetracaine Hydrochloride, and Levonordefrin in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$), not less than 95.0 percent and not more than 105.0 percent of the labeled amount of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levonordefrin ($C_9H_{13}NO_3$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

Labeling—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

Color and clarity—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Mepivacaine Hydrochloride and Levonordefrin Injection*.

Identification—

A: Mix 10 mL of Injection with 10 mL of 3 N hydrochloric acid, cool to 0° in an ice bath, add 5 mL of sodium nitrite solution (1 in 5), stirring gently, add 2 mL of a solution of 0.1 g of 2-naphthol in 5 mL of 1 N sodium hydroxide, and observe immediately: a bright orange-red precipitate is formed (*presence of a primary aminophenyl group*).

B: It responds to the tests for *Identification Tests—General* ⟨191⟩, *Chloride*.

C: To about 25 mL of Injection add 1 drop of ferric chloride TS: the solution immediately turns a light green and changes to a light blue within 1 minute. Add 2 drops of 3 N hydrochloric acid: the color reverts to light green (*presence of levonordefrin*).

Bacterial Endotoxins Test ⟨ 85 ⟩—It contains not more than 0.6 USP Endotoxin Unit per mg of procaine hydrochloride.

pH ⟨ 791 ⟩: between 3.5 and 5.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for procaine hydrochloride—Transfer 25.0 mL of Injection to a 250-mL beaker, add 10 mL of dilute hydrochloric acid (1 in 2), 15 mL of water, and 1.0 mL of formaldehyde solution, and allow to stand for 3 minutes. Titrate with 0.1 M sodium nitrite VS, using starch iodide paper as an external indicator. Perform a blank determination, and make any necessary correction. Calculate the quantity, in mg, of $C_{13}H_{20}N_2O_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(272.78/25)(V_2M_2 - 0.5V_1M_1)$$

in which V_2 is the volume, in mL, and M_2 is the exact molarity of the 0.1 M sodium nitrite solution used in the titration, V_1 and M_1 are as defined under *Assay for tetracaine hydrochloride*, and 272.78 is the molecular weight of procaine hydrochloride.

Assay for tetracaine hydrochloride—Transfer 50.0 mL of Injection to a 100-mL beaker, add 5 g of sodium acetate and 5 mL of isopropyl alcohol, and stir until the sodium acetate is dissolved. Add 1.5 mL of potassium thiocyanate solution (75 in 100), and stir while cooling in an ice bath. Keep in the ice bath for 1 hour, stirring about four times during the period. Do not stir excessively. Prepare a filtering crucible with double suitable filter paper, connect to a suction flask, and moisten the paper with a few drops of the clear supernatant. Remove the excess liquid by suction. Filter the mixture, and dry by suction. Transfer the entire contents of the crucible to the original beaker, and rinse the crucible with 10 mL of dilute hydrochloric acid (1 in 2), followed by 30 mL of water. Transfer the rinsings to the beaker. Cool in ice, and titrate with 0.02 M sodium nitrite VS, using starch iodide paper as an external indicator. Perform a blank determination, using 10 mL of

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dilute hydrochloric acid (1 in 2) and 30 mL of water, and make any necessary correction. Calculate the quantity, in mg, of $C_{15}H_{24}N_2O_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(300.83/50)(V_1M_1)$$

in which V_1 is the volume, in mL, and M_1 is the exact molarity of the 0.02 M sodium nitrite used in the titration, and 300.83 is the molecular weight of tetracaine hydrochloride.

Assay for levonordefrin—

Ferro-citrate solution, *Buffer solution*, and *Standard preparation*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

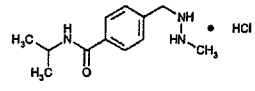
Assay preparation—Proceed with Injection as directed for *Assay Preparation* under *Epinephrine Assay* ⟨391⟩, except to read "levonordefrin" where "epinephrine" is specified.

Procedure—Proceed as directed for *Procedure* under *Epinephrine Assay* ⟨391⟩. Calculate the quantity, in mg, of levonordefrin ($C_9H_{13}NO_3$) in each mL of the Injection taken by the formula:

$$(183.21/333.30)(0.05C/V)(A_U/A_S)$$

in which 183.21 and 333.30 are the molecular weights of levonordefrin and epinephrine bitartrate, respectively, C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*, and V is the volume, in mL, of Injection taken. ▲ (USP 1-May-2020)

Procarbazine Hydrochloride



$C_{12}H_{19}N_3O \cdot HCl$ 257.76

Benzamide, *N*-(1-methylethyl)-4-[(2-methylhydrazino)methyl]-, monohydrochloride.

N-Isopropyl-α-(2-methylhydrazino)-*p*-toluamide monohydrochloride [366-70-1].

» Procarbazine Hydrochloride contains not less than 98.5 percent and not more than 100.5 percent of $C_{12}H_{19}N_3O \cdot HCl$.

[**CAUTION**—*Handle Procarbazine Hydrochloride with exceptional care, since it is a highly potent agent.*]

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Procarbazine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
[NOTE—Use low-actinic glassware throughout this test.]
Solution: 10 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 232 nm do not differ by more than 3.0%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Assay—Dissolve about 0.75 g of Procarbazine Hydrochloride, accurately weighed, in 100 mL of water. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically, using a calomel-glass electrode system. Each mL of 0.1 N sodium hydroxide is equivalent to 25.78 mg of $C_{12}H_{19}N_3O \cdot HCl$.

Procarbazine Hydrochloride Capsules

» Procarbazine Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of procarbazine ($C_{12}H_{19}N_3O$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Procarbazine Hydrochloride RS

Identification—The polarogram of the solution employed for measurement in the *Assay* exhibits a half-wave potential ($E_{1/2}$) within ±0.03 volt of that of USP Procarbazine Hydrochloride RS, similarly measured ($E_{1/2}$ is about –0.16 volt against a saturated calomel electrode).

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of procarbazine ($C_{12}H_{19}N_3O$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 233 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Procarbazine Hydrochloride RS in the same medium.
Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{12}H_{19}N_3O$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
pH 12 buffer—Dissolve 5.43 mL of phosphoric acid, 4.60 mL of glacial acetic acid, and 4.95 g of boric acid in water to make 2000 mL. To 100 mL of this solution add 100 mL of 0.2 N sodium hydroxide. Prior to use, deaerate this solution by bubbling with scrubbed nitrogen.
Procedure— [NOTE—Use low-actinic glassware throughout this procedure.] Weigh the contents of not less than 20 Capsules, and determine the average weight per capsule. Mix the combined contents to obtain a homogeneous sample. Transfer an accurately weighed portion of this powder, equivalent to about 60 mg of procarbazine hydrochloride, to a 100-mL volumetric flask that previously has been flushed with nitrogen. Dissolve in *pH 12 buffer*, dilute with *pH 12 buffer* to volume, and centrifuge a portion of this solution at about 1500 rpm for about 3 minutes. Transfer 10 mL to 15 mL of the solution to a polarographic cell that is regulated at 25 ± 0.1°.

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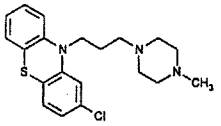
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Deaerate by bubbling scrubbed nitrogen through the solution for 5 minutes. Insert the dropping mercury electrode of a suitable polarograph, which is capable of measuring a current of 10 microamperes, using an average capillary, a mercury column height of 56 cm, and a drop rate of approximately 4 per second. Record the polarogram from –0.75 volt to +0.25 volt, using a saturated calomel electrode as the reference electrode. Determine the height of the current at a point 200 mV anodic of the half-wave potential. Calculate the quantity, in mg, of $C_{12}H_{19}N_3O$ in the portion of Capsule contents taken by the formula:

$$100(0.8585C)[i_U/i_S]$$

in which 0.8585 is the ratio of the molecular weight of procarbazine to that of procarbazine hydrochloride, i_U is the observed current of the solution from the Capsule contents and i_S is that determined similarly in a solution of USP Procarbazine Hydrochloride RS, the concentration of which is C mg per mL (about 600 µg per mL).

Prochlorperazine



$C_{20}H_{24}ClN_3S$ 373.94

10*H*-Phenothiazine, 2-chloro-10-[3-(4-methyl-1-piperazinyl) propyl]-;
2-Chloro-10-[3-(4-methyl-1-piperazinyl)propyl] phenothiazine [58-38-8].

DEFINITION

Prochlorperazine contains NLT 98.0% and NMT 101.0% of prochlorperazine ($C_{20}H_{24}ClN_3S$).

[NOTE—Throughout the following procedures, protect the sample, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES ⟨181⟩:** Meets the requirements when USP Prochlorperazine Maleate RS is used as the standard for comparison

ASSAY

- **PROCEDURE**

Sample: 400 mg of Prochlorperazine

Analysis: Transfer the *Sample* to a 125-mL conical flask. Add 30 mL of glacial acetic acid, and warm on a steam bath to dissolve. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination (see *Titrimetry ⟨541⟩*), and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 18.70 mg of prochlorperazine ($C_{20}H_{24}ClN_3S$).

Acceptance criteria: 98.0%–101.0%

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORDINARY IMPURITIES ⟨466⟩**

Standard solution: Methanol

Sample solution: Methanol

Eluant: Methanol and ammonium hydroxide (100:1)

Visualization: 1

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Prochlorperazine Maleate RS

Prochlorperazine Oral Solution

DEFINITION

Prochlorperazine Oral Solution contains an amount of Prochlorperazine Edisylate equivalent to NLT 92.0% and NMT 108.0% of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$).

[NOTE—Throughout the following procedures, protect the sample, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

- **A.**

Analysis: To 2 mL of Oral Solution add 3 mL of water and 3 or 4 drops of ferric chloride TS.

Acceptance criteria: A stable red color is produced.

- **B.**

Analysis: To 1 mL of Oral Solution add 10 mL of bromine TS, previously warmed to room temperature.

Acceptance criteria: Essentially no color change occurs (distinction from chlorpromazine hydrochloride, which immediately produces a green color).

ASSAY

- **PROCEDURE**

Ion-pairing solution: Dissolve 4.33 g of sodium 1-octanesulfonate in 500 mL of water. Add 4.0 mL of glacial acetic acid, and dilute with water to 1 L.

Mobile phase: Acetonitrile, methanol, and *Ion-pairing solution* (40:10:50)

Diluent: A mixture containing 1000 mL of distilled water, 8.6 mL of concentrated hydrochloric acid, and 1000 mL of methanol

Internal standard solution: 0.9 mg/mL of trifluoperazine hydrochloride in *Diluent*

Standard stock solution: 1.0 mg/mL of USP Prochlorperazine Maleate RS in *Diluent*

Standard solution: 0.09 mg/mL of trifluoperazine hydrochloride from *Internal standard solution* and 0.1 mg/mL of USP Prochlorperazine Maleate RS from *Standard stock solution*, in *Diluent*

Sample solution: Transfer a quantity of Oral Solution, equivalent to 10.0 mg of prochlorperazine, to a 100-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for prochlorperazine and trifluoperazine are 1.0 and 1.3, respectively.]

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Suitability requirements
Resolution: NLT 2.0 between prochlorperazine and the internal standard
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of prochlorperazine to the internal standard from the *Sample solution*
R_S = peak response ratio of prochlorperazine to the internal standard from the *Standard solution*
C_S = concentration of USP Prochlorperazine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prochlorperazine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of prochlorperazine, 373.94
M_{r2} = molecular weight of prochlorperazine maleate, 606.09

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for Oral Solution packaged in multi-unit containers
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for Oral Solution packaged in single-unit containers

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prochlorperazine Maleate RS

Prochlorperazine Suppositories

DEFINITION
Prochlorperazine Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$).
[NOTE—Throughout the following procedure, protect the sample, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION
• **A.**
Analysis: Place a quantity of Suppositories, equivalent to 5 mg of prochlorperazine, in a test tube. Add 4 mL of dilute hydrochloric acid (1 in 2), warm on a steam bath to melt the solid, and swirl to mix.
Acceptance criteria: A pink color develops in the aqueous layer.
• **B.**
Analysis: To the solution from *Identification* test *A*, add 10 mL of bromine TS.
Acceptance criteria: Essentially no color change occurs (distinction from chlorpromazine, which immediately produces a green color).

ASSAY
• **PROCEDURE**
Standard solution: Transfer 40 mg of USP Prochlorperazine Maleate RS to a 250-mL separator containing 75 mL of ether. Add 0.5 mL of 6 N ammonium hydroxide, and extract with four 65-mL portions of dilute hydrochloric acid (1 in 100), collecting the aqueous extracts in a 500-mL volumetric flask. Aerate the combined extracts for 15–20 min to remove dissolved ether. Add dilute hydrochloric acid (1 in 100) to volume. Pass a portion of the solution through filter paper, discarding the first 25 mL of the filtrate. To 25.0 mL of the subsequent filtrate add dilute hydrochloric acid (1 in 100) to make 200.0 mL.
Sample solution: Weigh, mash, and then mix NLT 15 Suppositories. Transfer a weighed quantity of the mass, equivalent to 25 mg of prochlorperazine, to a 100-mL beaker. Dissolve in 50 mL of ether, and transfer to a 250-mL separator with the aid of three 25-mL portions of ether. Proceed as directed for the *Standard solution*, beginning with "Extract with four 65-mL portions of dilute hydrochloric acid (1 in 100)".
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelengths: 254 and 278 nm
Cell: 1 cm
Blank: Dilute hydrochloric acid (1 in 100)
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Determine the absorbances of the *Standard* and *Sample solutions*, and calculate the differences in the absorbances of the two solutions at 254 and 278 nm.
Calculate the percentage of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$) in the portion of Suppositories taken:

$$\text{Result} = A_U/A_S \times (M_{r1}/M_{r2}) \times (W_S/W_U) \times 100$$

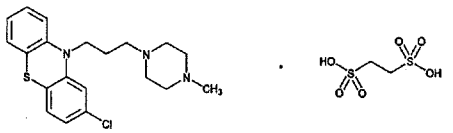
A_U = difference in the absorbances of the *Sample solution* at 254 nm and at 278 nm ($A_{254} - A_{278}$)
A_S = difference in the absorbances of the *Standard solution* at 254 nm and at 278 nm ($A_{254} - A_{278}$)
M_{r1} = molecular weight of prochlorperazine, 373.94
M_{r2} = molecular weight of prochlorperazine maleate, 606.09
W_S = weight of USP Prochlorperazine Maleate RS in the *Standard solution* (mg)
W_U = nominal amount of prochlorperazine in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers at a temperature below 37°. Do not expose the unwrapped Suppositories to sunlight.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prochlorperazine Maleate RS

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Prochlorperazine Edisylate



$C_{20}H_{24}ClN_3S \cdot C_2H_6O_6S_2$ 564.14

10*H*-Phenothiazine, 2-chloro-10-[3-(4-methyl-1-piperazinyl) propyl]-,1,2-ethanedisulfonate (1:1);
2-Chloro-10-[3-(4-methyl-1-piperazinyl)propyl] phenothiazine 1,2-ethanedisulfonate (1:1) [1257-78-9].

DEFINITION

Prochlorperazine Edisylate contains NLT 98.0% and NMT 101.5% of prochlorperazine edisylate ($C_{20}H_{24}ClN_3S \cdot C_2H_6O_6S_2$), calculated on the dried basis.

[NOTE—Throughout the following procedures, protect the sample, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩: Meets the requirements when USP Prochlorperazine Maleate RS is used as the standard for comparison
- **B. IDENTIFICATION TESTS—GENERAL,** *Sulfite* ⟨191⟩
  Sample: Fuse 100 mg with a few pellets of sodium hydroxide.
  Acceptance criteria: The cooled melt meets the requirements.

ASSAY

- **PROCEDURE**
  Sample solution: Transfer 750 mg of Prochlorperazine Edisylate to a separator containing 40 mL of water, and shake to dissolve.
  Analysis: Render the *Sample solution* alkaline with ammonium hydroxide, and extract with three 25-mL portions of ether. Wash the combined ether extracts once with 25 mL of water, discard the washing, and evaporate the ether solution on a steam bath to dryness. Dissolve the residue in 60 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, using crystal violet TS as the indicator. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 28.21 mg of prochlorperazine edisylate ($C_{20}H_{24}ClN_3S \cdot C_2H_6O_6S_2$).
  Acceptance criteria: 98.0%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **SELENIUM** ⟨291⟩
  Sample: 100 mg of Prochlorperazine Edisylate, mixed with 100 mg of magnesium oxide
  Acceptance criteria: NMT 30 ppm
- **ORDINARY IMPURITIES** ⟨466⟩
  Standard solution: Methanol and ammonium hydroxide (9:1)
  Sample solution: Methanol and ammonium hydroxide (9:1)
  Eluant: Chloroform, toluene, alcohol, and ammonium hydroxide (50: 50: 50: 0.5)
  Spray reagent: Mix 2 mL of chloroplatinic acid solution (1 in 10) and 50 mL of potassium iodide solution (4 in 100). Dilute with water to 100 mL, and acidify with 1 N hydrochloric acid.
  Visualization: 1, followed by *Spray reagent*

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample under vacuum at 100° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Prochlorperazine Maleate RS

Prochlorperazine Edisylate Injection

DEFINITION

Prochlorperazine Edisylate Injection is a sterile solution of Prochlorperazine Edisylate in Water for Injection. It contains an amount of prochlorperazine edisylate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$).

[NOTE—Throughout the following procedures, protect the sample, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩: Meets the requirements when USP Prochlorperazine Maleate RS is used as the standard for comparison

ASSAY

- **PROCEDURE**
  Buffer: 2.68 g/L of dibasic sodium phosphate heptahydrate in water, adjusted with phosphoric acid to a pH of 3.0
  Mobile phase: Acetonitrile and *Buffer* (7:3)
  Standard solution: Equivalent to 0.1 mg/mL of prochlorperazine, from USP Prochlorperazine Maleate RS in *Mobile phase*
  Sample solution: Transfer a portion of Injection, equivalent to 10 mg of prochlorperazine, to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; packing L1
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 3000 theoretical plates
  Tailing factor: NMT 2.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of prochlorperazine in the *Standard solution* (mg/mL)

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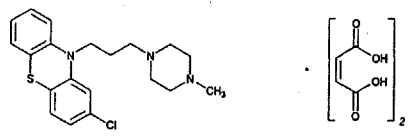
C_U = nominal concentration of prochlorperazine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 17.9 USP Endotoxin Units/mg of prochlorperazine
- **pH** ⟨791⟩: 4.2–6.2
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Prochlorperazine Maleate RS

Prochlorperazine Maleate



$C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$ 606.09

10*H*-Phenothiazine, 2-chloro-10-[3-(4-methyl-1-piperazinyl) propyl]-, (*Z*)-2-butenedioate (1:2);
2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl] phenothiazine maleate (1:2) [84-02-6].

DEFINITION
Prochlorperazine Maleate contains NLT 98.0% and NMT 102.0% of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$), calculated on the dried basis.
[NOTE—Throughout the following procedures, protect the samples, the Reference Standard, and solutions containing them from light, and conduct the procedures without delay.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K.▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Ion-pairing solution: Dissolve 4.33 g of sodium 1-octanesulfonate in 500 mL of water. Add 4.0 mL of glacial acetic acid, and dilute with water to 1 L.
Mobile phase: Acetonitrile, methanol, and *Ion-pairing solution* (40:15:45)
Standard solution: 0.2 mg/mL of USP Prochlorperazine Maleate RS in *Mobile phase*. Sonicate to dissolve, if needed, prior to final dilution.
Sample solution: 0.2 mg/mL of Prochlorperazine Maleate in *Mobile phase*. Sonicate to dissolve, if needed, prior to final dilution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1 or 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$) in the portion of Prochlorperazine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of prochlorperazine from the *Sample solution*
r_S = peak response of prochlorperazine from the *Standard solution*
C_S = concentration of USP Prochlorperazine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Prochlorperazine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer: 1.36 g/L of sodium acetate trihydrate in water (0.01 M). Add 2.0 mL of triethylamine and 6.0 mL of glacial acetic acid per L of solution.
Solution A: *Buffer*
Solution B: Acetonitrile
Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system for about 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
20	65	35
25	65	35
55	35	65
65	35	65

Diluent: Acetonitrile and water (40:60)
Impurity stock solution: 0.16 mg/mL of USP Prochlorperazine Related Compound A RS in *Diluent*
Standard stock solution: 0.16 mg/mL of USP Prochlorperazine Maleate RS in *Diluent*
System suitability solution: 1.6 µg/mL each of USP Prochlorperazine Maleate RS and USP Prochlorperazine Related Compound A RS in *Diluent* from the *Standard stock solution* and the *Impurity stock solution*, respectively
Standard solution: 6.4 µg/mL of USP Prochlorperazine Maleate RS in *Diluent* from the *Standard stock solution*
Sensitivity solution: 0.32 µg/mL of USP Prochlorperazine Maleate RS in *Diluent* from the *Standard solution*
Sample solution: 0.64 mg/mL of Prochlorperazine Maleate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50 ± 5°
Flow rate: 2.0 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between prochlorperazine related compound A and prochlorperazine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Prochlorperazine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of prochlorperazine from the *Standard solution*
C_S = concentration of USP Prochlorperazine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Prochlorperazine Maleate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of prochlorperazine, 373.94
M_{r2} = molecular weight of prochlorperazine maleate, 606.09
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)
Maleic acid	0.07	—	Disregard
Prochlorperazine sulfoxide[b]	0.20	0.38	0.15
Perazine[c]	0.66	1.0	0.15
Prochlorperazine 4-chloro isomer (prochlorperazine related compound A)[d]	0.97	0.61	0.15
Prochlorperazine	1.00	—	—
4-Chlorophenothiazine[e]	2.01	1.9	0.1
2-Chlorophenothiazine[f]	2.08	2.1	0.1
Specified unknown 1	2.64	1.0	0.50
Specified unknown 2	2.79	1.0	0.50
Specified unknown 3	2.88	1.0	0.20
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	1.5

[a] *F* values are based on the response of prochlorperazine (free base).
[b] 2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine sulfoxide.
[c] 10-[3-(4-Methylpiperazin-1-yl)propyl]-10*H*-phenothiazine.
[d] 4-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine.
[e] 4-Chloro-10*H*-phenothiazine.
[f] 2-Chloro-10*H*-phenothiazine.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 60° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prochlorperazine Maleate RS
USP Prochlorperazine Related Compound A RS
4-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine dihydrochloride.
$C_{20}H_{24}ClN_3S \cdot 2HCl$ 443.47

Prochlorperazine Maleate Tablets

DEFINITION
Prochlorperazine Maleate Tablets contain an amount of Prochlorperazine Maleate equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$).
[NOTE—Throughout the following procedures, protect the samples, Reference Standards, and solutions from light, and conduct the procedures without delay.]

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**
Ion-pairing solution: Dissolve 4.33 g of sodium 1-octanesulfonate in 500 mL of water. Add 4.0 mL of glacial acetic acid, and dilute with water to 1 L.
Mobile phase: Acetonitrile, methanol, and *Ion-pairing solution* (40:15:45)
Standard solution: 0.2 mg/mL of USP Prochlorperazine Maleate RS in *Mobile phase*
Sample solution: ▲Nominally equivalent to 0.12 mg/mL of prochlorperazine in *Mobile phase* prepared as follows.▲ (USP 1-Aug-2019) Transfer an equivalent to about 12 mg of prochlorperazine from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask. Add 60 mL of *Mobile phase*, sonicate for 3 min, and shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume, and filter, discarding the first 10 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ (USP 1-Aug-2019)
Column: 3.9-mm × 30-cm; ▲10 µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT ▲1.0%▲ (USP 1-Aug-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response ▲of prochlorperazine▲ (USP 1-Aug-2019) from the *Sample solution*
r_S = peak response ▲of prochlorperazine▲ (USP 1-Aug-2019) from the *Standard solution*
C_S = concentration of USP Prochlorperazine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prochlorperazine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of prochlorperazine, 373.94
M_{r2} = molecular weight of prochlorperazine maleate, 606.09

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: USP Prochlorperazine Maleate RS at a known concentration in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 254 nm
▲**Analysis**
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$) dissolved:

$$\text{Results} = (A_U/A_S) \times C_S \times D \times (1/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Prochlorperazine Maleate RS in the *Standard solution*
D = dilution factor for *Sample solution*, if needed
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL▲ (USP 1-Aug-2019)

Tolerances: NLT 75% (Q) of the labeled amount of prochlorperazine ($C_{20}H_{24}ClN_3S$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Buffer solution: 1.36 g/L of sodium acetate trihydrate in water (0.01 M). Add 2.0 mL of triethylamine and 6.0 mL of glacial acetic acid per liter of solution.
Solution A: *Buffer solution*
Solution B: Acetonitrile
Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system for about 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
20	65	35
25	65	35
55	35	65
65	35	65

Diluent: Acetonitrile and water (40:60)
Impurity stock solution: 0.16 mg/mL of USP Prochlorperazine Related Compound A RS in *Diluent*
Standard stock solution: 0.16 mg/mL of USP Prochlorperazine Maleate RS in *Diluent*
System suitability solution: 1.6 µg/mL of USP Prochlorperazine Maleate RS and 1.6 µg/mL of USP Prochlorperazine Related Compound A RS in *Diluent* from the *Standard stock solution* and the *Impurity stock solution*, respectively
Standard solution: 0.0064 mg/mL of USP Prochlorperazine Maleate RS in *Diluent* from the *Standard stock solution*
Sample solution: Nominally equivalent to 0.4 mg/mL of prochlorperazine in *Diluent*, prepared as follows. Transfer 20 Tablets to a suitable volumetric flask, using a 250-mL volumetric flask for 5-mg Tablets and a 500-mL volumetric flask for 10-mg Tablets. Add *Diluent* to about 80% of the final flask volume, sonicate with occasional swirling for 10 min ▲or shake by mechanical means for 20 min, and▲ (USP 1-Aug-2019) dilute with *Diluent* to volume. Centrifuge a portion of the solution, and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50 ± 5°
Flow rate: 2.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between prochlorperazine related compound A and prochlorperazine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual ▲specified or unspecified▲ (USP 1-Aug-2019) impurity in the portion of Tablets taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each ▲corresponding▲ (USP 1-Aug-2019) impurity from the *Sample solution*
r_S = peak response of prochlorperazine from the *Standard solution*
C_S = concentration of USP Prochlorperazine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prochlorperazine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of prochlorperazine, 373.94
M_{r2} = molecular weight of prochlorperazine maleate, 606.09
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid	0.07	—	—[a]
Prochlorperazinesulfoxide[b]	0.20	0.38	0.5
Perazine[c, d]	0.66	—	—
▲▲ (USP 1-Aug-2019) Prochlorperazine related compound A[d] ▲▲ (USP 1-Aug-2019)	0.97	—	—
Prochlorperazine	1.00	—	—
4-Chlorophenothiazine[d, e]	2.01	—	—
2-Chlorophenothiazine[d, f]	2.08	—	—
Specified unknown 1[d]	2.64	—	—
Specified unknown 2[d]	2.79	—	—
Specified unknown 3[d]	2.88	—	—
Any individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	2.0

▲▲ (USP 1-Aug-2019)
[a] Disregard.
[b] 2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine sulfoxide.
[c] 10-[3-(4-Methylpiperazin-1-yl)propyl]-10*H*-phenothiazine.
[d] Process impurity controlled in the drug substance. It is included for identification purposes only. It should not be reported for the drug product, and should not be included in the total impurities.
[e] 4-Chloro-10*H*-phenothiazine.
[f] 2-Chloro-10*H*-phenothiazine.

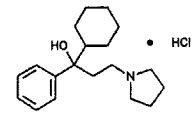
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Prochlorperazine Maleate RS
  USP Prochlorperazine Related Compound A RS
  4-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine dihydrochloride.
  $C_{20}H_{24}ClN_3S \cdot 2HCl$ ▲446.86▲ (USP 1-Aug-2019)

Procyclidine Hydrochloride



$C_{19}H_{29}NO \cdot HCl$ 323.90
1-Pyrrolidinepropanol, α-cyclohexyl-α-phenyl-, hydrochloride.
α-Cyclohexyl-α-phenyl-1-pyrrolidinepropanol hydrochloride [1508-76-5].

» Procyclidine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of $C_{19}H_{29}NO \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a dry place.
USP Reference standards ⟨11⟩—
USP Procyclidine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Dissolve about 250 mg in 10 mL of water in a separator, render alkaline with 6 N ammonium hydroxide, and extract with three 10-mL portions of ether. Filter the ether extracts slowly through a layer of about 2 g of anhydrous granular sodium sulfate supported on glass wool, evaporate the ether with a current of warm air, and scratch the surface of the container to induce crystallization of the residue: the procyclidine so obtained melts between 83° and 87°, the procedure for *Class I* being used (see *Melting Range or Temperature* ⟨741⟩).

C: A solution (1 in 100) responds to the tests for *Chloride* ⟨191⟩.

pH ⟨791⟩: between 5.0 and 6.5, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Related compounds—Dissolve approximately 200 mg of Procyclidine Hydrochloride in 20 mL of water, and render the solution alkaline by adding 1.5 mL of 6 N ammonium hydroxide. Extract with three 15-mL portions of chloroform, wash the combined extracts with 20 mL of water, discard the water washing, and filter the chloroform solution through a layer of 3 to 4 g of anhydrous granular sodium sulfate supported on glass wool. Reduce the volume to 5 mL by evaporating with the aid of gentle heat and a current of air. Inject 2 µL of this solution into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, and record the chromatogram to 2.5 relative to the retention time of the principal (procyclidine) peak. Under typical conditions, the instrument contains a 1-m × 2-mm glass column packed with 10% polyethylene glycol 20,000 and 2% potassium hydroxide on packing S1A. The column is maintained at a temperature of about 180°, the injection port is maintained at 210°, the detector block is maintained at about 220°, and dry helium is used as the carrier gas at a flow rate of about 60 mL per minute. From the total area under the curve, excluding the solvent peak, calculate the percentage of total impurities by area normalization: not more than 4.0% is found.

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Assay—Dissolve about 700 mg of Procyclidine Hydrochloride, accurately weighed, in 75 mL of glacial acetic acid in a 250-mL beaker, warming, if necessary, to effect solution. Cool, add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.39 mg of $C_{19}H_{29}NO \cdot HCl$.

Procyclidine Hydrochloride Tablets

» Procyclidine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{19}H_{29}NO \cdot HCl$.

Packaging and storage—Preserve in tight containers, and store in a dry place.
USP Reference standards ⟨11⟩—
USP Procyclidine Hydrochloride RS

Identification—
A: Triturate a portion of finely powdered Tablets, equivalent to about 10 mg of procyclidine hydrochloride, with 20 mL of chloroform, filter, evaporate the filtrate on a steam bath to dryness, and dry the residue at 105° for 1 hour: the IR absorption spectrum of a potassium bromide dispersion of the procyclidine hydrochloride so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Procyclidine Hydrochloride RS.
B: A portion of finely powdered Tablets, equivalent to about 50 mg of procyclidine hydrochloride, responds to *Identification* test *B* under *Procyclidine Hydrochloride.*

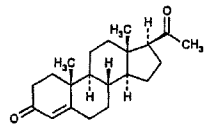
Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{19}H_{29}NO \cdot HCl$ dissolved, employing the procedure set forth in the *Assay,* making any necessary modifications.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{19}H_{29}NO \cdot HCl$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Related compounds—Using a portion of powdered Tablets, equivalent to 200 mg of procyclidine hydrochloride, proceed as directed in the test for *Related compounds* under *Procyclidine Hydrochloride.*

Assay—
Bromocresol purple solution—Dissolve 250 mg of bromocresol purple in dilute glacial acetic acid (1 in 50) to make 1000 mL.
Standard preparation—Transfer about 25 mg of USP Procyclidine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Bromocresol purple solution* to volume, and mix. The concentration of the *Standard preparation* is about 25 µg per mL.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2.5 mg of procyclidine hydrochloride, to a 100-mL volumetric flask, add 10.0 mL of water, and mix. Dilute with *Bromocresol purple solution* to volume, mix, and allow the undissolved particles to settle. Use the supernatant as directed in the *Procedure.*
Procedure—Transfer 5.0 mL each of the *Standard preparation* and the *Assay preparation* to individual 60-mL separators. Transfer 0.5 mL of water and 4.5 mL of *Bromocresol purple solution* to a third separator to provide the blank. Extract each solution with 20.0 mL of chloroform, and filter each extract, discarding the first 5 mL of the filtrate. Concomitantly determine the absorbance of each subsequent filtrate in a 1-cm cell at the wavelength of maximum absorbance at about 405 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{19}H_{29}NO \cdot HCl$ in the portion of Tablets taken by the formula:

$$0.1C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Procyclidine Hydrochloride RS in the *Standard preparation,* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Progesterone



$C_{21}H_{30}O_2$ 314.46
Pregn-4-ene-3,20-dione.
Progesterone [57-83-0].

» Progesterone contains not less than 97.0 percent and not more than 103.0 percent of $C_{21}H_{30}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Progesterone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: methanol.
Melting range ⟨741⟩: between 126° and 131°. It may exist also in a polymorphic modification, melting at about 121°.
Specific rotation ⟨781S⟩: between +175° and +183°.
Test solution: 20 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.5% of its weight.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of water and isopropyl alcohol (72:28). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

USP Monographs

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Internal standard solution—Transfer about 66 mg of methyltestosterone to a 10-mL volumetric flask, add dilute alcohol (85 in 100) to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Progesterone RS in dilute alcohol (85 in 100) to obtain a solution having a known concentration of about 2.5 mg per mL. Transfer 4.0 mL of this solution to a 10-mL volumetric flask, add 1.0 mL of *Internal standard solution*, then add dilute alcohol (85 in 100) to volume, and mix to obtain a solution having a known concentration of about 1 mg of USP Progesterone RS per mL.

Assay preparation—Transfer about 10 mg of Progesterone, accurately weighed, to a 10-mL volumetric flask, add 1.0 mL of *Internal standard solution*, then add dilute alcohol (85 in 100) to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the analyte and internal standard peaks is not less than 3.5; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2.0 for progesterone and 1.0 for methyltestosterone. Calculate the quantity, in mg, of $C_{21}H_{30}O_2$ in the portion of Progesterone taken by the formula:

$$10C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Progesterone RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Progesterone Injection

» Progesterone Injection is a sterile solution of Progesterone in a suitable solvent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{30}O_2$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type III glass.

USP Reference standards ⟨11⟩—
USP Progesterone RS

Identification—Insert a pledget of fine glass wool into the base of a chromatographic tube of about 200 × 25 mm. Mix 8.0 mL of nitromethane with 7.0 g of purified siliceous earth in a 150-mL beaker until uniform, and transfer to the chromatographic tube, packing lightly with a suitable tamping rod. Pack a pledget of glass wool on the top of the column. Dilute 1 mL of the Injection with *n*-heptane to obtain a solution having a concentration of about 1 mg of progesterone per mL. Transfer 4.0 mL of this solution to the prepared column. Pass 300 mL of *n*-heptane through the column, discarding the first 120 mL of the eluate. Collect the subsequent eluate in a 250-mL beaker. Evaporate the solution under a stream of nitrogen on a steam bath to about 50 mL, transfer to a 100-mL beaker, and evaporate to dryness. Remove the last traces of *n*-heptane by adding 1 mL of methanol and again drying. Dry the specimen over silica gel for 4 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Progesterone RS.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a degassed mixture of alcohol and water (11:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Progesterone RS in 20 mL of tetrahydrofuran, and dilute quantitatively, and stepwise if necessary, with alcohol to obtain a solution having a known concentration of about 0.08 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of progesterone, to a 100-mL volumetric flask, add 20 mL of tetrahydrofuran to dissolve, and dilute with alcohol to volume. Transfer 8 mL of this solution to a 100-mL volumetric flask, dilute with alcohol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at about 40°. Chromatograph a sample of dimethyl sulfoxide, and identify the retention time, t_a, of this nonretarded compound to calculate the capacity factor, *k'*. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, for progesterone is not less than 2.0; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—The run time for the *Assay preparation* must be at least twice that of the *Standard preparation*.] Calculate the quantity, in mg, of progesterone ($C_{21}H_{30}O_2$) in each mL of Injection taken by the formula:

$$1250(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Progesterone RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses for progesterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Progesterone Intrauterine Contraceptive System

» Progesterone Intrauterine Contraceptive System contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{30}O_2$. It is sterile.

Packaging and storage—Preserve in sealed, single-unit containers.

USP Reference standards ⟨11⟩—
USP Progesterone RS

Identification—Cut off and discard the sealed ends of the drug-containing cores of 2 Systems, and force the contents of

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the tubes into a small centrifuge tube. Add 3 mL of methanol, insert the stopper in the tube, mix, centrifuge, and transfer the clear, supernatant to a small beaker. Evaporate the methanol to dryness, wash the residue with two 4-mL portions of cyclohexane, and discard the washings. Dry the residue in vacuum at 50° to constant weight: the IR absorption spectrum of a mineral oil dispersion of the dried residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Progesterone RS.

Sterility Tests ⟨71⟩: meets the requirements.

Uniformity of dosage units ⟨905⟩: meets the requirements.

Chromatographic purity—

Test solution—Remove the drug-containing core from 1 System, as directed in the *Assay*, transferring it to a small flask with 25 mL of methanol. Shake vigorously for several minutes, and allow the insoluble portion to settle. The resulting supernatant is the *Test solution*.

Procedure—Divide a 20- × 20-cm thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel mixture, into sections 2 cm apart. In successive sections of the plate, on a line 2 cm from the lower edge of the plate and parallel to it, apply 1 µL, 2 µL, 3 µL, and 100 µL of the *Test solution*. Develop the plate in a suitable pre-equilibrated chromatographic chamber with a solvent system consisting of a mixture of chloroform and ethyl acetate (2:1) until the solvent front has moved 10 cm above the point of application of the spots. Remove the plate, and allow to air-dry. Observe the dried plate under short-wavelength UV light (254 nm). If spots other than the principal spot are observed in the lane of the 100-µL specimen, estimate the concentration of each by comparison with the 1-µL (1%), 2-µL (2%), and 3-µL (3%) spots. The requirement is met if the sum of impurities in the 100-µL specimen does not exceed 3%.

Drug release pattern—Remove the attached sutures from 10 Systems, and secure each system to a corrosion-resistant wire of sufficient length such that the systems are completely immersed during the shaking operation but do not touch the bottoms of the flasks. Suspend each system by the attached wire from the arm of a mechanical shaker designed to travel 2.5 cm in each direction in a vertically reciprocating cycle, at a speed of 2.5 cycles per second, so that each system is immersed in a separate 250-mL volumetric flask containing 230 mL of water, pre-equilibrated to 60 ± 0.1°. Immerse the volumetric flasks in an insulated constant-temperature water bath, maintained at 60 ± 0.1° and having a suitable means of maintaining the water level, so that the water level of the bath is above the water level in the flasks. Employ a rack or other suitable means of support for the flasks in the water bath.

Operate the shaker under the conditions described above for 23.5 hours, then remove the flasks and the systems from the bath. Remove the systems from the flasks, and immerse each system in a different flask containing 230 mL of water, pre-equilibrated to 60 ± 0.1°, and immerse these flasks in the water bath. Repeat this shaking operation daily for 12 days, using different flasks each day.

Determine the quantity of progesterone in the solutions from each of the 12 days of testing as follows. Immediately add 15 mL of methanol to each solution, allow to cool to room temperature, dilute with water to volume, and mix. Concomitantly determine the UV absorbances of each test solution and of a solution of USP Progesterone RS in the same medium, having a known concentration of about 7 µg per mL, in 2-cm cells at the wavelength of maximum absorbance at about 248 nm, with a suitable spectrophotometer, against a blank of water and methanol (47:3). Calculate the progesterone release rate, in mg per day, in the solutions taken by the formula:

$$(A_U/A_S)(24/23.5)0.25C$$

in which A_U and A_S are the absorbances of the test solution and the Standard solution, respectively; and C is the concentration, in µg per mL, of USP Progesterone RS in the Standard solution. For the time points specified, the drug-release pattern conforms to *Acceptance Table 1* under *Drug Release* ⟨724⟩.

Day	Release Rate (mg per day)
6	1.05–1.45
9	0.95–1.35
12	0.90–1.30

Assay—Cut off the lower sealed end of the drug-containing core of a number of Progesterone Intrauterine Contraceptive Systems, sufficient to provide about 400 mg of progesterone, forcing the viscous liquid core into a 1000-mL volumetric flask. Cut the core sections in half lengthwise, using a sharp blade, taking precautions not to contaminate either the core material or the outside of the membranes. Transfer all of these sections of the systems to the flask containing the core material. Add about 500 mL of methanol to the flask, shake vigorously for 5 to 10 minutes, dilute with methanol to volume, and centrifuge a portion of the solution. Dilute 10.0 mL of the clear, supernatant with methanol to 250 mL, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Progesterone RS, previously dried and accurately weighed, in methanol having a known concentration of about 16 µg of progesterone per mL, in 1-cm cells at the wavelength of maximum absorbance at about 241 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_{21}H_{30}O_2$ in each System taken by the formula:

$$25(C/N)(A_U/A_S)$$

which C is the concentration, in µg per mL, of USP Progesterone RS in the Standard solution; N is the number of Systems taken; and A_U and A_S are the absorbances of the test solution and the Standard solution, respectively.

Progesterone Injectable Suspension

» Progesterone Injectable Suspension is a sterile suspension of Progesterone in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{21}H_{30}O_2$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—
USP Methyltestosterone RS
USP Progesterone RS

Identification—Filter a volume of well-shaken Injectable Suspension, equivalent to not less than 100 mg of progesterone, through a medium-porosity, sintered-glass crucible, filtering again through the same crucible if the fluid is not clear. Wash with several 5-mL portions of water until 2 mL of the last washing, evaporated on a steam bath, leaves no weighable residue: the washed solid, dried at 105° to constant weight, melts between 126° and 131°, and responds to *Identification* test *A* under *Progesterone*.

USP Monographs

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pH ⟨791⟩: between 4.0 and 7.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and isopropyl alcohol (72:28). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩.)

Diluent—Prepare a mixture of alcohol and water (85 in 100).

Internal standard solution—Dissolve an accurately weighed quantity of USP Methyltestosterone RS in *Diluent* to obtain a solution containing about 6.6 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Progesterone RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 2.5 mg per mL.

Standard curve—To four separate polytef-lined, screw-capped centrifuge tubes, pipet 3-, 4-, 5-, and 6-mL portions, respectively, of the *Standard preparation*. Add *Diluent* to each tube to make about 8 mL of solution. Transfer 1.0 mL of *Internal standard solution* to each tube, and mix.

Assay preparation—Transfer an accurately measured volume of Injectable Suspension, equivalent to about 25 mg progesterone, to a polytef-lined, screw-capped, 25 mL test tube. Add 16 mL of *Diluent*, and shake until clear. Add 2.0 mL of *Internal standard solution*, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 2.1-mm × 1-m column that contains packing L2. The chromatographic conditions are such that retention times of the internal standard and progesterone are between 3 and 6 minutes and 7 and 11 minutes, respectively. Chromatograph any solution of the *Standard curve*, and record the peak areas as directed for *Procedure:* the resolution, *R*, between progesterone and methyltestosterone is not less than 3.5; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (5.0 µL) of each solution of the *Standard curve* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Plot the progesterone to methyltestosterone peak ratio versus the concentration, in mg per mL, of progesterone in the *Standard curve* solutions. Extrapolate the progesterone to methyltestosterone peak ratio of the *Assay preparation* in the *Standard curve* so obtained, and determine the progesterone concentration, *C*, in mg per mL, in the *Assay preparation*. Calculate the amount, in mg, of progesterone ($C_{21}H_{30}O_2$) in each mL of the Injectable Suspension taken by the formula:

$$V/A(C)$$

in which *V* is the total volume of the *Assay preparation; A* is the volume in mL of Injectable Suspension taken; and *C* is the concentration of progesterone in the *Assay preparation* calculated above.

Progesterone Compounded Vaginal Inserts

DEFINITION

Progesterone Compounded Vaginal Inserts contain NLT 90.0% and NMT 110.0% of the labeled amount of progesterone ($C_{21}H_{30}O_2$).

Prepare Compounded Progesterone Vaginal Inserts in Fatty Acid Base or Polyethylene Glycol Base as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Progesterone (micronized)	25–600 mg
Fatty Acid Base or Polyethylene Glycol Base, a sufficient quantity to make	1 Insert

Calibrate the actual molds with the *Base* that is used for preparing the Inserts, and adjust the formula accordingly. Heat the *Base* slowly and evenly until melted. Slowly add the *Progesterone* powder to the melted base, with stirring. Mix thoroughly, and pour into molds. If preparing Inserts in *Fatty Acid Base*, cool in a refrigerator until solidified, trim, and wrap. If preparing Inserts in *Polyethylene Glycol Base*, cool, trim, and wrap.

ASSAY

• **Inserts in Fatty Acid Base**

Solution A: Dehydrated alcohol, isopropyl alcohol, and methanol (90:5:5)

Mobile phase: *Solution A* and water (55:45). Filter, and degas.

Diluent: *Solution A* and water (70:30)

System suitability stock solution A: 0.4 mg/mL of USP Methyltestosterone RS in *Mobile phase*

System suitability stock solution B: 0.4 mg/mL of USP Progesterone RS in *Mobile phase*

System suitability solution: Transfer 2.0 mL each of *System suitability stock solution A* and *System suitability stock solution B* to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

Standard solution A: Prepare 0.25 mg/mL of USP Progesterone RS in *n*-propyl alcohol. Mix 5.0 mL of the solution with 10.0 mL of *Diluent*.

Standard solution B: Prepare 3 mg/mL of USP Progesterone RS in *n*-propyl alcohol. Transfer 3.0 mL of the solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution A: Transfer 1 Insert, containing NMT 100 mg of progesterone, to a 100-mL volumetric flask. Dissolve in 90 mL of *n*-propyl alcohol, heat at 45° for 4 min, and sonicate for 10 min. Cool, and dilute with *n*-propyl alcohol to volume. Dilute quantitatively, and stepwise if necessary, with *n*-propyl alcohol, sonicating if necessary, to obtain a solution containing a nominal concentration of 0.25 mg/mL of progesterone. Transfer 5.0 mL of this solution to a 50-mL centrifuge tube, add 10.0 mL of *Diluent*, sonicate for 1 min, and centrifuge for 10 min at 2000 rpm. Pass the supernatant through a filter of 0.45-µm or finer pore size, discarding the first 4 mL of the filtrate.

Sample solution B: Transfer 1 Insert, containing more than 100 mg of progesterone, to a 200-mL volumetric flask. Dissolve in 180 mL of *n*-propyl alcohol, heat at 45° for 8 min, and sonicate for 5 min. Cool, and dilute with *n*-propyl alcohol to volume. Dilute quantitatively, and stepwise if necessary, with *n*-propyl alcohol, sonicating each dilution for 1 min, to obtain a solution with a nominal concentration of 0.09 mg/mL of progesterone. Transfer 15 mL of this solution to a 50-mL centrifuge tube, and centrifuge for 10 min at 2000 rpm. Pass the supernatant through a filter of 0.45-µm or finer pore size, discarding the first 4 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 3.9-mm × 30-cm; packing L1

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Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for methyltestosterone and progesterone are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between methyltestosterone and progesterone
Tailing factor: NMT 2.0 for the progesterone peak
Relative standard deviation: NMT 2.0% for replicate injections for progesterone
Analysis
Samples: *Standard solution A* and *Sample solution A* or *Standard solution B* and *Sample solution B*
Calculate the percentage of the labeled amount of progesterone ($C_{21}H_{30}O_2$) in the Insert taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Progesterone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of progesterone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%
• **INSERTS IN POLYETHYLENE GLYCOL BASE**
Solution A, Mobile phase, System suitability stock solution A, System suitability stock solution B, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* for *Inserts in Fatty Acid Base.*
Standard solution: 0.1 mg/mL of USP Progesterone RS in *Mobile phase*
Sample solution: Dissolve 1 Insert in 200 mL of *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution with a nominal concentration of 0.1 mg/mL of progesterone. Pass a 10-mL portion of the mixture through a filter of 0.45-µm or finer pore size, discarding the first 4 mL of the filtrate.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of progesterone ($C_{21}H_{30}O_2$) in the Insert taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

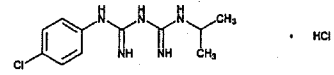
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Progesterone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of progesterone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Weight Variation*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in light-resistant containers, and store in a refrigerator. Store Inserts in Fatty Acid Base in well-closed containers. Store Inserts in Polyethylene Glycol Base in tight containers, and do not dispense or store in polystyrene containers.
• **BEYOND-USE DATE:** NMT 90 days after the date on which they were compounded when stored in a refrigerator
• **LABELING:** Label Inserts to state whether they are Progesterone Vaginal Inserts in a Fatty Acid Base or in a Polyethylene Glycol Base. Label to state the amount of progesterone, in mg, in each Insert. Label to state that they are to be stored in a refrigerator. Label to state that wrappers are to be removed before use. If necessary, Inserts in Polyethylene Glycol Base may be moistened before insertion.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methyltestosterone RS
USP Progesterone RS

Proguanil Hydrochloride



$C_{11}H_{16}ClN_5 \cdot HCl$ 290.19
Biguanide, 1-(4-chlorophenyl)-5-isopropyl, hydrochloride; 1-(*p*-Chlorophenyl)-5-isopropylbiguanide hydrochloride [637-32-1].

DEFINITION
Proguanil Hydrochloride contains NLT 98.5% and NMT 101.0% of proguanil hydrochloride ($C_{11}H_{16}ClN_5 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY
• **PROCEDURE**
Solution A: 0.65 g/L of 1-pentanesulfonic acid sodium salt and 7.0 g/L of sodium perchlorate in water. Adjust with 1% trifluoroacetic acid to a pH of 3.0.
Solution B: 0.65 g/L of 1-pentanesulfonic acid sodium salt and 7.0 g/L of sodium perchlorate in a mixture of methanol, acetonitrile, and water (400:400:200). Add 4 mL of 1% trifluoroacetic acid to 1 L of this solution.
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
20	40	60
25	75	25
35	75	25

Diluent: Acetonitrile, methanol, and water (200:200:600)
Standard solution: 0.2 mg/mL of USP Proguanil Hydrochloride RS in *Diluent*
Sample solution: 0.2 mg/mL of Proguanil Hydrochloride in *Diluent*

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of proguanil hydrochloride ($C_{11}H_{16}ClN_5 \cdot HCl$) in the portion of Proguanil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of proguanil from the *Sample solution*
r_S = peak response of proguanil from the *Standard solution*
C_S = concentration of USP Proguanil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Proguanil Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
• **Limit of Chloroaniline**
Solution A: 3.45 g/L solution of sodium nitrite
Solution B: 50 g/L solution of ammonium sulfamate
Solution C: 1 mg/mL of naphthylethylenediamine dihydrochloride in water. Prepare immediately before use.
Standard solution: 1.25 mg/L solution of chloroaniline
Sample solution: Dissolve 100 mg in 1 mL of 2 N hydrochloric acid, and dilute with water to 20 mL.
Analysis: Cool the *Sample solution* to 5°. Add 1 mL of *Solution A*, and allow to stand at 5° for 5 min. Add 2 mL of *Solution B*, and allow to stand for 10 min. Add 2 mL of *Solution C*, dilute with water to 50 mL, and allow to stand for 30 min.
Acceptance criteria: Any red color produced is not more intense than that of a standard prepared at the same time and in the same manner, using 20 mL of *Standard solution* (250 ppm).
• **Organic Impurities, Procedure 1**
[Note—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended when proguanil related compound A, proguanil related compound E, proguanil related compound F, and proguanil related compound G (see *Table 3*) may be present.]
Mobile phase: Dissolve 3.78 g of sodium hexanesulfonate in a mixture of 1200 mL of methanol, 800 mL of water, and 10 mL of glacial acetic acid.
Identification solution: 0.5 µg/mL of USP Proguanil Related Compound C RS in *Mobile phase*
System suitability stock solution: 0.5 µg/mL of USP Proguanil Related Compound D RS in *Mobile phase*
System suitability solution: Dilute 1 mL of the *Standard stock solution* with *Mobile phase* to 200 mL. To 1 mL of the resulting solution, add 1 mL of *System suitability stock solution*.
Standard stock solution: 0.1 mg/mL of USP Proguanil Hydrochloride RS in *Mobile phase*
Standard solution: 0.2 µg/mL of USP Proguanil Hydrochloride RS from the *Standard stock solution* in *Mobile phase*
Sample solution: 0.1 mg/mL of Proguanil Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 and 254 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1, or 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: At least 5 times the retention time of the proguanil peak at both wavelengths
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5 between proguanil related compound D and proguanil, *System suitability solution* at 230 nm
Relative standard deviation: NMT 10.0%, *Standard solution* at 230 nm
Analysis
Samples: *Identification solution*, *System suitability stock solution*, *Standard solution*, and *Sample solution*
[Note—The relative retention times for proguanil related compound D, proguanil, and proguanil related compound C are about 0.46, 1.0, and 2.5, respectively.]
Identify the components based on their relative retention times, and measure the responses for the major peaks.
Calculate the percentage of proguanil related compound C and proguanil related compound D, as detected at 230 nm, in the portion of Proguanil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of proguanil related compound C or proguanil related compound D from the *Sample solution*
r_S = peak response of proguanil from the *Standard solution*
C_S = concentration of USP Proguanil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Proguanil Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity, as detected at 230 nm, in the portion of Proguanil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity at 230 nm from the *Sample solution*
r_S = peak response of proguanil at 230 nm from the *Standard solution*
C_S = concentration of USP Proguanil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Proguanil Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity, as detected at 254 nm, in the portion of Proguanil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of each impurity at 254 nm from the *Sample solution*
r_S = peak response of proguanil at 254 nm from the *Standard solution*
C_S = concentration of USP Proguanil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Proguanil Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of total impurities as the sum of the calculated percentage contents of known and unknown impurities, considering each peak at the wavelength at which the peak shows the higher value.

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Proguanil related compound D[a]	0.46	0.2
Proguanil	1.0	—
Proguanil related compound C[b]	2.5	0.2
Any other impurity[c]	—	0.1
Total impurities	—	0.5

[a] 1,5-Diisopropylbiguanide or 1,5-Bis(1-methylethyl)biguanide.
[b] 1,5-Bis(4-chlorophenyl)biguanide.
[c] Disregard any peak below 0.05%.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A, Solution B, Mobile phase, and **Diluent:** Proceed as directed in the *Assay*.

Standard stock solution: 0.2 mg/mL each of USP Proguanil Related Compound A RS, USP Proguanil Related Compound C RS, USP Proguanil Related Compound D RS, USP Proguanil Related Compound E RS, USP Proguanil Related Compound F RS, and USP Proguanil Related Compound G RS in *Diluent*

Standard solution: 0.2 µg/mL of USP Proguanil Hydrochloride RS and 0.2 µg/mL each of USP Proguanil Related Compound A RS, USP Proguanil Related Compound C RS, USP Proguanil Related Compound D RS, USP Proguanil Related Compound E RS, USP Proguanil Related Compound F RS, and USP Proguanil Related Compound G RS in *Diluent* from the *Standard stock solution*

System suitability solution: 0.2 mg/mL of USP Proguanil Hydrochloride RS and 2 µg/mL each of USP Proguanil Related Compound A RS, USP Proguanil Related Compound C RS, USP Proguanil Related Compound D RS, USP Proguanil Related Compound E RS, USP Proguanil Related Compound F RS, and USP Proguanil Related Compound G RS in *Diluent* from the *Standard stock solution*

Sample solution: 0.2 mg/mL of Proguanil Hydrochloride in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between proguanil and proguanil related compound G
Tailing factor: NMT 2.0 for proguanil
Relative standard deviation: NMT 2.0% for the proguanil peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each proguanil related compound and any other impurity in the portion of Proguanil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the proguanil related compound or any other impurity from the *Sample solution*
r_S = peak response of the corresponding proguanil related compound or proguanil (for calculating any other impurity) from the *Standard solution*
C_S = concentration of the corresponding proguanil related compound or proguanil hydrochloride (for calculating any other impurity) in the *Standard solution* (mg/mL)
C_U = concentration of Proguanil Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Proguanil related compound A[a]	0.11	0.1
Proguanil related compound D[b]	0.34	0.1
Proguanil related compound E[c]	0.51	0.1
Proguanil	1.00	—
Proguanil related compound G[d]	1.05	0.1
Proguanil related compound F[e]	1.38	0.1
Proguanil related compound C[f]	1.49	0.1
Any other impurity	—	0.10
Total impurities	—	0.5

[a] 1-Cyano-3-isopropylguanidine.
[b] 1,5-Diisopropylbiguanide or 1,5-Bis(1-methylethyl) biguanide.
[c] 1-(4-Chlorophenyl)-3-cyanoguanidine.
[d] 1-(3-Chlorophenyl)-5-isopropylbiguanide.
[e] 1-(3,4-Dichlorophenyl)-5-isopropylbiguanide.
[f] 1,5-Bis(4-chlorophenyl)biguanide.

SPECIFIC TESTS

• **ACIDITY OR ALKALINITY**
Sample: 400 mg
Analysis: Add 0.4 mL of methyl red–methylene blue TS to 35 mL of water maintained at 60°–65°. Neutralize with either 0.01 N sodium hydroxide or 0.01 N hydrochloric acid to a gray color. Add the *Sample*, and stir until completely dissolved.
Acceptance criteria: The solution is gray or green. NMT 0.2 mL of 0.01 N hydrochloric acid is required to change the color of the solution to reddish-violet.

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 0.5%

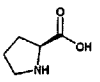
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at room temperature.

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- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Proguanil Hydrochloride RS
  USP Proguanil Related Compound A RS
  1-Cyano-3-isopropylguanidine.
  $C_5H_{10}N_4$ 126.16
  USP Proguanil Related Compound C RS
  1,5-Bis(4-chlorophenyl)biguanide.
  $C_{14}H_{13}Cl_2N_5$ 322.19
  USP Proguanil Related Compound D RS
  1,5-Bis(1-methylethyl)biguanide or
  1,5-Diisopropylbiguanide.
  $C_8H_{19}N_5$ 185.27
  USP Proguanil Related Compound E RS
  1-(4-Chlorophenyl)-3-cyanoguanidine.
  $C_8H_7ClN_4$ 194.62
  USP Proguanil Related Compound F RS
  1-(3,4-Dichlorophenyl)-5-isopropylbiguanide.
  $C_{11}H_{15}Cl_2N_5$ 288.18
  USP Proguanil Related Compound G RS
  1-(3-Chlorophenyl)-5-isopropylbiguanide.
  $C_{11}H_{16}ClN_5$ 253.73

Proline



$C_5H_9NO_2$ 115.13
L-Proline [147-85-3].

DEFINITION
Proline contains NLT 98.5% and NMT 101.5% of L-proline ($C_5H_9NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample: 100 mg of Proline
  Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Endpoint detection: Potentiometric
  Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the *Blank* determination.
  Calculate the percentage of proline ($C_5H_9NO_2$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = volume of *Titrant* consumed by the *Sample* (mL)
V_B = volume of *Titrant* consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 115.1 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

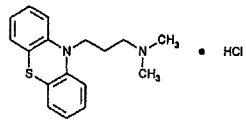
IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.4%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Standard solution: 0.50 mL of 0.020 N hydrochloric acid
  Sample: 0.73 g of Proline
  Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Standard solution: 0.10 mL of 0.020 N sulfuric acid
  Sample: 0.33 g of Proline
  Acceptance criteria: NMT 0.03%
- **IRON** ⟨241⟩**:** NMT 30 ppm
- **RELATED COMPOUNDS**
  System suitability solution: 0.4 mg/mL each of USP L-Proline RS and USP L-Threonine RS in 0.1 N hydrochloric acid
  Standard solution: 0.05 mg/mL of USP L-Proline RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]
  Sample solution: 10 mg/mL of Proline in 0.1 N hydrochloric acid
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)
  Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
  System suitability
  Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.
  Analysis
  Samples: *System suitability solution*, *Standard solution*, and *Sample solution*
  After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
  Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
  Individual impurities: NMT 0.5%
  Total impurities: NMT 2.0%

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 40 mg/mL in water
  Acceptance criteria: −84.3° to −86.3°
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.4% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Proline RS
  USP L-Threonine RS

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Promazine Hydrochloride



$C_{17}H_{20}N_2S \cdot HCl$ 320.88

10*H*-Phenothiazine-10-propanamine, *N*,*N*-dimethyl-, monohydrochloride.

10-3-(Dimethylamino)propylphenothiazine monohydrochloride [53-60-1].

» Promazine Hydrochloride, dried at 105° for 2 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_{17}H_{20}N_2S \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Promazine Hydrochloride RS

Completeness and clarity of solution—A solution of it (1 in 10) and a 1 in 10 solution of it in chloroform are practically clear and show not more than a light yellow color.

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Determine the absorbance of the assay solution employed for measurement of absorbance in the *Assay* at 301 nm, using 0.1 N hydrochloric acid as the blank. Similarly determine the absorbance of a 1 in 10 dilution of this solution, prepared with the same acid, at the wavelength of maximum absorbance at about 252 nm: the ratio $10(A_{252}/A_{301})$ is between 7.1 and 7.9.

C: It responds to the tests for *Chloride* ⟨191⟩.

Melting range, *Class I* ⟨741⟩: between 172° and 182°, but the range between beginning and end of melting does not exceed 3°.

pH ⟨791⟩: between 4.2 and 5.2, in a solution (1 in 20).

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Selenium ⟨291⟩—The absorbance of the solution from the *Test Solution*, prepared with 100 mg of Promazine Hydrochloride and 200 mg of magnesium oxide, is not greater than one-half that from the *Standard Solution* (0.003%).

Chromatographic purity—[NOTE—Perform this test under conditions of subdued light and with no unnecessary delays between the preparation of the solutions and the development of the chromatographic plate.]

Developing solvent—Mix 95 volumes of toluene with 15 volumes of alcohol and 1 volume of ammonium hydroxide.

Standard preparations—Dissolve an accurately weighed quantity of USP Promazine Hydrochloride RS in methanol to obtain *Standard preparation A* having a known concentration of 0.4 mg per mL. Dilute quantitatively with methanol to obtain *Standard preparations* having the following compositions:

Standard preparation	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimens)
A	(undiluted)	400	2.0
B	(1 in 2)	200	1.0
C	(3 in 10)	120	0.6
D	(1 in 10)	40	0.2

Test preparation—Dissolve an accurately weighed quantity of Promazine Hydrochloride in methanol to obtain a solution containing 20 mg per mL.

Procedure—Apply separately 10 µL of the *Test preparation* and 10 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Position the plate in a chromatographic chamber and develop the chromatograms in the *Developing solvent* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate by air drying for 15 minutes. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations:* the sum of the intensities of secondary spots obtained from the *Test preparation* corresponds to not more than 2.0% of related compounds, with no single impurity corresponding to more than 1.0%.

Assay— [NOTE—Use low-actinic glassware.] Transfer about 50 mg of Promazine Hydrochloride, previously dried and accurately weighed, to a 1000-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Without delay, concomitantly determine the absorbances of this solution and of a Standard solution of USP Promazine Hydrochloride RS in the same medium having a known concentration of about 50 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 301 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{17}H_{20}N_2S \cdot HCl$ in the portion of Promazine Hydrochloride taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Promazine Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution of Promazine Hydrochloride and the Standard solution, respectively.

Promazine Hydrochloride Injection

» Promazine Hydrochloride Injection is a sterile solution of Promazine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{20}N_2S \cdot HCl$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Promazine Hydrochloride RS

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[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: It meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

B: It responds to *Identification* test *B* under *Promazine Hydrochloride.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.8 USP Endotoxin Units per mg of promazine hydrochloride.

pH ⟨791⟩: between 4.0 and 5.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay— [NOTE—Use low-actinic glassware.] Transfer a volume of Injection, equivalent to about 50 mg of promazine hydrochloride, to a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 10.0 mL of the solution to a 250-mL separator, add 20 mL of water, render alkaline with ammonium hydroxide, and extract with four 25-mL portions of ether. Extract the combined ether extracts with five 15-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 100-mL volumetric flask. Aerate to remove residual ether, dilute with 0.1 N hydrochloric acid to volume, and mix. Without delay, concomitantly determine the absorbances of this solution and of a Standard solution of USP Promazine Hydrochloride RS in the same medium having a known concentration of about 50 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 301 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{17}H_{20}N_2S \cdot HCl$ in each mL of the Injection taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Promazine Hydrochloride RS in the Standard solution, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Promazine Hydrochloride Oral Solution

» Promazine Hydrochloride Oral Solution contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{20}N_2S \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Promazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: Dilute a volume of Oral Solution, equivalent to about 50 mg of promazine hydrochloride, with 0.01 N hydrochloric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator": the Oral Solution meets the requirements of the test.

B: It responds to *Identification* test *B* under *Promazine Hydrochloride.*

pH ⟨791⟩: between 5.0 and 5.5.

Assay— [NOTE—Use low-actinic glassware.] Transfer an accurately measured volume of Oral Solution, or a quantitative dilution of it in water, equivalent to about 10 mg of promazine hydrochloride, to a 250-mL separator. Add water to adjust the volume to about 45 mL, add 3 mL of sodium hydroxide solution (1 in 10), mix, and extract the promazine with five 25-mL portions of ether. Wash the combined ether extracts with 25 mL of water, and discard the aqueous washings. Extract the combined ether extract with one 50-mL and four 25-mL portions of 0.1 N hydrochloric acid. Filter the acid extracts through a pledget of cotton washed with 0.1 N hydrochloric acid into a 250-mL volumetric flask, dilute with the same acid to volume, and mix. Without delay, concomitantly determine the absorbances of this solution and of a Standard solution of USP Promazine Hydrochloride RS in the same medium having a known concentration of about 40 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 301 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{17}H_{20}N_2S \cdot HCl$ in each mL of the Oral Solution taken by the formula:

$$(0.25C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Promazine Hydrochloride RS in the Standard solution, V is the volume, in mL, of Oral Solution taken, and A_U and A_S are the absorbances from the assay solution and the Standard solution, respectively.

Delete the following:

▲Promazine Hydrochloride Syrup

» Promazine Hydrochloride Syrup contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{20}N_2S \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩ —

USP Promazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—It responds to *Identification* test *B* under *Promazine Hydrochloride.*

Assay— [NOTE—Use low-actinic glassware.] Transfer a volume of Syrup, equivalent to about 10 mg of promazine hydrochloride, to a 250-mL separator. Proceed as directed in the *Assay* under *Promazine Hydrochloride Oral Solution,* beginning with "Add water to adjust the volume to about 45 mL." Calculate the quantity, in mg, of $C_{17}H_{20}N_2S \cdot HCl$ in each mL of the Syrup taken by the formula:

$$(0.25C/V)(A_U/A_S)$$

USP Monographs

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in which C is the concentration, in µg per mL, of USP Promazine Hydrochloride RS in the Standard solution, V is the volume, in mL, of Syrup taken, and A_U and A_S are the absorbances of the solution from the Syrup and the Standard solution, respectively. ▲ (USP 1-May-2020)

Promazine Hydrochloride Tablets

» Promazine Hydrochloride Tablets contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{20}N_2S \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Promazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: Shake a portion of powdered Tablets, equivalent to about 50 mg of promazine hydrochloride, with 25 mL of 0.01 N hydrochloric acid for 5 minutes, and filter: the solution meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

B: It responds to *Identification* test *B* under *Promazine Hydrochloride*.

Disintegration ⟨701⟩: 30 minutes, with disks.

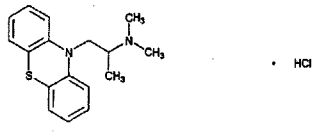
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay— [NOTE—Use low-actinic glassware.] Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of promazine hydrochloride, to a 100-mL volumetric flask. Add 50 mL of 0.1 N hydrochloric acid, and shake by mechanical means for about 1 hour. Dilute with 0.1 N hydrochloric acid to volume, mix, and centrifuge a portion of the mixture. Transfer 10.0 mL of the clear, supernatant to a 250-mL separator, and proceed as directed in the *Assay* under *Promazine Hydrochloride Injection*, beginning with "add 20 mL of water." Calculate the quantity, in mg, of $C_{17}H_{20}N_2S \cdot HCl$ in the portion of Tablets taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Promazine Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

Promethazine Hydrochloride



$C_{17}H_{20}N_2S \cdot HCl$ 320.88

10*H*-Phenothiazine-10-ethanamine, *N*,*N*,α-trimethyl-, monohydrochloride, (±)-;
(±)-10-[2-(Dimethylamino)propyl]phenothiazine monohydrochloride [58-33-3].

DEFINITION

Promethazine Hydrochloride contains NLT 97.0% and NMT 101.5% of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$), calculated on the dried basis.

[NOTE—Throughout the following procedures, protect the samples, the Reference Standards, and the solutions containing them, by conducting the procedures without delay under subdued light or using low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Dissolve 8.2 mL of hydrochloric acid in 1000 mL of water.

Mobile phase: Acetonitrile, water, and triethylamine (850:270:1)

System suitability stock solution: 1.2 mg/mL of USP Promethazine Related Compound B RS in *Diluent*. Sonicate to dissolve.

Standard solution: 0.1 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*. Sonicate to dissolve.

System suitability solution: 0.09 mg/mL of USP Promethazine Hydrochloride RS and 0.12 mg/mL of USP Promethazine Related Compound B RS in *Diluent* from the *Standard solution* and *System suitability stock solution*, respectively

Sample solution: 0.1 mg/mL of Promethazine Hydrochloride in *Diluent*. Sonicate to dissolve.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2.5 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention time of promethazine

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for promethazine related compound B and promethazine are 0.82 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between promethazine and promethazine related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Promethazine Hydrochloride taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Promethazine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.5% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Organic Impurities**

Diluent: Methanol and triethylamine (999:1)
Buffer: 3.7 g/L of ammonium acetate in water
Solution A: Acetonitrile and *Buffer* (300:700)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	60	40
18	60	40
18.1	100	0
25	100	0

System suitability stock solution: 0.5 mg/mL of USP Promethazine Related Compound B RS in *Diluent*
Standard stock solution: 0.5 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*
System suitability solution: 5 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS from the *Standard stock solution* and *System suitability stock solution*, respectively
Standard solution: 5 µg/mL of USP Promethazine Hydrochloride RS from the *Standard stock solution*
Sensitivity solution: 0.25 µg/mL of USP Promethazine Hydrochloride RS from the *Standard solution*
Sample solution: 0.5 mg/mL of Promethazine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 234 and 249 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.4 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[Note—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between promethazine and promethazine related compound B, *System suitability solution*
Relative standard deviation: NMT 3.0% at 234 and 249 nm, *Standard solution*
Signal-to-noise ratio: NLT 10 at 234 and 249 nm, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of promethazine sulfoxide in the portion of Promethazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of promethazine sulfoxide at 234 nm from the *Sample solution*
r_S = peak response of promethazine hydrochloride at 234 nm from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Promethazine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of all other impurities in the portion of Promethazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity at 249 nm from the *Sample solution*
r_S = peak response of promethazine hydrochloride at 249 nm from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Promethazine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine sulfoxide[a]	0.28	2.1	0.1
Desmethyl promethazine[b]	0.71	1.0	0.2
Promethazine	1.0	—	—
Promethazine related compound B	1.3	1.0	0.8
Phenothiazine	1.7	2.0	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.2

[a] *N*,*N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[b] *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 50 mg/mL of Promethazine Hydrochloride
Acceptance criteria: 4.0–5.0

- **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Promethazine Hydrochloride RS
USP Promethazine Related Compound B RS
Isopromethazine hydrochloride;
N,N-Dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine hydrochloride.
$C_{17}H_{20}N_2S \cdot HCl$ 320.88

Promethazine Hydrochloride Injection

DEFINITION
Promethazine Hydrochloride Injection is a sterile solution of Promethazine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).
[NOTE—Throughout the following procedures, protect the samples, the Reference Standards, and the solutions containing them, by conducting the procedures without delay under subdued light or using low-actinic glassware.]

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 3.7 g/L of ammonium acetate in water
Solution A: Acetonitrile and *Buffer* (30:70)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)
0	100	0
10	60	40
18	60	40
18.1	100	0
25	100	0

Diluent: 0.1% triethylamine in methanol
System suitability solution: 1.0 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS in *Diluent*
Standard solution: 0.05 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.05 mg/mL of promethazine hydrochloride from a volume of Injection in *Diluent*
[NOTE—Sonication may be used in the preparation of *System suitability solution*, *Standard solution*, and *Sample solution*.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 4°
Flow rate: 1.4 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for promethazine and promethazine related compound B are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 5.0 between the promethazine and promethazine related compound B peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 1.0 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS in *Diluent*
Sample solution: Nominally 500 µg/mL of promethazine hydrochloride from a volume of Injection in *Diluent*
[NOTE—Sonication may be used in the preparation of *Standard solution* and *Sample solution*.]
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between the promethazine and promethazine related compound B peaks
Relative standard deviation: NMT 2.0% for promethazine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of promethazine from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

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Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine sulfoxide[a]	0.3	0.29	2.6
Desmethyl promethazine[b]	0.7	1.1	0.2
Promethazine	1.0	—	—
Promethazine related compound B[c]	1.3	—	—
Phenothiazine[d]	1.7	2.3	0.2
Any individual unspecified degradation product	—	1.0	0.1
Total degradation products	—	—	2.8

[a] *N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[b] *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.
[c] This is a process impurity that is controlled in the drug substance and is included for identification only.
[d] 10*H*-Phenothiazine.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 5.0 USP Endotoxin Units/mg of promethazine hydrochloride
- **PH** ⟨791⟩: 4.0–5.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Promethazine Hydrochloride RS
  USP Promethazine Related Compound B RS
  Isopromethazine hydrochloride;
  N,N-Dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine hydrochloride.
  $C_{17}H_{20}N_2S \cdot HCl$ 320.88

Promethazine Hydrochloride Oral Solution

DEFINITION

Promethazine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).

[NOTE—Throughout the following procedures, protect the samples, the Reference Standards, and the solutions containing them, by conducting the procedures without delay, under subdued light or using low-actinic glassware.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 3.7 g/L of ammonium acetate in water

Solution A: Acetonitrile and *Buffer* (30:70)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	60	40
18	60	40
18.1	100	0
25	100	0

Diluent: 0.1% triethylamine in methanol
System suitability solution: 1.0 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS in *Diluent*
Standard solution: 0.05 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.05 mg/mL of promethazine hydrochloride from a volume of Oral Solution in *Diluent*. Centrifuge for 10 min and use the supernatant.
[NOTE—Sonication may be used in the preparation of the *System suitability solution*, *Standard solution*, and *Sample solution*.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification* test *B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 4°
Column: 30°
Flow rate: 1.4 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for promethazine and promethazine related compound B are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 5.0 between promethazine and promethazine related compound B peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

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IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1.0 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS in *Diluent*

Sample solution: Nominally 500 µg/mL of promethazine hydrochloride from a volume of Oral Solution in *Diluent*. Centrifuge for 10 min and use the supernatant. [NOTE—Sonication may be used in the preparation of the *Standard solution* and the *Sample solution*.]

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between promethazine and promethazine related compound B peaks

Relative standard deviation: NMT 5.0% for promethazine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of promethazine from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine sulfoxide[a]	0.3	0.26	1.0
Desmethyl promethazine[b]	0.6	1.0	0.2
Promethazine	1.0	—	—
Promethazine related compound B[c]	1.3	—	—
Phenothiazine[d]	1.5	2.2	0.2
Any individual unspecified degradation product	—	1.0	0.2

[a] *N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.

[b] *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.

[c] This is a process impurity which is controlled in the drug substance and is included in the table for identification only.

[d] 10*H*-Phenothiazine.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Promethazine Hydrochloride RS

USP Promethazine Related Compound B RS

Isopromethazine hydrochloride;
N,N-Dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine hydrochloride.
$C_{17}H_{20}N_2S \cdot HCl$ 320.88

Promethazine Hydrochloride Suppositories

» Promethazine Hydrochloride Suppositories contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{20}N_2S \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a cold place.

USP Reference standards ⟨11⟩—

USP Promethazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—Transfer a number of Suppositories, equivalent to about 50 mg of promethazine hydrochloride, to a 250-mL separator. Add 75 mL of solvent hexane and 25 mL of 0.01 N hydrochloric acid; shake to dissolve the solids. Using the aqueous phase, filtered through paper if necessary, proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator."

Assay—

Palladium chloride solution—Add 5 mL of hydrochloric acid to a beaker containing 500 mg of palladium chloride. Warm on a steam bath to obtain a complete solution. Slowly add 200 mL of hot water. (If necessary, continue warming and add additional hydrochloric acid to maintain complete solution.) Transfer the solution to a 500-mL volumetric flask, and dilute with water to volume. To 50 mL of this solution add 250 mL of 2 M sodium acetate, and adjust with hydrochloric acid to a pH of 4.0. Transfer the solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation—Using an accurately weighed quantity of USP Promethazine Hydrochloride RS, prepare a solution in 0.05 N hydrochloric acid containing about 0.1 mg in each mL. Protect the solution from light.

Assay preparation—Weigh 10 Suppositories and calculate the average. Carefully melt them, avoiding the use of excessive heat, and mix. Add 30 mL of hexanes to an accurately weighed portion, equivalent to about 50 mg of promethazine hydrochloride. Warm gently to dissolve, and transfer to a low-actinic separator. Rinse the transfer container with several small portions of the hexanes and 0.05 N hydrochloric acid, and add the rinsings to the separator. Extract with five 20-mL portions of 0.05 N hydrochloric acid shaking gently to avoid emulsions. Drain through glass wool prewashed with 0.05 N hydrochloric acid. Collect in a 500-mL low-actinic volumetric flask. Rinse the glass wool with additional 0.05 N hydrochloric acid, dilute the combined filtrate with 0.05 N hydrochloric acid to volume, and mix.

Procedure—Pipet 2.0 mL of the *Standard preparation*, *Assay preparation*, and 0.05 N hydrochloric acid into separate test tubes. Add 3.0 mL of *Palladium chloride solution* to each, and mix. Concomitantly determine the absorbances of the

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Standard preparation and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 450 nm, with a suitable spectrophotometer, using the hydrochloric acid-palladium chloride solution as the reagent blank in the reference cell. Calculate the quantity, in mg, of $C_{17}H_{20}N_2S \cdot HCl$ in the portion of Suppositories taken by the formula:

$$500C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Promethazine Hydrochloride RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Promethazine Hydrochloride Tablets

DEFINITION

Promethazine Hydrochloride Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).

[NOTE—Throughout the following procedures, protect the samples, the Reference Standards, and the solutions containing them, by conducting the procedures without delay under subdued light or using low-actinic glassware.]

IDENTIFICATION

• **A.**

Standard solution: In a separator, dissolve 50 mg of USP Promethazine Hydrochloride RS in 40 mL of dilute hydrochloric acid (1 in 1000).

Sample solution: Shake a quantity of powdered Tablets, equivalent to 50 mg of promethazine hydrochloride, with 30 mL of chloroform, and filter into a beaker. Evaporate the chloroform, dissolve the residue in 40 mL of dilute hydrochloric acid (1 in 1000), and transfer the liquid to a separator.

Analysis: Separately treat the *Sample solution* and the *Standard solution* as follows. Add 2 mL of 1 N sodium hydroxide and 15 mL of carbon disulfide to the separators, and shake for 2 min. Centrifuge if necessary to clarify the lower phase, and pass through a dry filter, collecting the filtrate from each separator in a small flask provided with a glass stopper. Reduce the volume of the carbon disulfide extracts to 4–5 mL, and proceed as directed in *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Determine the absorption spectra".

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Dissolve 8.2 mL of hydrochloric acid in 1000 mL of water.

Mobile phase: Acetonitrile, water, and triethylamine (850:270:1)

System suitability stock solution: 1.2 mg/mL of USP Promethazine Related Compound B RS in *Diluent*. Sonicate to dissolve.

Standard solution: 0.1 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*. Sonicate to dissolve.

System suitability solution: 0.09 mg/mL of USP Promethazine Hydrochloride RS and 0.12 mg/mL of USP Promethazine Related Compound B RS in *Diluent* from the *Standard solution* and *System suitability stock solution*, respectively

Sample stock solution: Nominally 2.5–5.0 mg/mL of promethazine hydrochloride prepared as follows. Transfer 20 Tablets to a volumetric flask of an appropriate size and add 50% of the flask volume of *Diluent*. Sonicate with swirling for NLT 20 min, or until the Tablets have fully disintegrated. Shake the flask for NLT 15 min and dilute with *Diluent* to volume.

Sample solution: Nominally 0.1 mg/mL of promethazine hydrochloride in *Diluent* from the *Sample stock solution*. Pass a portion through a filter of 0.45-µm pore size and use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2.5 mL/min

Injection volume: 20 µL

Run time: NLT 2.5 times the retention time of promethazine

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for promethazine related compound B and promethazine are 0.82 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between promethazine and promethazine related compound B, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: Prepare a solution with a known concentration of USP Promethazine Hydrochloride RS in *Medium*.

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: Absorption maximum at about 249 nm

Analysis

Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*

Tolerances: NLT 75% (Q) of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 15 min
Standard solution: (L/900) mg/mL of USP Promethazine Hydrochloride RS in *Medium*, where L is the label claim (mg/Tablet)
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: Absorption maximum at about 249 nm
Cell: 0.2 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Diluent: Methanol and triethylamine (999:1)
Buffer: 3.7 g/L of ammonium acetate in water
Solution A: *Buffer* and acetonitrile (700:300)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	60	40
18	60	40
18.1	100	0
25	100	0

System suitability stock solution: 0.5 mg/mL of USP Promethazine Related Compound B RS in *Diluent*
Standard stock solution: 0.5 mg/mL of USP Promethazine Hydrochloride RS in *Diluent*
System suitability solution: 5 µg/mL each of USP Promethazine Hydrochloride RS and USP Promethazine Related Compound B RS from the *Standard stock solution* and *System suitability stock solution*, respectively
Standard solution: 5 µg/mL of USP Promethazine Hydrochloride RS from the *Standard stock solution*
Sensitivity solution: 0.25 µg/mL of USP Promethazine Hydrochloride RS from the *Standard solution*
Sample solution: Nominally 0.5 mg/mL of promethazine hydrochloride from powdered Tablets (NLT 20) prepared as follows. Transfer a quantity of powdered Tablets, equivalent to 50 mg of promethazine hydrochloride, to a volumetric flask of appropriate size and add 75% of the flask volume of *Diluent*. Shake the flask for NLT 5 min and dilute with *Diluent* to volume. Pass a portion through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 234 and 249 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.4 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[Note—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between promethazine and promethazine related compound B, *System suitability solution*
Relative standard deviation: NMT 3.0% at 234 and 249 nm, *Standard solution*
Signal-to-noise ratio: NLT 10 at 234 and 249 nm, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of promethazine sulfoxide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of promethazine sulfoxide at 234 nm from the *Sample solution*
r_S = peak response of promethazine hydrochloride at 234 nm from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

USP Monographs

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Calculate the percentage of all other degradation products in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product at 249 nm from the *Sample solution*
r_S = peak response of promethazine hydrochloride at 249 nm from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine sulfoxide[a]	0.28	2.1	0.5
Desmethyl promethazine[b]	0.71	1.0	0.5
Promethazine	1.0	—	—
Promethazine related compound B[c]	1.3	—	—
Phenothiazine	1.7	2.0	0.5
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.0

[a] *N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[b] *N*-Methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine.
[c] This is a process impurity and is included for identification only. It is not to be reported and not to be included in the total degradation products.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Promethazine Hydrochloride RS
  USP Promethazine Related Compound B RS
  Isopromethazine hydrochloride;
  N,N-Dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine hydrochloride.
  $C_{17}H_{20}N_2S \cdot HCl$ 320.88

Promethazine and Phenylephrine Hydrochloride Oral Solution

DEFINITION

Promethazine and Phenylephrine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) and phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$). It may contain suitable preservatives.

IDENTIFICATION

- **A.** The retention times of the promethazine and phenylephrine peaks of the *Sample solutions* correspond to those of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- **▲B.** The UV absorption spectrum of both promethazine and phenylephrine peaks of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*. ▲ (USP 1-May-2019)

ASSAY

- **PROCEDURE**

Protect the sample and Standard solutions by using low-actinic glassware.

Solution A: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptane sulfonic acid sodium salt monohydrate in 950 mL of water, and carefully add 50 mL of acetonitrile. Adjust with phosphoric acid to an apparent pH of 3.0.

Solution B: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptane sulfonic acid sodium salt monohydrate in 500 mL of water, and add 500 mL of acetonitrile. Adjust with phosphoric acid to an apparent pH of 3.0.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1.0	100	0
50.0	0	100
50.1	100	0
55.0	100	0

Standard stock solution A: 1.8 mg/mL of USP Promethazine Hydrochloride RS and 1.4 mg/mL of USP Phenylephrine Hydrochloride RS in *Solution A*

Standard stock solution B: 1.4 mg/mL of USP Sodium Benzoate RS, 2.5 mg/mL of USP Methylparaben RS, and 0.28 mg/mL of USP Propylparaben RS. Prepare by dissolving appropriate quantities of USP Methylparaben RS, USP Propylparaben RS, and USP Sodium Benzoate RS into a volume of acetonitrile equivalent to 40% of the total volume of the flask and diluting with *Solution A* to volume.

Standard solution: 0.18 mg/mL of USP Promethazine Hydrochloride RS, 0.14 mg/mL of USP Phenylephrine Hydrochloride RS, 0.14 mg/mL of USP Sodium Benzoate RS, 0.25 mg/mL of USP Methylparaben RS, and 0.028 mg/mL of USP Propylparaben RS. Prepare by adding 5.0 mL each of *Standard stock solution A* and *Standard stock solution B* to a 50-mL volumetric flask and diluting with *Solution A* to volume.

Promethazine hydrochloride sample solution: Nominally equivalent to 0.18 mg/mL of promethazine hydrochloride in *Solution A*

Phenylephrine hydrochloride sample solution: Nominally equivalent to 0.14 mg/mL of phenylephrine hydrochloride in *Solution A*

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]
Suitability requirements
Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben
Relative standard deviation: NMT 2.0% for both promethazine and phenylephrine
Analysis
Samples: *Standard solution* and *Promethazine hydrochloride sample solution*
Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of promethazine from the *Promethazine hydrochloride sample solution*
r_S = peak response of promethazine from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Promethazine hydrochloride sample solution* (mg/mL)

Samples: *Standard solution* and *Phenylephrine hydrochloride sample solution*
Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenylephrine from the *Phenylephrine hydrochloride sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenylephrine hydrochloride in the *Phenylephrine hydrochloride sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% for both promethazine hydrochloride and phenylephrine hydrochloride

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 1**

Protect the sample and Standard solutions by using low-actinic glassware.

Solution C: Dissolve 4.36 g of dibasic potassium phosphate in 1 L of water, add 2.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 6.4.
5 N phosphoric acid: Dilute 12 mL of phosphoric acid with water to 100 mL.
Diluent: Methanol, water, and 5 N phosphoric acid (5:95:1)
Mobile phase: Acetonitrile, methanol, and *Solution C* (30:20:50)
Standard solution: 3.5 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*
Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Diluent* from the *Standard solution*. Prepare at time of use.
Promethazine hydrochloride sample solution: Nominally equivalent to 175 µg/mL of promethazine hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: ▲NLT▲ (USP 1-May-2019) 2 times the retention time of promethazine for Standards; ▲NLT▲ (USP 1-May-2019) 6 times the retention time of promethazine for samples
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 1.5 for promethazine, *Standard solution*
Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*
Signal-to-noise ratio: NLT 10 for promethazine, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Promethazine hydrochloride sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Promethazine hydrochloride sample solution*
r_S = peak response of promethazine hydrochloride from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Promethazine hydrochloride sample solution* (µg/mL)
F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine	1.0	1.0	—
Phenothiazine	2.4	2.0	0.5
Methylphenothiazine[a]	4.2	2.0	0.5

▲[a] 10-Methyl-10*H*-phenothiazine.▲ (USP 1-May-2019)

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Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 2**

Protect the sample and Standard solutions by using low-actinic glassware.

Solution A, Solution B, Mobile phase, Standard stock solution B, and **Phenylephrine hydrochloride sample solution:** Prepare as directed in the *Assay*.

Standard stock solution A: 88 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*

Standard solution: 8.8 µg/mL of USP Promethazine Hydrochloride RS, 140 µg/mL of USP Sodium Benzoate RS, 250 µg/mL of USP Methylparaben RS, and 28 µg/mL of USP Propylparaben RS. Prepare by adding 5.0 mL of *Standard stock solution A* and 5.0 mL of *Standard stock solution B* into a 50-mL volumetric flask, and dilute with *Solution A* to volume.

Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Solution A* from *Standard stock solution A*. Prepare at time of use.

Promethazine hydrochloride sample solution: Prepare as directed in the *Assay*. Use this solution to determine the promethazine related compounds and specified and unknown impurities.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]

Suitability requirements

Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben, *Standard solution*

Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*

Signal-to-noise ratio: NLT 10 for promethazine, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Phenylephrine hydrochloride sample solution*

Identify the phenylephrine impurities using the relative retention times given in *Table 3*.

Calculate the percentage of each phenylephrine impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Phenylephrine hydrochloride sample solution*

r_S = peak response of promethazine hydrochloride from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of phenylephrine hydrochloride in the *Phenylephrine hydrochloride sample solution* (µg/mL)

F = relative response factor for each impurity (see *Table 3*)

Samples: *Standard solution* and *Promethazine hydrochloride sample solution*

Using the data in *Table 4*, calculate the percentage of promethazine specified and unidentified impurities and any unspecified impurities in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Promethazine hydrochloride sample solution*

r_S = peak response of promethazine hydrochloride from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Promethazine hydrochloride sample solution* (µg/mL)

F = relative response factor for each impurity (see *Table 4*)

Acceptance criteria

Individual impurities: See *Table 2*, *Table 3*, and *Table 4*.

Total impurities: NMT 16.0%. Sum of the total phenylephrine impurities from *Table 3* and total promethazine impurities from *Table 4*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Unidentified impurity 1	0.32	1.4	0.6
Unidentified impurity 2	0.33	1.4	0.5
3-Hydroxyindole dione[a]	0.34	1.4	2.6
Norphenylephrine[b]	0.37	1.4	3.5
7-Hydroxyindole trione[c]	0.38	1.4	3.5
Phenylephrine	0.40	—	—
Unidentified impurity 3	0.42	1.4	0.2
Unidentified impurity 4	0.43	1.4	0.5
4-Hydroxyindole trione and pyrocatecholyl phenylephrine[d]	0.44	1.4	0.6
Unidentified impurity 5	0.45	1.4	0.5
Unidentified impurity 6	0.46	1.4	0.5
Benzyl phenylephrine[e]	0.69	1.0	0.5
Benzyladrianone[f]	0.73	—	—
Promethazine	1.0	—	—
Any other unspecified impurity	—	1.0	0.2
Total phenylephrine impurities	—	—	10.0

[a] 3-Hydroxy-1-methyl-2,3-dihydro-1*H*-indole-5,6-dione.

[b] (*R*)-3-(2-Amino-1-hydroxyethyl)phenol.

[c] 7-Hydroxy-1-methyl-1*H*-indole-3,5,6(2*H*)-trione.

[d] 4-Hydroxy-1-methyl-1*H*-indole-3,5,6(2*H*)-trione, and two ▲(*R*)-▲ (USP 1-May-2019) 3-{[2-hydroxy-2-(3-hydroxyphenyl)ethyl](methyl)amino}benzene-1,2-diol isomers.

[e] (*R*)-3-{2-[Benzyl(methyl)amino]-1-hydroxyethyl}phenol.

[f] Phenylephrine synthetic impurity. Do not quantify.

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Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenothiazine sulfoxide[a]	0.71	1.0	0.5
Promethazine sulfoxide[b]	0.72	3.3	2.8
Unidentified impurity 1	0.77	1.0	0.2
Methylphenothiazine sulfoxide[c]	0.81	1.0	0.5
Unidentified impurity 2	0.85	1.0	0.2
Phenothiazinone[d]	0.98	10.0	0.2
Desmethyl promethazine[e]	0.99	1.0	0.2
Promethazine	1.0	—	—
Isopromethazine[f]	1.01	—	—
Unidentified impurity 3	1.09	1.0	0.2
Unidentified impurity 4	1.16	1.0	0.2
Any unspecified impurity	—	1.0	0.2
Total promethazine impurities	—	—	5.0[g]

[a] 10*H*-Phenothiazine sulfoxide.
[b] *N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[c] 10-Methyl-10*H*-phenothiazine sulfoxide.
[d] 1*H*-Phenothiazin-1-one.
[e] *N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[f] Promethazine synthetic impurity. Do not quantify.
[g] Includes phenothiazine and methylphenothiazine from *Procedure 1*.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial limit does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 10 cfu/mL. It meets the requirements for absence of *Escherichia coli*.
- **pH** ⟨791⟩: 3.7–4.7
- **ALCOHOL DETERMINATION** ⟨611⟩, *Procedures, Method II—Gas Chromatographic Method* (if present): 90.0%–110.0% of the labeled quantity of alcohol (C_2H_5OH).
- **DELIVERABLE VOLUME** ⟨698⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methylparaben RS
  USP Phenylephrine Hydrochloride RS
  USP Promethazine Hydrochloride RS
  USP Propylparaben RS
  USP Sodium Benzoate RS

Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution

DEFINITION

Promethazine and Phenylephrine Hydrochloride and Codeine Phosphate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$), phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$), and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$). It may contain suitable preservatives.

IDENTIFICATION

- **A.** The retention times of the promethazine hydrochloride, phenylephrine hydrochloride, and codeine phosphate peaks from the *Sample solutions* correspond to those from the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

At all times, protect the sample and the Standard solutions by using low-actinic glassware.

Solution A: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptanesulfonic acid sodium salt monohydrate in 950 mL of water, and carefully add 50 mL of acetonitrile. Adjust with phosphoric acid to an apparent pH of 3.0.

Solution B: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of heptanesulfonic acid sodium salt monohydrate in 500 mL of water, and carefully add 500 mL of acetonitrile. Adjust with phosphoric acid to an apparent pH of 3.0.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1.0	100	0
50.0	0	100
50.1	100	0
55.0	100	0

Standard stock solution A: 1.8 mg/mL of USP Promethazine Hydrochloride RS, 1.4 mg/mL of USP Phenylephrine Hydrochloride RS, and 2.8 mg/mL of USP Codeine Phosphate RS in *Solution A*

Standard stock solution B: 1.4 mg/mL of USP Sodium Benzoate RS, 2.5 mg/mL of USP Methylparaben RS, and 0.28 mg/mL of USP Propylparaben RS. Prepare by dissolving appropriate quantities of USP Methylparaben RS, USP Propylparaben RS, and USP Sodium Benzoate RS in a volume of acetonitrile equivalent to 40% of the total volume of the flask and diluting with *Solution A* to volume.

Standard solution: 0.18 mg/mL of USP Promethazine Hydrochloride RS, 0.14 mg/mL of USP Phenylephrine Hydrochloride RS, 0.28 mg/mL of USP Codeine Phosphate RS, 0.14 mg/mL of USP Sodium Benzoate RS, 0.25 mg/mL of USP Methylparaben RS, and 0.028 mg/mL of USP Propylparaben RS. Prepare by adding 5.0 mL each of *Standard stock solution A* and *Standard stock solution B* to a 50-mL volumetric flask and diluting with *Solution A* to volume.

Promethazine hydrochloride sample solution: Equivalent to 0.18 mg/mL, based on the label claim, of promethazine hydrochloride in *Solution A*

Phenylephrine hydrochloride sample solution: Equivalent to 0.14 mg/mL, based on the label claim, of phenylephrine hydrochloride in *Solution A*

Codeine phosphate sample solution: Equivalent to 0.28 mg/mL, based on the label claim, of codeine phosphate in *Solution A*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

USP Monographs

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Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]
Suitability requirements
Resolution: NLT 4.5 between benzoate and methylparaben; NLT 1.5 between promethazine and propylparaben
Relative standard deviation: NMT 2.0% for promethazine, phenylephrine, and codeine
Analysis
Samples: *Standard solution* and *Promethazine hydrochloride sample solution*
Calculate the percentage of the labeled amount of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = promethazine peak response from the *Promethazine hydrochloride sample solution*
r_S = promethazine peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Promethazine hydrochloride sample solution* (mg/mL)

Samples: *Standard solution* and *Phenylephrine hydrochloride sample solution*
Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = phenylephrine peak response from the *Phenylephrine hydrochloride sample solution*
r_S = phenylephrine peak response from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenylephrine hydrochloride in the *Phenylephrine hydrochloride sample solution* (mg/mL)

Samples: *Standard solution* and *Codeine phosphate sample solution*
Calculate the percentage of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = codeine peak response from the *Codeine phosphate sample solution*
r_S = codeine peak response from the *Standard solution*
C_S = concentration of USP Codeine Phosphate RS, on the dried basis, in the *Standard solution* (mg/mL)
C_U = nominal concentration of codeine phosphate in the *Codeine phosphate sample solution* (mg/mL)
M_{r1} = molecular weight of codeine phosphate, 406.37
M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0% for promethazine hydrochloride, phenylephrine hydrochloride, and codeine phosphate

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1**
At all times, protect the sample and the Standard solutions by using low-actinic glassware.
Solution C: Dissolve 4.36 g of dibasic potassium phosphate in 1 L of water, add 2.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 6.4.
5 N phosphoric acid: Dilute 12 mL of phosphoric acid with water to 100 mL.
Diluent: Methanol, water, and *5 N phosphoric acid* (5:95:1)
Mobile phase: Acetonitrile, methanol, and *Solution C* (30:20:50)
Standard solution: 3.5 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*
Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Diluent*, prepared from *Standard solution*. Prepare at time of use.
Sample solution: Equivalent to 175 µg/mL of promethazine hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of promethazine, *Standard solution*; 6 times the retention time of promethazine, *Sample solution*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 1.5 for promethazine, *Standard solution*
Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*
Signal-to-noise ratio: NLT 10 for promethazine, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

r_U = impurity peak response from the *Sample solution*
r_S = promethazine hydrochloride peak response from the *Standard solution*
C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of promethazine hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

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Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Promethazine	1.0	1.0	—
Phenothiazine	2.4	2.0	0.5
Methylphenothiazine[a]	4.2	2.0	0.5

[a] 10-Methylphenothiazine.

• **ORGANIC IMPURITIES, PROCEDURE 2**

At all times, protect the sample and the Standard solutions by using low-actinic glassware.

Solution A, Solution B, Mobile phase, Standard stock solution B, Codeine phosphate sample solution, and **Phenylephrine hydrochloride sample solution:** Proceed as directed in the *Assay*.

Standard stock solution A: 88 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*

Standard solution: 8.8 µg/mL of USP Promethazine Hydrochloride RS, 140 µg/mL of USP Sodium Benzoate RS, 250 µg/mL of USP Methylparaben RS, and 28 µg/mL of USP Propylparaben RS. Prepare by adding 5.0 mL of *Standard stock solution A* and 5.0 mL of *Standard stock solution B* to a 50-mL volumetric flask and diluting with *Solution A* to volume.

Sensitivity solution: 0.18 µg/mL of USP Promethazine Hydrochloride RS in *Solution A*, prepared from *Standard stock solution A*. Prepare at time of use.

Promethazine hydrochloride sample solution: Proceed as directed in the *Assay*. Use this solution to determine promethazine related compounds and specified and unknown impurities.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution* and *Sensitivity solution*

[NOTE—The relative retention times for benzoate, methylparaben, and propylparaben are 0.59, 0.64, and 1.05, respectively.]

Suitability requirements

Resolution: NLT 4.5 between benzoate and methylparaben, and NLT 1.5 between promethazine and propylparaben, *Standard solution*

Relative standard deviation: NMT 7.5% for promethazine, *Standard solution*

Signal-to-noise ratio: NLT 10 for promethazine, *Sensitivity solution*

Analysis

Samples: *Phenylephrine hydrochloride sample solution* and *Standard solution*

Identify the phenylephrine impurities using the relative retention times listed in *Table 3*. Calculate the percentage of each phenylephrine impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

r_U = peak response of each impurity from the *Sample solution*

r_S = promethazine hydrochloride peak response from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of phenylephrine hydrochloride in the *Phenylephrine hydrochloride sample solution* (µg/mL)

F = relative response factor for each impurity (see *Table 3*)

Samples: *Standard solution* and *Promethazine hydrochloride sample solution*

Using the data in *Table 4*, calculate the percentage of each promethazine impurity, unspecified impurity, and unidentified impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

r_U = peak response of each impurity from the *Sample solution*

r_S = promethazine hydrochloride peak response from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of promethazine hydrochloride in the *Promethazine hydrochloride sample solution* (µg/mL)

F = relative response factor for each impurity (see *Table 4*)

Samples: *Codeine phosphate sample solution* and *Standard solution*

Identify the codeine impurities using the relative retention times given in *Table 4*. Calculate the percentage of each codeine impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

r_U = peak response of each impurity from the *Sample solution*

r_S = promethazine hydrochloride peak response from the *Standard solution*

C_S = concentration of USP Promethazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of codeine phosphate in the *Codeine phosphate sample solution* (µg/mL)

F = relative response factor for each codeine impurity (see *Table 4*)

Acceptance criteria: See *Table 3* and *Table 4*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Unidentified impurity 1	0.32	1.4	0.6
Unidentified impurity 2	0.33	1.4	0.5
3-Hydroxyindole dione[a]	0.34	1.4	2.6
Norphenylephrine[b]	0.37	1.4	3.5
7-Hydroxyindole trione[c]	0.38	1.4	3.5
Phenylephrine	0.40	—	—
Unidentified impurity 3	0.42	1.4	0.2
Unidentified impurity 4	0.43	1.4	0.5

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Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Hydroxyindole trione and pyrocatecholyl phenylephrine[d]	0.44	1.4	0.6
Unidentified impurity 5	0.45	1.4	0.5
Unidentified impurity 6	0.46	1.4	0.5
Benzyl phenylephrine[e]	0.69	1.0	0.5
Benzyladrianone[f]	0.73	—	—
Promethazine	1.0	—	—
Any other unspecified impurity	—	1.0	0.2
Total phenylephrine impurities	—	—	10.0

[a] 3-Hydroxy-1-methyl-2,3-dihydro-1*H*-indole-5,6-dione.
[b] (*R*)-3-(2-Amino-1-hydroxyethyl)phenol.
[c] 7-Hydroxy-1-methyl-1*H*-indole-3,5,6(2*H*)-trione.
[d] 4-Hydroxy-1-methyl-1*H*-indole-3,5,6(2*H*)-trione, and two 3-{[2-hydroxy-2-(3-hydroxyphenyl)ethyl](methyl)amino}benzene-1,2-diol isomers.
[e] (*R*)-3-(2-[Benzyl(methyl)amino]-1-hydroxyethyl}phenol.
[f] Phenylephrine synthetic impurity. Do not quantify.

Table 4

Name	Source	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Codeine *N*-oxide[a]	Codeine	0.52	0.83	0.2
Norcodeine[b]	Codeine	0.53	0.83	0.2
Codeine	—	0.54	—	—
Codeinone[c]	Codeine	0.61	0.63	0.3
Codeine methyl ether[d]	Codeine	0.67	—	—
Total codeine impurities	Codeine	—	—	1.0
Phenothiazine sulfoxide[e]	Promethazine	0.71	1.0	0.5
Promethazine sulfoxide[f]	Promethazine	0.72	3.3	2.8
Unidentified impurity 1	Promethazine	0.77	1.0	0.2
Methylphenothiazine sulfoxide[g]	Promethazine	0.81	1.0	0.5
Unidentified impurity 2	Promethazine	0.85	1.0	0.2
Phenothiazinone[h]	Promethazine	0.98	10.0	0.2
Desmethyl promethazine[i]	Promethazine	0.99	1.0	0.2
Promethazine	Promethazine	1.0	—	—
Isopromethazine[j]	Promethazine	1.01	—	—
Unidentified impurity 3	Promethazine	1.09	1.0	0.2
Unidentified impurity 4	Promethazine	1.16	1.0	0.2
Any unspecified impurity	—	—	1.0	0.2
Total promethazine impurities	Promethazine	—	—	5.0[k]

Table 4 *(continued)*

Name	Source	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	Codeine, Promethazine, and Phenylephrine	—	—	17.0

[a] Morphinan-6-ol, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl,17-oxide-(5α,6α).
[b] Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-(5α,6α).
[c] Morphinan-6-one, 7-8-didehydro-4,5-epoxy-3-methoxy-17-methyl-(5α).
[d] Codeine synthetic impurity. Do not quantitate.
[e] 10*H*-Phenothiazine sulfoxide.
[f] *N*,*N*-Dimethyl-1(10*H*-phenothiazin-10-yl)propan-2-amine sulfoxide.
[g] 10-Methyl-10*H*-phenothiazine sulfoxide.
[h] 1*H*-Phenothiazin-1-one.
[i] *N*-Methyl-1(10*H*-phenothiazin-10-yl)propan-2-amine.
[j] Promethazine synthetic impurity. Do not quantify.
[k] Includes phenothiazine and methylphenothiazine from *Procedure 1*.

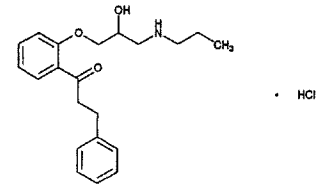
SPECIFIC TESTS

- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 10^1 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.
- **pH** ⟨791⟩: 3.7–4.7
- **Alcohol Determination,** *Method II* ⟨611⟩ (if present): 90.0%–110.0% of the labeled amount of C_2H_5OH
- **Deliverable Volume** ⟨698⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Codeine Phosphate RS
  USP Methylparaben RS
  USP Phenylephrine Hydrochloride RS
  USP Promethazine Hydrochloride RS
  USP Propylparaben RS
  USP Sodium Benzoate RS

Propafenone Hydrochloride



$C_{21}H_{27}NO_3 \cdot HCl$ 377.90

1-Propanone, 1-[2-[2-hydroxy-3-(propylamino)propoxy] phenyl]-3-phenyl-, hydrochloride;
2′-[2-Hydroxy-3-(propylamino)propoxy]-3-phenylpropiophenone hydrochloride [34183-22-7].

DEFINITION

Propafenone Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{21}H_{27}NO_3 \cdot HCl$, calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** Dissolve 0.5 g of Propafenone Hydrochloride in 50 mL of water with heating. Adjust with 0.1 N sodium hydroxide to a pH of 9.5–10.0: a precipitate is formed. Cool the mixture, and filter. Add 1 mL of 6 N nitric acid and 2–3 drops of 0.1 N silver nitrate to the filtrate: a precipitate is formed, which dissolves upon the addition of a few drops of ammonium hydroxide.

ASSAY

- **PROCEDURE**

Sample solution: Transfer 250 mg of Propafenone Hydrochloride to a 125-mL flask. Dissolve in 30 mL of methanol, and add 15 mL of mercuric acetate TS.

Analysis: Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 37.79 mg of $C_{21}H_{27}NO_3 \cdot HCl$.

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

- **Procedure**

Solvent A: 3.42 g/L of dipotassium hydrogen phosphate trihydrate. Adjust with phosphoric acid to a pH of 2.5.

Solvent B: Acetonitrile

Mobile phase: See the gradient table below.

Time (min)	Solvent A (%)	Solvent B (%)
0	65	35
8	65	35
20	30	70
30	30	70
31	65	35
36	65	35

Diluent: *Solvent A* and *Solvent B* (13:7)

System suitability solution: 0.1 mg/mL of USP Propafenone Hydrochloride RS and USP Propafenone Related Compound B RS in *Diluent*

Standard solution: 0.001 mg/mL of USP Propafenone Hydrochloride RS in *Diluent*

Sample solution: 1 mg/mL of Propafenone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection size: 20 µL

Run time: NLT six times the retention time of propafenone

Suitability requirements

Resolution: NLT 3 between propafenone and propafenone related compound B

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Propafenone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of propafenone from the *Standard solution*

C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution*

C_U = concentration of Propafenone Hydrochloride in the *Sample solution*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.3%. [NOTE—Disregard any peak below 0.03%.]

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Propafenone related compound B[a]	0.8	0.1
Impurity D[b]	2.3	0.1
Impurity G[c]	3.6	0.1
Impurity C[d]	4.1	0.1
Impurity F[e]	5.3	0.1
Individual unspecified impurities	—	0.1

[a] (2*E*)-1-[2-[(2*RS*)-2-Hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylprop-2-en-1-one.

[b] 1-[2-[(2*RS*)-2,3-Dihydroxypropoxy]phenyl]-3-phenylpropan-1-one.

[c] 1,1′[Propyliminobis(2-hydroxypropane-3,1-diyl)oxy-2,1-phenylene]bis(3-phenylpropan-1-one.

[d] 1-[2-[[(2*RS*)-Oxiranyl]methoxy]phenyl]-3-phenylpropan-1-one.

[e] 1,1′-[2-Hydroxypropane-1,3-diylbis(oxy-2,1-phenylene)]bis(3-phenylpropan-1-one).

SPECIFIC TESTS

- **CLARITY OF SOLUTION:** Dissolve 1.0 g in 30 mL of hot water, and observe without delay: the solution initially is clear.
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 171°–175°
- **pH** ⟨791⟩: 5.0–6.2, in a solution (1 in 200)
- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at a temperature between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Propafenone Hydrochloride RS

USP Propafenone Related Compound B RS

(*RS*,*E*)-1-[2-[2-Hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylprop-2-en-1-one.

$C_{21}H_{25}NO_3$ 339.43

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Propafenone Hydrochloride Extended-Release Capsules

DEFINITION

Propafenone Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 1.36 g/L of monobasic potassium phosphate in water, and adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Mobile phase: Methanol and *Buffer* (50:50)
Diluent: 50% methanol in water
Standard solution: 0.1 mg/mL of USP Propafenone Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 1 mg/mL of propafenone hydrochloride prepared as follows. Transfer a suitable amount of finely powdered contents from NLT 20 Capsules to an appropriate volumetric flask. Add about 60% of the final volume of *Diluent*, and sonicate with occasional swirling until the contents are completely disintegrated. Dilute with *Diluent* to volume and pass through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally 0.1 mg/mL of propafenone hydrochloride in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of propafenone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propafenone from the *Sample solution*
r_S = peak response of propafenone from the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of propafenone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1
Acid stage
Medium: 0.08 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 1 h
Diluent: 6.8 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 6.8.
Standard solution: (L/1000) mg/mL of USP Propafenone Hydrochloride RS in *Diluent*, where L is the label claim in mg/Capsule
Sample solution: At the specified time point, withdraw about 10 mL of the solution and pass through a suitable filter of 0.45-µm pore size. Discard at least the first 4 mL of the filtrate. Analyze the *Sample solution* immediately.
Instrumental conditions
Mode: UV
Analytical wavelengths: 305 and 375 nm
Cell: 0.2 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Measure and subtract the absorbance at 375 nm from the absorbance at 305 nm to obtain the absorbances for the *Sample solution* and *Standard solution*.
Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved:

$$\text{Result}_1 = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: See *Table 1*.
Buffer stage
Proceed as directed in the *Acid stage*, except for the following parameters.
Buffer: Dissolve 108.88 g of monobasic potassium phosphate in water, add 14.4 g of sodium hydroxide, mix to dissolve, and dilute with water to 1 L. Adjust with 2 N sodium hydroxide to a pH of 6.8.
Solution A: *Buffer* and 2 N sodium hydroxide (64:36)
Medium: At 2 h of dissolution time, add 100 mL of *Solution A*, preheated at 37°, to 900 mL of 0.08 N hydrochloric acid.
Times: 4 and 12 h
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

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Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved at each time point (*i*):

$$Result_2 = C_2 \times V \times (1/L) \times 100$$
$$Result_3 = \{[C_3 \times (V - V_S)] + (C_2 \times V_S)\} \times (1/L) \times 100$$

C_i = concentration of propafenone hydrochloride in the portion of sample withdrawn at time point (*i*) (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Capsule)
V_S = volume of *Medium* taken (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	5–25
2	4	40–70
3	12	NLT 75

The percentages of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Acid stage

Acid stage medium: 0.08 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm, with sinkers
Time: 1 h
Standard stock solution: 0.42 mg/mL of USP Propafenone Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Propafenone Hydrochloride RS to a suitable volumetric flask. Add methanol, NMT 10% of the final volume, and sonicate to dissolve. Dilute with *Acid stage medium* to volume.
Standard solution: 0.021 mg/mL of USP Propafenone Hydrochloride RS in *Acid stage medium* from the *Standard stock solution*
Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size. Dilute with *Acid stage medium* to a concentration similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 305 nm
Cell: 1 cm
Blank: *Acid stage medium*
Analysis
After 1 h in the *Acid stage medium* and the collection of the *Sample solution*, replace the portion of solution withdrawn with an equal volume of *Acid stage medium*. Continue for an additional 1 h in *Acid stage medium*.
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved:

$$Result_1 = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor (mL/mL)
V = volume of *Acid stage medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 2*.

Buffer stage

Proceed as directed in the *Acid stage*, except for the following parameters.
Buffer stage medium: After 2 h in the *Acid stage*, add 100 mL of phosphate buffer (68 g of monobasic potassium phosphate and 42 g of sodium hydroxide in 1000 mL of water), preheated at 37°, to 900 mL of *Acid stage medium*; 1000 mL.
Times: 6 and 15 h
Standard stock solution: 0.48 mg/mL of USP Propafenone Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Propafenone Hydrochloride RS to a suitable volumetric flask. Add methanol, NMT 10% of the final volume, and sonicate to dissolve. Dilute with *Buffer stage medium* to volume.
Standard solution: 0.048 mg/mL of USP Propafenone Hydrochloride RS in *Buffer stage medium* from *Standard stock solution*
Sample solution: Withdraw a 10-mL aliquot at each time point. Pass the solution through a suitable filter of 0.45-µm pore size. Dilute with *Buffer stage medium* to a concentration similar to that of the *Standard solution*.
Blank: *Buffer stage medium*
Instrumental conditions: See *Acid stage*.
Analysis
At the specified time points, replace the portion of solution withdrawn with 10 mL of *Buffer stage medium*.
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$Result_i = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor (mL/mL)

Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved at each time point (*i*):

$$Result_2 = \{[C_2 \times V] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$Result_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of propafenone hydrochloride in the portion of sample withdrawn at time point (*i*) (mg/mL)
V = volume of *Buffer stage medium*, 1000 mL
V_S = volume of the *Sample solution* withdrawn from the *Buffer stage medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 2*.

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Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	5–25
2	6	45–65
3	15	NLT 80

The percentages of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Acid stage

Acid stage medium: 0.08 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm, with sinkers

Time: 2 h

Phosphate buffer: Dissolve 190.06 g of tribasic sodium phosphate in 1 L of water.

Diluent: *Phosphate buffer* and *Acid stage medium* (12:88). Adjust the pH to 6.8 with phosphoric acid or sodium hydroxide if necessary.

Standard solution: (L/1000) mg/mL of USP Propafenone Hydrochloride RS, where L is the label claim in mg/Capsule, prepared as follows. Transfer a suitable amount of USP Propafenone Hydrochloride RS to a suitable volumetric flask. Dissolve with 10% of final volume of methanol with aid of sonication. Dilute with *Diluent* to volume.

Sample solution: At the specified time point, withdraw 10 mL of the solution under test and centrifuge. Use the supernatant.

Instrumental conditions

Mode: UV

Analytical wavelengths: 305 nm

Cell: 0.2 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved:

$$\text{Result}_1 = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Acid stage medium*, 900 mL

Tolerances: See *Table 3*.

Buffer stage

Proceed as directed in the *Acid stage*, except for the following parameters.

Buffer stage medium: After the samples are withdrawn at 2 h, add 110 mL or appropriate amount of *Phosphate buffer*, preheated at 37°, to *Acid stage medium*, and adjust the pH to 6.8 with phosphoric acid or sodium hydroxide if necessary; 1000 mL.

Times: 4 and 12 h

Sample solution: At the specified time points, withdraw 10 mL of the solution and centrifuge. Use the supernatant.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of propafenone hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Buffer stage medium*, 1000 mL
V_S = volume of *Sample solution* withdrawn (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 30
2	4	35–60
3	12	NLT 80

The percentages of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 4:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.

Acid stage

Acid stage medium: 0.08 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 2 h

Diluent: Dissolve 6.8 g of monobasic potassium phosphate with 1 L of water. Adjust with sodium hydroxide to a pH of 6.8.

Standard solution: 0.325 mg/mL of USP Propafenone Hydrochloride RS in *Diluent*

[NOTE—Sonication may be needed for dissolution.]

Sample solution: At the specified time point, withdraw 5 mL of the solution and pass through a suitable filter. Replace the portion of solution withdrawn with an equal volume of *Medium*.

Instrumental conditions

Mode: UV

Analytical wavelengths: 305 and 375 nm

Cell: 0.2 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

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Measure and subtract the absorbance at 375 nm from the absorbance at 305 nm to obtain the absorbances for the *Sample solution* and *Standard solution*.
Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved:

$$Result_i = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: See *Table 4*.

Buffer stage
Proceed as directed in the *Acid stage*, except for the following parameters.
Buffer: Dissolve 108.88 g of monobasic potassium phosphate and 14.4 g of sodium hydroxide with 1 L of water. Adjust with 2 N sodium hydroxide to a pH of 6.8.
Solution A: *Buffer* and 2 N sodium hydroxide (64:36)
Medium: At 2 h of dissolution time, add 100 mL of *Solution A*, preheated at 37°, to the vessel containing 900 mL of 0.08 N hydrochloric acid.
Times: 3, 6, and 12 h
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$Result_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved at each time point (i):

$$Result_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$Result_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$Result_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of propafenone hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Medium*, 1000 mL
V_S = volume of *Medium* taken, 5 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	15–35
2	3	26–46
3	6	56–76
4	12	NLT 80

The percentages of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*. ▲(RB 8-Mar-2019)

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Keep all solutions containing propafenone hydrochloride in amber glassware.
Solution A: 0.015 M dibasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.5 ± 0.2.
Solution B: Acetonitrile
Mobile phase: See *Table* ▲*5*. ▲(RB 8-Mar-2019)

Table ▲**5**▲ (RB 8-Mar-2019)

Time (min)	Solution A (%)	Solution B (%)
0	65	35
8	65	35
20	30	70
30	30	70
31	65	35
36	65	35

Diluent: 50% methanol in water
System suitability solution: 0.1 mg/mL each of USP Propafenone Hydrochloride RS and USP Propafenone Related Compound B RS in *Diluent*
Standard solution: 2.0 µg/mL of USP Propafenone Hydrochloride RS in *Diluent*. Sonicate if necessary.
Sensitivity solution: 0.3 µg/mL of USP Propafenone Hydrochloride RS in *Diluent* from the *Standard solution*
Sample solution: Nominally 1 mg/mL of propafenone hydrochloride, prepared as follows. Transfer a suitable amount of finely powdered contents from NLT 20 Capsules to an appropriate volumetric flask. Add about 40% of the final volume of *Diluent* and sonicate for about 15 min. Dilute with *Diluent* to volume and pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 3.0 between propafenone related compound B and propafenone, *System suitability solution*

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Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of propafenone from the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of propafenone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table* ▲6▲ (RB 8-Mar-2019) Disregard any peaks below 0.03% (peak area less than that of the *Sensitivity solution*).

Table ▲6▲ (RB 8-Mar-2019)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Propafenone related compound B[a,b]	0.81	—
Propafenone	1.00	—
Propafenone glycerol analog[a,c]	2.53	—
Flavone[a,d]	2.83	—
Propafenone dimer[a,e]	2.88	—
Propafenone chloroglycerol analog[a,f]	2.91	—
Propafenone glycidyl analog[a,g]	2.96	—
Propafenone phenol[a,h]	3.29	—
Propafenone glycerol dimer[a,i]	3.80	—
Any unspecified degradation product	—	0.15
Total degradation products	—	0.50

[a] Process impurities; do not include in total degradation products.
[b] (*RS,E*)-1-(2-[2-Hydroxy-3-(propylamino)propoxy]phenyl}-3-phenylprop-2-en-1-one.
[c] 1-[2-[(2*RS*)-2,3-Dihydroxypropoxy]phenyl]-3-phenylpropan-1-one.
[d] 2-Phenylchroman-4-one.
[e] 1,1′-[Propyliminobis(2-hydroxypropane-3,1-diyl)oxy-2,1-phenylene]bis(3-phenylpropan-1-one).
[f] 1-[2-(3-Chloro-2-hydroxypropoxy)phenyl]-3-phenylpropan-1-one.
[g] 1-[2-[[(*RS*)-Oxiranyl]methoxy]phenyl]-3-phenylpropan-1-one.
[h] 1-(2-Hydroxyphenyl)-3-phenylpropan-1-one.
[i] 1,1′-(2,2′-(2-Hydroxypropane-1,3-diyl)bis(oxy)bis(2,1-phenylene))bis(3-phenylpropan-1-one).

Change to read:

- **CONTENT OF PROPAFENONE RELATED COMPOUND A**

Buffer: Dissolve 3.4 g of dibasic potassium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 2.5 ± 0.05.
Solution A: Methanol and *Buffer* (45:55); pass through a suitable filter of 0.2-µm pore size.
Solution B: Methanol and *Buffer* (75:25); pass through a suitable filter of 0.2-µm pore size.
Mobile phase: See *Table* ▲7▲ (RB 8-Mar-2019)

Table ▲7▲ (RB 8-Mar-2019)

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4.0	100	0
7.0	50	50
10.0	0	100
12.0	0	100
12.5	100	0
15.0	100	0

Diluent: Methanol and water (80:20)
Standard solution: 2.0 µg/mL of USP Propafenone Related Compound A RS in *Diluent*
Sensitivity solution: 0.2 µg/mL of USP Propafenone Related Compound A RS in *Diluent* from the *Standard solution*
Sample solution: Nominally 1 mg/mL of propafenone hydrochloride prepared as follows. Transfer a suitable amount of finely powdered contents from NLT 20 Capsules to an appropriate volumetric flask. Add about 75% of the final volume of *Diluent* and sonicate with intermittent shaking for 20 min. Dilute with *Diluent* to volume and pass through a suitable filter of 0.45-µm pore size. Discard the first 4 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 60°
Flow rate: 0.4 mL/min
Injection volume: 4 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 6.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of propafenone related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propafenone related compound A from the *Sample solution*
r_S = peak response of propafenone related compound A from the *Standard solution*
C_S = concentration of USP Propafenone Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of propafenone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table* ▲8.▲ (RB 8-Mar-2019)

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Table ▲8▲ (RB 8-Mar-2019)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Propafenone	1.0	—
Propafenone related compound A[a]	1.9	0.20

[a] *N*-{2-Hydroxy-3-[2-(3-phenylpropanoyl)phenoxy]propyl}-*N*-propylformamide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Keep in tight containers and store at controlled room temperature.
- **LABELING:** When more than one test for *Dissolution* is given, the *Labeling* section states the test for *Dissolution* used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Propafenone Hydrochloride RS
  USP Propafenone Related Compound A RS
  N-{2-Hydroxy-3-[2-(3-phenylpropanoyl)phenoxy]propyl}-*N*-propylformamide.
  $C_{22}H_{27}NO_4$ 369.45
  USP Propafenone Related Compound B RS
  (*RS,E*)-1-{2-[2-Hydroxy-3-(propylamino)propoxy]phenyl}-3-phenylprop-2-en-1-one.
  $C_{21}H_{25}NO_3$ 339.43

Propafenone Hydrochloride Tablets

DEFINITION

Propafenone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A.** The ▲▲ 2S (USP41) retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ 2S (USP41)

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 0.1 M monobasic potassium phosphate prepared as follows. Dissolve 13.6 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.1, and then dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (32:68)

Standard solution: 0.15 mg/mL of USP Propafenone Hydrochloride RS in *Mobile phase*

Sample stock solution: Nominally 1.5 mg/mL of propafenone hydrochloride ▲▲ 2S (USP41) in *Mobile phase* prepared as follows. ▲Transfer an appropriate quantity of propafenone hydrochloride from NLT 20 powdered Tablets to a suitable volumetric flask, and ▲ 2S (USP41) add *Mobile phase* to ▲▲ 2S (USP41) 90% of the total volume. Sonicate for 10 min. Cool to room temperature, and dilute with *Mobile phase* to volume. Stir for 10 min, pass through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Sample solution: Nominally 0.15 mg/mL of propafenone hydrochloride from *Sample stock solution* in *Mobile phase*. Pass through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm. ▲ 2S (USP41)
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 45°
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of the propafenone peak

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propafenone from the *Sample solution*
r_S = peak response of propafenone from the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of propafenone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 30 min

Standard solution: 0.17 mg/mL of USP Propafenone Hydrochloride RS in 0.1 N hydrochloric acid prepared as follows. To a suitable quantity of USP Propafenone Hydrochloride RS in a suitable volumetric flask, add 0.1 N hydrochloric acid to fill 75% of the total volume. Place the flask in a water bath at 37° with stirring until completely dissolved. Remove the flask from the water bath, cool to room temperature, and dilute with 0.1 N hydrochloric acid to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Instrumental conditions
Mode: UV
Analytical wavelength: 250 nm

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) dissolved:

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$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of propafenone from the *Sample solution*
A_S = absorbance of propafenone from the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim for propafenone hydrochloride (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$) is dissolved.

Change to read:

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements▲ ▲2S (USP41)

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Solution A: 2.61 g/L of dipotassium hydrogen phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	71	29
8	71	29
20	29	71
30	29	71
31	71	29
36	71	29

Diluent: *Solution A* and *Solution B* (65:35)
System suitability solution: 0.1 mg/mL each of USP Propafenone Hydrochloride RS and USP Propafenone Related Compound B RS in *Diluent*
▲Standard stock solution: 0.05 mg/mL each of USP Propafenone Hydrochloride RS and USP Propafenone Related Compound B RS in *Diluent*▲ 2S (USP41)
Standard solution: 1 µg/mL each of USP Propafenone Hydrochloride RS and USP Propafenone Related Compound B RS ▲from *Standard stock solution*▲ 2S (USP41) in *Diluent*
Sample solution: Nominally 1 mg/mL of propafenone hydrochloride in *Diluent* prepared ▲as follows. Transfer an appropriate quantity of propafenone hydrochloride from NLT 20 powdered Tablets to a suitable volumetric flask, and▲ 2S (USP41) add *Diluent* to ▲ ▲2S (USP41) 90% of the total volume, sonicate for 10 min, and cool to room temperature. Dilute with *Diluent* to volume, and stir for 10 min. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
▲[NOTE—See *Table 2* for relative retention times.]▲ 2S (USP41)
Suitability requirements
Resolution: NLT 3.0 between propafenone and propafenone related compound B, *System suitability solution*
Tailing factor: NMT 2.0 for the propafenone peak, *Standard solution*
Relative standard deviation: NMT 5.0% for the propafenone ▲and propafenone related compound B peaks,▲ 2S (USP41) *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of propafenone related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propafenone related compound B from the *Sample solution*
r_S = peak response of propafenone related compound B from the *Standard solution*
C_S = concentration of USP Propafenone Related Compound B RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of propafenone hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of dealkyl propafenone and any ▲unspecified degradation product▲ 2S (USP41) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dealkyl propafenone or any ▲unspecified degradation product▲ 2S (USP41) from the *Sample solution*
r_S = peak response of propafenone from the *Standard solution*
C_S = concentration of USP Propafenone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of propafenone hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dealkyl propafenone[a]	0.53	0.1
Propafenone related compound B ▲ ▲2S (USP41)	0.78	0.2
Propafenone	1.00	—
Any ▲unspecified degradation product▲ 2S (USP41)	—	0.2
Total impurities▲ ▲2S (USP41)	—	0.4

[a] 2′-(2-Hydroxy-3-aminopropoxy)-3-phenylpropiophenone.
▲ ▲2S (USP41)

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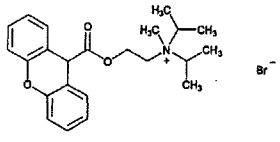
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store at controlled room temperature in tightly closed containers at a relative humidity below 60%.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Propafenone Hydrochloride RS
  USP Propafenone Related Compound B RS
  ▲(*RS,E*)-1-[2-[2-Hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylprop-2-en-1-one. ▲ 25 (USP41)
  $C_{21}H_{25}NO_3$ 339.43

Propantheline Bromide



$C_{23}H_{30}BrNO_3$ 448.39

2-Propanaminium, *N*-methyl-*N*-(1-methylethyl)-*N*-[2-[(9*H*-xanthen-9-ylcarbonyl)oxy]ethyl]-, bromide; (2-Hydroxyethyl)diisopropylmethylammonium bromide xanthene-9-carboxylate [50-34-0].

DEFINITION

Propantheline Bromide contains NLT 98.0% and NMT 102.0% of propantheline bromide ($C_{23}H_{30}BrNO_3$), calculated on the dried basis.

IDENTIFICATION

- **A.**
  Sample solution: 6 mg/mL of Propantheline Bromide in chloroform
  Analysis: In a well-ventilated hood, apply 2 mL of *Sample solution* dropwise to a salt plate while continuously evaporating the solvent with the aid of an IR heat lamp and a current of dry air. Heat the residue at 105° for 15 min.
  Acceptance criteria: The IR absorption spectrum of the residue on the single salt plate exhibits maxima only at the same wavelengths as those of a similar preparation of USP Propantheline Bromide RS, treated in the same manner.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
  Standard solution: 6 mg/mL USP Propantheline Bromide RS in chloroform
  Sample solution: 6 mg/mL of Propantheline Bromide in chloroform
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel
  Application volume: 5 µL
  Developing solvent system: Acetone and 1 N hydrochloric acid (1:1)
  Spray reagent: Potassium–bismuth iodide TS
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Develop the chromatogram until the solvent front has moved three-fourths the length of the plate. Dry the plate at 105° for 5 min. Spray the plate with *Spray reagent*, and heat at 105° for 5 min.
  Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Bromide* ⟨191⟩
  Sample solution: 10 mg/mL
  Analysis: Add 2 mL of 2 N nitric acid to 5 mL of *Sample solution*.
  Acceptance criteria: This solution meets the requirements of the tests for bromide, except that in the test *A*, the chloroform layer may be yellow.

ASSAY

- **PROCEDURE**
  Sample solution: 600 mg
  Analysis: Dissolve the *Sample* in a mixture of 20 mL of glacial acetic acid and 15 mL of mercuric acetate TS, warming slightly if necessary to dissolve. Cool to room temperature. Titrate the *Sample solution* with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 44.84 mg of propantheline bromide ($C_{23}H_{30}BrNO_3$).
  Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Buffer: 17.3 g of sodium dodecyl sulfate in 1000 mL of water containing 10 mL of phosphoric acid in a 2000-mL volumetric flask. Add 250 mL of 0.5 M sodium hydroxide, and while stirring, adjust with 0.5 M sodium hydroxide or dilute phosphoric acid (1 in 10) to a pH of 3.5 ± 0.05. Dilute with water to volume.
  Mobile phase: Acetonitrile and *Buffer* (55:45)
  Standard solution: 6.0 µg/mL of USP Propantheline Bromide Related Compound A RS, 1.5 µg/mL of USP Xanthanoic Acid RS, and 1.5 µg/mL of USP Xanthone RS in *Mobile phase*
  Sample solution: 0.3 mg/mL of Propantheline Bromide in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; packing L7
  Flow rate: 2.0 mL/min
  Run time: NLT 1.5 times the retention time of the propantheline bromide peak
  Injection volume: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 1.2 between the least resolved peaks
  Relative standard deviation: NMT 6.0% for each component
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of xanthanoic acid, xanthone, and propantheline bromide related compound A in the portion of Propantheline Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of xanthanoic acid, xanthone, or propantheline bromide related compound A from the *Sample solution*

r_S = peak response of xanthanoic acid, xanthone, or propantheline bromide related compound A from the *Standard solution*

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C_S = concentration of xanthanoic acid, xanthone, or propantheline bromide related compound A in the *Standard solution* (µg/mL)
C_U = concentration of Propantheline Bromide in the *Sample solution* (µg/mL)

Calculate the percentage of all unknown impurities in the portion of Propantheline Bromide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each unknown impurity
r_T = sum of the responses of all the measured peaks

Acceptance criteria: Disregard any peak less than 0.1%.
Individual impurities: NMT 2.0% for propantheline bromide related compound A; NMT 0.5% each for xanthone and xanthanoic acid
Total impurities: NMT 3.0%

SPECIFIC TESTS
• **CONTENT OF BROMIDE**
Sample solution: 500 mg
Analysis: Dissolve the *Sample* in 40 mL of water, and add 10 mL of glacial acetic acid and 40 mL of methanol. Titrate the *Sample solution* with 0.1 N silver nitrate VS, using eosin Y TS as an indicator. Each mL of 0.1 N silver nitrate is equivalent to 7.990 mg of bromide.
Acceptance criteria: 17.5%–18.2% of bromide, calculated on the dried basis
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Propantheline Bromide RS
USP Propantheline Bromide Related Compound A RS
9-Hydroxypropantheline bromide.
$C_{23}H_{30}BrNO_4$ 464.39
USP Xanthanoic Acid RS
$C_{14}H_{10}O_3$ 226.23
USP Xanthone RS
$C_{13}H_8O_2$ 196.21

Propantheline Bromide Tablets

DEFINITION
Propantheline Bromide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of propantheline bromide ($C_{23}H_{30}BrNO_3$).

IDENTIFICATION
• **A.**
Standard solution: 6 mg/mL of USP Propantheline Bromide RS in chloroform
Sample solution: Triturate the equivalent to 90 mg of propantheline bromide, from finely powdered Tablets, with 10 mL of chloroform. Filter, and wash the filter with 10 mL of chloroform, collecting the filtrate and washing in a separator. Add 10 mL of water, shake, and discard the chloroform layer. Wash the aqueous layer with two 10-mL portions of ether, and discard the ether washings. Filter the aqueous solution, and evaporate on a steam bath with the aid of a current of dry air to dryness. Dissolve the residue in 5 mL of chloroform.
Analysis: In a well-ventilated hood, apply 2 mL each of the *Standard solution* and *Sample solution* dropwise to separate salt plates while continuously evaporating the solvent with the aid of an IR heat lamp and a current of dry air. Heat the residues at 105° for 15 min.
Acceptance criteria: The IR absorption spectrum of the residue on the single salt plate exhibits maxima only at the same wavelengths as those of a similar preparation of USP Propantheline Bromide RS, treated in the same manner.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 17.3 g of sodium dodecyl sulfate in 1000 mL of water containing 10 mL of phosphoric acid in a 2000-mL volumetric flask. Add 250 mL of 0.5 M sodium hydroxide, and while stirring, adjust with 0.5 M sodium hydroxide or dilute phosphoric acid (1 in 10) to a pH of 3.5 ± 0.05. Dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (55:45)
Standard solution: 0.3 mg/mL of USP Propantheline Bromide RS in *Mobile phase*
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder equivalent to 15 mg of propantheline bromide to a 50-mL volumetric flask, and dissolve in *Mobile phase*. Dilute with *Mobile phase* to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of propantheline bromide ($C_{23}H_{30}BrNO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Propantheline Bromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of propantheline bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: pH 4.5 ± 0.05 acetate buffer prepared by mixing 1.64 g of anhydrous sodium acetate and 1.25 mL of glacial acetic acid with 500 mL of water, and diluting with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: USP Propantheline Bromide RS at a known concentration in *Medium*

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Sample solution: Filtered portion of solution under test
Buffer, Mobile phase, Chromatographic system, and System suitability: Prepare as directed in the *Assay*.
Analysis: Determine the amount of propantheline bromide ($C_{23}H_{30}BrNO_3$) dissolved by proceeding as directed in the *Assay*.
Tolerances: NLT 75% (*Q*) of the labeled amount of propantheline bromide ($C_{23}H_{30}BrNO_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Buffer: 17.3 g of sodium dodecyl sulfate in 1000 mL of water containing 10 mL of phosphoric acid in a 2000-mL volumetric flask. Add 250 mL of 0.5 M sodium hydroxide, and while stirring, adjust with 0.5 M sodium hydroxide or dilute phosphoric acid (1 in 10) to a pH of 3.5 ± 0.05. Dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (55:45)
Standard solution: 12.0 µg/mL of USP Propantheline Bromide Related Compound A RS, 3.0 µg/mL of USP Xanthanoic Acid RS, and 3.0 µg/mL of USP Xanthone RS in *Mobile phase*
Sample solution: Prepare as directed in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between the least resolved peaks
Relative standard deviation: NMT 6.0% for each component
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of xanthanoic acid, xanthone, and propantheline bromide related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

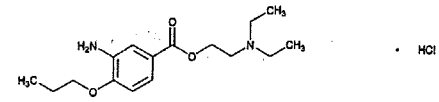
r_U = peak response for xanthanoic acid, xanthone, or propantheline bromide related compound A from the *Sample solution*
r_S = peak response for xanthanoic acid, xanthone, or propantheline bromide related compound A from the *Standard solution*
C_S = concentration of xanthanoic acid, xanthone, or propantheline bromide related compound A in the *Standard solution* (µg/mL)
C_U = nominal concentration of propantheline bromide in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 4.0% of propantheline bromide related compound A; NMT 1.0% each of xanthone and xanthanoic acid. Disregard any peak less than 0.1%.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Propantheline Bromide RS
USP Propantheline Bromide Related Compound A RS
9-Hydroxypropantheline bromide.
$C_{23}H_{30}BrNO_4$ 464.39
USP Xanthanoic Acid RS
$C_{14}H_{10}O_3$ 226.23
USP Xanthone RS
$C_{13}H_8O_2$ 196.21

Proparacaine Hydrochloride



$C_{16}H_{26}N_2O_3 \cdot HCl$ 330.85
Benzoic acid, 3-amino-4-propoxy-, 2-(diethylamino)ethyl ester, monohydrochloride;
2-(Diethylamino)ethyl 3-amino-4-propoxybenzoate monohydrochloride [5875-06-9].

DEFINITION

Proparacaine Hydrochloride contains NLT 97.0% and NMT 103.0% of proparacaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

• **A. Identification—Organic Nitrogenous Bases** ⟨181⟩: Meets the requirements
• **B. Ultraviolet Absorption**
Buffer: Proceed as directed for pH 6.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*). Dilute with water to prepare a 10% solution.
Analytical wavelength: 310 nm
Sample stock solution: 0.2 mg/mL of Proparacaine Hydrochloride
Sample solution: 0.02 mg/mL of Proparacaine Hydrochloride prepared as follows. To a volume of the *Sample stock solution* equivalent to 10% of the flask volume, add 2% of the flask volume of *Buffer*, and dilute with water to volume.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of a similarly prepared solution of USP Proparacaine Hydrochloride RS, concomitantly measured, and the respective absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
• **C. Identification Tests—General,** *Chloride* ⟨191⟩
Sample solution: 20 mg/mL of Proparacaine Hydrochloride
Analysis: Proceed as directed in the chapter using the procedure for test *B*.
Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**
Sample solution: Transfer 250 mg of Proparacaine Hydrochloride to a 250-mL conical flask. Add 80 mL of a 1-in-20 solution of acetic anhydride in glacial acetic acid, and heat on a steam bath for 10 min. Cool to room temperature, and add 10 mL of mercuric acetate TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Visual
Analysis: Titrate with *Titrant* to a blue-green endpoint using 1 or 2 drops of crystal violet TS as indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.09 mg of proparacaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$).
Acceptance criteria: 97.0%–103.0% on the dried basis

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IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.15%
- **ORDINARY IMPURITIES** ⟨466⟩
  Standard solution and **Test solution:** Methanol
  Eluant: Butyl alcohol, glacial acetic acid, and water (5:1:3)
  Visualization: 1; 17
  Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 178°–185°, but the range between beginning and end of melting does not exceed 2°.
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Proparacaine Hydrochloride RS

Proparacaine Hydrochloride Ophthalmic Solution

DEFINITION
Proparacaine Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Proparacaine Hydrochloride. It contains NLT 95.0% and NMT 110.0% of the labeled amount of proparacaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$).

IDENTIFICATION
- **A.**
  Solution A: Dilute hydrochloric acid (1 in 100)
  Solution B: 100 mg/mL of sodium nitrite
  Solution C: 20 mg/mL of 2-naphthol in 1 N sodium hydroxide
  Sample solution: 1 mL of Ophthalmic Solution
  Analysis 1: To the *Sample solution* in a test tube add 5 mL of *Solution A*, mix, and cool in an ice bath for 2 min. Add 2 drops of *Solution B*, stir, and cool again for 2 min. Add 1 mL of *Solution C*.
  Acceptance criteria 1: A scarlet-red precipitate is formed.
  Analysis 2: Add 5 mL of acetone to the test tube contents of *Analysis 1*.
  Acceptance criteria 2: The precipitate does not dissolve.

ASSAY
- **PROCEDURE**
  Buffer: 6.8 g/L of monobasic potassium phosphate. Add 5 mL of triethylamine, and adjust with 5 N potassium hydroxide to a pH of 7.5. Pass through a filter of 0.5-µm or finer pore size, and degas.
  Mobile phase: Acetonitrile and *Buffer* (40:60)
  Standard stock solution: 1 mg/mL of USP Proparacaine Hydrochloride RS
  Standard solution: 0.1 mg/mL of USP Proparacaine Hydrochloride RS in *Mobile phase* from the *Standard stock solution*. Use this solution within 6 h.
  Sample solution: Nominally equivalent to 0.1 mg/mL of proparacaine hydrochloride from a measured volume of Ophthalmic Solution in *Mobile phase*. Use this solution within 6 h.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 270 nm
  Column: 4.6-mm × 15-cm; 5-µm spherical packing L10
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 3000 theoretical plates
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of proparacaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

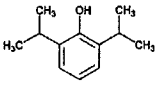
r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Proparacaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of proparacaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 3.5–6.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label it to indicate that it is to be stored in a refrigerator after the container is opened.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Proparacaine Hydrochloride RS

Propofol



$C_{12}H_{18}O$ 178.27
Phenol, 2,6-bis(1-methylethyl);
2,6-Diisopropylphenol [2078-54-8].

DEFINITION
Propofol contains NLT 98.0% and NMT 102.0% of propofol ($C_{12}H_{18}O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE 1**
  [NOTE—This is to be performed in conjunction with *Organic Impurities, Procedure 1*.]
  Standard solution: 10 mg/mL of USP Propofol RS in methanol
  Sample solution: 10 mg/mL of Propofol in methanol
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)

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Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m; coated with a 1.2-µm phase G16
Temperatures
Detector: 300°
Injection port: 250°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
145	—	145	20
145	5	200	5

Carrier gas: Helium
Flow rate: 8 mL/min
Injection volume: 1.0 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 1.5% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of propofol ($C_{12}H_{18}O$) in the portion of Propofol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Propofol RS in the Standard solution (mg/mL)
C_U = concentration of Propofol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

• **PROCEDURE 2**
[NOTE—This is to be performed in conjunction with *Organic Impurities, Procedure 2*.]
Mobile phase: Hexane, acetonitrile, and alcohol (990: 7.5: 1)
Standard solution: 2.4 mg/mL of USP Propofol RS in hexane
Sample solution: 2.4 mg/mL of Propofol in hexane
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 20-cm; 5-µm packing L3
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of propofol ($C_{12}H_{18}O$) in the portion of Propofol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Propofol RS in the *Standard solution* (mg/mL)
C_U = concentration of Propofol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1**
[NOTE—On the basis of knowledge of the manufacturing process, either (1) *Organic Impurities, Procedure 1* is performed in conjunction with *Limit of Propofol Related Compound A, Limit of Propofol Related Compound B, Procedure 1*, and *Assay, Procedure 1*; or (2) *Organic Impurities, Procedure 2* is performed in conjunction with *Limit of Propofol Related Compound B, Procedure 2* and the *Assay, Procedure 2*.]
System suitability solution: 100 mg/mL of USP Propofol Resolution Mixture RS in methanol
Standard solution: 0.1 mg/mL of USP Propofol RS in methanol
Sample solution: 100 mg/mL of Propofol in methanol
Chromatographic system: Proceed as directed in the *Assay, Procedure 1*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2 between propofol and 2-isopropyl-6-*n*-propylphenol, *System suitability solution*
Column efficiency: NLT 5000 theoretical plates, *Standard solution*
Relative standard deviation: NMT 3.5% for six replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Propofol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity in the *Sample solution*
r_S = peak response for propofol in the *Standard solution*
C_S = concentration of USP Propofol RS in the *Standard solution* (mg/mL)
C_U = concentration of Propofol in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Propofol related compound C[a]	0.18	0.1
Propofol	1.0	—
2-Isopropyl-6-*n*-propylphenol	1.1	0.1
Any other individual impurity	—	0.1
Total impurities	—	0.3

[a] 2,6-Diisopropylphenyl isopropyl ether.

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• **ORGANIC IMPURITIES, PROCEDURE 2**
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay, Procedure 2*.
System suitability solution: 0.1 µL/mL of USP Propofol RS and 0.3 µL/mL of USP Propofol Related Compound B RS in hexane
Peak identification solution: 0.25 mg/mL of USP Propofol Related Compound A RS, 100 µL/mL of the Propofol that is under test, and 5 µL/mL of USP Propofol Related Compound C RS in hexane
Sample solution: 100 mg/mL of Propofol in hexane
Reference solution: 0.1 mg/mL of Propofol in hexane from the *Sample solution*
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between propofol related compound B and propofol
Analysis
Samples: *Sample solution* and *Reference solution*
Calculate the percentage of each impurity in the portion of Propofol taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times D \times 100$$

r_U = peak response of each impurity in the *Sample solution*
r_S = peak response of propofol in the *Reference solution*
F = relative response factor (see *Table 3*)
D = dilution factor used to prepare the *Reference solution*, 0.001

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Propofol related compound C[a]	0.5	0.2	0.2
Propofol related compound B[b]	0.8	1.0	0.05
Propofol	1.0	—	—
Propofol related compound A[c]	5.0	4.0	0.01
Any other individual impurity	—	1.0	0.05
Total impurities	—	—	0.3

[a] 2,6-Diisopropylphenyl isopropyl ether.
[b] 2,6-Diisopropylbenzoquinone.
[c] 3,3′-5,5′-Tetraisopropyldiphenol.

• **LIMIT OF PROPOFOL RELATED COMPOUND A**
[NOTE—This test is to be performed in conjunction with *Organic Impurities, Procedure 1*.]
Mobile phase: Acetonitrile, methanol, and water (50:10:40)
Standard solution: 20 µg/mL of USP Propofol Related Compound A RS in methanol
Sample solution: 20 mg/mL of Propofol in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 6000 theoretical plates
Relative standard deviation: NMT 15% for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of propofol related compound A in the portion of Propofol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propofol related compound A from the *Sample solution*
r_S = peak response of propofol related compound A from the *Standard solution*
C_S = concentration of USP Propofol Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Propofol in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1% of propofol related compound A

• **LIMIT OF PROPOFOL RELATED COMPOUND B, PROCEDURE 1**
[NOTE—This is to be performed in conjunction with *Organic Impurities, Procedure 1*.]
Sample: Propofol
Instrumental conditions
Mode: UV
Analytical wavelength: 330 nm
Blank: Air
Analysis: Measure the absorbance of the *Sample* using air as the blank.
Acceptance criteria: NMT 0.1%; the absorbance of the *Sample* is NMT 0.4 absorbance units.

• **LIMIT OF PROPOFOL RELATED COMPOUND B, PROCEDURE 2**
[NOTE—This is to be performed in conjunction with *Organic Impurities, Procedure 2*.]
Mobile phase: Prepare as directed in the *Assay, Procedure 2*.
Standard solution: 5 µg/mL of USP Propofol Related Compound B RS in hexane
Sample solution: 50 mg/mL of Propofol in hexane
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 20-cm; 5-µm packing L3
Flow rate: 2 mL/min
Injection volume: 20 µL
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for propofol related compound B and propofol are about 0.8 and 1.0, respectively.]
Calculate the percentage of propofol related compound B in the portion of Propofol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propofol related compound B from the *Sample solution*
r_S = peak response of propofol related compound B from the *Standard solution*
C_S = concentration of USP Propofol Related Compound B RS in the *Standard solution* (mg/mL)

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C_U = concentration of Propofol in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.05% of propofol related compound B

SPECIFIC TESTS

• **REFRACTIVE INDEX** ⟨831⟩: 1.5125–1.5145 at 20°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers under an atmosphere of inert gas, and protect from light. Store at room temperature.

• **LABELING:** The labeling indicates the *Organic Impurities* procedure with which the article complies if a procedure other than *Organic Impurities, Procedure 1* is used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Propofol RS

USP Propofol Related Compound A RS
3,3′-5,5′-Tetraisopropyldiphenol.
$C_{24}H_{34}O_2$ 354.53

USP Propofol Related Compound B RS
2,6-Diisopropyl-1,4-benzoquinone.
$C_{12}H_{16}O_2$ 192.25

USP Propofol Related Compound C RS
2,6-Diisopropylphenyl isopropyl ether.
$C_{15}H_{24}O$ 220.35

USP Propofol Resolution Mixture RS
Propofol and 2-isopropyl-6-*n*-propylphenol.

Propofol Injectable Emulsion

DEFINITION

Propofol Injectable Emulsion contains Propofol in a 10% (w/v) oil-in-water sterile emulsion. The aqueous component contains glycerol, a suitable antimicrobial agent, and Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of propofol ($C_{12}H_{18}O$). It contains a suitable emulsifying agent.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Wavelength range: 200–450 nm
Standard solution: 100 µg/mL
Sample solution: Dilute a volume of Injectable Emulsion, equivalent to 10 mg of propofol, with isopropyl alcohol to 100 mL.
Medium: Isopropyl alcohol

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Diluent: Dilute 68 mL of water with isopropyl alcohol to 1 L.
Mobile phase: Tetrahydrofuran and water (4:6)
Standard solution: 0.8 mg/mL of USP Propofol RS in *Diluent*
Sample solution: Transfer a volume of well-shaken Injectable Emulsion, equivalent to about 40 mg of propofol, to a 50-mL volumetric flask. Dissolve in and dilute with isopropyl alcohol to volume, and mix. The solution contains about 0.8 mg/mL of propofol.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 5-mm × 10-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of propofol ($C_{12}H_{18}O$) in the portion of Injectable Emulsion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Propofol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of propofol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **LIMIT OF FREE FATTY ACIDS**
[NOTE—To obtain a titer of sufficient sensitivity for the blank reagent, it is necessary to add stearic acid at a concentration of approximately 1.0 mmol/L.]
Blank titration: Weigh about 142.3 mg of stearic acid into a 500-mL volumetric flask. Dissolve in and dilute with dehydrated alcohol to volume to obtain the blank solution. Pipet 10 mL of the blank solution into a 100-mL beaker, and proceed as directed in the *Analysis*, beginning with "Add 0.5 mL of 0.05 N hydrochloric acid".
Calculate the blank correction, *B*, in mmol/L:

$$\text{Result} = (NV_{TB}/V_B) - N_{SA}$$

N = concentration of the titrant (mmol/L)
V_{TB} = volume of the titrant added between the first and second inflection points for the *Blank titration* (mL)
V_B = volume of the blank solution used for the analysis (mL)
N_{SA} = concentration of stearic acid (mmol/L)

Analysis
Sample: A volume of Injectable Emulsion, nominally equivalent to 100 mg of propofol
Transfer the *Sample* to a 100-mL beaker. Add 0.5 mL of 0.05 N hydrochloric acid, then dilute with a solution of alcohol and water (45:20) to 60 mL. Titrate with 0.05 M sodium hydroxide VS to a potentiometric endpoint, using a suitable electrode.
Calculate the free fatty acid content, in mmol/L:

$$\text{Result} = (NV_T/V_S) - B$$

N = concentration of the titrant (mmol/L)
V_T = volume of the titrant added between the first and second inflection points (mL)
V_S = volume of Injectable Emulsion used for the analysis (mL)
B = blank correction factor (mmol/L), calculated as shown above

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Acceptance criteria: NMT 7 mmol/L

• **ORGANIC IMPURITIES**

Diluent, Mobile phase, and **Sample solution:** Proceed as directed in the *Assay*.

System suitability solution: Dissolve quantities of USP Propofol RS, USP Propofol Related Compound A RS, and USP Propofol Related Compound B RS in *Diluent* to obtain a solution with concentrations of 0.8 mg/mL of propofol, and 0.002 and 0.0008 mg/mL of propofol related compound A and propofol related compound B, respectively.

Standard solution: 0.002 mg/mL of USP Propofol Related Compound A RS and 0.0008 mg/mL of USP Propofol Related Compound B RS in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*, except that detection is at 254 nm.

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for propofol related compound B, propofol, and propofol related compound A are about 0.8, 1.0, and 2.5, respectively.]

Suitability requirements

Resolution: NLT 2.5 between propofol and propofol related compound B

Column efficiency: NLT 1000 theoretical plates based on the propofol peak

Tailing factor: NMT 1.5 for the propofol peak

Relative standard deviation: NMT 2% for the propofol peak

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the content of propofol related compound A and propofol related compound B, as a percentage of the label content of propofol, in the portion of Injectable Emulsion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propofol related compound A or propofol related compound B from the *Sample solution*

r_S = peak response of propofol related compound A or propofol related compound B from the *Standard solution*

C_S = concentration of USP Propofol Related Compound A RS or USP Propofol Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of propofol in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5% of propofol related compound A; NMT 0.5% of propofol related compound B

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.33 USP Endotoxin Units/mg of propofol.

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **PH** ⟨791⟩: 4.5–8.5

• **GLOBULE SIZE DISTRIBUTION IN LIPID INJECTABLE EMULSIONS** ⟨729⟩: Meets the requirements

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve under an inert atmosphere of nitrogen. Store at controlled room temperature. Do not freeze.

• **LABELING:** Label it to include the following: Shake well before use. Do not use if there is evidence of excessive creaming or aggregation, if large droplets are visible, or if there are other forms of phase separation indicating that the stability of the product has been compromised. Slight creaming, which should disappear after shaking, may be visible upon prolonged standing.

• **USP REFERENCE STANDARDS** ⟨11⟩

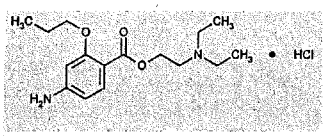
USP Propofol RS

USP Propofol Related Compound A RS
3,3′-5,5′-Tetraisopropyldiphenol.

USP Propofol Related Compound B RS
2,6-Diisopropyl-1,4-benzoquinone.

Delete the following:

▲Propoxycaine Hydrochloride



$C_{16}H_{26}N_2O_3 \cdot HCl$ 330.85

Benzoic acid, 4-amino-2-propoxy-, 2-(diethylamino)ethyl ester, monohydrochloride.

2-(Diethylamino)ethyl 4-amino-2-propoxybenzoate monohydrochloride [550-83-4].

» Propoxycaine Hydrochloride, dried at 105° for 3 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_{16}H_{26}N_2O_3 \cdot HCl$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨ 11 ⟩ —
USP Propoxycaine Hydrochloride RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 10 µg per mL.

Medium: water.

Absorptivities at 303 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: A solution (1 in 100) responds to the tests for *Chloride* ⟨191⟩.

Melting range, *Class I* ⟨ 741 ⟩ : between 146° and 151°.

Loss on drying ⟨ 731 ⟩ —Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨ 281 ⟩ : not more than 0.2%.

Chromatographic purity—

Solvent—Prepare a mixture of chloroform and methanol (9:1).

Standard preparations—Dissolve a quantity of USP Propoxycaine Hydrochloride RS in *Solvent*, and dilute quantitatively, and stepwise if necessary, with *Solvent* to obtain a solution containing 0.5 mg per mL. Dilute quantitatively with *Solvent* to obtain Standard preparations having the following compositions:

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Standard preparation	Dilution	Concentration (mg RS per mL)	Percentage (%, for comparison with test specimen)
A	1 in 2	0.25	0.5
B	1 in 5	0.10	0.2
C	1 in 10	0.05	0.1

Test preparation—Dissolve an accurately weighed quantity of Propoxycaine Hydrochloride in *Solvent* to obtain a solution containing 50 mg per mL.

Procedure—Apply separately 5 µL of the *Test preparation* and 5 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of chloroform, methanol, and isopropylamine (96:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate, with the aid of warm circulating air. Examine the plate under short-wavelength UV light, and again after exposing the plate to iodine vapors for a few minutes and spraying it with 7 N sulfuric acid. In each instance, compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations*: no secondary spot is more intense than the principal spot obtained from *Standard preparation A* (0.5%), and the sum of the intensities of all secondary spots obtained from the *Test preparation* does not exceed 1.0%.

Assay—Dissolve about 500 mg of Propoxycaine Hydrochloride, previously dried and accurately weighed, in 200 mL of ice-cold water containing 1 g of potassium bromide and 2.5 mL of hydrochloric acid, and titrate with 0.1 M sodium nitrite VS, using starch iodide paper as an external indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M sodium nitrite is equivalent to 33.09 mg of $C_{16}H_{26}N_2O_3 \cdot HCl$. ▲ (USP 1-May-2020)

Delete the following:

▲Propoxycaine and Procaine Hydrochlorides and Levonordefrin Injection

» Propoxycaine and Procaine Hydrochlorides and Levonordefrin Injection is a sterile solution of Propoxycaine Hydrochloride, Procaine Hydrochloride, and Levonordefrin in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of propoxycaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$), not less than 95.0 percent and not more than 105.0 percent of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levonordefrin ($C_9H_{13}NO_3$).

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.

Labeling—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards ⟨ 11 ⟩ —
USP Procaine Hydrochloride RS
USP Propoxycaine Hydrochloride RS

Color and clarity—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Mepivacaine Hydrochloride and Levonordefrin Injection*.

Identification—

A: Cool a mixture of 10 mL of Injection and 10 mL of 3 N hydrochloric acid in an ice bath to 0°, add 5 mL of sodium nitrite solution (1 in 5), stirring gently, and 2 mL of a solution of 0.1 g of 2-naphthol in 5 mL of 1 N sodium hydroxide, and observe immediately: a bright orange-red precipitate is formed (*presence of a primary aminophenyl group*).

B: It responds to the tests for *Identification Tests—General* ⟨191⟩, *Chloride*.

C: To about 25 mL of Injection add 1 drop of ferric chloride TS: the solution immediately turns a light green and changes to a light blue within 1 minute. Add 2 drops of 3 N hydrochloric acid: the color reverts to light green (*presence of levonordefrin*).

Bacterial Endotoxins Test ⟨ 85 ⟩ —It contains not more than 0.8 USP Endotoxin Unit per mg of propoxycaine hydrochloride.

pH ⟨ 791 ⟩ : between 3.5 and 5.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for propoxycaine and procaine hydrochlorides —

Standard propoxycaine hydrochloride preparation—Prepare, by quantitative and stepwise dilution, a solution in dilute hydrochloric acid (1 in 6) containing about 50 µg of USP Propoxycaine Hydrochloride RS, accurately weighed, in each mL.

Standard procaine hydrochloride preparation—Prepare, by quantitative and stepwise dilution, a solution in dilute hydrochloric acid (1 in 6) containing about 200 µg of USP Procaine Hydrochloride RS, accurately weighed, in each mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of procaine hydrochloride, to a 125-mL separator containing 25 mL of chloroform and 10 mL of water. Add 5 mL of sodium carbonate TS, shake vigorously, allow the layers to separate, and transfer the chloroform layer to a second separator. Extract the aqueous layer with two 10-mL portions of chloroform, adding the extracts to the second separator. Extract the combined chloroform extracts with 35 mL of dilute hydrochloric acid (1 in 6), transfer the chloroform to a third separator, and transfer the acid extract to a 100-mL volumetric flask. Extract the chloroform with two 10-mL portions of dilute hydrochloric acid (1 in 6), add the acid extracts to the volumetric flask, add dilute hydrochloric acid (1 in 6) to volume, and mix. Transfer 15.0 mL of this solution to a second 100-mL volumetric flask, add dilute hydrochloric acid (1 in 6) to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* in 1-cm cells at 296 and 272 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of

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propoxycaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$(0.667C/V)(A_{296}A_4 - A_{272}A_3)/(A_1A_4 - A_2A_3)$$

in which C is the concentration, in µg per mL, of USP Propoxycaine Hydrochloride RS in the *Standard propoxycaine hydrochloride preparation*.

Calculate the quantity, in mg, of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$) in each mL of the Injection taken by the formula:

$$(0.667C/V)(A_{272}A_1 - A_{296}A_2)/(A_1A_4 - A_2A_3)$$

in which C is the concentration, in µg per mL, of USP Procaine Hydrochloride RS in the *Standard procaine hydrochloride preparation*, V is the volume, in mL, of Injection taken, A_1 and A_2 are the absorbances of the *Standard propoxycaine hydrochloride preparation* at 296 and 272 nm, respectively, A_3 and A_4 are the absorbances of the *Standard procaine hydrochloride preparation* at 296 and 272 nm, respectively, and A_{296} and A_{272} are the absorbances of the *Assay preparation* at 296 and 272 nm, respectively.

Assay for levonordefrin—

Ferro-citrate solution, *Buffer solution*, and *Standard preparation*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Assay preparation—Proceed with Injection as directed for *Assay Preparation* under *Epinephrine Assay* ⟨391⟩, except to read "levonordefrin" where "epinephrine" is specified.

Procedure—Proceed as directed for *Procedure* under *Epinephrine Assay* ⟨391⟩. Calculate the quantity, in mg, of levonordefrin ($C_9H_{13}NO_3$) in each mL of the Injection taken by the formula:

$$(183.21/333.30)(0.05C/V)(A_U/A_S)$$

in which 183.21 and 333.30 are the molecular weights of levonordefrin and epinephrine bitartrate, respectively, C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*, and V is the volume, in mL, of Injection taken. ▲ (USP 1-May-2020)

Delete the following:

▲Propoxycaine and Procaine Hydrochlorides and Norepinephrine Bitartrate Injection

» Propoxycaine and Procaine Hydrochlorides and Norepinephrine Bitartrate Injection is a sterile solution of Propoxycaine Hydrochloride, Procaine Hydrochloride, and Norepinephrine Bitartrate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of propoxycaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$), not less than 95.0 percent and not more than 105.0 percent of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$), and an amount of norepinephrine bitartrate ($C_8H_{11}NO_3 \cdot C_4H_6O_6 \cdot H_2O$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norepinephrine ($C_8H_{11}NO_3$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

Labeling—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards ⟨ 11 ⟩ —
USP Norepinephrine Bitartrate RS
USP Procaine Hydrochloride RS
USP Propoxycaine Hydrochloride RS

Color and clarity—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Norepinephrine Bitartrate Injection*.

Identification—

A: Cool a mixture of 10 mL of Injection and 10 mL of 3 N hydrochloric acid in an ice bath to 0°, add 5 mL of sodium nitrite solution (1 in 5), stirring gently, and 2 mL of a solution of 0.1 g of 2-naphthol in 5 mL of 1 N sodium hydroxide, and observe immediately: a bright orange-red precipitate is formed (*presence of a primary aminophenyl group*).

B: It responds to the tests for *Identification Tests— General* ⟨191⟩, *Chloride*.

C: To about 5 mL of Injection add 1 drop of ferric chloride TS: a green color develops (*presence of norepinephrine bitartrate*).

Bacterial Endotoxins Test ⟨ 85 ⟩ —It contains not more than 0.8 USP Endotoxin Unit per mg of propoxycaine hydrochloride.

pH ⟨ 791 ⟩ : between 3.5 and 5.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for propoxycaine and procaine hydrochlorides —

Standard propoxycaine hydrochloride preparation—Prepare, by quantitative and stepwise dilution, a solution in 2 N hydrochloric acid containing about 50 µg of USP Propoxycaine Hydrochloride RS, accurately weighed, in each mL.

Standard procaine hydrochloride preparation—Prepare, by quantitative and stepwise dilution, a solution in 2 N hydrochloric acid containing about 200 µg of USP Procaine Hydrochloride RS, accurately weighed, in each mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of procaine hydrochloride, to a 125-mL separator containing 25 mL of chloroform and 10 mL of water. Add 5 mL of sodium carbonate TS, shake vigorously, allow the layers to separate, and transfer the chloroform layer to a second separator. Extract the aqueous layer with two 10-mL portions of chloroform, adding the extracts to the second separator. Extract the combined chloroform extracts with 35 mL of 2 N hydrochloric acid, transfer the chloroform to a third separator, and transfer the acid extract to a 100-mL volumetric flask. Extract the chloroform with two 10-mL portions of 2 N hydrochloric acid, add the acid extracts to the volumetric flask, dilute with 2 N hydrochloric acid to volume, and mix. Transfer 15.0 mL of this solution to a second 100-mL volumetric flask, dilute with 2 N hydrochloric acid to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation*, in 1-cm

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cells, at 296 nm and 272 nm, with a suitable spectrophotometer, using water as the blank.

Calculate the quantity, in mg, of propoxycaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$(0.667C/V)(A_{296}A_4 - A_{272}A_3)/(A_1A_4 - A_2A_3)$$

in which C is the concentration, in µg per mL, of USP Propoxycaine Hydrochloride RSin the *Standard propoxycaine hydrochloride preparation*, V is the volume, in mL, of Injection taken, A_1 and A_2 are the absorbances of the *Standard propoxycaine hydrochloride preparation* at 296 nm and 272 nm, respectively, A_3 and A_4 are the absorbances of the *Standard procaine hydrochloride preparation* at 296 nm and 272 nm, respectively, and A_{296} and A_{272} are the absorbances of the *Assay preparation* at 296 nm and 272 nm, respectively.

Calculate the quantity, in mg, of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$) in each mL of the Injection taken by the formula:

$$(0.667C/V)(A_{272}A_1 - A_{296}A_2)/(A_1A_4 - A_2A_3)$$

in which C is the concentration, in µg per mL, of USP Procaine Hydrochloride RS in the *Standard procaine hydrochloride preparation*, and V is the volume, in mL, of Injection taken.

Assay for norepinephrine—

Ferro-citrate solution, *Buffer solution*, and *Standard preparation*—Prepare as directed under *Epinephrine Assay* ⟨391⟩, except to read " USP Norepinephrine Bitartrate RS" for "USP Epinephrine Bitartrate RS" throughout.

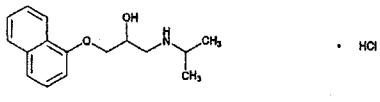
Assay preparation—Prepare as directed for *Assay Preparation* under *Epinephrine Assay* ⟨391⟩ except to read "norepinephrine" where "epinephrine" is specified.

Procedure—Proceed as directed for *Procedure* under *Epinephrine Assay* ⟨391⟩. Calculate the quantity, in mg, of norepinephrine ($C_8H_{11}NO_3$) in each mL of the Injection taken by the formula:

$$(169.18/337.28)(0.05C/V)(A_U/A_S)$$

in which 169.18 and 337.28 are the molecular weights of norepinephrine and norepinephrine bitartrate monohydrate, respectively; C is the concentration, in µg per mL, of USP Norepinephrine Bitartrate RS in the *Standard preparation*; and V is the volume, in mL, of Injection taken. ▲(USP 1-May-2020)

Propranolol Hydrochloride



$C_{16}H_{21}NO_2 \cdot HCl$ 295.80

2-Propanol, 1-[(1-methylethyl)amino]-3-(1-naphthalenyloxy)-, hydrochloride, (±)-; (±)-1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride [318-98-9].

DEFINITION

Change to read:

Propranolol Hydrochloride contains NLT 98.0% and NMT ▲102.0%▲ (USP 1-May-2020) of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

ASSAY

Change to read:

- **PROCEDURE**

▲Mobile phase: Mix 1.6 g of sodium dodecyl sulfate and 0.31 g of tetrabutylammonium phosphate in a mixture of 1 mL of sulfuric acid, 450 mL of water, and 550 mL of acetonitrile. Adjust with 2 N sodium hydroxide solution to a pH of 3.3.

Standard solution: 0.2 mg/mL of USP Propranolol Hydrochloride RS in *Mobile phase*. Sonication may be needed to aid dissolution.

Sample solution: 0.2 mg/mL of Propranolol Hydrochloride in *Mobile phase*. Sonication may be needed to aid dissolution.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 292 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.8 mL/min
Injection volume: 20 µL
Run time: NLT 7 times the retention time of propranolol

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$) in the portion of Propranolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propranolol from the *Sample solution*
r_S = peak response of propranolol from the *Standard solution*
C_S = concentration of USP Propranolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Propranolol Hydrochloride in the *Sample solution* (mg/mL)▲ (USP 1-May-2020)

Acceptance criteria: 98.0%–▲102.0%▲ (USP 1-May-2020) on the dried basis

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IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Add the following:

▲• **ORGANIC IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.002 mg/mL of USP Propranolol Related Compound A RS and 2 mg/mL of USP Propranolol Hydrochloride RS in *Mobile phase*

Standard solution: 0.002 mg/mL of USP Propranolol Hydrochloride RS in *Mobile phase*

Sensitivity solution: 0.001 mg/mL of USP Propranolol Hydrochloride RS in *Mobile phase* from *Standard solution*

Sample solution: 2 mg/mL of Propranolol Hydrochloride in *Mobile phase*. Sonication may be needed to aid dissolution.

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—The relative retention times for the impurities are listed in *Table 1*.]

Suitability requirements

Resolution: NLT 3.0 between propranolol and propranolol related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified and unspecified impurity in the portion of Propranolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified and unspecified impurity from the *Sample solution*

r_S = peak response of propranolol from the *Standard solution*

C_S = concentration of USP Propranolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Propranolol Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Propranolol related compound A	0.6	1.4	0.1
Propranolol	1.0	1.0	—
Propranolol dimer[a]	4.8	1.3	0.1
Dinaphthyl glycerol[b]	5.7	1.9	0.1
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.4

[a] 3,3′-(Isopropylazanediyl)bis[1-(naphthalen-1-yloxy)propan-2-ol].

[b] 1,3-Bis(naphthalen-1-yloxy)propan-2-ol. ▲(USP 1-May-2020)

SPECIFIC TESTS

Delete the following:

▲• **MELTING RANGE OR TEMPERATURE,** *Class Ia* ⟨741⟩: 162°–165°▲ (USP 1-May-2020)

Delete the following:

▲• **OPTICAL ROTATION** ⟨781S⟩, *Specific Rotation*

Sample solution: 40 mg/mL in water

Acceptance criteria: −1.0° to +1.0°▲ (USP 1-May-2020)

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Propranolol Hydrochloride RS

▲USP Propranolol Related Compound A RS

3-(Naphthalen-1-yloxy)propane-1,2-diol.

$C_{13}H_{14}O_3$ 218.25▲ (USP 1-May-2020)

Propranolol Hydrochloride Extended-Release Capsules

DEFINITION

Propranolol Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$).

IDENTIFICATION

Change to read:

• ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Sample: Transfer the contents of a number of Capsules, equivalent to 160 mg of propranolol hydrochloride, to a glass mortar. Add 5 mL of water, and triturate the mixture with a glass pestle. Transfer the suspension to a centrifuge tube with the aid of 10 mL of water. Add 1 mL of 1 N sodium hydroxide. Add 15 mL of ether, and shake by mechanical means for 5 min. Centrifuge the mixture, and transfer as much of the ether layer as possible to a second centrifuge tube. Add 0.1 mL of hydrochloric acid to the ether extract, and shake. Centrifuge, and discard the ether layer. Add 15 mL of ether to the precipitate, and shake by mechanical means for 5 min. Centrifuge, and discard the ether layer. Dry the precipitate in vacuum at 45° for 30 min. Transfer a small amount of the dried precipitate to a mortar, and grind to a fine powder.

ASSAY

• **PROCEDURE**

Buffer: 6.8 mg/mL of monobasic potassium phosphate. Pass the solution through a filter of 0.5-µm or finer pore size before use.

Mobile phase: Acetonitrile and *Buffer* (7:13)

Diluent: Acetonitrile and water (7:13)

Standard stock solution: 200 µg/mL of USP Propranolol Hydrochloride RS in methanol

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Standard solution: 20 µg/mL in *Diluent* from *Standard stock solution*

Sample stock solution Transfer the contents of Capsules (NLT 10) to a suitable volumetric flask. Add methanol (60% of the volume of the flask), and swirl by mechanical means for 2 h. Allow to stand for 16 h, then sonicate for 30 min, and swirl for 30 min. Dilute with methanol to volume, and centrifuge a portion of the solution. Use the clear supernatant for further use.

Sample solution: Nominally equivalent to 20 µg/mL of propranolol hydrochloride in *Diluent* from *Sample stock solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 15-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for propranolol is about 5–9 min.]
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 3
Relative standard deviation: NMT 2%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{21}NO_2 \cdot HCl$ in each Capsule taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Propranolol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of propranolol hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
pH 1.2 buffer solution: Dissolve 2.0 g of sodium chloride in water, add 7.0 mL of hydrochloric acid, and dilute with water to 1 L.
pH 6.8 buffer solution: 21.72 mg/mL of anhydrous dibasic sodium phosphate and 4.94 mg/mL of citric acid monohydrate in water
Media: Proceed as directed under *Method B* for *Delayed-Release Dosage Forms*, using 900 mL of *pH 1.2 buffer solution* during the *Acid stage*, and conduct the test for 1.5 h. For the *Buffer stage*, use 900 mL of *pH 6.8 buffer solution*, conduct the test for 2.5 h (this is the 4-h time point: 1.5 h in *Acid stage* plus 2.5 h in *Buffer stage*), conduct the test for the additional time points, always considering T_1 = 1.5 h, and use the acceptance criteria given under *Tolerances*.
Apparatus 1: 100 rpm
Times: 1.5, 4, 8, 14, and 24 h
Standard solution: USP Propranolol Hydrochloride RS at a known concentration in water
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Maximum absorbance at 320 nm, with respect to a baseline drawn from 355 nm through 340 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: The percentages of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1.5	NMT 30%
4	35%–60%
8	55%–80%
14	70%–95%
24	81%–110%

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
pH 1.2 buffer solution: Dissolve 2.0 g of sodium chloride in water, add 7.0 mL of hydrochloric acid, and dilute with water to 1 L.
pH 7.5 buffer solution: Dissolve 6.8 g of monobasic potassium phosphate and 1.6 g of sodium hydroxide in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 7.5, and dilute with water to 1 L.
Media: Proceed as directed under *Method B* for *Delayed-Release Dosage Forms*, using 900 mL of *pH 1.2 buffer solution* during the *Acid stage*, and conduct the test for 1 h. For the *Buffer stage*, use 900 mL of *pH 7.5 buffer solution*, conduct the test for 2 h (this is the 3-h time point: 1 h in *Acid stage* plus 2 h in *Buffer stage*), conduct the test for the additional time points, always considering T_1 = 1 h, and use the acceptance criteria given under *Tolerances*.
Apparatus 1: 50 rpm
Times: 1, 3, 6, and 12 h
Standard solution: USP Propranolol Hydrochloride RS at a known concentration in water
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Spectrometric conditions and **Analysis:** Proceed as directed under *Test 1*.
Tolerances: The percentages of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	NMT 20%
3	20%–45%
6	45%–80%
12	NLT 80%

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Acid stage medium: *pH 1.2 buffer solution* (prepared by dissolving 2.0 g of sodium chloride in water, adding 7.0 mL of hydrochloric acid, and diluting with water to 1000 mL); 900 mL
Buffer stage medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 1: 100 rpm

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Standard stock solution: 1 mg/mL of USP Propranolol Hydrochloride RS in water
Working standard solution: Quantitatively dilute the *Standard stock solution* with water to obtain a final concentration of about (L/1000) mg per mL, where L is the Capsule label claim in mg.
Analysis: Conduct the test in *Acid stage medium* for 1.5 h, sample, and pass through a suitable filter of 0.45-µm or finer pore size. Replace the *Acid stage medium* with the *Buffer stage medium*, and conduct the test for 2.5 h (this is the 4-h time point: 1.5 h in *Acid stage medium* plus 2.5 h in *Buffer stage medium*), conduct the test for the additional time points, always considering T_1 = 1.5 h, and use the acceptance criteria given under *Tolerances*. Determine the amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved, using UV absorbances at the wavelength of maximum absorbance at about 320 nm, with respect to a baseline drawn from 355 nm through 340 nm, using a 1-cm cell and water as the blank.
Determine the percentage of propranolol hydrochloride dissolved using the spectrophotometric procedure as directed for *Test 1*.
Tolerances: The percentages of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1.5	NMT 15%
4	NMT 30%
8	25%–60%
14	55%–85%
24	NLT 75%

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Acid stage medium: *pH 1.2 buffer solution* (prepared by dissolving 2.0 g of sodium chloride in water, adding 7.0 mL of hydrochloric acid, and diluting with water to 1000 mL); 900 mL, deaerated
Buffer stage medium: pH 6.8 phosphate buffer; 900 mL, deaerated
Apparatus 1: 100 rpm
Times: 1.5 h in acid stage; 4, 8, 14, and 24 h in buffer stage
Standard solution: 0.18 mg/mL of USP Propranolol Hydrochloride RS in water
Analysis: Conduct the test in *Acid stage medium* for 1.5 h, sample, and pass through a suitable filter of 10-µm or finer pore size. Replace the *Acid stage medium* with the *Buffer stage medium*, and conduct the test for 2.5 h (this is the 4-h time point: 1.5 h in *Acid stage medium* plus 2.5 h in *Buffer stage medium*), conduct the test for the additional time points, always considering T_1 = 1.5 h, and use the acceptance criteria given under *Tolerances*. Determine the amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved, using UV absorbances at the wavelength of maximum absorbance at about 320 nm, with respect to a baseline drawn from 355 nm through 340 nm, using a 1-cm cell and water as the blank.
Tolerances: The percentages of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1.5	NMT 30%
4	27%–52%
8	52%–77%
14	70%–95%
24	81%–110%

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
Procedure for content uniformity
Standard solution: 40 µg/mL of USP Propranolol Hydrochloride RS in methanol
Sample stock solution: Transfer the contents of 1 Capsule to a suitable volumetric flask. Add methanol (70% of the volume of the flask), swirl occasionally for 30 min, sonicate for 1 min, and then swirl occasionally for an additional 30 min. Dilute with methanol to volume, and centrifuge a portion of the solution. Use the clear supernatant for preparing the *Sample solution*.
Sample solution: Equivalent to 40 µg/mL in methanol from *Sample stock solution*
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 290 nm
Cell: 1 cm
Blank: Methanol
Calculate the percentage of $C_{16}H_{21}NO_2 \cdot HCl$ in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Propranolol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** The labeling states the *Dissolution Test* with which the product complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Propranolol Hydrochloride RS

Propranolol Hydrochloride Injection

DEFINITION
Propranolol Hydrochloride Injection is a sterile solution of Propranolol Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of Propranolol Hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 0.5 g of sodium dodecyl sulfate in 18 mL of 0.15 M phosphoric acid. Add 90 mL of acetonitrile and 90 mL of methanol, dilute with water to make 250 mL, and pass through a filter of 0.5-µm pore size.

Standard stock solution: 1 mg/mL of USP Propranolol Hydrochloride RS in methanol

Standard solution: 0.2 mg/mL of USP Propranolol Hydrochloride RS in methanol from *Standard stock solution*. Pass through a suitable filter of 0.7-µm or finer pore size.

System suitability stock solution: 0.25 mg/mL of procainamide hydrochloride in methanol

System suitability solution: Dilute with methanol, 5 mL each of *System suitability stock solution* and *Standard stock solution* in a nominally 25-mL volumetric flask, to volume.

Sample solution: Equivalent to 0.2 mg/mL of propranolol hydrochloride from a volume of Injection diluted with methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for procainamide and propranolol are 0.6 and 1.0, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 2.0 between procainamide and propranolol, *System suitability solution*

Tailing factor: NMT 3.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propranolol from the *Sample solution*

r_S = peak response of propranolol from the *Standard solution*

C_S = concentration of USP Propranolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of propranolol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 55.6 USP Endotoxin Units/mg of propranolol hydrochloride

• **pH** ⟨791⟩: 2.8–4.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose, light-resistant containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Propranolol Hydrochloride RS

Propranolol Hydrochloride Tablets

» Propranolol Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Propranolol Hydrochloride RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for propranolol, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Dissolution ⟨711⟩—

Medium: dilute hydrochloric acid (1 in 100); 1000 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 289 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Propranolol Hydrochloride RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Transfer 1 Tablet to a 100-mL volumetric flask, add 5 mL of dilute hydrochloric acid (1 in 100), and let stand, swirling occasionally, until it is disintegrated. Add about 70 mL of methanol, and sonicate for about 1 minute. Dilute with methanol to volume, mix, and centrifuge a portion of the solution. Dilute an aliquot of the clear solution quantitatively with methanol to provide a solution containing about 40 µg of propranolol hydrochloride per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Propranolol Hydrochloride RS in methanol, at a known concentration of about 40 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 290 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_{16}H_{21}NO_2 \cdot HCl$ in the Tablet by the formula:

$$(T/D)C(A_U/A_S)$$

in which T is the labeled quantity, in mg, of propranolol hydrochloride in the Tablet, D is the concentration, in µg per mL, of the solution from the Tablet, based on the labeled quantity per Tablet and the extent of dilution, C is the concentration, in µg per mL, of USP Propranolol Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—

Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Propranolol Hydrochloride*.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of propranolol hydrochloride, to a 50-mL volumetric flask, add 40 mL of methanol, shake, and sonicate for 5 minutes. Dilute with methanol to volume, mix, and filter through a 0.7-µm or finer

USP Monographs

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porosity filter. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Propranolol Hydrochloride*. Calculate the quantity, in mg, of $C_{16}H_{21}NO_2 \cdot HCl$ in the portion of Tablets taken by the formula:

$$250C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Propranolol Hydrochloride in the *Standard preparation*, and r_U and r_S are the propranolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Propranolol Hydrochloride and Hydrochlorothiazide Tablets

» Propranolol Hydrochloride and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73
USP Hydrochlorothiazide RS
USP Propranolol Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)—
Test specimen—Transfer a quantity of finely powdered Tablets, equivalent to about 100 mg of propranolol hydrochloride, to a 50-mL centrifuge tube, add 15 mL of water and 1 mL of 1 N sodium hydroxide, and mix. Add 20 mL of ether, cap the tube, and shake by mechanical means for 5 minutes. Centrifuge the mixture, and transfer as much of the ether layer as possible to a second centrifuge tube. Add 0.05 mL of hydrochloric acid to the ether extract, and shake. Centrifuge, and discard the ether layer. Add 20 mL of ether to the residue in the tube, and shake by mechanical means for 5 minutes. Centrifuge, and discard the ether layer. Dry the residue in the tube in vacuum at about 50° for 30 minutes. Transfer a small amount of the dried residue to a mortar, and grind to fine powder.
Standard specimen: a similar preparation of USP Propranolol Hydrochloride RS.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)—
Test specimen—Transfer a quantity of finely powdered Tablets, equivalent to about 100 mg of hydrochlorothiazide, to a 35-mL centrifuge tube, add 30 mL of acetone, mix, and allow to stand for 30 minutes, with occasional shaking. Centrifuge, and decant the acetone extract into a beaker, discarding the residue in the centrifuge tube. Evaporate the acetone extract on a steam bath to dryness, add 10 mL of 0.1 N sodium hydroxide, and mix, using a spatula to dislodge any residue from the beaker. Transfer the suspension to a 125-mL separator, and wash the beaker with about 5 mL of water, adding the washing to the separator. Add 50 mL of ether to the separator, shake for 2 minutes, and allow the phases to separate. Draw off the clear lower layer, filtering it into a beaker. Add 1 N hydrochloric acid dropwise with stirring until a pH of about 2 is reached. [NOTE—Precipitation occurs during the addition of the acid.] When precipitation is complete, decant the supernatant, and wash the precipitate with 5 mL of water. Dry the precipitate at 105° for 30 minutes.
Standard specimen: a similar preparation of USP Hydrochlorothiazide RS.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Filter a portion of the solution under test, transfer 10.0 mL of the filtrate to a suitable capped bottle, and add 5.0 mL of water, 1.0 mL of 5 N sodium hydroxide, and 25.0 mL of *n*-heptane. Cap the bottle, shake by mechanical means for 5 minutes, and allow the layers to separate, centrifuging if necessary, to obtain clear upper (*n*-heptane) and lower (aqueous) extracts. Determine the quantity, in µg, of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$) in each mL of the *n*-heptane extract by employing UV absorption at the wavelength of maximum absorbance at about 293 nm, in comparison with an *n*-heptane Standard solution obtained by similarly treating and extracting a mixture of 5.0 mL of an aqueous solution having a known concentration of USP Propranolol Hydrochloride RS and 5.0 mL of a 0.005 N sodium hydroxide solution having a known concentration of USP Hydrochlorothiazide RS, using a blank consisting of the *n*-heptane extract obtained by similarly treating and extracting 10.0 mL of water. Determine the quantity, in µg, of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in each mL of the aqueous extract by employing UV absorption at the wavelength of maximum absorbance at about 273 nm, in comparison with the aqueous extract remaining from the preparation of the *n*-heptane Standard solution, using as a blank the aqueous extract remaining from the preparation of the *n*-heptane blank extract. Calculate the quantity, in mg, of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved by multiplying the quantity, in µg, of propranolol hydrochloride in each mL of the *n*-heptane extract from the solution under test by 2.25. Calculate the quantity, in mg, of $C_7H_8ClN_3O_4S_2$ dissolved by multiplying the quantity, in µg, of hydrochlorothiazide in each mL of the aqueous extract from the solution under test by 1.44.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{16}H_{21}NO_2 \cdot HCl$ and not less than 80% *(Q)* of the labeled amount of $C_7H_8ClN_3O_4S_2$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Content Uniformity* with respect to propranolol hydrochloride and hydrochlorothiazide.
Procedure for content uniformity—Transfer a Tablet to a suitable container, and add 500.0 mL of 0.1 N hydrochloric acid. Shake until the Tablet has disintegrated, sonicate for 30 seconds, shake by mechanical means for 30 minutes, and then repeat the sonication and shaking. Centrifuge a portion of the solution, and transfer 6.0 mL of the clear supernatant and 15.0 mL of water to a suitable capped bottle. Add 1.0 mL of 5 N sodium hydroxide and 25.0 mL of *n*-heptane, cap the bottle, shake by mechanical means for 5 minutes, and allow the layers to separate, centrifuging, if necessary, to obtain clear upper (*n*-heptane) and lower (aqueous) layers (test solutions). Prepare a similar Standard solution by mixing 6.0 mL of 0.1 N

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hydrochloric acid, 3.0 mL of water, 6.0 mL of an aqueous solution having a known concentration of USP Propranolol Hydrochloride RS, and 6.0 mL of a 0.04 N sodium hydroxide solution having a known concentration of USP Hydrochlorothiazide RS, and proceeding as directed for the test solutions, beginning with "Add 1.0 mL of 5 N sodium hydroxide." Prepare similar blank *n*-heptane and aqueous extracts by adding 6.0 mL of 0.1 N hydrochloric acid to 15.0 mL of water, and proceeding as directed for the test solutions, beginning with "Add 1.0 mL of 5 N sodium hydroxide". Concomitantly determine the absorbances of the *n*-heptane test solution and the *n*-heptane Standard solution at the wavelength of maximum absorbance at about 293 nm, using the *n*-heptane blank extract to set the instrument. Calculate the quantity, in mg, of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$) in the Tablet taken by the formula:

$$(12.5/6)(C)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Propranolol Hydrochloride RS in the *n*-heptane Standard solution; and A_U and A_S are the absorbances at 293 nm of the *n*-heptane test solution and the *n*-heptane Standard solution, respectively. Concomitantly determine the absorbances of the aqueous test solution and the aqueous Standard solution at the wavelength of maximum absorbance at about 273 nm, using the aqueous blank extract to set the instrument. Calculate the quantity, in mg, of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the Tablet taken by the formula:

$$(11/6)(C)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Hydrochlorothiazide RS in the aqueous Standard solution; and A_U and A_S are the absorbances at 273 nm of the aqueous test solution and the aqueous Standard solution, respectively.

Related compounds—

Tetrabutylammonium hydroxide solution, Buffer, Mobile phase, and *Chromatographic system*—Proceed as directed under *Assay.*

Standard solution—Dissolve an accurately weighed quantity of USP Benzothiadiazine Related Compound A RS in methanol to obtain a solution having a known concentration of about 0.5 mg per mL. Transfer an accurately measured volume of this solution, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.5 µg per mL.

Test solution—Use the *Assay preparation* prepared as directed in the *Assay.*

Chromatographic system—Proceed as directed under *Assay,* except to chromatograph the *Standard solution:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken by the formula:

$$50(C/L)(r_U/r_S)$$

in which C is the concentration in µg per mL, of USP Benzothiadiazine Related Compound A RS in the *Standard solution; L* is the amount, in mg, of hydrochlorothiazide in the portion of Tablets taken, based on the labeled amount; and r_U and r_S are the peak areas of benzothiadiazine related compound A obtained from the *Test solution* and *Standard solution,* respectively: not more than 1.0% is found.

Assay—

Tetrabutylammonium hydroxide solution—Use a suitable aqueous or methanolic solution having a known concentration of tetrabutylammonium hydroxide.

Buffer—Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water in a 2000-mL volumetric flask. Add 3.4 mL of phosphoric acid and a volume of *Tetrabutylammonium hydroxide solution* equivalent to about 2.6 g of tetrabutylammonium hydroxide, dilute with water to volume, and mix. Adjust, if necessary, with phosphoric acid or 10 N potassium hydroxide to a pH of 2.5 ± 0.1, and pass through a filter having a 0.5-µm or finer porosity.

Mobile phase—Prepare a suitable mixture of *Buffer* and methanol (850:150). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩) so that the retention time of propranolol is between 12 and 25 minutes.

Standard hydrochlorothiazide stock solution—Transfer about 25 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 100-mL volumetric flask, add 15 mL of methanol, and mix to dissolve. Dilute with *Buffer* to volume, and mix.

Standard propranolol hydrochloride stock solution—Dissolve an accurately weighed quantity of USP Propranolol Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.25J mg per mL, J being the ratio of the labeled quantity, in mg, of propranolol hydrochloride to the labeled quantity, in mg, of hydrochlorothiazide per Tablet.

Standard preparation—Transfer 5.0 mL of *Standard hydrochlorothiazide stock solution* and 5.0 mL of *Standard propranolol hydrochloride stock solution* to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 50 µg of hydrochlorothiazide and 50J µg of propranolol hydrochloride per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 25 mg of hydrochlorothiazide, to a 500-mL volumetric flask. Add 5 mL of water, mix, and allow to stand for 5 minutes, with occasional swirling. Add 75 mL of methanol, mix, and sonicate for 10 minutes, with occasional swirling, adding ice to the bath, if necessary, to maintain the temperature at not more than 20°. Add about 350 mL of *Buffer* to the flask, and sonicate for 10 minutes, with occasional swirling, maintaining the temperature of the bath at not more than 20°. Dilute with *Buffer* to volume, and mix. Centrifuge a portion of this solution, if necessary, to obtain a clear solution (*Assay preparation*).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 4-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the propranolol peak is not less than 2500 theoretical plates; the tailing factor for the propranolol and hydrochlorothiazide peaks is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—The relative retention times are about 0.25 for benzothiadiazine related compound A, 0.4 for hydrochlorothiazide, and 1.0 for propranolol.]

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken by the same formula:

$$0.5C(r_U/r_S)$$

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in which C is the concentration, in µg per mL, of the appropriate Reference Standard in the *Standard preparation;* and r_U and r_S are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Propylene Glycol



$C_3H_8O_2$ 76.09
1,2-Propanediol;
Propane-1,2-diol [57-55-6].

DEFINITION
Propylene Glycol contains NLT 99.5% of $C_3H_8O_2$.

IDENTIFICATION
[NOTE—Compliance is determined by meeting the requirements of *Identification* tests *A*, *B*, and *C*.]

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197F▲** (CN 1-May-2020)
[NOTE—Undried specimen.]
- **B. LIMIT OF DIETHYLENE GLYCOL AND ETHYLENE GLYCOL**
Diluent: Methanol
Standard solution: 2.0 mg/mL of USP Propylene Glycol RS, 0.050 mg/mL of USP Ethylene Glycol RS, 0.050 mg/mL of USP Diethylene Glycol RS, and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol
Sample solution: 50 mg/mL of Propylene Glycol and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica coated with 3.0-µm G43 stationary phase, and a deactivated split liner with glass wool
Temperature
Injector: 220°
Detector: 250°
Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	4
100	50	120	10
120	50	220	6

Carrier gas: Helium
Injection size: 1.0 µL
Flow rate: 4.5 mL/min
Injection type: The split flow ratio is about 10:1.
System suitability
Sample: *Standard solution*
[NOTE—For informational purposes only. See *Impurity Table 1* for relative retention times for ethylene glycol, internal standard, and diethylene glycol. The retention time for propylene glycol is 4 min.]

Impurity Table 1

Component	Relative Retention Time
Ethylene glycol	0.8
Propylene glycol	1.0
Internal standard	1.7
Diethylene glycol	2.4

Suitability requirements
Resolution: NLT 5 between ethylene glycol and propylene glycol
Analysis
Sample: *Sample solution*
Acceptance criteria
Diethylene glycol: If a peak at the retention time for diethylene glycol is present in the *Sample solution*, the peak response ratio relative to 2,2,2-trichloroethanol is NMT the peak response ratio for diethylene glycol relative to 2,2,2-trichloroethanol in the *Standard solution*: NMT 0.10% for diethylene glycol.
Ethylene glycol: If a peak at the retention times for ethylene glycol is present in the *Sample solution*, the peak response ratio relative to 2,2,2-trichloroethanol is not more than the peak response ratio for ethylene glycol relative to 2,2,2-trichloroethanol in the *Standard solution*: NMT 0.10% for ethylene glycol is found.
- **C.** Examine the chromatograms obtained in *Identification* test *B*. The retention time of the propylene glycol peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
- **PROCEDURE**
Sample: Propylene Glycol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Thermal conductivity
Column: 1-m × 4-mm; 5% phase G16; support S5
Temperature
Injector: 240°
Detector: 250°
Column: Increase from 120° to 200° at a rate of 5°/min.
Carrier gas: Helium
Injection size: 10 µL
[NOTE—The approximate retention time for propylene glycol is 5.7 min, and the approximate retention times for the three isomers of dipropylene glycol, when present, are 8.2, 9.0, and 10.2 min, respectively.]
Analysis: Calculate the percentage of $C_3H_8O_2$ in the *Sample* by dividing the area under the propylene glycol peak by the sum of the areas under all of the peaks, excluding those due to air and water, and multiplying by 100:

$$\text{Result} = [r_U/(r_U + \Sigma r_U)] \times 100$$

r_U = peak response for Propylene Glycol from the *Sample*
Σr_U = sum of individual impurity peak responses, excluding those due to air and water, from the *Sample*

Acceptance criteria: NLT 99.5%

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IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩

Sample: 50 g

Analysis: Heat the *Sample* in a tared 100-mL shallow dish until it ignites, and allow it to burn without further application of heat in a place free from drafts. Cool, moisten the residue with 0.5 mL of sulfuric acid, and ignite to constant weight.

Acceptance criteria: The weight of the residue is NMT 3.5 mg.

- **Chloride and Sulfate,** *Chloride* ⟨221⟩: A 1-mL portion shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (70 ppm).
- **Chloride and Sulfate,** *Sulfate* ⟨221⟩: A 5.0-mL portion shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (60 ppm).

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 1.035–1.037
- **ACIDITY**

Sample: 10 mL of Propylene Glycol

Analysis: Add 1 mL of phenolphthalein TS to 50 mL of water, then add 0.1 N sodium hydroxide until the solution remains pink for 30 s. Add the *Sample*, and titrate with 0.10 N sodium hydroxide until the original pink color returns and remains for 30 s.

Acceptance criteria: NMT 0.20 mL of 0.10 N sodium hydroxide

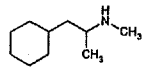
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Diethylene Glycol RS
USP Ethylene Glycol RS
USP Propylene Glycol RS

Propylhexedrine



$C_{10}H_{21}N$ 155.28

Cyclohexaneethanamine, *N*,α-dimethyl-, (±)-.
(±)-*N*,α-Dimethylcyclohexaneethylamine [101-40-6].

» Propylhexedrine contains not less than 98.0 percent and not more than 101.0 percent of $C_{10}H_{21}N$.

Packaging and storage—Preserve in tight containers.

Identification—

A: To 3 mL of water contained in a small flask add about 0.1 mL of it and 0.5 mL of 1 N hydrochloric acid, and agitate the mixture until clear. Add 20 mL of trinitrophenol TS, insert the stopper in the flask, shake vigorously for a few minutes, and allow to stand for 2 hours. Filter, wash the precipitate with about 20 mL of cold water, and dry in vacuum at 60° for 4 hours: the picrate so obtained melts between 108° and 110° (see *Melting Range or Temperature* ⟨741⟩).

[**CAUTION**—Picrates may explode.]

B: A solution, prepared as directed in *Identification* test *A*, yields a brown precipitate with iodine TS and a white precipitate with mercuric-potassium iodide TS.

Specific gravity ⟨841⟩: between 0.848 and 0.852.

Assay—Tare a glass-stoppered conical flask containing about 15 mL of water, add quickly about 0.5 mL of Propylhexedrine, and again weigh. Add to the contents of the flask 30 mL of neutralized alcohol, then add methyl red TS, and titrate with 0.1 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sulfuric acid is equivalent to 15.53 mg of $C_{10}H_{21}N$.

Propylhexedrine Inhalant

» Propylhexedrine Inhalant consists of cylindrical rolls of suitable fibrous material impregnated with Propylhexedrine, usually aromatized, and contained in a suitable inhaler. The inhaler contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{10}H_{21}N$.

Packaging and storage—Preserve in tight containers (inhalers), and avoid exposure to excessive heat.

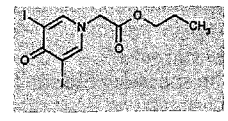
Identification—Place the contents of 1 inhaler in a glass-stoppered flask, add 50 mL of methanol, and allow to stand for 1 hour with frequent agitation. Filter, pressing out the roll on the filter. Add to the filtrate 1 N hydrochloric acid until it is slightly acid to moistened litmus paper, then add 30 mL of water, and evaporate to about 20 mL. Cool, transfer to a small separator, and shake with 10 mL of ether. Withdraw the water layer, warm it on a steam bath to expel any ether, and dilute to about 25 mL. From 10 mL of the solution, precipitate the propylhexedrine with trinitrophenol TS as directed in *Identification* test *A* under *Propylhexedrine*: the propylhexedrine picrate so obtained melts between 108° and 110° (see *Melting Range or Temperature* ⟨741⟩).

[**CAUTION**—Picrates may explode.]

Assay—Place the contents of 2 inhalers of Inhalant in the thimble of a continuous-extraction apparatus, and quickly assemble the apparatus. Rinse each of the emptied inhalers with about 20 mL of methanol, pouring the rinsings through the condenser into the extraction flask. Add through the condenser 20 mL to 30 mL of methanol, and extract for 15 to 20 cycles. Cool the extract, transfer it completely with the aid of small portions of methanol to a 100-mL volumetric flask, dilute with methanol to volume, and mix. To 50.0 mL of the solution add 25.0 mL of 0.1 N sulfuric acid VS, and evaporate to about 40 mL. Cool, add methyl red TS, and titrate the excess acid with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sulfuric acid is equivalent to 15.53 mg of $C_{10}H_{21}N$.

Delete the following:

▲Propyliodone



$C_{10}H_{11}I_2NO_3$ 447.01

1(4*H*)-Pyridineacetic acid, 3,5-diiodo-4-oxo-, propyl ester.
Propyl 3,5-diiodo-4-oxo-1(4*H*)pyridineacetate [587-61-1].

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» Propyliodone contains not less than 99.0 percent and not more than 101.0 percent of $C_{10}H_{11}I_2NO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Identification—

A: Heat 100 mg with a few drops of sulfuric acid: violet vapors are evolved.

B: Reflux 1 g with 10 mL of 1 N sodium hydroxide for 30 minutes, add 10 mL of water, and acidify to litmus paper with hydrochloric acid: the precipitate of 3,5-diiodo-4-oxo-1(4*H*)-pyridineacetic acid, after being washed with water and dried at 105°, melts at about 245°.

Melting range 〈 741 〉: between 187° and 190°.

Acidity—Dissolve 1.0 g in 40 mL of hot *n*-propyl alcohol previously neutralized to phenolphthalein TS, cool, and allow to stand in an ice bath for 15 minutes with frequent shaking. Filter, wash the residue with neutralized *n*-propyl alcohol, combine the filtrate and washings, add phenolphthalein TS, and titrate with 0.050 N sodium hydroxide to a pink color that persists for 15 seconds: not more than 0.15 mL of 0.050 N sodium hydroxide is required for neutralization.

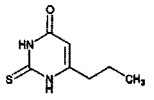
Loss on drying 〈 731 〉—Dry it at 105° to constant weight: it loses not more than 0.5% of its weight.

Residue on ignition 〈 281 〉: not more than 0.1%.

Iodine and iodide—Shake 2.4 g with 30 mL of water for 15 minutes, filter, and to 10 mL of filtrate add 1 mL of 2 N nitric acid, 1 mL of sodium nitrite solution (1 in 500), and 2 mL of chloroform. Shake, and centrifuge: any purple color in the chloroform layer is not darker than that obtained with a mixture of 6 mL of water and 4 mL of potassium iodide solution (2.6 in 100,000) treated in the same manner (0.01% of I).

Assay—Using about 15 mg of Propyliodone, accurately weighed, proceed as directed in the *Assay* under *Iodoquinol*. Each mL of 0.02 N sodium thiosulfate is equivalent to 0.7450 mg of $C_{10}H_{11}I_2NO_3$. ▲(USP 1-May-2020)

Propylthiouracil



$C_7H_{10}N_2OS$ 170.23

4(1*H*)-Pyrimidinone, 2,3-dihydro-6-propyl-2-thioxo-; 6-Propyl-2-thiouracil [51-52-5].

DEFINITION

Propylthiouracil contains NLT 98.0% and NMT 100.5% of propylthiouracil ($C_7H_{10}N_2OS$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** 〈197〉, *Infrared Spectroscopy*: **197K**▲(CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample: 300 mg

Analysis: Transfer the *Sample* to a 500-mL conical flask, and add 30 mL of water. Add from a buret 30 mL of 0.1 N sodium hydroxide VS, heat to boiling, and agitate until solution is complete. Wash down any particles on the wall of the flask with a few mL of water, add 50 mL of 0.1 N silver nitrate while mixing, and boil gently for 7 min. Cool to room temperature, and continue to titrate with 0.1 N sodium hydroxide VS determining the endpoint potentiometrically, using a glass-calomel electrode system. Each mL of 0.1 N sodium hydroxide is equivalent to 8.512 mg of propylthiouracil ($C_7H_{10}N_2OS$).

Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** 〈281〉: NMT 0.1%
- **SELENIUM** 〈291〉

Sample: 200 mg

Acceptance criteria: 0.003%

- **ORDINARY IMPURITIES** 〈466〉

Solvent: Methanol

Application volume: 10 µL

Eluant: Toluene, ethyl acetate, and formic acid (50:45:5) in a nonequilibrated chamber

Visualization: 1

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** 〈741〉: 218°–221°
- **LOSS ON DRYING** 〈731〉

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** 〈11〉

USP Propylthiouracil RS

Propylthiouracil Compounded Oral Suspension

DEFINITION

Propylthiouracil Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$).

Prepare Propylthiouracil Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈795〉).

Propylthiouracil tablets[a] equivalent to	500 mg of propylthiouracil
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Propylthiouracil 50-mg tablets, West-Ward Pharmaceutical Corp., Eatontown, NJ.

[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Propylthiouracil tablets* in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a propylthiouracil liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

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ASSAY

• **PROCEDURE**

Mobile phase: 5 mM 1-heptanesulfonic acid, acetic acid (1% v/v), and methanol (40:45:15). Pass through a nylon 66 filter of 0.45-µm pore size, and degas.

Internal standard solution: 1.0 mg/mL of 6-methyl-2-thiouracil in methanol

Standard stock solution: 1.75 mg/mL of USP Propylthiouracil RS in methanol

Standard solution: Pipet 0.4 mL of the *Standard stock solution* into a 10-mL volumetric flask, and add 0.25 mL of the *Internal standard solution*. Dilute with *Mobile phase* to volume to obtain a nominal concentration of 70 µg/mL of propylthiouracil and 25 µg/mL of 6-methyl-2-thiouracil, and centrifuge.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 2 mL of Oral Suspension into a 10-mL volumetric flask, and dilute with methanol to volume to obtain a concentration of 1 mg/mL. Transfer 0.7 mL of the diluted solution to a 10-mL volumetric flask, and add 0.25 mL of the *Internal standard solution*. Dilute with *Mobile phase* to volume to obtain a nominal concentration of 70 µg/mL of propylthiouracil and 25 µg/mL of 6-methyl-2-thiouracil, and centrifuge.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 3.0-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 0.4 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for 6-methyl-2-thiouracil and propylthiouracil are about 0.25 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10.0 between propylthiouracil and 6-methyl-2-thiouracil

Column efficiency: NLT 10,000 theoretical plates

Tailing factor: NMT 2.0 for the propylthiouracil peak

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of propylthiouracil to the internal standard from the *Sample solution*

R_S = peak response ratio of propylthiouracil to the internal standard from the *Standard solution*

C_S = concentration of USP Propylthiouracil RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of propylthiouracil in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator, and NMT 60 days after the date on which it was compounded, when stored at controlled room temperature

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Propylthiouracil RS

Propylthiouracil Tablets

DEFINITION

Propylthiouracil Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Boil a quantity of finely powdered Tablets equivalent to 100 mg of propylthiouracil with 10 mL of alcohol under a reflux condenser for 20 min. Filter while hot, and evaporate the filtrate on a steam bath to dryness. Use the residue.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Transfer 3.40 g of monobasic potassium phosphate to a 1000-mL beaker. Add 500 mL of water, and stir until dissolved. Adjust with phosphoric acid or 0.1 N sodium hydroxide to a pH of 4.6, and add 500 mL of water. Adjust the resulting solution with phosphoric acid or 0.1 N sodium hydroxide to a pH of 4.6.

Mobile phase: Acetonitrile and *Buffer* (20:80)

Standard stock solution: 0.5 mg/mL of USP Propylthiouracil RS prepared as follows. Transfer 25 mg of USP Propylthiouracil RS to a 50-mL volumetric flask, add 5 mL of methanol, and sonicate for 5 min. Add 25 mL of water, and shake by mechanical means for 15 min. Dilute with water to volume.

Standard solution: 50 µg/mL of USP Propylthiouracil RS from the *Standard stock solution* in water

Sample stock solution: Nominally 0.5 mg/mL of propylthiouracil from Tablets prepared as follows. Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of propylthiouracil to a 100-mL volumetric flask, add 10 mL of methanol, and sonicate for 5 min. Add 50 mL of water, and shake by mechanical means for 20 min. Dilute with water to volume and pass through a suitable filter. Use the filtrate.

Sample solution: Nominally 50.0 µg/mL of propylthiouracil from the *Sample stock solution* in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 272 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

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Suitability requirements
Column efficiency: NLT 3500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propylthiouracil from the *Sample solution*
r_S = peak response of propylthiouracil from the *Standard solution*
C_S = concentration of USP Propylthiouracil RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of propylthiouracil in the *Sample solution* (µg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: USP Propylthiouracil RS at a known concentration in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter and dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 274 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of propylthiouracil ($C_7H_{10}N_2OS$) dissolved from the UV absorbances.
Tolerances: NLT 85% (*Q*) of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Propylthiouracil RS

Protamine Sulfate

DEFINITION
Protamine Sulfate is a purified mixture of peptides obtained from the sperm or testes of Salmonidae, which has the property of neutralizing heparin. Each mg of Protamine Sulfate, calculated on the dried basis, neutralizes NLT 100 USP Heparin Units. It contains NLT 90.0% and NMT 110.0% of protamine sulfate, calculated on the dried basis.

IDENTIFICATION
• **A.** The retention times of the four major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sulfate*: Meets the requirements
• **C. BIOIDENTITY**
Sample solution A: 0.15 mg/mL of Protamine Sulfate in water
Sample solution B: Dilute 2.0 mL of *Sample solution A* with water to 3.0 mL.
Sample solution C: Dilute 1.0 mL of *Sample solution A* with water to 3.0 mL.
Titrant: USP Heparin Sodium for Assays RS in water (about 80–120 USP Heparin Units/mL)
Analysis: [NOTE—Titrate each *Sample solution* in duplicate.] Transfer a volume of each *Sample solution* to the analytical cell of a suitable colorimeter, and set the apparatus for measurement at a suitable wavelength (none is critical) in the visible range. Add *Titrant* in small volumes until there is a sharp increase in the absorbance, and note the volume of *Titrant* added. Perform the entire *Analysis* in triplicate for a total of 18 determinations.
Calculate the number of USP Heparin Units per mg of Protamine Sulfate in the volume of *Titrant* added at the endpoint.
Calculate the USP Heparin Units neutralized per mg of Protamine Sulfate taken:

$$\text{Result} = (V_T \times C_T)/(V_S \times C_U)$$

V_T = volume of *Titrant* added (mL)
C_T = concentration of *Titrant* (USP Heparin Units/mL)
V_S = volume of each *Sample solution* (mL)
C_U = concentration of Protamine Sulfate in each *Sample solution* (mg/mL)

Calculate the potency of the Protamine Sulfate as the average of the 18 values. Calculate the three standard deviations for the results obtained with each *Sample solution*. Calculate the three standard deviations for the results obtained with each of the three independent assays. The test is valid if each of the six standard deviations is NMT 5% of the average result.
Acceptance criteria: Each mg of Protamine Sulfate neutralizes NLT 100 USP Heparin Units on the dried basis.

ASSAY
• **PROCEDURE**
Solution A: 0.1 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 1.8. Pass through a membrane filter of 0.45-µm pore size, and degas before use.
Solution B: *Solution A* and acetonitrile (93.5: 6.5)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
15	55	45
25	55	45
30	85	15

[NOTE—Initial gradient composition may be adjusted as appropriate to obtain sufficient resolution. The end of the gradient can be increased to re-equilibrate the column for the next injection.]
Standard solution: 0.5 mg/mL of USP Protamine Sulfate RS in 0.01 M hydrochloric acid
Sample solution: 0.5 mg/mL of Protamine Sulfate in 0.01 M hydrochloric acid

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 55°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Retention time: The chromatogram of the *Standard solution* must show four major peaks (in increasing elution order: protamine peptides 1, 2, 3, and 4) with protamine peptide 4 eluting no later than 15 min. [NOTE—See the reference chromatogram provided with the USP Protamine Sulfate RS certificate.]
Resolution: NLT 2.0 between protamine peptides 1 and 2, calculated by the tangent method
Relative standard deviation: NMT 2.0% for the total integrated areas of at least six replicate injections, using vertical drop down integration
Analysis
Samples: *Standard solution* and *Sample solution*
Separately inject equal volumes of the *Standard solution* (at least six injections) and the *Sample solution*, record the chromatograms for approximately 30 min, and measure the responses for all the peaks using a full scale comparable to the height of the largest peak and using vertical drop down integration.
Calculate the percentage of protamine sulfate in the portion of Protamine Sulfate taken:

$$\text{Result} = \Sigma[(r_U/r_S) \times (C_S/C_U)] \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Protamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Protamine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% on the dried basis

IMPURITIES
• **RESIDUAL SOLVENTS** ⟨467⟩**:** Meets the requirements
• **CHROMATOGRAPHIC PURITY**
Solution A, Solution B, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Proceed as directed in the *Assay*.
Calculate the percentage purity of Protamine Sulfate from the four protamine peptide peaks:

$$\text{Result} = [(r_{U1} + r_{U2} + r_{U3} + r_{U4})/r_T] \times 100$$

r_{U1} = peak response of protamine peptide 1 from the *Sample solution*
r_{U2} = peak response of protamine peptide 2 from the *Sample solution*
r_{U3} = peak response of protamine peptide 3 from the *Sample solution*
r_{U4} = peak response of protamine peptide 4 from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: NLT 92%

• **IRON** ⟨241⟩**:** NMT 10 ppm
• **METHYLMERCURY:** NMT 10 ppm, using a validated analytical procedure. [NOTE—Methylmercury determination is not necessary when the content for total mercury is less than the limit for methylmercury.]

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 7.0 USP Endotoxin Units/mg
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 5%
• **ULTRAVIOLET ABSORBANCE**
Sample solution: 1.0% Solution of Protamine Sulfate in water
Instrumental conditions
Mode: UV
Wavelength range: 260–280 nm
Blank: Water
Acceptance criteria: The difference between the absorbance of the *Sample solution* at 260–280 nm and the *Blank* is NMT 0.1.
• **SULFATE**
Sample: 150 mg
Analysis: Dissolve the *Sample* in 75 mL of water, add 5 mL of 3 N hydrochloric acid, heat to boiling, and while maintaining the boiling point, slowly add 10 mL of barium chloride TS. Cover the vessel, and allow the mixture to stand on a steam bath for 1 h. Filter, wash the precipitate with several portions of hot water, dry, and ignite to constant weight. The weight of the barium sulfate, multiplied by 0.4117, represents the weight of sulfate in the portion of Protamine Sulfate taken.
Acceptance criteria: 16%–22% on the dried basis

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight and tamper-proof containers. Store at controlled room temperature or at 2°–8°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Endotoxin RS
USP Heparin Sodium for Assays RS
USP Protamine Sulfate RS

Protamine Sulfate Injection

DEFINITION
Protamine Sulfate Injection is a sterile, isotonic solution of Protamine Sulfate. Protamine Sulfate used in the manufacture of Protamine Sulfate Injection complies with the compendial requirements stated in the Protamine Sulfate monograph. Each mg of Protamine Sulfate, used in the manufacture of the Injection, neutralizes NLT 100 USP Heparin Units, calculated on the dried basis. It contains NLT 90.0% and NMT 120.0% of the labeled amount of protamine sulfate.

IDENTIFICATION
• **A.** The retention times of the four major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sulfate*: Meets the requirements

ASSAY
• **PROCEDURE**
Solution A: 0.1 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 1.8. Pass the solution

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through a membrane filter of 0.45-µm pore size, and degas before use.
Solution B: *Solution A* and acetonitrile (93.5: 6.5)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
15	55	45
25	55	45
30	85	15

[NOTE—Initial gradient composition may be adjusted as appropriate to obtain sufficient resolution. The end of the gradient can be increased to re-equilibrate the column for the next injection.]
Standard solution: 0.5 mg/mL of USP Protamine Sulfate RS in 0.01 M hydrochloric acid
Sample solution: 0.5 mg/mL of protamine sulfate in 0.01 M hydrochloric acid
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 55°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Retention time: The chromatogram of the *Standard solution* must show four major peaks (in increasing elution order: protamine peptides 1, 2, 3, and 4), with protamine peptide 4 eluting no later than 15 min. [NOTE—See the reference chromatogram provided with the USP Protamine Sulfate RS certificate.]
Resolution: NLT 2.0 between protamine peptides 1 and 2, calculated by the tangent method
Relative standard deviation: NMT 2.0% for the total integrated areas of the six chromatograms of the *Standard solution*, using vertical drop-down integration
Analysis
Samples: *Standard solution* and *Sample solution*
Separately inject equal volumes of the *Standard solution* (at least six injections) and the *Sample solution* into the chromatograph. Record the chromatograms for approximately 30 min, and measure the responses for all the peaks observed using a full-scale comparable to the height of the largest peak and using vertical drop-down integration.
Calculate the percentage of the labeled amount of protamine sulfate in the portion of Injection taken:

$$\text{Result} = \Sigma[(r_U/r_S) \times (C_S/C_U)] \times 100$$

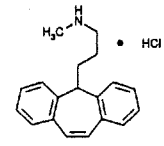
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Protamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of protamine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 7.0 USP Endotoxin Units/mg of protamine sulfate.
- **PH** ⟨791⟩: 6.0–7.0
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature or at 2°–8°.
- **LABELING:** Label it to indicate the approximate neutralization capacity in USP Heparin Units.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Protamine Sulfate RS

Protriptyline Hydrochloride



$C_{19}H_{21}N \cdot HCl$ 299.84
5*H*-Dibenzo[*a*,*d*]cycloheptene-5-propanamine, *N*-methyl-, hydrochloride.
N-Methyl-5*H*-dibenzo[*a*,*d*]cycloheptene-5-propylamine hydrochloride [1225-55-4].

» Protriptyline Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of $C_{19}H_{21}N \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Protriptyline Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: 0.1 N methanolic hydrochloric acid.
Absorptivities at 292 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: It responds to tests *A* and *B* in *Identification Tests—General* ⟨191⟩ *Chemical Identification Tests, Chloride.*
D: Its X-ray diffraction pattern (see *X-ray Diffraction* ⟨941⟩) conforms to that of USP Protriptyline Hydrochloride RS.
pH ⟨791⟩: between 5.0 and 6.5, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry it at a pressure below 5 mm of mercury at 60° to constant weight: it loses not more than 0.3% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

USP Monographs

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Assay—Transfer about 700 mg of Protriptyline Hydrochloride, accurately weighed, to a 125-mL conical flask, and dissolve in 30 mL of glacial acetic acid. Add crystal violet TS and 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 29.98 mg of $C_{19}H_{21}N \cdot HCl$.

Protriptyline Hydrochloride Tablets

DEFINITION

Protriptyline Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of protriptyline hydrochloride ($C_{19}H_{21}N \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 11 g of monobasic sodium phosphate and 1.9 g of sodium 1-hexanesulfonate in 900 mL of water. Dilute with water to 1 L and adjust with phosphoric acid to a pH of 2.9.
Solution A: Acetonitrile and *Buffer* (17:83)
Solution B: Acetonitrile and *Buffer* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	0	100
20	0	100
25	100	0
30	100	0

Diluent: Mix 200 mL of alcohol and 40 mL of 2.5 N hydrochloric acid, and dilute with water to 1000 mL.
Standard solution: 200 µg/mL of USP Protriptyline Hydrochloride RS in *Diluent*
Sample solution: Nominally 200 µg/mL of protriptyline hydrochloride prepared as follows. Transfer NLT 5 Tablets to an appropriate volumetric flask. Add *Diluent* to about 80% of the flask volume, sonicate, and swirl as necessary to dissolve. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size and discard the first 8 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of protriptyline hydrochloride ($C_{19}H_{21}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Protriptyline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of protriptyline hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Protriptyline Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute the filtrate with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 290 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of protriptyline hydrochloride ($C_{19}H_{21}N \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Protriptyline Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of protriptyline hydrochloride ($C_{19}H_{21}N \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.2 µg/mL of USP Protriptyline Hydrochloride RS in *Diluent*
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%

USP Monographs

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Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

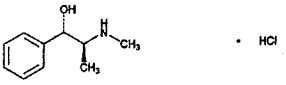
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of protriptyline hydrochloride from the *Standard solution*
C_S = concentration of USP Protriptyline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of protriptyline hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: Disregard any impurity peaks less than 0.05%.
Any individual, unspecified degradation product: NMT 0.2%
Total degradation products: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Protriptyline Hydrochloride RS

Pseudoephedrine Hydrochloride



$C_{10}H_{15}NO \cdot HCl$ 201.69
Benzenemethanol, α-[1-(methylamino)ethyl]-, [*S*-(*R**,*R**)]-, hydrochloride;
(+)-Pseudoephedrine hydrochloride [345-78-8].

DEFINITION
Pseudoephedrine Hydrochloride contains NLT 98.0% and NMT 102.0% of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY
- **PROCEDURE**
  Triethylamine–phosphoric acid solution: Mix 5 mL of triethylamine with 1 L of water. Adjust with phosphoric acid to a pH of 6.8.
  Mobile phase: Methanol and *Triethylamine–phosphoric acid solution* (10:90)
  System suitability solution: 0.1 mg/mL of USP Pseudoephedrine Hydrochloride RS and 0.002 mg/mL of USP Ephedrine Sulfate RS in water
  Standard solution: 0.1 mg/mL of USP Pseudoephedrine Hydrochloride RS in water
  Sample solution: 0.1 mg/mL of pseudoephedrine hydrochloride in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 206 nm
  Column: 3.0-mm × 15-cm; packing L11
  Flow rate: 0.6 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for ephedrine and pseudoephedrine are approximately 0.9 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2 between the pseudoephedrine and ephedrine peaks
  Tailing factor: NMT 2.0 for the pseudoephedrine peak
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Pseudoephedrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Pseudoephedrine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Sample solution: Proceed as directed in the *Assay*.
  Analysis: Using the chromatogram of the *Sample solution*, calculate the percentage of each impurity in the portion of Pseudoephedrine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria: NMT 2.0%, total impurities

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩: 182°–186°. The range between beginning and end of melting does not exceed 2°.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 50 mg/mL
  Acceptance criteria: +61.0° to +62.5°
- **pH** ⟨791⟩: 4.6–6.0, in a 50-mg/mL solution
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample at 105° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ephedrine Sulfate RS
  USP Pseudoephedrine Hydrochloride RS

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Pseudoephedrine Hydrochloride Extended-Release Capsules

DEFINITION

Pseudoephedrine Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Mix a portion of Capsule contents equivalent to 180 mg of pseudoephedrine hydrochloride, filter with 10 mL of chloroform, and collect using vacuum filtration. Maintain the vacuum until no further filtrate can be collected, and evaporate the chloroform on a steam bath, taking care to avoid overheating. Recrystallize the residue from a small amount of dehydrated alcohol.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Alcohol and 4 mg/mL of ammonium acetate solution (17:3)

Standard solution: 1.2 mg/mL of USP Pseudoephedrine Hydrochloride RS in alcohol

Sample solution: 1.2 mg/mL of pseudoephedrine hydrochloride in 0.01 N hydrochloric acid, prepared by transferring an appropriate amount of the contents of NLT 20 Capsules to a suitable volumetric flask and dissolving in 10% of the flask volume of 0.01 N hydrochloric acid by sonicating for 10 min. Cool to room temperature, dilute with 0.01 N hydrochloric acid to volume, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; packing L3

Flow rate: 0.7 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 3, 6, and 12 h

Standard solution: USP Pseudoephedrine Hydrochloride RS of a known concentration similar to that of the *Sample solution*, in *Medium*

Sample solution: Filtered portion of sample

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved, using the *Mobile phase* and *Chromatographic system* in the *Assay*.

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved
3	20%–50%
6	45%–75%
12	NLT 75%

The percentages of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Pseudoephedrine Hydrochloride RS

Pseudoephedrine Hydrochloride Oral Solution

DEFINITION

Pseudoephedrine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

- **A. MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩

Sample: A volume of Oral Solution equivalent to 120 mg of pseudoephedrine hydrochloride

Analysis: Extract the *Sample* with two 30-mL portions of ether. Discard the extracts, and add 4 mL of 1 N sodium hydroxide. Extract with 30 mL of chloroform, and evaporate the chloroform on a steam bath, avoiding overheating.

Acceptance criteria: The pseudoephedrine so obtained melts at 118°.

- **B.**

Sample solution: 5 mg/mL of pseudoephedrine from the *Sample* in *Identification* test *A* in 0.1 N hydrochloric acid

Acceptance criteria: The *Sample solution* is dextrorotatory.

ASSAY

- **PROCEDURE**

Mobile phase: Alcohol and 0.40% ammonium acetate solution (17:3)

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Standard solution: 1.2 mg/mL of USP Pseudoephedrine Hydrochloride RS in 0.01 N hydrochloric acid
Sample solution: 1.2 mg/mL of pseudoephedrine hydrochloride from an appropriate volume of Oral Solution in 0.01 N hydrochloric acid; filter the solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements for Oral Solution packaged in single-unit containers
• **Deliverable Volume** ⟨698⟩: Meets the requirements for Oral Solution packaged in multiple-unit containers

SPECIFIC TESTS
• **Reaction:** It is acid to litmus.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Pseudoephedrine Hydrochloride RS

Pseudoephedrine Hydrochloride Tablets

DEFINITION
Pseudoephedrine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of pseudo ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Standard solution: 3 mg/mL of USP Pseudoephedrine Hydrochloride RS in water
Sample solution: Shake or mix 1 Tablet in 10 mL of water until the Tablet completely disintegrates. Sonicate for 5 min, centrifuge for 5 min, and pass through a nylon filter.
Chromatographic system
Developing solvent system: Butyl alcohol, glacial acetic acid, and water (8:2:2)
Analysis: Proceed as directed in the chapter.
Acceptance criteria: The R_f value and appearance of the principal spot from the *Sample solution* correspond to that from the *Standard solution*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Alcohol and 0.40% ammonium acetate solution (17:3)
Standard solution: 1.2 mg/mL of USP Pseudoephedrine Hydrochloride RS in 0.01 N hydrochloric acid
Sample solution: Nominally 1.2 mg/mL of pseudoephedrine hydrochloride in 0.01 N hydrochloric acid, prepared by transferring an appropriate amount of the contents of NLT 20 Tablets to a suitable volumetric flask and dissolving in 0.01 N hydrochloric acid by sonicating. Cool to room temperature, dilute with 0.01 N hydrochloric acid to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% from five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Alcohol and 0.40% ammonium acetate solution (17:3)
Standard solution: USP Pseudoephedrine Hydrochloride RS of a known concentration similar to that of the *Sample solution*, in *Medium*
Sample solution: Filtered portion of sample suitably diluted with *Medium*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 214 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 1.5 mL/min
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% from five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Mobile phase: Alcohol and 0.40% ammonium acetate solution (17:3)
Standard solution: 0.15 mg/mL of USP Pseudoephedrine Hydrochloride RS in a mixture of methanol and 0.01 N hydrochloric acid (1:4)
Sample solution: Transfer 1 Tablet to a 100-mL volumetric flask, add 20 mL of methanol, and shake for 30 min. Add 25 mL of 0.01 N hydrochloric acid, and sonicate to dissolve. Cool to room temperature, dilute with 0.01 N hydrochloric acid to volume, and filter.
Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements for chewable tablets

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Pseudoephedrine Hydrochloride RS

Pseudoephedrine Hydrochloride Extended-Release Tablets

DEFINITION

Pseudoephedrine Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Triturate a number of Tablets, nominally equivalent to 180 mg of pseudoephedrine hydrochloride. Filter with about 10 mL of chloroform collected using vacuum filtration. Maintain the vacuum until no further filtrate can be collected, and evaporate the chloroform on a steam bath, taking care to avoid overheating. Recrystallize the residue from a small amount of dehydrated alcohol.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Mobile phase: Alcohol and ammonium acetate solution (1 in 250) (850:150). Filter, and degas
Standard solution: 1.2 mg/mL of USP Pseudoephedrine Hydrochloride RS in alcohol
Sample stock solution: Transfer NLT 20 Tablets to a suitable container, add 500 mL of alcohol, and homogenize until the Tablets are dispersed. Quantitatively transfer the contents of the container to a 1000-mL volumetric flask, dilute with alcohol to volume, mix, and allow to stand for the solids to settle.
Sample solution: Transfer 25.0 mL of the supernatant from the *Sample stock solution* into a 50-mL volumetric flask, dilute with alcohol to volume, and mix. Pass a portion of this solution through a filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; packing L3
Flow rate: 0.7 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

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PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

For Tablets labeled for dosing every 12 h

Test 1

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 1, 3, and 6 h
Mobile phase and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.13 mg/mL of USP Pseudoephedrine Hydrochloride RS in water
Sample solution: Filter a portion of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; packing *L3*
Flow rate: 0.7 mL/min
Injection volume: 50 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved.
Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	25–45
3	50–75
6	NLT 75

The percentages of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 1, 3, and 6 h
Standard solution: A known concentration of USP Pseudoephedrine Hydrochloride RS in *Medium*.
Sample solution: Pass portions of the solution under test through a filter of 0.45-µm pore size, and suitably dilute with *Medium*.
Analysis: Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved by comparing the maximum absorbance of *Sample solution* with that of *Standard solution* at about 214 nm.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	25–45
3	60–80
6	NLT 80

The percentages of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium, Apparatus, and **Times:** Proceed as directed in *Test 1*.
Solution A: Acetonitrile and water (45:55)
Mobile phase: 2.5 g/L of docusate sodium in *Solution A*. Add 1.0 mL of phosphoric acid. Adjust with ammonia water, 25% to a pH of 3.2.
Standard solution: (*L*/900) mg/mL of USP Pseudoephedrine Hydrochloride RS in *Medium*, where *L* is the label claim in mg/Tablet.
Sample solution: Withdraw a portion of the solution under test from each vessel at the specified time point and pass through a suitable filter of 0.45-µm pore size. Replace the portion of solution withdrawn with an equal volume of *Medium* previously equilibrated to 37.0° ± 0.5°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 1.5 times the retention time of pseudoephedrine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the sample withdrawn from the vessel at each time point *i*:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution*

Calculate the percentages of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of pseudoephedrine hydrochloride in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

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Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	27–47
2	3	53–73
3	6	NLT 80

The percentages of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*. ▲(RB 1-Mar-2019)

For Tablets labeled for dosing every 24 h

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.9% sodium chloride in water; 50 mL

Apparatus 7 (see *Drug Release* ⟨724⟩)**:** 30 cycles/min; 2–3 cm amplitude. To prepare the sample, see *Figure 1*.

Times: 2, 8, 14, and 24 h

Solution A: Transfer 200 mL of water to a 1000-mL volumetric flask. Add 3.4 mL of phosphoric acid and 5 mL of triethylamine. Add water to almost 900 mL. Adjust with 1 N sodium hydroxide to a pH of about 6.8, dilute with water to volume, and mix.

Mobile phase: Methanol and *Solution A* (100:900)

System suitability solution: 0.4 mg/mL of USP Pseudoephedrine Hydrochloride RS in water

Sample solution: Solution under test

Standard solution: Known concentrations of USP Pseudoephedrine Hydrochloride RS in water, in a range around the expected concentration of the *Sample solution* at each time interval.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 5-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Sample solution* and *Standard solution*

Measure the major peak responses of the *Standard solution* and *Sample solution*. Construct a calibration curve by plotting the peak response versus concentrations of the *Standard solution*. Determine the amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at each time interval from a linear regression analysis of the calibration curve.

Tolerances: See *Table* ▲4▲ (RB 1-Mar-2019).

Table ▲4▲ (RB 1-Mar-2019)

Time (h)	Amount Dissolved (%)
2	20–35
8	40–65
14	60–90
24	NLT 85

The percentages of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Pseudoephedrine Hydrochloride RS

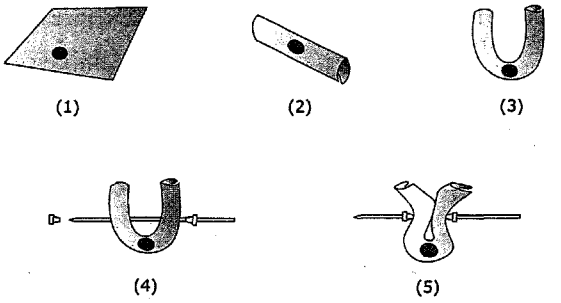


Figure 1. Steps to prepare the sample. (1) Place 1 Tablet on a 5-cm × 5-cm nylon netting. (2) Fold the netting over the Tablet. Continue folding until the Tablet is enclosed in the netting. (3) Fold the netting so that the two open ends meet. The Tablet should be enveloped in the center of the netting. (4) Insert a rod (see Drug Release ⟨724⟩, ▲Figure 5c)▲ (ERR 1-May-2019) through the netting to secure the Tablet. (5) Secure the netting with HPLC plastic ferrules or other appropriate device. Trim the excess netting. Attach each sample holder to the vertically reciprocating sample holder. (Pseudoephedrine Hydrochloride Extended-Release Tablets)

USP Monographs

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Pseudoephedrine Hydrochloride, Carbinoxamine Maleate, and Dextromethorphan Hydrobromide Oral Solution

DEFINITION

Pseudoephedrine Hydrochloride, Carbinoxamine Maleate, and Dextromethorphan Hydrobromide Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$), dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

- **A.** The retention times of the carbinoxamine maleate and dextromethorphan hydrobromide peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay* for *Carbinoxamine Maleate and Dextromethorphan Hydrobromide*.
- **B.** The retention time of the pseudoephedrine hydrochloride peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Pseudoephedrine Hydrochloride*.

ASSAY

- **CARBINOXAMINE MALEATE AND DEXTROMETHORPHAN HYDROBROMIDE**

Buffer: Dissolve about 4.4 g of dibasic potassium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 5.5.

Mobile phase: Methanol and *Buffer* (600:400)

Standard solution: 0.1 mg/mL of carbinoxamine maleate and 0.3 mg/mL of dextromethorphan hydrobromide from USP Carbinoxamine Maleate RS and USP Dextromethorphan Hydrobromide RS in water

Sample solution: Nominally 0.1 mg/mL of carbinoxamine maleate and 0.3 mg/mL of dextromethorphan hydrobromide from a suitable volume of Oral Solution in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; packing L9

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for dextromethorphan and carbinoxamine are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between carbinoxamine and dextromethorphan

Tailing factor: NMT 2.0 for the dextromethorphan peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$) and of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the appropriate analyte from the *Sample solution*

r_S = peak response of the appropriate analyte from the *Standard solution*

C_S = concentration of the appropriate Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the appropriate analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$) and 90.0%–110.0% of the labeled amount of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr$)

- **PSEUDOEPHEDRINE HYDROCHLORIDE**

Buffer: Dissolve about 4.4 g of dibasic potassium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 5.5.

Mobile phase: Methanol and *Buffer* (600:400)

Standard solution: 1.2 mg/mL of pseudoephedrine hydrochloride from USP Pseudoephedrine Hydrochloride RS in water

Sample solution: Nominally 1.2 mg/mL of pseudoephedrine hydrochloride from a suitable volume of Oral Solution in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 4.6-mm × 25-cm; packing L9

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pseudoephedrine from the *Sample solution*

r_S = peak response of pseudoephedrine from the *Standard solution*

C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$)

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers

Acceptance criteria: Meets the requirements

- **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/g, the total combined molds and yeasts count does not exceed 10^1 cfu/g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

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- **pH** ⟨791⟩: 3.0–5.0
- **Alcohol Determination,** *Method II* ⟨611⟩ (if present): 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH) is found.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Carbinoxamine Maleate RS
  USP Dextromethorphan Hydrobromide RS
  USP Pseudoephedrine Hydrochloride RS

Pseudoephedrine Sulfate

$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ 428.54
Benzenemethanol, α-[1-(methylamino)ethyl]-, [*S*-(*R**,*R**)]-, sulfate (2:1) (salt).
(+)-Pseudoephedrine sulfate (2:1) (salt) [7460-12-0].

» Pseudoephedrine Sulfate contains not less than 98.0 percent and not more than 100.5 percent of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Pseudoephedrine Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 500 µg per mL.
Medium: water.
Absorptivities at 257 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: A solution of it responds to the test for *Sulfate* ⟨191⟩.
Melting range, *Class I* ⟨741⟩: between 174° and 179°, but the range between beginning and end of melting does not exceed 2°.
Specific rotation ⟨781S⟩: between +56.0° and +59.0°.
Test solution: 50 mg per mL, in water.
pH ⟨791⟩: between 5.0 and 6.5 in a solution (1 in 20).
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 2.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chloride ⟨221⟩—A 200-mg portion shows no more chloride than corresponds to 0.4 mL of 0.02 N hydrochloric acid (0.14%).

Ordinary impurities ⟨466⟩—
Test solution: alcohol.
Standard solution: alcohol.
Eluant: a mixture of alcohol, glacial acetic acid, and water (10:3:1).
Visualization: Expose the plate for 24 hours to iodine vapors in a pre-equilibrated closed chamber, on the bottom of which there are iodine crystals.

Assay—Dissolve about 150 mg of Pseudoephedrine Sulfate, accurately weighed, in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 42.85 mg of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

Psyllium Hemicellulose

DEFINITION
Psyllium Hemicellulose is the alkali soluble fraction of the husk from *Plantago ovata* Forssk. It consists of a combination of highly substituted arabinoxylan polysaccharides. These polysaccharides are linear chains of xylose units (β-(1→4)-xylan) to which are attached single units of arabinose and additional xylose. Rhamnose, galactose, glucose, and rhamnosyluronic acid residues are also present as minor constituents. It contains NLT 75.0% of dietary soluble fiber, calculated on the dried basis.

IDENTIFICATION
- **A.** The powdered mucilage stains red with ruthenium red TS and lead acetate TS.
- **B.** It meets the requirements in *Specific Tests* for *Swell Volume.*

COMPOSITION
- **Content of Soluble Dietary Fiber**
  Alcohol solution: Transfer 82.0 mL of alcohol to a 100-mL volumetric flask, and dilute with water to volume.
  Buffer solution A: Dissolve 1.95 g of 2-(*N*-morpholino)-ethanesulfonic acid and 1.22 g of tris(hydroxymethyl) aminomethane in 170 mL of water. Adjust with 6 N sodium hydroxide to a pH of 8.2, and dilute with water to 200 mL. [Note—It is important to adjust the pH to 8.2 at 24°. If the *Buffer solution* temperature is 20°, adjust the pH to 8.3; if the temperature is 28°, adjust the pH to 8.1. For deviations at 20°–28°, adjust by interpolation.]
  Acid solution: Prepare 0.561 N hydrochloric acid by mixing 9.35 mL of 6 N hydrochloric acid in 70 mL of water. Dilute with water to 100.0 mL.
  Buffer solution B: Prepare a pH 6.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*).
  Protease solution: Dissolve 5 mg of protease in 0.1 mL of *Buffer solution B*
  Enzyme purity: To ensure the absence of undesirable enzymatic activities and the presence of desirable enzymatic activities, proceed as directed for *Sample solution* and *Analysis* using the substrates listed in *Table 1* in place of Psyllium Hemicellulose.

Table 1

Substrate	Weight (g)	Activity Tested
Pectin	0.2	Pectinase
Arabinogalactan	0.2	Hemicellulase
β-Glucan	0.2	β-Glucanase
Wheat starch	1.0	α-Amylase and amyloglucosidase
Corn starch	1.0	α-Amylase and amyloglucosidase
Casein	0.3	Protease

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The enzyme preparation is suitable if more than 90% of the original weight of pectin, arabinogalactan, and β-glucan is recovered; NMT 2% of the original weight of casein and corn starch is recovered; and NMT 1% of the original weight of wheat starch is recovered. [NOTE—Test the enzyme purity of every new lot of enzyme and at 6-month intervals thereafter.]

Blank solution: Using two 400-mL tall-form beakers, appropriately labeled, proceed as directed for *Analysis* without Psyllium Hemicellulose.

Sample solution: Weigh, in duplicate, approximately 0.2 g of Psyllium Hemicellulose, previously milled to a very fine powder. [NOTE—Duplicates should differ by less than 1 mg in weight.] Transfer duplicate samples to appropriately labeled tall-form 400-mL beakers, and proceed as directed for *Analysis*.

Analysis: Treat each solution in the following manner. Add 40 mL of *Buffer solution A* to the beaker. [NOTE—For the *Sample solution*, stir until the Psyllium Hemicellulose is completely dispersed.]

Add 125 µL of heat-stable α-amylase solution, and stir to ensure uniform mixing. Cover the beaker with aluminum foil, and incubate over a water bath maintained at 95°–100° for 15 min, with continuous agitation. [NOTE—Start timing once the water bath temperature reaches 95°. A total time of 35 min is usually sufficient.]

Remove the beaker from the water bath, and cool to 60°. Remove the aluminum foil, scrape any ring from inside the beaker, and disperse any gels in the bottom of the beaker with a spatula. Rinse the walls of the beaker and the spatula with 10 mL of water, collecting the rinsings in the beaker. Add 500 µL of *Protease solution*. Cover with aluminum foil, and incubate over a water bath maintained at 60 ± 3° for 30 min with continuous agitation. [NOTE—Start timing when the bath temperature reaches 60°.]

Remove the foil, and transfer 5 mL of *Acid solution* while stirring. Adjust, if necessary, with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.28 ± 0.07 at 60°. [NOTE—It is important to adjust the pH to 4.28 while the solution in the beaker is maintained at 60°; otherwise, the pH will increase at lower temperatures.]

Add 150 µL of amyloglucosidase solution while stirring. Cover with aluminum foil, and incubate over a water bath maintained at 60 ± 3° for 30 min with constant agitation. [NOTE—Start timing once the water bath reaches 60°.]

Transfer 40 mL of the beaker contents to a 50-mL centrifuge tube, and sonicate the tube contents for 3 min.[1] Centrifuge at 10,000–14,000 rpm for 10 min. Carefully pour the supernatant into a labeled 600-mL tared beaker. Do not disturb any pellet in the bottom of the centrifuge tube. Add the remaining sample from the original 400-mL beaker into the centrifuge tube still containing the pellet. Rinse the 400-mL beaker with 15–20 mL of water, and add the rinsing to the 50-mL centrifuge tube. Centrifuge the sample at 10,000–14,000 rpm for 10 min. Carefully pour the supernatant into the 600-mL beaker containing the first supernatant. Add 390 mL (measured before heating) of alcohol at 60° to the 600-mL beaker. Cover the beaker, and allow to stand for at least 1 h to form a precipitate.

Place 3 g of chromatographic siliceous earth into a clean air-dried crucible with a fritted disk. Heat the crucible containing chromatographic siliceous earth at 525° in a muffle furnace for at least 4 h. Cool. Pass deionized water through the crucible while applying constant suction. Rinse with acetone, and allow to air-dry. Store the crucible in a convection oven at 130° for at least 2 h before use. Weigh the prepared crucible to 0.1 mg before use. Wet the chromatographic siliceous earth in the crucible using a stream of *Alcohol solution* from a washing bottle, and apply suction to evenly distribute the chromatographic siliceous earth over the fritted disk. Maintaining the suction, transfer the supernatant and precipitate from the beaker to the crucible, and filter. Transfer any solid residue in the beaker with the aid of *Alcohol solution*. [NOTE—In some cases, gums may form during filtration, trapping liquid in the residue. If so, break the surface film with a spatula to improve filtration.]

Wash the residue in the crucible sequentially with 30 mL of *Alcohol solution*, 20 mL of alcohol, and 20 mL of acetone. Dry the crucible containing the residue at 100° in a convection oven for at least 4 h, and cool to room temperature in a desiccator.

Determine the weight of the residue (*R*).

Use one of the duplicate residues from the *Sample solution* and one of the blank residues from the *Blank solution* to determine the protein content, in mg, by placing the residue in a 500-mL Kjeldahl flask, and proceeding as directed for *Nitrogen Determination* ⟨461⟩, *Method I*. The protein content is determined by multiplying the content of nitrogen found by 6.25. Incinerate the residue from the remaining duplicate of the *Sample solution* and the *Blank solution* as directed for *Articles of Botanical Origin* ⟨561⟩, *Total Ash* at a reduced temperature of 525°, and determine the ash content as directed.

Calculate the corrected average weight of the blank (*B*), in mg:

$$B = R_B - P_B - A_B$$

R_B = weight of the average blank residue for duplicate blank determinations (mg)
P_B = content found in the test for protein in the blank (mg)
A_B = content of ash found in the blank (mg)

Calculate the percentage of soluble dietary fiber:

$$\text{Result} = [(R_U - P_U - A_U - B)/W_U] \times 100$$

R_U = weight of average residue for the duplicate *Sample solution* (mg)
P_U = content of protein found in the Psyllium Hemicellulose (mg)
A_U = content of ash found in the Psyllium Hemicellulose (mg)
B = average weight of the blank as calculated above
W_U = average weight of the Psyllium Hemicellulose taken (mg)

Acceptance criteria: NLT 75.0% of dietary soluble fiber on the dried basis

CONTAMINANTS

• LIMIT OF ALCOHOL

Internal standard solution: *n*-Propyl alcohol and water (1:99)

Standard stock solution: 5.0 mL of absolute alcohol at 20 ± 2°, diluted with water to 500.0 mL

Standard solution: 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution*, diluted with water to 100.0 mL

Sample solution: 0.5 g of Psyllium Hemicellulose in 90 mL of water. Stir rapidly for 3 h using a magnetic stirrer. Add

[1] A suitable sonicator is Sonifier 250 (or equivalent), equipped with a 12-mm tip, from Branson Ultrasonic Corp., Danbury, CT, in which an output control value of 3 and a cycle time of 75% generate a power output of 43 W.

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10.0 mL of the *Internal standard solution*. Pass the *Sample solution* through a filter having a 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused silica analytical; coated with 3.0-µm G43 stationary phase. [NOTE—A 0.53-mm × 2-m fused silica guard column may be used.]
Temperature: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	10	230	3

Injector: 250°
Detector: 300°
Carrier gas: Helium
Flow rate: 4 mL/min
Injection size: 0.5 µL
Injection type: Split flow ratio, 10:1

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%, for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alcohol in the portion of Psyllium Hemicellulose taken:

$$\text{Result} = (R_U/R_S) \times C_S \times (V/W) \times 100$$

R_U = ratio of the peak response of alcohol/peak response of *n*-propyl alcohol from the *Sample solution*
R_S = ratio of the peak response of alcohol/peak response of *n*-propyl alcohol from the *Standard solution*
C_S = concentration of alcohol in the *Standard stock solution* (mg/mL)
V = volume of the *Standard stock solution* (mL)
W = weight of Psyllium Hemicellulose taken to prepare the *Sample solution* (mg)

Acceptance criteria: NMT 12.0% (w/w)

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

SPECIFIC TESTS

- **SWELL VOLUME**

Sample: 0.50 g of Psyllium Hemicellulose
Analysis: Add the *Sample* to a glass-stoppered, 100-mL graduated mixing cylinder. To avoid material clumping, hold the cylinder at a 45° angle, and gently rotate it while using a wash bottle to forcefully add 30 mL of water. Add water to bring the total volume to 100 mL, and cap the cylinder. Invert the cylinder several times until a uniform suspension is achieved, and allow to stand. Gently invert the cylinder several times again at 4–8 h after the initial *Sample solution* preparation, and allow to stand. Allow the gel to settle for 16 h. Determine the volume of the gel.
Acceptance criteria: NLT 80 mL/g of Psyllium Hemicellulose

- **TOTAL ACIDITY:** Transfer 40 mL of the supernatant as obtained in the test for *Swell Volume* without disturbing the gel. Titrate with 0.03 N sodium hydroxide, using 1 mL of phenolphthalein TS as indicator.

Acceptance criteria: NMT 1.8 mL of 0.03 N sodium hydroxide is consumed

- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 12.0% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨561⟩**:** NMT 5.0%
- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨561⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

Psyllium Husk

DEFINITION
Psyllium Husk is the cleaned, dried seed coat (epidermis) separated by winnowing and thrashing from the seeds of *Plantago ovata* Forssk., known in commerce as Blond Psyllium, Indian Psyllium, or Ispaghula, or from *Plantago arenaria* Waldst. & Kit. (*Plantago psyllium* L.) known in commerce as Spanish or French Psyllium (Fam. *Plantaginaceae*), in whole or in powdered form.

IDENTIFICATION

- **A.** Mounted in cresol, cells, viewed microscopically, are composed of polygonal prismatic cells having 4–6 straight or slightly wavy walls.
- **B.** Mounted in alcohol and irrigated with water, viewed microscopically, the mucilage in the outer part of the epidermal cells swells rapidly and goes into solution.

IMPURITIES

- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨561⟩**:** NMT 1.0%
- **ARTICLES OF BOTANICAL ORIGIN,** *Limits of Elemental Impurities* ⟨561⟩**:** Meets the requirements
- **ORGANIC IMPURITIES PROCEDURE 1: LIGHT EXTRANEOUS MATTER**

[NOTE—Perform this test in a well-ventilated hood.]
Sample: 99–101 g of Psyllium Husk, weighed to 0.1 g accuracy
Analysis: Transfer the *Sample* to a 1000-mL tall-form beaker. Add 800 mL of trichloroethylene, previously adjusted to a temperature of 24°–26°, and maintain this temperature throughout the test. Stir the husk for 5 s, and allow it to settle while protecting the surface from drafts in the hood. Remove the floating material with a spoon made of 50-mesh screen, and transfer the material to a piece of filter paper in a dish. Stir the husk mixture again, allow it to settle, remove the floating material again, and combine it with the material on the paper. Repeat this procedure until no more material appears on the surface. Dry the removed material with the paper in a hood and then in an oven at 40° for 3 h. Cool to room temperature. Weigh the filter paper with the material. Brush the material off the paper, weigh the paper, and then calculate the percentage of light extraneous matter.
Acceptance criteria: NMT 5%

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• **ORGANIC IMPURITIES PROCEDURE 2: INSECT INFESTATION**
Sample: 25 g
Analysis: Transfer the *Sample* to a 250-mL beaker, add sufficient solvent hexane to saturate, add an additional 75–100 mL of solvent hexane, and allow to stand for 10 min, stirring occasionally with a stirring rod. Wet a sheet of filter paper with alcohol, and filter the mixture with the aid of vacuum. Discard the filtrate. Transfer the residue to the original beaker with the aid of alcohol. Add alcohol to bring the volume to 150 mL above the level of the transferred residue. Boil for 10 min. Filter through alcohol-wetted paper as above. Prepare a trap flask consisting of a 2000-mL graduated, narrow-mouth conical flask into which is inserted a rubber disk supported on a stiff metal rod 4 mm in diameter and longer than the height of the flask, the rod being threaded at the lower end and furnished with nuts and washers to hold the disk in place, and the disk being of the proper shape and size to prevent liquid in the body of the flask from spilling when it is pressed up against the neck from the inside. Transfer the residue to the trap flask, completing the transfer with the aid of hot water. Add sufficient hot water to bring the volume to 1000 mL. Add 20 mL of hydrochloric acid. Raise the rod, and support it so that the rubber disk is held above the liquid level. Rinse the rubber disk with hot water. Spray the inside of the neck of the flask with an antifoam spray. Boil for 30 min, and cool to room temperature. Add 40 mL of solvent hexane, and agitate for 1 min by tilting the flask and moving the rod vertically with wrist action. Allow to stand for 5 min. Add water to bring the level of liquid to the neck of the flask, and allow to stand for 20 min. Simultaneously rotate the disk to free it from settled material, and raise it as far as possible into the neck of the flask. Prepare a sheet of ruled filter paper with lines approximately 5 mm apart for filtration by moistening it with water and placing it on a vacuum funnel. Transfer the material trapped in the neck of the flask to the filter with the aid of water. If necessary, wash the paper with alcohol to remove traces of hexane. Place the paper on a 100-mm petri dish that has been wetted with a solution containing equal volumes of glycerin and alcohol. Add 35 mL of solvent hexane to the flask, and gently stir with the trapping rod. Add water to bring the liquid level into the neck of the flask. Allow to stand for 15 min. Using the same technique as before, transfer the trapped material onto a separate paper. Examine the papers at 30× magnification.
Acceptance criteria
Powdered Psyllium Husk: NMT 400 insect fragments, including mites and psocids
Whole Psyllium Husk: NMT 100 insect fragments, including mites and psocids
• **ARTICLES OF BOTANICAL ORIGIN,** *Pesticide Residue Analysis* ⟨561⟩: Meets the requirements

SPECIFIC TESTS
• **BOTANIC CHARACTERISTICS**
Histology—Husk: The epidermis is composed of large cells having transparent walls filled with mucilage, and the cells swell rapidly in aqueous mounts and appear polygonal to slightly rounded in a surface view when viewed from above (from below they appear elongated to rectangular). The swelling takes place mainly in the radial direction. The mucilage of the epidermal cells stains red with ruthenium red and lead acetate TS. The very occasional starch granules that are present in some of the epidermal cells and that may be found embedded in the mucilage are small and simple or compounded with four or more components.
Powdered psyllium seed husk: It is a pale to medium buff-colored powder having a slight pinkish tinge and a weak characteristic odor. Occasional single and 2- to 4-compound starch granules, the individual grains being spheroidal plano to angular convex from 2–10 µm in diameter, are found embedded in the mucilage. Entire or broken epidermal cells are filled with mucilage. In surface view, the epidermal cells appear polygonal to slightly rounded. Mucilage stains red with ruthenium red TS and lead acetate TS. Some of the elongated and rectangular cells representing the lower part of epidermis and also radially swollen epidermal cells can be found.
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total combined molds and yeasts count does not exceed 1000 cfu/g, and it meets the requirements of the test for absence of *Salmonella* species and *Escherichia coli.*
• **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨561⟩: NMT 4.0%
• **WATER DETERMINATION,** *Method II* ⟨921⟩: NMT 12.0%
• **SWELL VOLUME**
Sample: 3.5 g of Psyllium Husk
Analysis: Transfer 250 mL of simulated intestinal fluid TS without enzymes to a glass-stoppered 500-mL graduated cylinder. Gradually, with shaking, add the *Sample* until a uniform, smooth suspension is obtained. Dilute with the same fluid to 500 mL. Shake the cylinder for 1 min every 30 min for 8 h. Allow the gel to settle for 16 h (total time is 24 h). Determine the volume of the gel.
Acceptance criteria
Powdered Psyllium Husk: NLT 40 mL/g
Whole Psyllium Husk: NLT 35 mL/g

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, secured against insect attack.

Psyllium Hydrophilic Mucilloid for Oral Suspension

DEFINITION
Psyllium Hydrophilic Mucilloid for Oral Suspension is a dry mixture of Psyllium Husk with suitable additives.

IDENTIFICATION
• **A. BOTANIC CHARACTERISTICS**
Microscopic: It shows occasional single and 2- to 4-compound starch granules, the individual grains being spheroidal plano to angular convex, 2–10 um in diameter, found embedded in the mucilage. Entire or broken epidermal cells are filled with mucilage. In surface view, the epidermal cells appear polygonal to slightly rounded. Mucilage stains red with ruthenium red TS and lead acetate TS. Some of the elongated and rectangular cells representing the lower part of epidermis and also radially swollen epidermal cells can be found.

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Salmonella* species and of *Escherichia coli.*
• **SWELL VOLUME**
Sample: Psyllium Hydrophilic Mucilloid for Oral Suspension, equivalent to 3.5 g of psyllium husk
Analysis: Transfer 250 mL of simulated intestinal fluid TS without enzymes to a glass-stoppered, 500-mL graduated cylinder. Gradually, with shaking, add the *Sample,* and shake until a uniform, smooth suspension is obtained. Dilute with the same fluid to 500 mL. Shake the cylinder for

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1 min every 30 min for 8 h. Allow the gel to settle for 16 h (total time, 24 h). Determine the volume of the gel.
Acceptance criteria: NLT 110 mL

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

Pumice

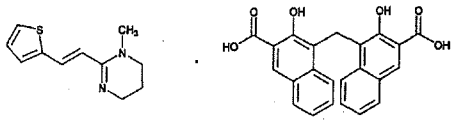
DEFINITION
Pumice is a substance of volcanic origin, consisting chiefly of complex silicates of aluminum, potassium, and sodium.

IMPURITIES
- **IRON**
  Sample solution: The remaining one-half of the filtrate from the test for *Water-Soluble Substances*
  Analysis: Acidify the *Sample solution* with hydrochloric acid, and add a few drops of potassium ferrocyanide TS.
  Acceptance criteria: No blue color is produced.

SPECIFIC TESTS
- **WATER-SOLUBLE SUBSTANCES**
  Sample: 10 g
  Analysis: Boil the *Sample* with 50 mL of water for 30 min, adding water from time to time to maintain approximately the original volume, and then filter. The filtrate is neutral to litmus. Evaporate one-half of this filtrate, dry at 105° for 1 h, and weigh the residue.
  Acceptance criteria: NMT 10 mg (NMT 0.20%)
- **ACID-SOLUBLE SUBSTANCES**
  Sample: 1 g
  Analysis: Boil the *Sample* with 25 mL of 3 N hydrochloric acid for 30 min, adding water from time to time to maintain approximately the original volume, then filter. Add five drops of sulfuric acid to the filtrate, evaporate to dryness, ignite, and weigh the residue.
  Acceptance criteria: NMT 60 mg (NMT 6.0%)
- **POWDERED PUMICE**
  Pumice flour or **superfine pumice:** NLT 97.0% of pumice flour or superfine pumice passes through a No. 200 standard mesh sieve.
  Fine pumice: NLT 95.0% of fine pumice passes through a No. 150 standard mesh sieve, and NMT 75.0% passes through a No. 200 standard mesh sieve.
  Coarse pumice: NLT 95.0% of coarse pumice passes through a No. 60 standard mesh sieve, and NMT 5.0% passes through a No. 200 standard mesh sieve.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label powdered Pumice to indicate, in descriptive terms, the fineness of the powder.

Pyrantel Pamoate



$C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6$ 594.68

Pyrimidine, 1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl) ethenyl]-, (*E*)-, compd. with 4,4′-methylenebis[3-hydroxy-2-naphthalenecarboxylic acid] (1:1);
(*E*)-1,4,5,6-Tetrahydro-1-methyl-2-[2-(2-thienyl)vinyl] pyrimidine 4,4′-methylenebis[3-hydroxy-2-naphthoate] (1:1) [22204-24-6].

DEFINITION
Pyrantel Pamoate contains NLT 97.0% and NMT 103.0% of $C_{34}H_{30}N_2O_6S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy:* **197K▲** (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy:* **197U▲** (CN 1-May-2020)
  Solution: 16 µg/mL in methanol
- **C.** The retention time of the major peaks due to pyrantel base and pamoic acid of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  [NOTE—Use low-actinic glassware in preparing solutions of pyrantel pamoate, or otherwise protect the solutions from unnecessary exposure to bright light. Complete the *Assay* without prolonged interruption.]
  Mobile phase: Acetonitrile, glacial acetic acid, diethylamine, and water (92.8: 3: 1.2: 3)
  [NOTE—Increasing the amount of acetonitrile in the *Mobile phase* increases retention times. Increasing the amount of acetic acid, water, and diethylamine decreases retention times. Should the *Mobile phase* need to be adjusted, maintain the ratios among acetic acid, diethylamine, and water (1: 0.4: 1).]
  Standard solution: 80 µg/mL of USP Pyrantel Pamoate RS in *Mobile phase*
  Sample solution: 80 µg/mL of Pyrantel Pamoate in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 288 nm
  Column: 4.6-mm × 25-cm; packing L3
  Flow rate: 1 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for pamoic acid and pyrantel are 0.6 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 10.0 between pyrantel and pamoic acid
  Column efficiency: NLT 8000 theoretical plates for the pyrantel peak
  Tailing factor: NMT 1.3 for the pyrantel peak
  Relative standard deviation: NMT 1.0% for the pyrantel peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Record the chromatograms obtained for a period of NLT 2.5 times the retention times of pyrantel, and measure the responses for the major peaks.

USP Monographs

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Calculate the percentage of $C_{34}H_{30}N_2O_6S$ in the portion of Pyrantel Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for pyrantel from the *Sample solution*
r_S = peak response for pyrantel from the *Standard solution*
C_S = concentration of USP Pyrantel Pamoate RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

OTHER COMPONENTS

• **CONTENT OF PAMOIC ACID**

Mobile phase: Acetonitrile, glacial acetic acid, diethylamine, and water (92.8: 3: 1.2: 3)

[NOTE—Increasing the amount of acetonitrile in the *Mobile phase* increases retention times. Increasing the amount of acetic acid, water, and diethylamine decreases retention times. Should the *Mobile phase* need to be adjusted, maintain the ratios among acetic acid, diethylamine, and water (1: 0.4: 1).]

Standard solution: 52 µg/mL of USP Pamoic Acid RS in *Mobile phase*

Sample solution: 80 µg/mL of Pyrantel Pamoate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 288 nm

Column: 4.6-mm × 25-cm; packing L3

Flow rate: 1 mL/min

Injection size: 20 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{23}H_{16}O_6$ in the portion of Pyrantel Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for pamoic acid from the *Sample solution*
r_S = peak response for pamoic acid from the *Standard solution*
C_S = concentration of USP Pamoic Acid RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 63.4%–67.3% of pamoic acid, calculated on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Limit of Iron** ⟨241⟩

Analysis: To the residue obtained in the test for *Residue on Ignition*, add hydrochloric acid and nitric acid (3:2), and evaporate on a steam bath to dryness. Dissolve the residue in 2 mL of hydrochloric acid with the aid of gentle heat. Add 18 mL of hydrochloric acid, dilute with water to 50 mL, and mix. Dilute 5 mL of this solution with water to 47 mL.

Acceptance criteria: The limit is 0.0075%.

• **Residue on Ignition** ⟨281⟩: NMT 0.5%

Sample: 1.33 g

ORGANIC IMPURITIES

• **Procedure**

Diluent: Glacial acetic acid, diethylamine, and water (5:2:5)

Mobile phase: Acetonitrile and *Diluent* (92.8: 7.2).

[NOTE—Prepare the solutions immediately before use, and protect from light at all stages.]

Stock standard solution: 0.8 mg/mL of USP Pyrantel Pamoate RS. Dissolve in *Diluent*, using 7% of the final volume, and diluting with acetonitrile to volume.

Standard solution: 4 µg/mL of USP Pyrantel Pamoate RS in *Mobile phase* from the *Stock standard solution*

System suitability solution: Dissolve a quantity of USP Pyrantel Related Compound A RS first in the *Stock standard solution*, using 5% of the final volume. Dilute with *Diluent* to volume to obtain a solution with a concentration of 0.2 mg/mL of pyrantel related compound A and 40 µg/mL of pyrantel pamoate. Transfer a suitable portion of this solution, and dilute quantitatively with *Diluent* to obtain a solution with a final concentration of about 4 µg/mL of pyrantel related compound A and 0.8 µg/mL of pyrantel pamoate.

Sample solution: 0.8 mg/mL of pyrantel pamoate prepared by dissolving in *Diluent*, using 7% of the final volume, and diluting with acetonitrile to volume

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 288 nm

Column: 4.6-mm × 25-cm; packing L3

Flow rate: 1.0 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between pyrantel and pyrantel related compound A

Tailing factor: NMT 1.5 for the pyrantel peak

Relative standard deviation: NMT 10.0% for the pyrantel peak

Analysis

Samples: *Standard solution*, *System suitability solution*, and *Sample solution*

[NOTE—Record the chromatograms for about 4 times the retention time of pyrantel. Disregard any peak in the *Sample solution* having an area less than one-tenth the principal peak in the *Standard solution*.]

Calculate the percentage of pyrantel related compound A in the portion of Pyrantel Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for pyrantel related compound A from the *Sample solution*
r_S = peak response for pyrantel related compound A from the *System suitability solution*
C_S = concentration of USP Pyrantel Related Compound A RS in the *System suitability solution* (µg/mL)
C_U = concentration of pyrantel pamoate in the *Sample solution* (µg/mL)

Calculate the percentage of *Impurity B* or any other unspecified impurity in the portion of Pyrantel Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for impurity B or any individual unspecified impurity from the *Sample solution*
r_S = peak response for pyrantel from the *Standard solution*
C_S = concentration of USP Pyrantel Pamoate RS in the *Standard solution* (µg/mL)

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C_U = concentration of pyrantel pamoate in the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pamoic acid[a]	0.5	—	—
Pyrantel(*E*-isomer)	1.0	—	—
Pyrantel related compound A[b]	1.3	—	0.5
Impurity B[c]	1.8	2.5	0.2
Individual unspecified impurities	—	1.0	0.1
Total unspecified impurities	—	—	0.3

[a] For identification purposes only.
[b] 1-Methyl-2-[(*Z*)-2-(thiophen-2-yl)ethenyl]-1,4,5,6-tetrahydropyrimidine.
[c] (*E*)-*N*-[3-(Methylamino)propyl]-3-(thiophen-2-yl)prop-2-enamide.

SPECIFIC TESTS
- **Loss on Drying** ⟨731⟩: Dry a sample in a vacuum at 60° for 3 h: it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Pamoic Acid RS
  $C_{23}H_{16}O_6$ 388.38
  USP Pyrantel Pamoate RS
  USP Pyrantel Related Compound A RS
  (*Z*)-1-Methyl-2-(2-(thiophen-2-yl)vinyl)-1,4,5,6-tetrahydropyrimidine.
  $C_{34}H_{30}N_2O_6S$ 594.69

Pyrantel Pamoate Oral Suspension

» Pyrantel Pamoate Oral Suspension is a suspension of Pyrantel Pamoate in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pyrantel ($C_{11}H_{14}N_2S$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Pyrantel Pamoate RS
Identification—[See *Note* in the *Assay*.]
A: Dilute a suitable volume of Oral Suspension with 0.05 N methanolic ammonium hydroxide to obtain a solution having a concentration of about 8 mg of pyrantel pamoate per mL. Similarly prepare a Standard solution of USP Pyrantel Pamoate RS. Shake both solutions by mechanical means, and centrifuge to obtain clear solutions. Apply a 100-µL portion of each solution to a 20- × 20-cm thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.50-mm layer of silica gel mixture. Develop the plate in a suitable chromatographic chamber containing the upper phase obtained by shaking together methyl isobutyl ketone, formic acid, and water (2:1:1). Develop the plate until the solvent front is about 2 cm from the top edge of the plate. Remove the plate, allow the solvent to evaporate, and examine the plate under UV light at about 365 nm: the R_F value of the principal spot from the test solution corresponds to that obtained from the Standard solution.
B: [Note—Prepare 0.05 N methanolic ammonium hydroxide by transferring 0.8 mL of ammonium hydroxide to a 250-mL volumetric flask containing 100 mL of methanol, diluting with methanol to volume, and mixing.] Dilute a suitable volume of Oral Suspension with 0.05 N methanolic ammonium hydroxide to obtain a solution having a concentration of about 16 mg of pyrantel pamoate per mL. Similarly prepare a Standard solution of USP Pyrantel Pamoate RS. Shake both solutions by mechanical means, and centrifuge to obtain clear solutions. Apply a 20-µL portion of each solution to an 18- × 24-cm sheet of chromatographic paper (Whatman No. 1 or equivalent) that previously has been prepared as follows. Impregnate the paper with a freshly prepared solution obtained by mixing 7 volumes of acetone and 3 volumes of glycine-sodium chloride-hydrochloric acid buffer solution (prepared by mixing 3 volumes of a solution that is 0.3 M with respect to both glycine and sodium chloride with 7 volumes of 0.3 M hydrochloric acid). Press the impregnated paper uniformly between white, nonfluorescent blotters to remove the excess solvent. Place the spotted chromatographic paper in a suitable chromatographic chamber, and develop by descending chromatography (see *Chromatography* ⟨621⟩), using as the solvent system the upper phase obtained by mixing ethyl acetate, butanol, and water (10:1:1). After developing for 20 hours, remove the paper from the chamber, air-dry for 10 minutes, transfer to an air-circulating oven, and dry at 60° for 30 minutes: the R_F value of the principal spot from the solution under test corresponds to that obtained from the Standard solution.
C: The retention times of the major peaks due to pyrantel base and pamoic acid in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Uniformity of dosage units ⟨905⟩—
FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.
pH ⟨791⟩: between 4.5 and 6.0.
Assay— [Note—Use low-actinic glassware in preparing solutions of pyrantel pamoate, and otherwise protect the solutions from unnecessary exposure to bright light. Complete the assay without prolonged interruption.]
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Pyrantel Pamoate.*
Assay preparation—Transfer by means of a pipet an accurately measured volume of Oral Suspension, equivalent to about 200 mg of pyrantel pamoate, into a 100-mL volumetric flask, disperse, and dilute with water to volume. While stirring the dispersion with a magnetic stirrer, transfer 1.0 mL of the aliquot to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, mix, and filter.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms obtained for a

USP Monographs

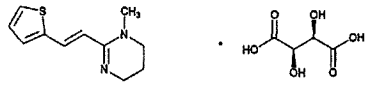
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period of not less than 2.5 times the retention times of pyrantel base, and measure the responses for the major peaks. The relative retention times for pamoic acid and pyrantel base are about 0.6 and 1.0, respectively. Calculate the quantity, in mg, of pyrantel ($C_{11}H_{14}N_2S$) in each mL of the Oral Suspension taken by the formula:

$$2500(0.347)(C/V)(r_U/r_S)$$

in which 0.347 is the ratio of the molecular weight of pyrantel to that of pyrantel pamoate; C is the concentration, in mg per mL, of USP Pyrantel Pamoate RS in the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken; and r_U and r_S are the peak responses for pyrantel base obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pyrantel Tartrate



$C_{11}H_{14}N_2S \cdot C_4H_6O_6$ 356.39

Pyrimidine, 1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl) ethenyl]-, (*E*)-, (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1); (*E*)-1,4,5,6-Tetrahydro-1-methyl-2-[2-(2-thienyl)vinyl] pyrimidine tartrate (1:1) [33401-94-4].

DEFINITION

Pyrantel Tartrate contains NLT 98.0% and NMT 102.0% of pyrantel tartrate ($C_{11}H_{14}N_2S \cdot C_4H_6O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
  [NOTE—Concomitantly prepare potassium bromide dispersions of the Standard and sample. Differences between sets of Standard/sample spectra may occur as a result of differing specimen preparation or environmental conditions.]
- **B. IDENTIFICATION TESTS—GENERAL, *Tartrate* ⟨191⟩**
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  [NOTE—Protect all solutions containing Pyrantel Tartrate from light.]
  Mobile phase: Acetonitrile, glacial acetic acid, diethylamine, and water (94: 2.5: 1: 2.5)
  Standard solution: 0.42 mg/mL of USP Pyrantel Tartrate RS in *Mobile phase*
  Sample solution: 0.42 mg/mL of Pyrantel Tartrate in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 316 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L3
  Flow rate: 1 mL/min
  Injection volume: 5 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of pyrantel tartrate ($C_{11}H_{14}N_2S \cdot C_4H_6O_6$) in the portion of Pyrantel Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pyrantel Tartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Pyrantel Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**
  [NOTE—Protect all solutions containing Pyrantel Tartrate from light.]
  Mobile phase: Acetonitrile, glacial acetic acid, diethylamine, and water (94: 2.5: 1: 2.5)
  Standard solution: 0.84 µg/mL of USP Pyrantel Tartrate RS in *Mobile phase*
  Sample solution: 0.42 mg/mL of Pyrantel Tartrate in *Mobile phase*
  System suitability solution: Expose a portion of the *Sample solution* to short-wavelength UV light for 30 min (Pyrantel Tartrate undergoes partial degradation to *cis*-pyrantel). A degradation level to at least 0.3% of *cis*-pyrantel must be obtained, as shown by the appearance of a corresponding peak in the chromatogram. If it is not obtained, again expose the solution to short-wavelength UV light.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 236 and 316 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L3
  Flow rate: 1 mL/min
  Run time: 2.5 times the retention time of pyrantel
  Injection volume: 100 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for pyrantel and *cis*-pyrantel are 1.0 and 1.2, respectively.]
  Suitability requirements
  System suitability requirements must be met at both 236 and 316 nm.
  Resolution: NLT 1.5 between pyrantel and *cis*-pyrantel
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of each impurity detected at 236 nm in the portion of Pyrantel Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity detected at 236 nm from the *Sample solution*
r_S = peak response detected at 236 nm from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

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Calculate the percentage of each impurity detected at 316 nm in the portion of Pyrantel Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

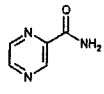
r_U = peak response of each individual impurity detected at 316 nm from the *Sample solution*
r_S = peak response detected at 316 nm from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria
Any individual impurity: NMT 0.2% of any individual impurity detected at 236 nm; NMT 0.2% of any individual impurity detected at 316 nm
Total impurities: NMT 1.0% of total impurities detected at 236 nm; NMT 1.0% of total impurities detected at 316 nm

SPECIFIC TESTS
- **pH** ⟨791⟩
Sample solution: 10.0 mg/mL in carbon dioxide-free water
Acceptance criteria: 3.3–3.7
- **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers protected from light, and store at room temperature.
- **Labeling:** Label it to indicate that it is for veterinary use only.
- **USP Reference Standards** ⟨11⟩
USP Pyrantel Tartrate RS

Pyrazinamide



$C_5H_5N_3O$ 123.11
Pyrazinecarboxamide.
Pyrazinecarboxamide [98-96-4].

» Pyrazinamide contains not less than 99.0 percent and not more than 101.0 percent of $C_5H_5N_3O$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Pyrazinamide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: water.
Absorptivities at 268 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.
C: Boil 20 mg with 5 mL of 5 N sodium hydroxide: the odor of ammonia is perceptible.
Melting range ⟨741⟩: between 188° and 191°.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Residue on ignition ⟨281⟩: not more than 0.1%.
Assay—Place about 300 mg of Pyrazinamide, accurately weighed, in a 500-mL Kjeldahl flask, dissolve in 100 mL of water, and add 75 mL of 5 N sodium hydroxide. Connect the flask by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 20 mL of boric acid solution (1 in 25) contained in a suitable receiver. Boil gently for 20 minutes, avoiding insofar as possible distilling any of the liquid, then boil vigorously to complete the distillation of the ammonia. Cool the liquid in the receiver if necessary, add methyl purple TS, and titrate with 0.1 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N hydrochloric acid is equivalent to 12.31 mg of $C_5H_5N_3O$.

Pyrazinamide Compounded Oral Suspension

DEFINITION
Pyrazinamide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of pyrazinamide ($C_5H_5N_3O$).
Prepare Pyrazinamide Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Pyrazinamide tablets[a] equivalent to	1 g of pyrazinamide
Vehicle: a 1:1 mixture of Ora-Sweet[b] (regular or sugar-free) and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Pyrazinamide 500-mg tablets, Mikart Inc., Atlanta, GA.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Pyrazinamide tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a pyrazinamide liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY
- **Procedure**
Solution A: 10 mM monobasic sodium phosphate, adjusted with phosphoric acid to a pH of 3.5
Mobile phase: Acetonitrile and *Solution A* (10:90). Filter and degas.
Standard stock solution: 1.0 mg/mL of USP Pyrazinamide RS in methanol
Standard solution: Pipet 1.0 mL of the *Standard stock solution* into a 10-mL volumetric flask, and dilute with

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methanol to volume to obtain a solution with a nominal concentration of 0.1 mg/mL of pyrazinamide.
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of sample into a 100-mL volumetric flask, and dilute with methanol to volume to obtain a solution having a nominal concentration of 0.1 mg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for pyrazinamide is about 6.5 min.]
Suitability requirements
Column efficiency: NLT 8000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pyrazinamide ($C_5H_5N_3O$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pyrazinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pyrazinamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.9–5.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Pyrazinamide RS

Pyrazinamide Tablets

» Pyrazinamide Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of pyrazinamide ($C_5H_5N_3O$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Pyrazinamide RS

Identification—
A: To a quantity of powdered Tablets, equivalent to about 1 g of pyrazinamide, add about 75 mL of isopropyl alcohol, heat on a steam bath, and filter while hot. Allow to cool, filter the crystals that form, and dry at 105° for 1 hour: the IR absorption spectrum of a mineral oil dispersion of the dried crystals so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Pyrazinamide RS. If a difference appears, dissolve portions of both the dried crystals and the Reference Standard in acetone, evaporate the solutions to dryness, and repeat the test on the residues.
B: The dried crystals obtained in *Identification* test *A* meet the requirements for *Identification* test *B* under *Pyrazinamide*.
C: To 20 mg of the dried crystals obtained in *Identification* test *A* add 5 mL of 5 N sodium hydroxide, and heat gently over an open flame: the odor of ammonia is perceptible.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_5H_5N_3O$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 268 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Pyrazinamide RS in the same *Medium*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_5H_5N_3O$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase—Prepare pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and adjust with phosphoric acid to a pH of 3.0. Mix 10 mL of acetonitrile with 1 L of this solution, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer an accurately weighed quantity of USP Pyrazinamide RS to a suitable volumetric flask, dissolve in water, sonicating to dissolve, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.1 mg per mL. Transfer 20.0 mL of the solution to a 50-mL volumetric flask, dilute with water to volume, and mix.
System suitability solution—Transfer 1 mL of hydrochloric acid to a 5-mL volumetric flask, dilute with *Standard preparation* to volume, and mix. Keep this solution on a boiling water bath for 5 minutes, and cool.
Assay preparation—Accurately weigh not fewer than 20 Tablets, and grind to a fine powder. Transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of pyrazinamide, to a 500-mL volumetric flask, add 300 mL of water, and sonicate for 10 minutes. Dilute with water to volume, and mix. Filter a portion of this solution, discarding the first few mL of the filtrate. Transfer 20.0 mL of this filtrate to a 100-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2500 theoretical plates; and the tailing factor for the pyrazinamide peak is not more than 1.3. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about

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0.45 for pyrazinoic acid and 1.0 for pyrazinamide; and the resolution, *R*, between pyrazinamide and pyrazinoic acid is not less than 6.0.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of pyrazinamide ($C_5H_5N_3O$) in the portion of Tablets taken by the formula:

$$2.5C(r_U/r_S)$$

in which *C* is the concentration, in µg, of USP Pyrazinamide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pyrethrum Extract

DEFINITION

Pyrethrum Extract is a mixture of three naturally occurring, closely related insecticidal esters of chrysanthemic acid (pyrethrins I: jasmolin I, cinerin I, and pyrethrin I) and three closely related esters of pyrethric acid (pyrethrins II: jasmolin II, cinerin II, and pyrethrin II). It contains NLT 90.0% and NMT 110.0% of the labeled amount of pyrethrins (sum of pyrethrins I and pyrethrins II). The ratio of pyrethrins I to pyrethrins II in the Extract is NLT 0.8 and NMT 2.8. It may contain pigments characteristic of chrysanthemum species, triglyceride oils, terpenoids, and carotenoid. It may also contain suitable solvents and antioxidants. It contains no other added substances.

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Hexanes and tetrahydrofuran (97.75: 2.25)

Standard solution: 0.5 mg/mL of USP Pyrethrum Extract RS in hexanes

Sample solution: Nominally 0.5 mg/mL of pyrethrins from Extract in hexanes

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L10

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

For relative retention times, see *Table 1*.

Table 1

Name	Relative Retention Time
Jasmolin I	0.78–0.80
Cinerin I	0.86–0.87
Pyrethrin I	1.0
Jasmolin II	1.95–2.15
Cinerin II	2.15–2.4
Pyrethrin II	2.55–2.88

Suitability requirements

Resolution: NLT 2.0 between individual pyrethrin peaks

Relative standard deviation: NMT 1.0%

Analysis 1

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pyrethrins (sum of pyrethrins I and pyrethrins II) in the portion of Extract taken:

$$\text{Result} = \{\Sigma[(r_U/r_S) \times (C_S/C_U)]\} \times 100$$

r_U = peak response of individual pyrethrin from the *Sample solution*

r_S = peak response of individual pyrethrin from the *Standard solution*

C_S = concentration of individual pyrethrin in the *Standard solution* (mg/mL)

C_U = nominal concentration of pyrethrins in the *Sample solution* (mg/mL)

Acceptance criteria 1: 90.0%–110.0%

Analysis 2

Sample: *Sample solution*

Calculate the ratio of pyrethrins I to pyrethrins II in the portion of Extract taken:

$$\text{Result} = A/B$$

A = sum of the quantities of pyrethrins I

B = sum of the quantities of pyrethrins II

Acceptance criteria 2: 0.8–2.8

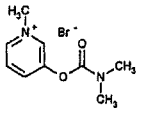
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Pyrethrum Extract RS

Pyridostigmine Bromide



$C_9H_{13}BrN_2O_2$ 261.12

Pyridinium, 3-[[(dimethylamino)carbonyl]oxy]-1-methyl-, bromide;

3-Hydroxy-1-methylpyridinium bromide dimethylcarbamate [101-26-8].

DEFINITION

Pyridostigmine Bromide contains NLT 98.0% and NMT 102.0% of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Bromide*

Sample solution: 20 mg/mL of Pyridostigmine Bromide
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 4.3 g/L of sodium dodecyl sulfate in water. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: Acetonitrile and *Buffer* (32:68)
System suitability solution: 2 µg/mL each of USP Pyridostigmine Bromide RS, USP Pyridostigmine Related Compound A RS, and USP Pyridostigmine Related Compound B RS in *Mobile phase*
Standard solution: 0.3 mg/mL of USP Pyridostigmine Bromide RS in *Mobile phase*
Sample solution: 0.3 mg/mL of Pyridostigmine Bromide in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2.6 times the retention time of pyridostigmine
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between pyridostigmine related compound A and pyridostigmine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$) in the portion of Pyridostigmine Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Pyridostigmine Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Pyridostigmine Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.2 µg/mL of USP Pyridostigmine Bromide RS in *Mobile phase*
Standard solution: 0.6 µg/mL each of USP Pyridostigmine Bromide RS, USP Pyridostigmine Related Compound A RS, and USP Pyridostigmine Related Compound B RS in *Mobile phase*
Sample solution: 1000 µg/mL of Pyridostigmine Bromide in *Mobile phase*
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between pyridostigmine related compound A and pyridostigmine, *Standard solution*
Relative standard deviation: NMT 5.0% for all the three peaks, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pyridostigmine related compound A and pyridostigmine related compound B in the portion of Pyridostigmine Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pyridostigmine related compound A or pyridostigmine related compound B from the *Sample solution*
r_S = peak response of pyridostigmine related compound A or pyridostigmine related compound B from the *Standard solution*
C_S = concentration of USP Pyridostigmine Related Compound A RS or USP Pyridostigmine Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of Pyridostigmine Bromide in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified impurity in the portion of Pyridostigmine Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of pyridostigmine from the *Standard solution*
C_S = concentration of USP Pyridostigmine Bromide RS in the *Standard solution* (µg/mL)
C_U = concentration of Pyridostigmine Bromide in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.02%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyridostigmine related compound B	0.78	0.06
Pyridostigmine related compound A	0.92	0.06
Pyridostigmine	1.0	—
Any individual unspecified impurity	—	0.06
Total impurities	—	0.5

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SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 100° for 4 h in a suitable vacuum drying tube, using phosphorus pentoxide as the desiccant.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Pyridostigmine Bromide RS
USP Pyridostigmine Related Compound A RS
Pyridin-3-yl dimethylcarbamate.
$C_8H_{10}N_2O_2$ 166.18
USP Pyridostigmine Related Compound B RS
3-Hydroxy-1-methylpyridin-1-ium bromide.
C_6H_8BrNO 190.04

Pyridostigmine Bromide Injection

» Pyridostigmine Bromide Injection is a sterile solution of Pyridostigmine Bromide in a suitable medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_9H_{13}BrN_2O_2$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Pyridostigmine Bromide RS

Identification—
A: The solution prepared for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Pyridostigmine Bromide RS, concomitantly measured.
B: To 2 mL of Injection add 1 mL of 2 N nitric acid: the solution so obtained responds to the tests for *Bromide* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 17.0 USP Endotoxin Units per mg of pyridostigmine bromide.
pH ⟨791⟩: between 4.5 and 5.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer to a suitable separator an accurately measured volume of Injection, equivalent to about 20 mg of pyridostigmine bromide. Add 10 mL of 1 N hydrochloric acid, and mix. Extract with four 20-mL portions and one 15-mL portion of ethyl ether, and discard the extracts. Transfer the aqueous layer to a 100-mL volumetric flask, using about 25 mL of water to aid the transfer. Place the flask on a steam bath and warm, with the aid of a stream of nitrogen, to evaporate any residual ether, then cool the flask to room temperature, dilute with water to volume, and mix. Dilute 20.0 mL of the resulting solution with 0.1 N hydrochloric acid to 100.0 mL, and mix. Dissolve an accurately weighed quantity of USP Pyridostigmine Bromide RS in 0.1 N hydrochloric acid to obtain a Standard solution having a known concentration of about 40 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 269 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_9H_{13}BrN_2O_2$ in each mL of the Injection taken by the formula:

$$(0.5C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Pyridostigmine Bromide RS in the Standard solution, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Pyridostigmine Bromide Oral Solution

» Pyridostigmine Bromide Oral Solution contains, in each 100 mL, not less than 1.08 g and not more than 1.32 g of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Pyridostigmine Bromide RS
Identification—To 5 mL of Oral Solution in a separator add 100 mL of 2.5 N hydrochloric acid, and mix. Extract with five 20-mL portions of chloroform, place 2 mL of the aqueous solution in a 50-mL volumetric flask, and add water to volume: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Pyridostigmine Bromide RS, concomitantly measured.

Assay—
Phosphate solution—Dissolve 38 g of monobasic sodium phosphate and 2 g of anhydrous dibasic sodium phosphate in water to make 1000 mL. Adjust the pH, if necessary, by slight variation of the ratio of the two ingredients, to 5.3 ± 0.1.
Bromocresol green solution—Dissolve 250 mg of bromocresol green sodium salt in 250 mL of *Phosphate solution.*
Standard preparation—Dissolve a suitable quantity of USP Pyridostigmine Bromide RS, accurately weighed, in water, to obtain a solution having a known concentration of about 1.2 mg per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 120 mg of pyridostigmine bromide, to a 100-mL volumetric flask, add water to volume, and mix.
Procedure—Transfer 10 mL each, accurately measured, of the *Assay preparation* and the *Standard preparation* to separate 125-mL separators. Into each separator pipet 10 mL of water, 20 mL of *Phosphate solution,* and 5 mL of *Bromocresol green solution.* Extract each solution with six 15-mL portions of chloroform, collecting the chloroform extracts in respective 100-mL volumetric flasks, then add chloroform to volume in each flask, and mix. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 415 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$) in the portion of Oral Solution taken by the formula:

$$100C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Pyridostigmine Bromide RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Pyridostigmine Bromide Tablets

DEFINITION

Pyridostigmine Bromide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Bromide*

Sample solution: Shake a quantity of finely powdered Tablets, equivalent to 100 mg of pyridostigmine bromide, with 20 mL of water for 5 min, and filter the mixture. Use the filtrate.

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 1 g of sodium 1-heptanesulfonate in 500 mL of water in a 1000-mL volumetric flask, and add 5.0 mL of triethylamine and 100 mL of acetonitrile. Dilute with water to volume, and mix. Adjust with phosphoric acid to a pH of 3.0.

Diluent: Mix 11.2 g of phosphoric acid with 500 mL of water, and adjust with a 50% sodium hydroxide solution to a pH of 7.0. Dilute with water to 1000 mL.

Standard solution: 0.25 mg/mL of USP Pyridostigmine Bromide RS in *Diluent*

Sample solution: Nominally 0.25 mg/mL of pyridostigmine bromide prepared as follows. Finely powder NLT 20 Tablets and transfer a portion of the powder, equivalent to about 50 mg of pyridostigmine bromide, to a suitable volumetric flask. Add about 50% of the flask volume of *Diluent*, and shake for 30 min. Dilute with *Diluent* to volume, mix, and centrifuge. Use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4-mm × 30-cm; packing *L1*

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pyridostigmine Bromide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pyridostigmine bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: USP Pyridostigmine Bromide RS in *Medium* at a known concentration approximately the same as that of the *Sample solution*

Sample solution: Dilute with *Medium* and filter to obtain a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 270 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Pyridostigmine Bromide RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim of pyridostigmine bromide (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: 4.3 g/L of sodium dodecyl sulfate in water. Adjust with phosphoric acid to a pH of 2.0.

Mobile phase: *Acetonitrile* and *Solution A* (30:70)

System suitability solution: 5 µg/mL each of USP Pyridostigmine Bromide RS and USP Pyridostigmine Related Compound A RS in *Mobile phase*

Sensitivity solution: 0.4 µg/mL of USP Pyridostigmine Bromide RS in *Mobile phase*

Standard solution 1: 0.005 mg/mL of USP Pyridostigmine Bromide RS in *Mobile phase*

Standard solution 2: 0.06 mg/mL of USP Pyridostigmine Bromide RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of pyridostigmine bromide prepared as follows. Transfer a portion of powdered Tablets equivalent to 100 mg of pyridostigmine bromide to a suitable volumetric flask with 100 mL of *Mobile phase*. Shake for 30 min, and pass a portion of the solution through a glass fiber filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L1*

Flow rate: 1.1 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of pyridostigmine

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution 1*

[NOTE—See *Table 1* for the relative retention times.]

System suitability requirements

Resolution: NLT 1.5 between pyridostigmine and pyridostigmine related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution 1*

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Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of pyridostigmine related compound A and any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pyridostigmine related compound A or any individual unspecified degradation product from the *Sample solution*

r_S = peak response of pyridostigmine from *Standard solution 1*

C_S = concentration of USP Pyridostigmine Bromide RS in *Standard solution 1* (mg/mL)

C_U = nominal concentration of pyridostigmine in the *Sample solution* (mg/mL)

Calculate the percentage of pyridostigmine related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pyridostigmine related compound B from the *Sample solution*

r_S = peak response of pyridostigmine from *Standard solution 2*

C_S = concentration of USP Pyridostigmine Bromide RS in *Standard solution 2* (mg/mL)

C_U = nominal concentration of pyridostigmine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak below 0.04%.

Table 1

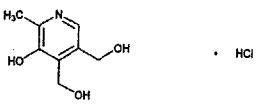
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyridostigmine related compound B[a]	0.75	0.2
Pyridostigmine related compound A	0.92	0.2
Pyridostigmine	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] 3-Hydroxy-1-methylpyridin-1-ium bromide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Pyridostigmine Bromide RS
  USP Pyridostigmine Related Compound A RS
  Pyridin-3-yl dimethylcarbamate.
  $C_8H_{10}N_2O_2$ 166.18

Pyridoxine Hydrochloride



$C_8H_{11}NO_3 \cdot HCl$ 205.64

3,4-Pyridinedimethanol, 5-hydroxy-6-methyl-, hydrochloride; Pyridoxol hydrochloride [58-56-0].

DEFINITION

Pyridoxine Hydrochloride contains NLT 98.0% and NMT 102.0% of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Mix 10 mL of glacial acetic acid, 0.6 g of sodium 1-hexanesulfonate, and 700 mL of water in a 1000-mL volumetric flask. Adjust with glacial acetic acid or 1 N sodium hydroxide to a pH of 3.0. Add 235 mL of methanol, and dilute with water to volume.

Internal standard solution: 5 mg/mL of *p*-hydroxybenzoic acid in *Mobile phase*

Standard solution: Prepare a 0.5-mg/mL solution of USP Pyridoxine Hydrochloride RS in *Mobile phase*. Transfer 10.0 mL of this solution and 1.0 mL of *Internal standard solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Prepare a 0.5-mg/mL solution of Pyridoxine Hydrochloride in *Mobile phase*. Transfer 10.0 mL of this solution and 1.0 mL of *Internal standard solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for pyridoxine and *p*-hydroxybenzoic acid are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between pyridoxine and *p*-hydroxybenzoic acid

Relative standard deviation: NMT 3.0% for the ratios of the pyridoxine peak area response to the internal standard peak area response

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) in the portion of Pyridoxine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

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R_U = internal standard ratio (peak response of pyridoxine/peak response of the internal standard) from the *Sample solution*
R_S = internal standard ratio (peak response of pyridoxine/peak response of the internal standard) from the *Standard solution*
C_S = concentration of USP Pyridoxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Pyridoxine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS

• **CONTENT OF CHLORIDE**

Sample: 500 mg of Pyridoxine Hydrochloride
Blank: 50 mL of methanol
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 50 mL of methanol. Add 5 mL of glacial acetic acid and 2–3 drops of eosin Y TS. Titrate with the *Titrant*. Perform a *Blank* determination. Calculate the percentage of chloride (Cl) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 16.9%–17.6% on the dried basis

• **LOSS ON DRYING** ⟨731⟩: Dry a sample in vacuum over silica gel for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pyridoxine Hydrochloride RS

Pyridoxine Hydrochloride Injection

» Pyridoxine Hydrochloride Injection is a sterile solution of Pyridoxine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of Pyridoxine Hydrochloride ($C_8H_{11}NO_3 \cdot HCl$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Pyridoxine Hydrochloride RS
Identification—Evaporate a volume of Injection, equivalent to about 50 mg of pyridoxine hydrochloride, on a steam bath to dryness. Add 5 mL of dehydrated alcohol, and again evaporate to dryness. Dry the residue at 105° for 3 hours: the residue so obtained responds to *Identification* tests *A* and *B* under *Pyridoxine Hydrochloride*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.4 USP Endotoxin Unit per mg of pyridoxine hydrochloride.
pH ⟨791⟩: between 2.0 and 3.8.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Ammonium chloride–ammonium hydroxide buffer—Dissolve 16 g of ammonium chloride in 70 mL of water, add 16 mL of ammonium hydroxide, dilute with water to 100 mL, mix, and filter.

Chlorimide solution—Dissolve 40 mg of 2,6-dichloroquinone-chlorimide in 100 mL of isopropyl alcohol. Store the solution in a refrigerator, and use within 1 month. Do not use the solution if it has become pink.

Standard stock solution—Dissolve a suitable quantity of USP Pyridoxine Hydrochloride RS, accurately weighed, in 0.1 N hydrochloric acid, quantitatively dilute with the same solvent to obtain a solution having a known concentration of about 0.1 mg per mL, and mix. Keep the solution in an amber bottle, in a cool place.

Standard preparation—In a 100-mL volumetric flask dilute 10.0 mL of the *Standard stock solution* with water to volume, and mix. Prepare this solution daily as needed.

Assay preparation—Dilute an accurately measured volume of Injection, equivalent to about 100 mg of pyridoxine hydrochloride, quantitatively and stepwise with water to a concentration of about 10 µg of pyridoxine hydrochloride per mL.

Procedure—

(a) Pipet 5 mL of the clear *Assay preparation* into a flask, add 25.0 mL of isopropyl alcohol, and mix. Pipet 5 mL of the isopropyl alcohol dilution into a glass-stoppered, 25-mL graduated cylinder or test tube; and add in succession, mixing after each addition, 1.0 mL of *Ammonium chloride–ammonium hydroxide buffer*, 1.0 mL of sodium acetate solution (1 in 5), and 1.0 mL of water. Cool to about 25°, then add 1.0 mL of *Chlorimide solution*, and shake vigorously for 10 seconds, accurately timed. Ninety seconds, accurately timed, after the addition of the *Chlorimide solution*, determine the absorbance at the wavelength of maximum absorbance at about 650 nm, with a suitable spectrophotometer, using water as the blank. [NOTE—Make the reading promptly to avoid errors due to fading of the color.] Designate the absorbance as A_U.

(b) Repeat procedure *(a)*, but substitute 1.0 mL of boric acid solution (1 in 20) for the 1.0 mL of water. Designate the absorbance as A_U'.

(c) Repeat procedure *(a)*, but substitute 5.0 mL of the *Standard preparation* for the 5.0 mL of the *Assay preparation*. Designate the absorbance as A_S.

(d) Repeat procedure *(c)*, but substitute 1.0 mL of boric acid solution (1 in 20) for the 1.0 mL of water. Designate the absorbance as A_S'.

Calculate the quantity, in mg, of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$10(C/V)(A_U - A_U')/(A_S - A_S')$$

in which C is the concentration, in µg per mL, of USP Pyridoxine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and the other terms are as defined above.

USP Monographs

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Pyridoxine Hydrochloride Tablets

DEFINITION

Pyridoxine Hydrochloride Tablets contain NLT 95.0% and NMT 115.0% of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$).

IDENTIFICATION

• **A. Reaction with Ferric Ion**

Sample: Equivalent to 100 mg of pyridoxine hydrochloride from a quantity of powdered Tablets

Analysis: Add 5 mL of water to the *Sample*. Shake the mixture, filter into a test tube, and add 2 or 3 drops of ferric chloride TS.

Acceptance criteria: An orange to deep red color is produced.

ASSAY

• **Procedure**

Buffer: Dissolve 16 g of ammonium chloride in 70 mL of water, add 16 mL of ammonium hydroxide, dilute with water to 100 mL, and filter.

Color reagent: 0.4 mg/mL of 2,6-dichloroquinone chlorimide in isopropyl alcohol. [Note—Store the solution in a refrigerator, and use within one month. Do not use the solution if it has become pink.]

Standard stock solution: 0.1 mg/mL of USP Pyridoxine Hydrochloride RS in 0.1 N hydrochloric acid. [Note—Keep the solution in an amber bottle, in a cool place.]

Standard solution: 10 µg/mL of USP Pyridoxine Hydrochloride RS from the *Standard stock solution* diluted with water. [Note—Prepare this solution daily as needed.]

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer, with the aid of water, a portion of the powdered Tablets to a conical flask. Add 0.5 mL hydrochloric acid per each mg of the nominal amount of pyridoxine hydrochloride taken, then dilute with water to about 0.04 mg/mL, and heat on a steam bath until disintegration is complete. Cool, dilute with water to 10 µg/mL, and centrifuge a portion of the mixture.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Visible

Analytical wavelength: 650 nm

Blank: Water

Analysis

Samples: *Standard solution* and *Sample solution*

Separately add 25.0 mL of isopropyl alcohol to 5 mL each of the *Standard solution* and the *Sample solution*. Transfer 5 mL of each of the resulting solutions to separate, glass-stoppered, 25-mL graduated cylinders or test tubes, and add in succession, mixing after each addition, 1.0 mL of *Buffer*, 1.0 mL of a 0.2-g/mL solution of sodium acetate, and 1.0 mL of water. Cool to 25°, add 1.0 mL of *Color reagent*, and shake vigorously for 10 s, accurately timed. Determine the absorbance 90 s after the addition of the *Color reagent*. Repeat the procedure both for the *Standard solution* and the *Sample solution*, but substitute 1.0 mL of a 50-mg/mL solution of boric acid solution for the 1.0 mL of water.

[Note—Make the reading promptly to avoid errors due to fading of the color.]

Calculate the percentage of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (A_U - A_U')/(A_S - A_S') \times (C_S/C_U) \times 100$$

A_U = absorbance of *Sample solution* with water

A_U' = absorbance of *Sample solution* with boric acid

A_S = absorbance of *Standard solution* with water

A_S' = absorbance of *Standard solution* with boric acid

C_S = concentration of USP Pyridoxine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pyridoxine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–115.0%

PERFORMANCE TESTS

• **Dissolution,** *Procedure for a Pooled Sample* ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 1.40 mg/mL of sodium 1-hexanesulfonate

Standard solution: Known concentration of USP Pyridoxine Hydrochloride RS in *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak area of pyridoxine from the *Sample solution*

r_S = peak area of pyridoxine from the *Standard solution*

C_S = concentration of USP Pyridoxine Hydrochloride RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

Content uniformity

Standard solution: 10 µg/mL of USP Pyridoxine Hydrochloride RS in dilute hydrochloric acid (1 in 100)

Sample solution: Transfer 1 Tablet, finely powdered, to a 500-mL volumetric flask containing 300 mL of water, shake for 30 min, and dilute with water to volume. Filter a portion of the mixture, discarding the first 25 mL of the filtrate. Dilute an aliquot of the filtrate with dilute hydrochloric acid (1 in 100) to obtain 10 µg/mL of pyridoxine hydrochloride.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 290 nm

Cell: 1 cm

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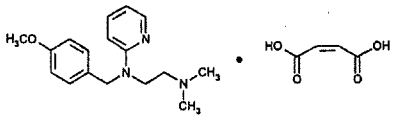
Blank: Dilute hydrochloric acid (1 in 100)
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the absorbances of the *Standard solution* and *Sample solution*.
Calculate the percentage of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Pyridoxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pyridoxine hydrochloride in the *Sample solution* (mg/mL)
L = label claim (mg/Tablet)

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Pyridoxine Hydrochloride RS

Pyrilamine Maleate



$C_{17}H_{23}N_3O \cdot C_4H_4O_4$ 401.46
1,2-Ethanediamine, *N*-[(4-methoxyphenyl)methyl]-*N*′,*N*′-dimethyl-*N*-2-pyridinyl-, (*Z*)-2-butenedioate (1:1).
2-[[2-(Dimethylamino)ethyl](*p*-methoxybenzyl)amino] pyridine maleate (1:1) [59-33-6].

» Pyrilamine Maleate, dried in vacuum over phosphorus pentoxide for 5 hours, contains not less than 98.0 percent and not more than 100.5 percent of $C_{17}H_{23}N_3O \cdot C_4H_4O_4$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Pyrilamine Maleate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: 0.5 N sulfuric acid.
Absorptivities at 236 nm and 312 nm, calculated on the dried basis, do not differ by more than 3.0%.
Melting range, *Class I* ⟨741⟩: between 99° and 103°.

Loss on drying ⟨731⟩—Dry it in vacuum over phosphorus pentoxide for 5 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—
TEST 1—
Standard solution—Dissolve an accurately weighed quantity of USP Pyrilamine Maleate RS in a mixture of methanol and ammonium hydroxide (200:1) to obtain a solution having a known concentration of about 0.4 mg per mL. Quantitatively dilute this solution with the mixture of methanol and ammonium hydroxide (200:1) to obtain *Standard solutions A*, *B*, and *C* having the following compositions:

Standard solution	Dilution	Concentration (mg of RS per mL)	Percentage (%, for comparison with test specimen)
A	(1 in 4)	0.1	0.5
B	(3 in 20)	0.06	0.3
C	(1 in 20)	0.02	0.1

Test solution—Dissolve an accurately weighed quantity of Pyrilamine Maleate in a mixture of methanol and ammonium hydroxide (200:1) to obtain a solution having a known concentration of about 20 mg per mL.
Eluant: ethyl acetate, diethylamine, *n*-hexane, and methanol (93:7:1:1).
Procedure—Apply separately 10 µL of the *Test solution* and 10 µL of each of the three *Standard solutions* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. [NOTE—The plate has been prewashed for 2 hours with *Eluant* and dried.] Allow the spots on the plate to dry. Place the plate in a chromatographic chamber and develop the chromatograms in *Eluant* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and air-dry the plate. View the plate under short-wavelength UV light and compare the intensities of any secondary spots from the chromatogram of the *Test solution* with those of the principal spots from the chromatograms of the *Standard solutions*. No secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot from *Standard solution A* (0.5%), and the sum of the intensities of all secondary spots from the *Test solution* corresponds to not more than 1.0%.
TEST 2—
Mobile phase—Prepare a filtered and degassed mixture of 0.01 M ammonium acetate, methanol, and triethylamine (40:60:0.1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP Pyrilamine Maleate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.5 µg per mL.
Test solution—Transfer about 50 mg of Pyrilamine Maleate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains packing L11. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, run the chromatograph for 25

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minutes, record the chromatograms, and measure the peak area responses, but do not measure the maleate peak area response, which elutes near the void volume. Calculate the percentage of each impurity in the portion of pyrilamine taken by the formula:

$$10{,}000(C/W)(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP Pyrilamine Maleate RS in the *Standard solution*; W is the weight, in mg, of the Pyrilamine Maleate taken to prepare the *Test solution*; r_i is the peak area response for each impurity; and r_S is the response of the *Standard solution*: not more than 0.3% of any individual impurity is found, and not more than 1.0% of total impurities is found.

Assay—Dissolve about 400 mg of Pyrilamine Maleate, previously dried and accurately weighed, in 50 mL of glacial acetic acid. Add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 20.07 mg of $C_{17}H_{23}N_3O \cdot C_4H_4O_4$.

Pyrilamine Maleate Tablets

» Pyrilamine Maleate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{17}H_{23}N_3O \cdot C_4H_4O_4$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Pyrilamine Maleate RS

Identification—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{17}H_{23}N_3O \cdot C_4H_4O_4$ dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{17}H_{23}N_3O \cdot C_4H_4O_4$ is dissolved in 45 minutes.

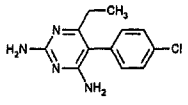
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—Proceed with Tablets as directed under *Salts of Organic Nitrogenous Bases* ⟨501⟩, determining the absorbance at the wavelength of maximum absorbance at about 312 nm. Calculate the quantity, in mg, of $C_{17}H_{23}N_3O \cdot C_4H_4O_4$ in the portion of Tablets taken by the formula:

$$0.05C(A_U/A_S)$$

in which C is the concentration, in µg per mL, calculated on the dried basis, of USP Pyrilamine Maleate RS in the *Standard Preparation*.

Pyrimethamine



$C_{12}H_{13}ClN_4$ 248.71

2,4-Pyrimidinediamine, 5-(4-chlorophenyl)-6-ethyl-.
2,4-Diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine [58-14-0].

» Pyrimethamine contains not less than 99.0 percent and not more than 101.0 percent of $C_{12}H_{13}ClN_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Pyrimethamine RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Mix about 100 mg with 500 mg of anhydrous sodium carbonate, and ignite the mixture. Cool, add 5 mL of hot water, heat for 5 minutes on a steam bath, filter, and neutralize the filtrate with nitric acid: the solution meets the requirements of the test for *Chloride* ⟨191⟩.

C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Melting range ⟨741⟩: between 238° and 242°.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Ordinary impurities ⟨466⟩—
Test solution: a mixture of methanol and chloroform (1:1).
Standard solution: a mixture of methanol and chloroform (1:1).
Eluant: a mixture of *n*-propyl alcohol, glacial acetic acid, and water (8:1:1).
Visualization: 2.

Assay—
0.1% Phosphoric acid solution—Add 1 mL of phosphoric acid to water, and dilute to 1000 mL.
Mobile phase—Prepare a suitable degassed and filtered mixture of *0.1% Phosphoric acid solution* and acetonitrile (83:17). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer an accurately weighed quantity of USP Pyrimethamine RS to a suitable volumetric flask, and dilute with *0.1% Phosphoric acid solution* to volume to obtain a solution having a known concentration of about 0.02 mg per mL.
Assay preparation—Transfer an accurately weighed quantity of Pyrimethamine to a suitable volumetric flask, and dilute with *0.1% Phosphoric acid solution* to volume to obtain a solution having a known concentration of about 0.02 mg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 2.0-mm × 10-cm column that contains 3-µm packing

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L11. The flow rate is about 0.3 mL per minute. Chromatograph replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, is not less than 2.5; the tailing factor is not more than 1.8; and the relative standard deviation is not more than 1.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in percentage, of $C_{12}H_{13}ClN_4$ in the portion of Pyrimethamine taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Pyrimethamine RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of Pyrimethamine in the *Assay preparation* based on the weighed amount; r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively; and 100 is the percent conversion factor.

Pyrimethamine Compounded Oral Suspension

DEFINITION

Pyrimethamine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of pyrimethamine ($C_{12}H_{13}ClN_4$).

Prepare Pyrimethamine Compounded Oral Suspension 2 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Pyrimethamine powder	200 mg
Vehicle: a 1:1 mixture of Methylcellulose 1% Solution, and Syrup, *NF,* a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. Place *Pyrimethamine powder* in a suitable mortar. Add 2 mL of the *Vehicle,* and triturate to make a smooth paste. Add an additional 10 mL of the *Vehicle* to the paste, and levigate to form a suspension. Add increasing volumes of *Vehicle* to make a liquid that is pourable. Transfer the contents of the mortar to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 50 mM anhydrous sodium acetate (40:60). Pass through a PVDF membrane filter of 0.45-µm pore size, and degas.

System suitability solution: 0.20 mg/mL of USP Pyrimethamine RS (previously dried) and 0.10 mg/mL of USP Phenacetin RS (previously dried) in methanol

Standard solution: 0.20 mg/mL of USP Pyrimethamine RS (previously dried) in methanol

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.20 mg/mL of pyrimethamine from Oral Suspension in methanol. Sonicate, and mix on a vortex mixer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Autosampler temperature: 10°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phenacetin and pyrimethamine are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6.0 between the pyrimethamine and phenacetin peaks, *System suitability solution*

Column efficiency: NLT 8000 theoretical plates, *System suitability solution*

Tailing factor: NMT 1.5, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pyrimethamine ($C_{12}H_{13}ClN_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pyrimethamine from the *Sample solution*

r_S = peak response of pyrimethamine from the *Standard solution*

C_S = concentration of USP Pyrimethamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pyrimethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 6.6–7.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature

• **LABELING:** Label it to indicate that it is to be well shaken before use, protect from light, and to state the *Beyond-Use Date.*

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Phenacetin RS

USP Pyrimethamine RS

Pyrimethamine Tablets

» Pyrimethamine Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of pyrimethamine ($C_{12}H_{13}ClN_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Pyrimethamine RS

USP Monographs

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Identification—

A: The UV absorption spectrum of the *Assay Preparation* exhibits maxima at the same wavelengths as that of a similar solution of USP Pyrimethamine RS.

B: To a quantity of powdered Tablets, equivalent to about 250 mg of pyrimethamine, add 25 mL of acetone, boil for 2 minutes, and filter through a sintered-glass crucible. Repeat this treatment three times with 25-mL portions of acetone. Evaporate the combined filtrates carefully on a steam bath with the aid of a current of air to dryness: the residue responds to *Identification* test *A* under *Pyrimethamine,* and melts between 237° and 242° (see *Melting Range or Temperature* ⟨741⟩).

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{12}H_{13}ClN_4$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 273 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Pyrimethamine RS in the same *Medium.*

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{12}H_{13}ClN_4$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Transfer 1 Tablet to a 100-mL volumetric flask, add 25 mL of 0.1 N hydrochloric acid, warm the flask on a steam bath for 5 minutes, cool, dilute with 0.1 N hydrochloric acid to volume, mix, and filter, rejecting the first few mL of the filtrate. Pipet a portion of the clear filtrate, equivalent to about 2.5 mg of pyrimethamine, into a 250-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Dissolve an accurately weighed quantity of USP Pyrimethamine RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with 0.1 N hydrochloric acid to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{12}H_{13}ClN_4$ in the Tablet taken by the formula:

$$(T/D)C(A_U/A_S)$$

in which T is the labeled quantity, in mg, of pyrimethamine in the Tablet; D is the concentration, in µg per mL, of pyrimethamine in the solution from the Tablet, on the basis of the labeled quantity per Tablet and the extent of dilution; C is the concentration, in µg per mL, of USP Pyrimethamine RS in the Standard solution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—Proceed with Tablets as directed under *Salts of Organic Nitrogenous Bases* ⟨501⟩, diluting 5.0 mL each of the *Standard Preparation* and the *Assay Preparation,* respectively, with 0.5 N sulfuric acid to 200.0 mL and determining the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 273 nm. Calculate the quantity, in mg, of pyrimethamine ($C_{12}H_{13}ClN_4$) in the portion of Tablets taken by the formula:

$$50C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Pyrimethamine RS in the *Standard Preparation;* and A_U and A_S are as defined in the chapter.

Pyroxylin

Cellulose, nitrate.
Pyroxylin [9004-70-0].

» Pyroxylin is a product obtained by the action of a mixture of nitric and sulfuric acids on cotton and consists chiefly of cellulose tetranitrate $(C_{12}H_{16}N_4O_{18})_n$.

[NOTE—Dry Pyroxylin is a light yellow, matted mass of filaments, resembling raw cotton in appearance, but harsh to the touch. *It is exceedingly flammable,* burning, when unconfined, very rapidly and with a luminous flame. When kept in well-closed bottles and exposed to light, it is decomposed with the evolution of nitrous vapors, leaving a carbonaceous residue.]

Pyroxylin available commercially is moistened with about 30 percent of alcohol or other suitable solvent. The alcohol or other solvent must be allowed to evaporate from the Pyroxylin to yield the dried substance described in this Pharmacopeia. Pyroxylin moistened with alcohol or other solvent may be used in the tests set forth in this monograph, provided the weight of test specimen taken corresponds to the specified amount of dry Pyroxylin.

Packaging and storage—Preserve loosely packed in cartons, protected from light.

Labeling—The label bears a caution statement to the effect that Pyroxylin is highly flammable.

Viscosity—Rotational Methods ⟨912⟩—Dissolve 48.8 g in a mixture of 88 g of alcohol and 193.2 g of toluene, and when solution is complete, add 70 g of ethyl acetate, and mix. Transfer the solution to the cup of a rotational type of viscometer, adjust the temperature to 25°, and determine the viscosity, making certain that the apparent viscosity reaches equilibrium before taking the final reading: the viscosity is between 110 and 147 poises.

Residue on ignition ⟨281⟩—Saturate about 500 mg, accurately weighed, with alcohol in a dish placed in cold water, and ignite the Pyroxylin at the top. When combustion is complete, heat the dish to redness, and cool: not more than 0.3% of residue remains.

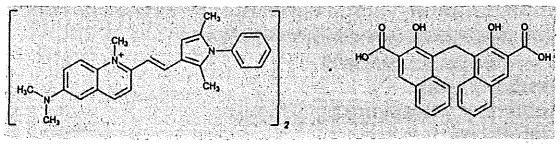
Acidity and water-soluble substances—Stir 1.0 g with 20 mL of water for 10 minutes, and filter: the filtrate does not have an acid reaction to litmus. Evaporate 10 mL of the filtrate on a steam bath to dryness, and dry the residue at 105° for 1 hour: not more than 1.5 mg of residue remains.

USP Monographs

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Delete the following:

▲Pyrvinium Pamoate



$C_{75}H_{70}N_6O_6$ 1151.39

Quinolinium, 6-(dimethylamino)-2-[2-(2,5-dimethyl-1-phenyl]-1*H*-pyrrol-3-yl)ethenyl]-1-methyl-, salt with 4,4′-methylenebis[3-hydroxy-2-naphthalenecarboxylic acid] (2:1);

6-(Dimethylamino)-2-[2-(2,5-dimethyl-1-phenylpyrrol-3-yl) vinyl]-1-methylquinolinium 4,4′-methylenebis[3-hydroxy-2-naphthoate] (2:1) [3546-41-6].

DEFINITION

Pyrvinium Pamoate contains NLT 96.0% and NMT 104.0% of pyrvinium pamoate ($C_{75}H_{70}N_6O_6$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197K⟩
- **B.**

Sample solution: Proceed as directed in the *Assay*.
Acceptance criteria: Exhibits absorbance maxima at about 358 and 505 nm, and the ratio A_{505}/A_{358} is 1.93–2.07

ASSAY

- **Procedure**

Use low-actinic flasks in preparing the solutions, and otherwise protect the solutions from unnecessary exposure to bright light. Complete the *Assay* without prolonged interruption.
Standard stock solution: 1 mg/mL of USP Pyrvinium Pamoate RS dissolved in glacial acetic acid using 50% of the final volume. Dilute with methanol to final volume.
Standard solution: 10 µg/mL of USP Pyrvinium Pamoate RS from the *Standard stock solution* in methanol
Sample stock solution: 1 mg/mL of Pyrvinium Pamoate dissolved in glacial acetic acid using 50% of the final volume. Dilute with methanol to final volume.
Sample solution: 10 µg/mL of Pyrvinium Pamoate from the *Sample stock solution* in methanol
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 505 nm
Cell: 1 cm
Blank: Methanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pyrvinium pamoate ($C_{75}H_{70}N_6O_6$) in the portion of Pyrvinium Pamoate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Pyrvinium Pamoate RS in the *Standard solution* (µg/mL)
C_U = concentration of Pyrvinium Pamoate in the *Sample solution* (µg/mL)

Acceptance criteria: 96.0%–104.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.5%

SPECIFIC TESTS

- **Water Determination** ⟨921⟩, *Method I*

Sample: 200 mg
Analysis: Use 10 mL each of methanol and chloroform as the solvent.
Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩

USP Pyrvinium Pamoate RS▲ (USP 1-May-2020)

Delete the following:

▲Pyrvinium Pamoate Oral Suspension

DEFINITION

Pyrvinium Pamoate Oral Suspension contains, in each 100 mL, an amount of pyrvinium pamoate equivalent to NLT 0.90 g and NMT 1.10 g of pyrvinium ($C_{26}H_{28}N_3^+$).

IDENTIFICATION

- **A.**

Standard solution and **Sample solution:** Prepare as directed in the *Assay*.
Analysis: Obtain the absorbance spectrum of the *Standard solution* and the *Sample solution* at 300–600 nm.
Acceptance criteria: Concomitantly measured, the *Sample solution* exhibits the same maxima and minima at the same wavelengths as the *Standard solution*.

ASSAY

- **Procedure**

Use low-actinic flasks in preparing the solutions, and otherwise protect the solutions from unnecessary exposure to bright light. Complete the *Assay* without prolonged interruption.
Standard solution: 9 µg/mL of USP Pyrvinium Pamoate RS prepared as follows. Dissolve in 4 mL of acetic acid per each 3 mg of USP Pyrvinium Pamoate RS. Dilute with methanol to final volume. Further dilute in methanol, if necessary.
Sample stock solution: Using a pipet calibrated "to contain", transfer 5 mL of Oral Suspension to a 250-mL volumetric flask. Complete the transfer by rinsing the pipet with 10 mL of methanol. Add 100 mL of glacial acetic acid, and mix to dissolve the pyrvinium pamoate. Dilute this solution with methanol to volume, and mix.
Sample solution: Transfer 3 mL of the resulting solution to a 100-mL volumetric flask, and dilute with methanol to volume.
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 505 nm
Cell: 1 cm
Blank: Methanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the labeled quantity of pyrvinium ($C_{26}H_{28}N_3^+$), in g per 100 mL of Oral Suspension:

$$\text{Result} = (A_U/A_S) \times C_S \times (M_{r1}/M_{r2}) \times F_1 \times F_2$$

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A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Pyrvinium Pamoate RS in the *Standard solution* (µg/mL)
M_{r1} = molecular weight of pyrvinium, 382.52
M_{r2} = half of the molecular weight of pyrvinium pamoate, 575.70
F_1 = 100 mL
F_2 = conversion factor, 10^{-6} g/µg

Acceptance criteria: 0.90–1.10 g

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 6.0–8.0, determined potentiometrically

PERFORMANCE TESTS
- **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements for oral suspension packaged in single-unit containers
- **Deliverable Volume** ⟨698⟩**:** Meets the requirements for oral suspension packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Pyrvinium Pamoate RS▲ (USP 1-May-2020)

Delete the following:

▲Pyrvinium Pamoate Tablets

DEFINITION
Pyrvinium Pamoate Tablets contain an amount of pyrvinium pamoate equivalent to NLT 92.0% and NMT 108.0% of the labeled amount of pyrvinium ($C_{26}H_{28}N_3^+$).

IDENTIFICATION
- **A.**
  Standard solution and **Sample solution:** Prepare as directed in the *Assay*.
  Analysis: Obtain the absorbance spectrum of the *Standard solution* and the *Sample solution* at 300–600 nm.
  Acceptance criteria: Concomitantly measured, the *Sample solution* exhibits the same maxima and minima at the same wavelengths as the *Standard solution*.

ASSAY
- **Procedure**
  Use low-actinic flasks in preparing the solutions, and otherwise protect the solutions from unnecessary exposure to bright light. Complete the *Assay* without prolonged interruption.
  Standard solution: 9 µg/mL of USP Pyrvinium Pamoate RS prepared as follows. Dissolve in 1 mL of acetic acid per each 3 mg of USP Pyrvinium Pamoate RS. Dilute with methanol to final volume. Further dilute in methanol, if necessary.
  Sample stock solution: Nominally 1 mg/L of pyrvinium prepared as follows. Place 500 mg of pyrvinium from a number of Tablets in a 500-mL volumetric flask, and add 25 mL of water and 25 mL of acetone. Completely disintegrate the Tablets by heating on a steam bath for 10 min with frequent mixing, allowing part of the acetone to boil off slowly. To the hot mixture add 250 mL of glacial acetic acid, and mix occasionally for 5 min without further heating to dissolve the pyrvinium pamoate. Dilute with methanol to volume at room temperature, and mix. Centrifuge a portion of the mixture until a clear solution is obtained. Use the clear solution to prepare the *Sample solution*.
  Sample solution: Nominally 6 µg/mL of pyrvinium from the *Standard stock solution* in methanol
  Instrumental conditions
  Mode: UV-Vis
  Analytical wavelength: 505 nm
  Cell: 1 cm
  Blank: Methanol
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of pyrvinium ($C_{26}H_{28}N_3^+$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

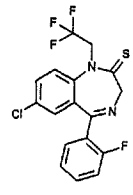
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Pyrvinium Pamoate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pyrvinium pamoate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of pyrvinium, 382.52
M_{r2} = half of the molecular weight of pyrvinium pamoate, 575.70

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS
- **Disintegration** ⟨701⟩
  Time: 30 min
  Acceptance criteria: Meet the requirements
- **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Pyrvinium Pamoate RS▲ (USP 1-May-2020)

Quazepam



$C_{17}H_{11}ClF_4N_2S$ 386.79
2*H*-1,4-Benzodiazepine-2-thione, 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-1-(2,2,2-trifluoroethyl)-.
7-Chloro-5-(*o*-fluorophenyl)-1,3-dihydro-1-(2,2,2-trifluoroethyl)-2*H*-1,4-benzodiazepine-2-thione [36735-22-5].

» Quazepam contains not less than 98.5 percent and not more than 101.5 percent of $C_{17}H_{11}ClF_4N_2S$, calculated on the dried basis.

USP Monographs

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Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Quazepam RS
USP Quazepam Related Compound A RS
7-Chloro-1-(2,2,2 trifluoroethyl)-5-(2-Fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepine-2-one.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The R_F value of the principal spot in the chromatogram of the *Test solution* obtained in the test for *Related compounds* corresponds to that in the chromatogram of *Standard solution A.*

Melting range ⟨741⟩: between 146° and 151°, but the range between beginning and end of melting does not exceed 2°.
Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

Related compounds—
Test solution—Prepare a solution of Quazepam in methylene chloride containing 20 mg per mL.
Standard solution A—Dissolve an accurately weighed quantity of USP Quazepam RS in methylene chloride to obtain a solution having a known concentration of about 20 mg per mL.
Standard solution B—Dissolve an accurately weighed quantity of USP Quazepam RS in methylene chloride to obtain a solution having a known concentration of about 0.04 mg per mL (0.2%).
Standard solution C—Dissolve an accurately weighed quantity of USP Quazepam Related Compound A RS in methylene chloride to obtain a solution having a known concentration of about 0.2 mg per mL (1%).
Procedure—Separately apply 5 µL of the *Test solution* and 5 µL of each of the *Standard solutions* to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of cyclohexane, ethyl acetate, and ether (170:40:25) in a paper-lined tank until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow to air-dry, and examine the plate under short-wavelength UV light. Compare the intensity of the secondary spot in the chromatogram of the *Test solution* having the same R_F value as that of the primary spot of *Standard solution C:* the spot is not larger or more intense than the principal spot in the chromatogram of *Standard solution C.* Compare the intensities of any additional secondary spots observed in the chromatogram of the *Test solution* with that of the principal spot in the chromatogram of *Standard solution B:* the sum of the intensities of the additional secondary spots obtained from the *Test solution* corresponds to not more than 0.2%.
Assay—Dissolve about 500 mg of Quazepam, accurately weighed, in 150 mL of acetic anhydride. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass-calomel electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 38.68 mg of $C_{17}H_{11}ClF_4N_2S$.

Quazepam Tablets

» Quazepam Tablets contain not less than 90 percent and not more than 110.0 percent of quazepam ($C_{17}H_{11}ClF_4N_2S$).

Packaging and storage—Preserve in a well-closed container.
USP Reference standards ⟨11⟩—
USP Ethylparaben RS
USP Quazepam RS
USP Quazepam Related Compound A RS
7-Chloro-1-(2,2,2 trifluoroethyl)-5-(2-Fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepine-2-one.

Identification—
A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* both relative to the internal standard as obtained in the *Assay.*
B: The R_F value of the principal spot in the chromatogram of the *Test solution* obtained in the test for *Related compounds* corresponds to that in the chromatogram of *Standard solution A.*

Dissolution ⟨711⟩—
Medium: 1% sodium lauryl sulfate; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{17}H_{11}ClF_4N_2S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 287 nm on filtered portions of the solution under test in comparison with a Standard solution having a known concentration of USP Quazepam RS dissolved in a small volume of methanol and diluted with *Dissolution Medium.*
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{17}H_{11}ClF_4N_2S$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Related compounds—
Test solution—Grind 10 Tablets to a fine powder. Dissolve an accurately weighed portion of the powder in methylene chloride to obtain a solution having a concentration of 10 mg of quazepam per mL. Mix for 30 minutes, and centrifuge.
Standard solution A—Dissolve an accurately weighed quantity of USP Quazepam RS in methylene chloride to obtain a solution having a known concentration of about 10 mg per mL.
Standard solution B—Dissolve an accurately weighed quantity of USP Quazepam RS in methylene chloride to obtain a solution having a known concentration of about 0.04 mg per mL (0.2%).
Standard solution C—Dissolve an accurately weighed quantity of USP Quazepam Related Compound A RS in methylene chloride to obtain a solution having a known concentration of about 0.3 mg per mL (1.5%).
Procedure—Separately apply 10 µL each of the *Test Solution* and *Standard solution A* and 5 µL each of *Standard solutions B* and C to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of cyclohexane, ethyl acetate, and ether (170:40:25) in a paper-lined tank until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow to air-dry, and examine the plate under short-wavelength UV

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light. Compare the intensity of the secondary spot in the chromatogram of the *Test solution* having the same R_F value as the principal spot in the chromatogram of *Standard solution C*. The spot is not larger or more intense than the principal spot obtained from *Standard solution C*. Compare the intensities of any additional secondary spots observed in the chromatogram of the *Test solution* with that of the principal spot in the chromatogram of *Standard solution B:* no additional secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from *Standard solution B,* and the sum of the intensities of the additional secondary spots obtained from the *Test solution* is not more than 0.6%.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol and water (7:3). Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve an accurately weighed quantity of USP Ethylparaben RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing about 0.19 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Quazepam RS in *Internal standard solution,* and dilute quantitatively, and stepwise if necessary, with *Internal standard solution* to obtain a solution having a known concentration of about 1.5 mg of quazepam per mL.

Assay preparation—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed portion of powder, equivalent to about 15 mg of quazepam, to a 50-mL screw-capped centrifuge tube. Add 10.0 mL of *Internal standard solution,* and centrifuge for 30 minutes.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between ethylparaben and quazepam is not less than 5.5; and the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—For identification purposes, the relative retention times are about 0.4 for ethylparaben and 1.0 for quazepam.]

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of quazepam ($C_{17}H_{11}ClF_4N_2S$) in the portion of Tablets taken by the formula:

$$10C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Quazepam RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak response of quazepam to that of ethylparaben obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Quetiapine Tablets

DEFINITION

Quetiapine Tablets contain an amount of quetiapine fumarate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)

Standard solution: Dissolve 10 mg of quetiapine fumarate in 10 mL of acetone. Sonicate for 10 min. Filter and evaporate the solvent. Dissolve the residue in 2 mL of chloroform. Filter and use 20 µL of the filtrate for analysis.

Sample solution: Finely powder 10 Tablets. Dissolve an amount of the powder equivalent to 10 mg of quetiapine fumarate in 10 mL of acetone, avoiding large pieces of Tablet coating, if any. Sonicate for 10 min. Filter and evaporate the solvent. Dissolve the residue in 2 mL of chloroform. Filter and use the filtrate for analysis.

Blank: Filter 10 mL of acetone and evaporate the solvent. Dissolve the residue using 2 mL of chloroform and filter.

Analysis

Samples: *Standard solution, Sample solution,* and *Blank*

Add drop-wise approximately 20 µL of each of the samples separately onto a clean IR transmission window, allowing each drop to dry before adding the next. Record the spectra.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: 1.4 g/L of monobasic potassium phosphate in water. To 1 L of this solution, add 1 mL of triethylamine and adjust with dilute phosphoric acid to a pH of 6.5 (1 in 10, v/v).

Mobile phase: Acetonitrile and *Buffer* (35:65)

Standard solution: 0.1 mg/mL of USP Quetiapine Fumarate RS in *Mobile phase*

Sample stock solution: Nominally 2.0 mg/mL of quetiapine prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add water to fill 5% of the final volume, and sonicate to disperse the Tablets. Add *Mobile phase* to fill 60% of the final volume, and sonicate for 30 min with intermittent shaking. Dilute with *Mobile phase* to volume. Centrifuge for 5 min. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Sample solution: Nominally 0.1 mg/mL of quetiapine prepared by diluting an aliquot of the *Sample stock solution* with *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: 2 times the retention time of quetiapine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times N \times (M_{r1}/M_{r2}) \times 100$$

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r_U = peak response of quetiapine from the *Sample solution*
r_S = peak response of quetiapine from the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quetiapine in the *Sample solution* (mg/mL)
N = number of moles of quetiapine/mole of quetiapine fumarate, 2
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 1.4 g/L of monobasic potassium phosphate in water. To 1 L of this solution, add 1 mL of triethylamine and adjust with dilute phosphoric acid to a pH of 6.0 (1 in 10).
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard stock solution: 3.3 mg/mL of USP Quetiapine Fumarate RS prepared as follows. Dissolve the Standard first in methanol using 10% of the final volume, and dilute with *Medium* to volume.
Standard solution: 0.03 mg/mL of USP Quetiapine Fumarate RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
Run time: 1.5 times the retention time of quetiapine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times N \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of quetiapine from the *Sample solution*
r_S = peak response of quetiapine from the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
N = number of moles of quetiapine/mole of quetiapine fumarate, 2
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) is dissolved.

Test 2

If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 1 g/L of sodium chloride in water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 20 min
Standard stock solution: 1.3 mg/mL of USP Quetiapine Fumarate RS prepared as follows. Transfer a suitable quantity of USP Quetiapine Fumarate RS to a suitable volumetric flask. Dissolve in about 25% of the flask volume of methanol. Dilute with *Medium* to volume.
Standard solution: (L/900) mg/mL of USP Quetiapine Fumarate RS from a suitable volume of the *Standard stock solution* in *Medium*, where L is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Blank: *Medium*
Instrumental conditions
Mode: UV
Analytical wavelength: 290 nm with background correction at 490 nm
Cell: See *Table 1*.

Table 1

Label claim, L (mg/Tablet)	Cell (cm)
25	1.0
50	1.0
100	0.5
150	0.2
200	0.2
300	0.1
400	0.1

Analysis
Samples: *Medium*, *Standard solution*, and *Sample solution*
Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times N \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
N = number of moles of quetiapine/mole of quetiapine fumarate, 2
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) is dissolved.

Test 3

If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm

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Times
For Tablets labeled to contain 25 mg: 30 min
For Tablets labeled to contain 50, 100, 200, 300, or 400 mg: 45 min
Standard solution: 0.34 mg/mL of USP Quetiapine Fumarate RS in 0.1 N hydrochloric acid
Dilute standard solution: 0.06 mg/mL of USP Quetiapine Fumarate RS from the *Standard solution* in 0.1 N hydrochloric acid
Sample solution: Withdraw a suitable portion of the solution under test. Transfer 9 mL of the portion to a 10-mL volumetric flask and dilute with 1 N hydrochloric acid to volume. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size and discard the first 2 mL.
Instrumental conditions
Mode: UV
Analytical wavelength: 282 nm
Cells
For Tablets labeled to contain 25 or 50 mg: 0.5 cm
For Tablets labeled to contain 100, 200, 300, or 400 mg: 0.1 cm
Blank: 0.1 N hydrochloric acid
Analysis
Samples: *Standard solution, Dilute standard solution, Sample solution,* and *Blank*
For Tablets labeled to contain 25 or 50 mg
Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times N \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Dilute standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Dilute standard solution* (mg/mL)
D = dilution factor, 1.1
N = number of moles of quetiapine/mole of quetiapine fumarate, 2
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

For Tablets labeled to contain 100, 200, 300, or 400 mg
Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times N \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
D = dilution factor, 1.1
N = number of moles of quetiapine/mole of quetiapine fumarate, 2
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances
For Tablets labeled to contain 25 mg: NLT 75% (Q) of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) is dissolved.
For Tablets labeled to contain 50, 100, 200, 300, or 400 mg: NLT 70% (Q) of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer: Dissolve 0.8 g of anhydrous dibasic sodium phosphate and 0.6 g of potassium dihydrogen orthophosphate in 1 L of water.
Solution A: Acetonitrile and *Buffer* (10:90). Adjust with dilute phosphoric acid (1 in 10, v/v) to a pH of 6.7.
Solution B: Acetonitrile
Diluent: Acetonitrile and *Solution A* (65:35)
Mobile phase: See *Table 2.*

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
20	75	25
30	65	35
35	35	65
55	25	75
56	80	20
65	80	20

System suitability solution: 0.5 mg/mL of USP Quetiapine System Suitability RS in *Diluent* prepared as follows. Transfer the required quantity of USP Quetiapine System Suitability RS to a suitable volumetric flask. Add 70% of the flask volume and sonicate to dissolve. Dilute with *Diluent* to volume.
Standard solution: 1.2 µg/mL of USP Quetiapine Fumarate RS in *Diluent.* Sonication may be used to aid in dissolution.
Sample solution: Nominally 0.5 mg/mL of quetiapine in *Diluent* from a portion of weighed and crushed Tablets (NLT 10). Sonicate for 10 min with intermittent shaking.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between quetiapine desethoxy and quetiapine peaks, *System suitability solution*
Tailing factor: NMT 1.5 for the quetiapine peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times N \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

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r_S = peak response of quetiapine from the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quetiapine in the *Sample solution* (mg/mL)
N = number of moles of quetiapine/mole of quetiapine fumarate, 2
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09

Acceptance criteria: See *Table 3*. Disregard any peak with an area below 0.05% in the *Sample solution*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Quetiapine *N*-oxide[a]	0.28	0.2
Quetiapine related compound B	0.39	0.2
Quetiapine related compound G	0.69	0.2
Quetiapine desethoxy[b, c]	0.88	—
Quetiapine	1.0	—
Bis(dibenzothiazepinyl) piperazine[b, d]	2.0	—
Any individual unspecified degradation product	—	0.2
Total impurities	—	0.5

[a] 4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)-1-[2-(2-hydroxyethoxy)ethyl]piperazine 1-oxide.
[b] Process impurities controlled in the drug substance. Included for identification purposes only. Not reported for the drug product and not included in the total impurities.
[c] 2-[4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)piperazin-1-yl]ethanol.
[d] 1,4-Bis(dibenzo[*b,f*][1,4]thiazepin-11-yl)piperazine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Quetiapine Fumarate RS
  USP Quetiapine System Suitability RS
  It contains quetiapine fumarate and at least 0.1% of each of the following impurities: Quetiapine related compound B: 11-(Piperazin-1-yl)dibenzo[*b,f*][1,4]thiazepine; Quetiapine related compound G: Dibenzo[*b,f*][1,4]thiazepin-11(10H)-one; and Quetiapine desethoxy: 2-[4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)piperazin-1-yl]ethanol.

Quetiapine Extended-Release Tablets

DEFINITION

Quetiapine Extended-Release Tablets contain quetiapine fumarate $[(C_{21}H_{25}N_3O_2S)_2 \cdot C_4H_4O_4]$ equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
  Standard solution: Transfer 10 mg of USP Quetiapine Fumarate RS to a suitable vial. Add 10 mL of acetone and cap the vial. Sonicate for about 10 min. Allow the solution to equilibrate to room temperature. Evaporate the acetone completely. Add 2 mL of chloroform. Gently swirl for several minutes. Pass through a suitable filter of 0.45-µm pore size. Use the filtrate.
  Sample solution: Grind NLT 10 Tablets. Transfer an amount of powder equivalent to NLT 10 mg of quetiapine fumarate to a suitable vial. Add 10 mL of acetone and cap the vial. Sonicate for about 10 min. Allow the solution to equilibrate to room temperature. Evaporate the acetone completely. Add 2 mL of chloroform. Gently swirl for several minutes. Pass through a suitable filter of 0.45-µm pore size. Use the filtrate.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  Buffer: Dissolve 2.6 g/L of dibasic ammonium phosphate in water.
  Mobile phase: Methanol, acetonitrile, and *Buffer* (54:7:39)
  Diluent: Acetonitrile and water (50:50)
  System suitability stock solution: 0.05 mg/mL of USP Quetiapine Related Compound H RS in *Mobile phase*
  System suitability solution: 0.005 mg/mL of USP Quetiapine Related Compound H RS and 0.5 mg/mL of USP Quetiapine System Suitability RS in *Mobile phase* prepared as follows. Transfer 5 mg of USP Quetiapine System Suitability RS to a 10-mL volumetric flask. Add 7 mL of *Mobile phase* and sonicate to dissolve. Transfer 1 mL of *System suitability stock solution* to the volumetric flask. Dilute with *Mobile phase* to volume.
  Standard solution: 0.2 mg/mL of USP Quetiapine Fumarate RS in *Mobile phase*
  Sample stock solution: Transfer NLT 5 Tablets to a homogenizer vessel. Add 50 mL of acetonitrile, swirl to wet, and allow to stand for approximately 10 min. Add an additional 160 mL of *Diluent* and extract for about 10 min. Transfer the contents of the homogenizer to a 500-mL volumetric flask. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size and use the filtrate.
  Sample solution: Nominally 0.16–0.18 mg/mL of quetiapine from the *Sample stock solution* in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Flow rate: 1.3 mL/min
  Injection volume: 30 µL
  Run time: NLT 2.5 times the retention time of quetiapine
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—See ▲*Table 8*▲ (RB 1-Apr-2019) for the relative retention times.]

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Suitability requirements
Resolution: NLT 1.5 between quetiapine related compound G and quetiapine related compound H; NLT 2.0 between the quetiapine desthoxy and quetiapine peaks; *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times N \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quetiapine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium 1: Citrate buffer, pH 4.8. Dissolve 9.6 g of anhydrous citric acid in 600 mL of water. Add 90 mL of 1 N sodium hydroxide. Dilute with water to 1 L; 900 mL.
Medium 2: Dissolve 17.9 g of dibasic sodium phosphate dodecahydrate in 400 mL of water. Add 460 mL of ▲1 N sodium hydroxide VS▲ (RB 1-Apr-2019) and dilute with water to 1 L; 100 mL.
[NOTE—It is recommended to check the pH of the mixture of 90 mL of *Medium 1* and 10 mL of *Medium 2*, which should be between 6.4 and 6.8. If the pH of the mixture is less than 6.4, 10 mL/L of ▲1 N sodium hydroxide VS▲ (RB 1-Apr-2019) may be added to *Medium 2*. If the pH of the mixture is greater than 6.8, 10 mL/L of ▲1 N hydrochloric acid VS▲ (RB 1-Apr-2019) may be added to *Medium 2*.]
Start the test with 900 mL of *Medium 1*. Add 100 mL of *Medium 2* to the vessel after 5 h of the test and continue the test.
Apparatus 1: 200 rpm
Times: 1, 6, 12, and 20 h
Diluent: *Medium 1* and *Medium 2* (90:10)
Standard solution: (L/400) mg/mL of USP Quetiapine Fumarate RS in *Diluent*, where L is the label claim in mg/Tablet
Sample solution: Pass a suitable portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Analytical wavelength: About 290 nm
Blank: *Diluent*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of quetiapine ($C_{21}H_{25}N_3O_2S$) in *Medium* (mg/mL) after time point (i):

$$C_i = (A_U/A_S) \times C_S \times (M_{r1}/M_{r2}) \times N$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of quetiapine fumarate in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of quetiapine in *Medium* in the portion of sample withdrawn at each time point (mg/mL)
V = volume of *Medium*, 900 mL for 1 h; 1000 mL for 6-, 12-, and 20-h time points
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	6	47–69
3	12	65–95
4	20	NLT 85

The percentages of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 4, 8, and 24 h
Standard solution: 0.03 mg/mL of USP Quetiapine Fumarate RS in water
Sample solution: Pass a suitable portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first few milliliters of filtrate. Replace the volume withdrawn with an equal volume of *Medium*. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 290 nm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of quetiapine ($C_{21}H_{25}N_3O_2S$) in *Medium* (mg/mL) after each time point (i):

$$C_i = (A_U/A_S) \times C_S \times D \times (M_{r1}/M_{r2}) \times N$$

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A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of quetiapine fumarate in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of quetiapine in *Medium* in the portion of sample withdrawn at each time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	5–25
2	4	20–45
3	8	45–75
4	24	NLT 85

The percentages of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: ▲0.1 N hydrochloric acid VS;▲ (RB 1-Apr-2019) 900 mL
Apparatus 2: 50 rpm
Times: 1, 4, and 8 h
Standard solution: USP Quetiapine Fumarate RS, equivalent to (L/900) mg/mL of quetiapine in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a suitable portion of the solution under test through a suitable full flow filter of 10-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: 295 nm
Cell
For 50-mg Tablets: 10 mm
For 150-, 200-, 300-, and 400-mg Tablets: 1 mm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times N \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of quetiapine fumarate in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (for 50-, 150-, and 200-mg Tablets) (%)	Amount Dissolved (for 300- and 400-mg Tablets) (%)
1	1	NMT 40	NMT 35
2	4	55–75	45–65
3	8	NLT 85	NLT 80

The percentages of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: Water; 900 mL
Apparatus 2: 100 rpm
Times: 1, 4, 8, and 16 h
Standard solution: USP Quetiapine Fumarate RS, equivalent to (L/900) mg/mL of quetiapine in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a suitable portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Analytical wavelength: 250 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times N \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of quetiapine fumarate in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Tolerances: See *Table 4*. The percentages of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

USP Monographs

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Table 4 (Quetiapine Extended-Release Tablets)

Time Point (*i*)	Time (h)	Amount Dissolved (for 50-mg Tablets) (%)	Amount Dissolved (for 150-mg Tablets) (%)	Amount Dissolved (for 200-mg Tablets) (%)	Amount Dissolved (for 300-mg Tablets) (%)	Amount Dissolved (for 400-mg Tablets) (%)
1	1	NMT 20	NMT 20	NMT 20	NMT 15	NMT 15
2	4	30–55	35–55	28–48	22–42	22–42
3	8	60–85	65–90	60–85	52–76	50–75
4	16	NLT 85	NLT 85	NLT 85	NLT 85	NLT 85

Medium, Apparatus 1, Times, Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Dissolution Test 2*.
Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	10–30
2	4	30–50
3	8	60–80
4	24	NLT 85

The percentages of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: pH 6.8 phosphate buffer (6.8 g/L of monobasic potassium phosphate and 0.9 g/L of sodium hydroxide in water. Adjust with 1 N sodium hydroxide VS or phosphoric acid to a pH of 6.8, and sonicate for NLT 10 min); 900 mL
Apparatus 2: 100 rpm, with sinker
Times: 1, 4, 8, and 16 h
Mobile phase: Methanol, trifluoroacetic acid, and water (40: 0.1: 60)
Standard solution: 0.1 mg/mL of USP Quetiapine Fumarate RS prepared as follows. Transfer an appropriate amount of USP Quetiapine Fumarate RS to a suitable volumetric flask, and add 5% of the final flask volume of methanol. Sonicate to dissolve, then dilute with *Medium* to volume.
Sample solution: Pass a suitable portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first few milliliters of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 5.0-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: NLT 2.0 times the retention time of quetiapine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i, of quetiapine ($C_{21}H_{25}N_3O_2S$) in *Medium* (mg/mL) after each time point (*i*):

$$C_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times N$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of quetiapine free base, 383.51
M_{r2} = molecular weight of quetiapine fumarate, 883.09
N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of quetiapine in *Medium* in the portion of sample withdrawn at each time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (*i*)	Time (h)	Amount Dissolved (for 50- and 150-mg Tablets) (%)	Amount Dissolved (for 200-, 300-, and 400-mg Tablets) (%)
1	1	NMT 15	NMT 10
2	4	21–41	21–41
3	8	56–76	51–71
4	16	NLT 80	NLT 80

The percentages of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 7:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

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Acid stage medium: Citrate buffer, pH 4.8 (9.6 g/L of anhydrous citric acid in water prepared as follows. Transfer a suitable quantity of anhydrous citric acid to an appropriate volumetric flask. Dissolve in 60% of the flask volume of water, then add 9% of the flask volume of 1 N sodium hydroxide VS. Dilute with water to volume); 900 mL, deaerated

0.05 M phosphate buffer solution: 17.9 g/L of dibasic sodium phosphate dodecahydrate solution prepared as follows. Transfer a suitable amount of dibasic sodium phosphate dodecahydrate to an appropriate volumetric flask containing 40% of the flask volume of water. Add 46% of the flask volume of 1 N sodium hydroxide VS and dilute with water to volume.

Buffer stage medium: Phosphate buffer, pH 6.6 (add 100 mL of *0.05 M phosphate buffer solution* to the *Acid stage medium*; adjust with 1 N sodium hydroxide VS or 1 N hydrochloric acid VS to obtain a pH of 6.6 ± 0.20, if necessary); 1000 mL

Apparatus 1: 20-mesh basket; 200 rpm

Times: 1 and 4 h in *Acid stage medium*; 6, 10, and 16 h in *Buffer stage medium*. The time in the *Buffer stage medium* includes the time in the *Acid stage medium*.

Procedure: Run the test in the *Acid stage medium* for the times specified. After 5 h, add 100 mL of *0.05 M phosphate buffer solution* and continue running the test in *Buffer stage medium* for the times specified.

Standard stock solution: 1.2 mg/mL of USP Quetiapine Fumarate RS prepared as follows. Transfer an appropriate amount of USP Quetiapine Fumarate RS into a suitable volumetric flask. Add 40% of the flask volume of methanol and sonicate to dissolve. Dilute with water to volume.

Acid stage standard solution: 0.03 mg/mL of USP Quetiapine Fumarate RS from *Standard stock solution* in *Acid stage medium*

Buffer stage standard solution: 0.03 mg/mL of USP Quetiapine Fumarate RS from *Standard stock solution* in *Buffer stage medium*

Acid stage sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first portion of filtrate if needed. Dilute the filtrate further with *Acid stage medium*, if needed. Replace the portion of solution removed from the vessel with an equivalent volume of warmed *Acid stage medium*.

Buffer stage sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first portion of filtrate if needed. Dilute the filtrate further with *Buffer stage medium*, if needed. Replace the portion of solution removed from the vessel with an equivalent volume of warmed *Buffer stage medium*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 290 nm

Blank: *Acid stage medium* or *Buffer stage medium*

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%, *Acid stage standard solution* and *Buffer stage standard solution*

Analysis

Samples: *Acid stage standard solution* and *Acid stage sample solution* or *Buffer stage standard solution* and *Buffer stage sample solution*

Calculate the concentration (C_i) of quetiapine ($C_{21}H_{25}N_3O_2S$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S \times D \times (M_{r1}/M_{r2}) \times N$$

A_U = absorbance of the *Acid stage sample solution* or *Buffer stage sample solution*

A_S = absorbance of the *Acid stage standard solution* or *Buffer stage standard solution*, corresponding to the related sample solution

C_S = concentration of quetiapine fumarate in *Acid stage standard solution* or *Buffer stage standard solution* (mg/mL)

D = dilution factor for the *Acid stage sample solution* or *Buffer stage sample solution*, if needed

M_{r1} = molecular weight of quetiapine free base, 383.51

M_{r2} = molecular weight of quetiapine fumarate, 883.09

N = number of moles of quetiapine free base per mole of quetiapine fumarate, 2

Calculate the percentage of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V_A \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V_A) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V_B) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V_B) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{(C_5 \times V_B) + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of quetiapine in *Acid stage medium* or *Buffer stage medium* in the portion of sample withdrawn at each time point (mg/mL)

V_A = volume of *Acid stage medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of the *Acid stage sample solution* or *Buffer stage sample solution* withdrawn from the vessel and replaced with *Acid stage medium* or *Buffer stage medium*, respectively (mL)

V_B = volume of *Buffer stage medium*, 1000 mL

Tolerances: See *Table 7*.

Table 7

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 15
2	4	28–48
3	6	40–60
4	10	62–82
5	16	NLT 80

The percentages of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*. ▲(RB 1-Apr-2019)

- **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

USP Monographs

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IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

[NOTE—See ▲*Table 8*▲ (RB 1-Apr-2019) for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between quetiapine related compound G and quetiapine related compound H; NLT 2.0 between the quetiapine desthoxy and quetiapine peaks

Analysis

Sample: *Sample solution*

[NOTE—See ▲*Table 8*▲ (RB 1-Apr-2019) for the relative retention times.]

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of quetiapine from the *Sample solution*

F = relative response factor for the corresponding degradation product from ▲*Table 8*▲ (RB 1-Apr-2019)

Acceptance criteria: See ▲*Table 8*▲ (RB 1-Apr-2019) Disregard peaks less than 0.05%.

▲**Table 8**▲ (RB 1-Apr-2019)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fumaric acid[a]	0.1	—	—
Quetiapine related compound G	0.48	1.4	0.2
Quetiapine related compound H	0.57	1.0	0.2
Quetiapine desethoxy[b]	0.87	—	—
Quetiapine	1.0	—	—
Quetiapine related compound B[b]	1.9	—	—
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	0.4

[a] Counter ion peak, not to be included in the total degradation products.

[b] Process impurity controlled in the drug substance. Included for identification purposes only. Not reported for the drug product and not included in the total degradation products.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Quetiapine Fumarate RS

USP Quetiapine Related Compound H RS

4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)-1-[2-(2-hydroxyethoxy)ethyl]piperazine 1-oxide.

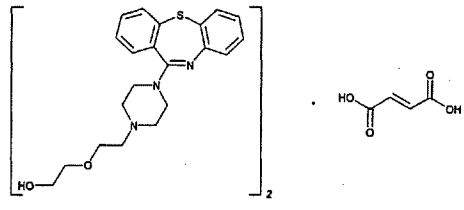
$C_{21}H_{25}N_3O_3S$ 399.51

USP Quetiapine System Suitability RS

It contains quetiapine fumarate and at least 0.1% of each of the following impurities:

Quetiapine related compound B: 11-(Piperazin-1-yl) dibenzo[*b,f*][1,4]thiazepine; Quetiapine related compound G: Dibenzo[*b,f*][1,4]thiazepin-11(10*H*)-one; and Quetiapine desethoxy: 2-[4-(Dibenzo[*b,f*][1,4] thiazepin-11-yl)piperazin-1-yl]ethanol.

Quetiapine Fumarate



$(C_{21}H_{25}N_3O_2S)_2 \cdot C_4H_4O_4$ 883.09

Ethanol, 2-[2-(4-dibenzo[*b,f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]-, (*E*)-2-butenedioate (2:1) (salt); 2-[2-(4-Dibenzo[*b,f*][1,4]thiazepin-11-yl-1-piperazinyl) ethoxy]ethanol fumarate (2:1) (salt) [111974-72-2].

DEFINITION

Quetiapine Fumarate contains NLT 98.0% and NMT 102.0% of quetiapine fumarate [$(C_{21}H_{25}N_3O_2S)_2 \cdot C_4H_4O_4$], calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 2.6 g/L of dibasic ammonium phosphate. Adjust with phosphoric acid to a pH of 6.5.

Mobile phase: Methanol, acetonitrile, and *Buffer* (54:7:39)

System suitability solution: 1.0 mg/mL of USP Quetiapine System Suitability RS in *Mobile phase*

Standard stock solution: 0.16 mg/mL of USP Quetiapine Fumarate RS in *Mobile phase*

Standard solution: 0.08 mg/mL of USP Quetiapine Fumarate RS from *Standard stock solution* in *Mobile phase*

Sample stock solution: 0.16 mg/mL of Quetiapine Fumarate in *Mobile phase*

Sample solution: 0.08 mg/mL of Quetiapine Fumarate from *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.3 mL/min

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Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for quetiapine desethoxy and quetiapine are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the quetiapine desethoxy and quetiapine peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of quetiapine fumarate $[(C_{21}H_{25}N_3O_2S)_2 \cdot C_4H_4O_4]$ in the portion of Quetiapine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Quetiapine Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer: 3.1 g/L of ammonium acetate in water. Add 2 mL of 25% ammonium hydroxide to each 1 L of solution. The pH of the resulting solution is NLT 9.2.
Solution A: Acetonitrile and *Buffer* (25:75)
Solution B: Acetonitrile
Diluent: *Solution A* and *Solution B* (86:14)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	100	0
60	29.3	70.7
60.1	100	0
68	100	0

Peak identification solution: 1 µg/mL of USP Quetiapine Fumarate RS, 10 µg/mL of USP Quetiapine Related Compound B RS, and 2 µg/mL of USP Quetiapine Related Compound G RS in *Diluent*
System suitability solution: 1 mg/mL of USP Quetiapine System Suitability RS in *Diluent*
Standard solution: 0.001 mg/mL of USP Quetiapine Fumarate RS in *Diluent*
Sample solution: 1.0 mg/mL of Quetiapine Fumarate in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Peak identification solution, System suitability solution,* and *Standard solution*
[NOTE—See *Table 2* for relative retention times. Quetiapine related compound B will be the largest peak in the *Peak identification solution* chromatogram.]
Suitability requirements
Resolution: NLT 4.0 between the quetiapine desethoxy and quetiapine peaks, *System suitability solution*; NLT 3.0 between quetiapine related compound B and quetiapine related compound G, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Quetiapine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F)\ 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of quetiapine from the *Standard solution*
C_S = concentration of USP Quetiapine Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Quetiapine Fumarate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks below 0.05% or with retention times less than 2 min.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fumaric acid[a]	0.08	—	—
Quetiapine quaternary salt[b, c]	0.27	0.62	0.15
Quetiapine related compound G	0.55	1.2	0.15
Quetiapine related compound B	0.67	1.2	0.15
Quetiapine desethoxy[d]	0.83	1.0	0.15
Quetiapine	1.0	—	—
Quetiapine tetraethylene glycol analog[b, e]	1.2	1.0	0.10
N-Ethyl quetiapine[b, f]	1.51	1.1	0.15
Bis(dibenzothiazepinyl) piperazine[b, g]	2.22	1.0	0.10
Any other unknown individual impurity	—	—	0.10
Total impurities	—	—	0.50

[a] Peak due to counter ion, included for peak identification purposes. Not to be included in total impurities.
[b] Process impurity specific to manufacturing process.
[c] 4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)-1,1-bis[2-(2-hydroxyethoxy)ethyl] piperazin-1-ium.
[d] 2-[4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)piperazin-1-yl]ethanol.
[e] 2-[2-(2-[2-[4-(Dibenzo[b,f][1,4]thiazepin-11-yl)piperazin-1-yl]ethoxy}ethoxy) ethoxy]ethanol.
[f] 11-(4-Ethylpiperazin-1-yl)dibenzo[*b,f*][1,4]thiazepine.
[g] 1,4-Bis(dibenzo[*b,f*][1,4]thiazepin-11-yl)piperazine.

USP Monographs

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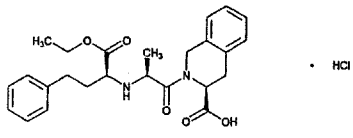
SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° to constant weight.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light.
- **USP Reference Standards** ⟨11⟩
  USP Quetiapine Fumarate RS
  USP Quetiapine Related Compound B RS
  11-(Piperazin-1-yl)dibenzo[*b,f*][1,4]thiazepine.
  $C_{17}H_{17}N_3S$ 295.40
  USP Quetiapine Related Compound G RS
  Dibenzo[*b,f*][1,4]thiazepin-11(10*H*)-one.
  $C_{13}H_9NOS$ 227.28
  USP Quetiapine System Suitability RS
  It contains quetiapine fumarate and NLT 0.1% of each of the following impurities: Quetiapine related compound B: 11-(Piperazin-1-yl)dibenzo[*b,f*][1,4]thiazepine; Quetiapine related compound G: Dibenzo[*b,f*][1,4]thiazepin-11(10H)-one; and Quetiapine desethoxy: 2-[4-(Dibenzo[*b,f*][1,4]thiazepin-11-yl)piperazin-1-yl]ethanol.

Quinapril Hydrochloride



$C_{25}H_{30}N_2O_5 \cdot HCl$ 474.98

3-Isoquinolinecarboxylic acid, 2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, monohydrochloride, [3*S*-[2[*R**(*R**)],3*R**]].
(*S*)-2-[(*S*)-*N*-[(*S*)-1-Carboxy-3-phenylpropyl]alanyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid, 1-ethyl ester, monohydrochloride [82586-55-8].

» Quinapril Hydrochloride contains not less than 98.5 percent and not more than 101.5 percent of $C_{25}H_{30}N_2O_5 \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Quinapril Hydrochloride RS
USP Quinapril Related Compound A RS
Ethyl [3*S*-[(2*R**), 3a, 11ab]]-1,3,4,6,11,11a-hexahydro-3-methyl-1,4-dioxo-a-(2-phenylethyl)-2*H*-pyrazino[1,2-b] isoquinoline-2-acetate.
$C_{25}H_{27}N_2O_4$ 419.49
USP Quinapril Related Compound B RS
3-Isoquinolinecarboxylic acid, 2-[2-[(1-carboxy-3-phenylpropyl)amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, [3*S*-[2[*R**(*R**)],3*R**]].
$C_{23}H_{26}N_2O_5$ 410.47

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Specific rotation ⟨781S⟩: between +14.4° and +15.4°.
Test solution: 20 mg per mL, in methanol.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Limit of residual solvents—

Standard stock solution—Transfer about 50 mL of dimethylformamide to a 200-mL volumetric flask. Add about 75 mg each of acetone and acetonitrile and 30 mg each of methylene chloride and toluene, each accurately weighed by difference, and mix. Dilute with dimethylformamide to volume, and mix.

System suitability solution 1—Transfer about 25 mL of dimethylformamide to a 50-mL volumetric flask. Add 35 µL of dehydrated alcohol and 25 µL of methylene chloride. Dilute with dimethylformamide to volume, and mix. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

System suitability solution 2—Transfer 2.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

Standard solution—Transfer 4.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

Test solution—Transfer about 60 mg of Quinapril Hydrochloride, accurately weighed, to a suitable headspace vial, add 5.0 mL of dimethylformamide, seal, and shake to dissolve.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a headspace sampler, a 0.53-mm × 30-m fused-silica column coated with a 1.0-µm film of phase G16, and a split injection system. The carrier gas is helium, flowing at a rate of 6 mL per minute. The split flow rate is about 100 mL per minute, with a back pressure of 3.5 psi. The oven temperature of the headspace sampler is maintained at 60°, and the vial pressure is maintained at 6.1 psi. The temperature of the headspace loop and transfer lines is maintained at 65°. The vials are equilibrated for 10 minutes prior to injection, and injection occurs every 36 minutes. The chromatograph is programmed as follows. Initially the column temperature is maintained at 35° for 10 minutes, then the temperature is increased at a rate of 7° per minute to 150°, and maintained at 150° for 4 minutes. The injection port temperature is maintained at 180°, and the detector is maintained at 240°. Chromatograph *System suitability solution 1,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.94 for methylene chloride and 1.0 for alcohol; the resolution, *R,* between methylene chloride and alcohol is not less than 1.2; the column efficiency, determined from the methylene chloride peak, is not less than 4900 theoretical plates; and the tailing factor for the methylene chloride peak is not more than 1.7. Chromatograph *System suitability solution 2,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 15.0%.

Procedure—Separately inject equal volumes (about 1 mL) of the gaseous headspace of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Separately

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calculate the percentages, by weight, of acetone, acetonitrile, methylene chloride, and toluene in the portion of Quinapril Hydrochloride taken by the formula:

$$0.2(W_S/W_Q)(r_U/r_S)$$

in which W_S is the weight, in mg, of the appropriate solvent taken to prepare the *Standard stock solution*; W_Q is the weight, in mg, of Quinapril Hydrochloride taken to prepare the *Test solution*; and r_U and r_S are the peak responses of the relevant solvent obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.25% each of acetone and acetonitrile is found; and not more than 0.1% each of methylene chloride and toluene is found.

Related compounds—

Diluent, *Mobile phase*, and *Chromatographic system*—Prepare as directed in the *Assay*.

System suitability solution—Prepare as directed for the *System suitability preparation* in the *Assay*.

Standard solution—Dissolve accurately weighed quantities of USP Quinapril Related Compound A RS and USP Quinapril Related Compound B RS in *Diluent* to obtain a solution having known concentrations of about 0.5 mg of each per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Test solution—Use the *Assay preparation*.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each quinapril related compound in the portion of Quinapril Hydrochloride taken by the formula:

$$100(V_U/W_U)C_S(r_U/r_S)$$

in which V_U is the volume, in mL, of the *Test solution*; W_U is the weight, in mg, of Quinapril Hydrochloride taken to prepare the *Test solution*; C_S is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard solution*; and r_U and r_S are the peak areas of the corresponding quinapril related compound obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.5% each of quinapril related compound A and quinapril related compound B is found. Calculate the percentage of each impurity in the portion of Quinapril Hydrochloride taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity obtained from the *Test solution*; and r_s is the sum of the responses of all the peaks obtained from the *Test solution*: not more than 0.2% of any individual impurity, other than quinapril related compound A and quinapril related compound B, is found; and not more than 2.0% of total impurities is found.

Content of chloride—Transfer about 100 mg of Quinapril Hydrochloride, accurately weighed, to a 100-mL beaker. Dissolve in 50 mL of water and 10 mL of alcohol. Acidify with nitric acid. Titrate with 0.01 N silver nitrate VS, and determine the endpoint potentiometrically, using suitable electrodes (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.01 N silver nitrate is equivalent to 0.3545 mg of chloride: not less than 7.2% and not more than 7.6% of chloride is found.

Assay—

Diluent—Prepare a mixture of pH 6.5, 0.025 M monobasic ammonium phosphate solution and acetonitrile (3:2).

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and methanesulfonic acid (72: 28: 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability preparation—Dissolve accurately weighed quantities of USP Quinapril Hydrochloride RS, USP Quinapril Related Compound A RS, and USP Quinapril Related Compound B RS in *Diluent* to obtain a solution having known concentrations of about 2 mg of USP Quinapril Hydrochloride RS per mL and 0.005 mg each of USP Quinapril Related Compound A RS and USP Quinapril Related Compound B RS per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Quinapril Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 2 mg per mL.

Assay preparation—Transfer about 100 mg of Quinapril Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector, a 4.6-mm × 3-cm guard column that contains 5-µm packing L10, and a 4.6-mm × 25-cm column that contains 5-µm packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the resolution between quinapril and quinapril related compound A is not less than 1.75; the resolution between quinapril and quinapril related compound B is not less than 3.5; the column efficiency is not less than 550 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the quinapril hydrochloride peaks. Calculate the quantity, in mg, of $C_{25}H_{30}N_2O_5 \cdot HCl$ in the portion of Quinapril Hydrochloride taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Quinapril Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Quinapril and Hydrochlorothiazide Tablets

DEFINITION

Quinapril and Hydrochlorothiazide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amounts of quinapril ($C_{25}H_{30}N_2O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The relative retention times of the major peaks from the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 1.36 g/L of monobasic potassium phosphate in water, add 2 mL of triethylamine, and adjust with phosphoric acid to a pH of 3.0. Pass through a suitable nylon filter of 0.45-µm pore size.

Mobile phase: See *Table 1*.

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Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	75	25
8	40	60
12	40	60
13	75	25
18	75	25

Diluent: Acetonitrile and *Buffer* (50:50)
Quinapril hydrochloride standard stock solution: 0.45 mg/mL of USP Quinapril Hydrochloride RS in *Diluent*
Hydrochlorothiazide standard stock solution: 0.5 mg/mL of USP Hydrochlorothiazide RS in *Diluent*
Standard solution: Dilute *Quinapril hydrochloride standard stock solution* and *Hydrochlorothiazide standard stock solution* with *Diluent* to final volume as given in *Table 2*.

Table 2

Tablet Strength Quinapril Hydrochloride/ Hydrochlorothiazide (mg/mg)	Quinapril Hydrochloride Standard Stock Solution (mL)	Hydrochlorothiazide Standard Stock Solution (mL)	Final Volume (mL)
10/12.5	5	5	100
20/12.5	10	5	200
20/25	5	5	100

Sample stock solution: Transfer 5 Tablets into a 250-mL volumetric flask, add 50 mL of *Diluent*, and sonicate for 15 min. Add about 50 mL of acetonitrile, and sonicate for 15 min with shaking. Add 50 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume.
Sample solution: 0.02 mg/mL of quinapril in *Diluent* from the *Sample stock solution*. [NOTE—The hydrochlorothiazide concentration may vary depending on the ratio of quinapril hydrochloride to hydrochlorothiazide in the Tablet.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column oven temperature: 35°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution*
Suitability requirements
Column efficiency: NLT 2500 theoretical plates from both quinapril and hydrochlorothiazide peaks
Tailing factor: NMT 2.0 for both quinapril and hydrochlorothiazide peaks
Relative standard deviation: NMT 2.0% for both quinapril and hydrochlorothiazide peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of quinapril ($C_{25}H_{30}N_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of quinapril from the *Sample solution*
r_S = peak response of quinapril from the *Standard solution*
C_S = concentration of USP Quinapril Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinapril in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of quinapril, 438.52
M_{r2} = molecular weight of quinapril hydrochloride, 474.98

Calculate the percentage of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Buffer, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 20 min
Quinapril hydrochloride standard stock solution: 0.45 mg/mL of USP Quinapril Hydrochloride RS in *Diluent*
Hydrochlorothiazide standard stock solution: 0.55 mg/mL of USP Hydrochlorothiazide RS in *Diluent*
Standard solution: Prepare dilutions of *Quinapril hydrochloride standard stock solution* and *Hydrochlorothiazide standard stock solution* in *Medium* as directed in *Table 3*.

Table 3

Tablet Strength Quinapril Hydrochloride/ Hydrochlorothiazide (mg/mg)	Quinapril Hydrochloride Standard Stock Solution (mL)	Hydrochlorothiazide Standard Stock Solution (mL)	Final Volume (mL)
10/12.5	5	5	200
20/12.5	10	5	200
20/25	5	5	100

Sample solution: Pass a portion of the solution under test through a suitable nylon filter of 0.45-µm pore size, discarding the first few mL.
Analysis
Calculate the percentage of quinapril ($C_{25}H_{30}N_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of quinapril from the *Sample solution*
r_S = peak response of quinapril from the *Standard solution*
C_S = concentration of USP Quinapril Hydrochloride RS in the *Standard solution*
L = label claim for quinapril hydrochloride (mg/Tablet)
V = volume of *Medium*, 900 mL

USP Monographs

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M_{r1} = molecular weight of quinapril, 438.52
M_{r2} = molecular weight of quinapril hydrochloride, 474.98

Calculate the percentage of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution*
L = label claim for hydrochlorothiazide (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of each quinapril ($C_{25}H_{28}N_6O$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer and **Diluent:** Proceed as directed in the *Assay*.
Mobile phase: See *Table 4*.

Table 4

Time (min)	Buffer (%)	Acetonitrile (%)
0	90	10
10	60	40
30	30	70
31	90	10
40	90	10

Standard stock solution: 0.45 mg/mL of each USP Quinapril Hydrochloride RS, USP Quinapril Related Compound A RS, and USP Quinapril Related Compound B RS, and 0.50 mg/mL of each USP Hydrochlorothiazide RS and USP Benzothiadiazine Related Compound A RS in *Diluent*
Standard solution: 0.45 µg/mL of quinapril hydrochloride, quinapril related compound A, and quinapril related compound B, and 0.5 µg/mL of hydrochlorothiazide and benzothiadiazine related compound A from *Standard stock solution* in *Diluent*
Sample stock solution: Transfer 5 Tablets into a 250-mL volumetric flask, add 50 mL of diluent, and sonicate for 10 min to disperse the Tablets. Add about 50 mL of acetonitrile, and sonicate for 15 min with shaking. Add 50 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume. Pass through a suitable nylon filter of 0.45-µm pore size.
Sample solution: For Tablet strengths of 10 mg/12.5 mg and 20 mg/12.5 mg of quinapril hydrochloride/hydrochlorothiazide, use *Sample stock solution* as is. For Tablet strength of 20 mg/25 mg of quinapril hydrochloride/hydrochlorothiazide, dilute 5 mL of *Sample stock solution* with *Diluent* to 10 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column oven temperature: 35°
Sample temperature: 5°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000 theoretical plates from both quinapril and hydrochlorothiazide peaks
Tailing factor: NMT 2.0 for both quinapril and hydrochlorothiazide peaks
Relative standard deviation: NMT 5.0% for both quinapril and hydrochlorothiazide peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of quinapril related compound A and quinapril related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of quinapril related compound A or quinapril related compound B from the *Sample solution*
r_S = peak response of USP Quinapril Related Compound A RS or USP Quinapril Related Compound B RS from the *Standard solution*
C_S = concentration of USP Quinapril Related Compound A RS and USP Quinapril Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinapril in the *Sample solution* (mg/mL)

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A from the *Standard solution*
C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each other impurity from the *Sample solution*
r_S = peak response of quinapril from the *Standard solution*
C_S = concentration of USP Quinapril Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinapril in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of quinapril, 438.52
M_{r2} = molecular weight of quinapril hydrochloride, 474.98

Acceptance criteria: See *Table 5*.

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Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A[a]	0.42	1.0
Chlorothiazide[b]	0.45	—
Hydrochlorothiazide[b]	0.49	—
5-Chlorohydrochlorothiazide[b]	0.65	—
Quinapril related compound B[c]	0.74	3.0
Hydrochlorothiazide dimer[b]	0.78	—
Quinapril methyl ester[b]	0.91	—
Quinapril[b]	1.00	—
Quinapril isopropyl ester[b]	1.10	—
Hexahydroquinapril[b]	1.23	—
Quinapril related compound A[d]	1.59	1.0
Quinapril, benzyl ester[b]	1.94	—
Any other individual unspecified impurity	—	0.2
Total impurities[e]	—	2.0

[a] 4-Amino-6-chloro-1,3-benzenedisulfonamide.
[b] Process related impurity, monitored in the drug substance.
[c] 3-Isoquinolinecarboxylic acid, 2-[2-[(1-carboxy-3-phenylpropyl)amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, [3*S*-[2[*R**(*R**)],3*R**]]].
[d] Ethyl[3*S*-[2(*R**),3a,11a beta]]-1,3,4,6,11,11a-hexahydro-3-methyl-1,4-dioxo-alpha-(2-phenylethyl)-2*H*-pyrazino[1,2-*b*]isoquinoline-2-acetate.
[e] Total impurities does not include quinapril related compound B and benzothiadiazine related compound A.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and protect from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzothiadiazine Related Compound A RS
  4-Amino-6-chloro-1,3-benzenedisulfonamide.
  $C_6H_8ClN_3O_4S_2$ 285.73
  USP Hydrochlorothiazide RS
  USP Quinapril Hydrochloride RS
  USP Quinapril Related Compound A RS
  Ethyl[3*S*-[2(*R**),3a,11a beta]]-1,3,4,6,11,11a-hexahydro-3-methyl-1,4-dioxo-alpha-(2-phenylethyl)-2*H*-pyrazino[1,2-*b*]isoquinoline-2-acetate.
  $C_{25}H_{28}N_2O_4$ 420.50
  USP Quinapril Related Compound B RS
  3-Isoquinolinecarboxylic acid, 2-[2-[(1-carboxy-3-phenylpropyl)amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, 3*S*-[2[*R**(*R**)],3*R**]]-.
  $C_{23}H_{26}N_2O_5$ 410.46

Quinapril Tablets

» Quinapril Tablets contain an amount of Quinapril Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of quinapril ($C_{25}H_{30}N_2O_5$).

Packaging and storage—Preserve in well-closed containers. Store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Quinapril Hydrochloride RS
USP Quinapril Related Compound A RS
Ethyl [3*S*-[(2*R**), 3a, 11ab]]-1,3,4,6,11,11a-hexahydro-3-methyl-1,4-dioxo-a-(2-phenylethyl)-2*H*-pyrazino[1,2-b] isoquinoline-2-acetate.
$C_{25}H_{27}N_2O_4$ 419.49
USP Quinapril Related Compound B RS
3-Isoquinolinecarboxylic acid, 2-[2-[(1-carboxy-3-phenylpropyl)amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, [3*S*-[2[*R**(*R**)],3*R**]].
$C_{23}H_{26}N_2O_5$ 410.47

Identification—

A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—Transfer a quantity of finely powdered Tablets, equivalent to about 10.0 mg of quinapril, to a 15-mL centrifuge tube; add 5 mL of methanol; mix; and centrifuge for 10 minutes.
Standard solution—Transfer about 10.8 mg of USP Quinapril Hydrochloride RS to a 15-mL centrifuge tube, add 5 mL of methanol, mix, and centrifuge for 10 minutes.
Application volume: 25 µL.
Developing solvent system: a mixture of methanol and ethyl acetate (1:1).
Procedure—Proceed as directed in the chapter, except to wash the plate in methanol and air-dry it prior to use.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{25}H_{30}N_2O_5$ dissolved by employing the procedure set forth in the *Assay,* using a filtered portion of the solution under test as the *Assay preparation,* using methanol to prepare the *Standard preparation,* and making any necessary volumetric adjustments with water.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{25}H_{30}N_2O_5$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.
PROCEDURE FOR CONTENT UNIFORMITY—
Solvent, Buffered solvent, Mobile phase, and *Chromatographic system*—Prepare as directed in the *Assay.*
Standard solution—Prepare as directed for *Standard preparation* in the *Assay.*
Test solution—Transfer 1 Tablet to a volumetric flask. Add a volume of *Solvent,* equivalent to about one-half the flask volume; sonicate for 5 minutes; and shake by mechanical means for about 15 minutes. Dilute with *Solvent* to volume, mix, and pass through a suitable filter, discarding the first portion of the filtrate. Dilute a portion of the filtrate quantitatively, and stepwise if necessary, with *Solvent* to obtain a solution containing about 0.108 mg of quinapril hydrochloride per mL.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of quinapril ($C_{25}H_{30}N_2O_5$) in each Tablet taken by the formula:

$$(438.52/474.98)C(L/D)(r_U/r_S)$$

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in which 438.52 and 474.98 are the molecular weights of quinapril and quinapril hydrochloride, respectively; *C* is the concentration, in mg per mL, of USP Quinapril Hydrochloride RS in the *Standard solution; L* is the labeled quantity, in mg, of quinapril in each Tablet; *D* is the concentration, in mg per mL, of quinapril hydrochloride in the *Test solution;* and r_U and r_S are the quinapril peak areas obtained from the *Test solution* and the *Standard solution,* respectively.

Related compounds—

Solvent, Buffered solvent, and *Mobile phase*—Prepare as directed in the *Assay.*

Resolution solution—Dissolve accurately weighed quantities of USP Quinapril Hydrochloride RS, USP Quinapril Related Compound A RS, and USP Quinapril Related Compound B RS in *Solvent* to obtain a solution having known concentrations of about 0.1 mg of USP Quinapril Hydrochloride RS and 0.005 mg each of USP Quinapril Related Compound A RS and USP Quinapril Related Compound B RS per mL.

Standard solution—Dissolve accurately weighed quantities of USP Quinapril Hydrochloride RS, USP Quinapril Related Compound A RS, and USP Quinapril Related Compound B RS in *Solvent,* and dilute quantitatively, and stepwise if necessary, to obtain a solution having known concentrations of about 0.5 µg each of USP Quinapril Hydrochloride RS, USP Quinapril Related Compound A RS, and USP Quinapril Related Compound B RS per mL.

Test solution—Use the *Assay preparation.*

Chromatographic system—Prepare as directed in the *Assay.* Chromatograph the *Resolution solution,* and record the peak areas as directed for *Procedure:* the relative retention times are about 0.6 for quinapril related compound B, 1.0 for quinapril, and 2.0 for quinapril related compound A; and the resolution, *R,* between quinapril and quinapril related compound A and between quinapril and quinapril related compound B is not less than 2.0. Chromatograph the *Standard solution,* and record the peak areas as directed for *Procedure:* the column efficiency is not less than 600 theoretical plates; the tailing factor for the quinapril and quinapril related compound A peaks is less than 1.5 and that for the quinapril related compound B peak is less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0% for quinapril and not more than 3.0% for each quinapril related compound.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of each quinapril related compound in the portion of Tablets taken by the formula:

$$500CD(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard solution; D* is the dilution factor used to prepare the *Test solution;* and r_U and r_S are the peak areas of the corresponding quinapril related compound obtained from the *Test solution* and the *Standard solution,* respectively: not more than 1.0% of quinapril related compound A is found; not more than 3.0% of quinapril related compound B is found; and not more than 3.6% of total impurities is found.

Assay—

Solvent—Prepare a mixture of water and acetonitrile (65:35).

Buffered solvent—Prepare a mixture of pH 6.5, 0.05 M dibasic potassium phosphate and acetonitrile (65:35).

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and methanesulfonic acid (65: 35: 0.2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Quinapril Hydrochloride RS in *Solvent* to obtain a solution having a known concentration of about 1.08 mg per mL. Quantitatively dilute with *Buffered solvent* to obtain a solution having a known concentration of about 0.108 mg per mL.

Assay preparation—Transfer 10 Tablets to a 500-mL volumetric flask, add about 300 mL of *Solvent,* and sonicate until the Tablets have disintegrated. Shake by mechanical means for about 15 minutes, dilute with *Solvent* to volume, mix, and centrifuge. Dilute quantitatively, and stepwise if necessary, with *Solvent* to obtain a solution having a concentration of about 0.1 mg of quinapril per mL; and pass through a suitable filter, discarding the first portion of the filtrate.

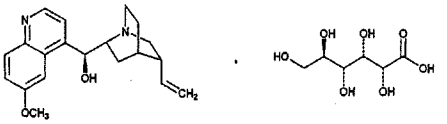
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 6.0-mm × 4-cm column that contains 3-µm packing L10. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the column efficiency is not less than 600 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of quinapril ($C_{25}H_{30}N_2O_5$) in the portion of Tablets taken by the formula:

$$500CD(438.52/474.98)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Quinapril Hydrochloride RS in the *Standard preparation; D* is the dilution factor used to prepare the *Assay preparation;* 438.52 and 474.98 are the molecular weights of quinapril and quinapril hydrochloride, respectively; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Quinidine Gluconate



$C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$ 520.57

Cinchonan-9-ol, 6′-methoxy-, (9*S*)-, mono-D-gluconate (salt); Quinidine mono-D-gluconate (salt) [7054-25-3].

DEFINITION

Quinidine Gluconate is the gluconate of an alkaloid that may be obtained from various species of *Cinchona* and their hybrids, from *Remijia pedunculata* Flückiger (Fam. Rubiaceae), or prepared from quinine. Quinidine Gluconate contains NLT 99.0% and NMT 100.5% of total alkaloid salt, calculated as quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$) on the dried basis.

USP Monographs

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IDENTIFICATION

• **A.**

Sample solution: 1-in-2000 solution of Quinidine Gluconate in dilute sulfuric acid (1 in 350)

Acceptance criteria: The *Sample solution* exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid, the fluorescence disappears.

• **B.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Organic Impurities*.

• **C.**

Sample solution: 20 mg/mL of Quinidine Gluconate

Acceptance criteria: The *Sample solution* is dextrorotatory.

• **D.**

Sample: Dissolve 700 mg of Quinidine Gluconate in 5 mL of water with the aid of heat. Add 1 mL of glacial acetic acid and 200 mg of phenylhydrazine hydrochloride.

Analysis: Heat in a water bath for 15 min, cool, and scratch the inner surface of the tube with a glass rod.

Acceptance criteria: Orange crystals are formed.

ASSAY

• **PROCEDURE**

Sample solution: Dissolve 150 mg of Quinidine Gluconate in 10 mL of glacial acetic acid, heating gently if necessary, and cool the solution.

Titrimetric system

Mode: Titrimetry

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: To the *Sample solution* add 20 mL of acetic anhydride and 4 drops of *p*-naphtholbenzein TS, and titrate with *Titrant* from a 10-mL microburet to a green endpoint. Perform a blank determination, and make any necessary corrections (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 26.03 mg of total alkaloid salt, calculated as quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$).

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.15%

• **ORGANIC IMPURITIES**

Standard solution A: 6 mg/mL of USP Quinidine Gluconate RS in diluted alcohol

Standard solution B: 0.06 mg/mL of USP Quinidine Gluconate RS in diluted alcohol from *Standard solution A*

Standard solution C: 0.04 mg/mL of USP Quininone RS (corresponding to 0.06 mg of the gluconate) and 0.08 mg/mL of cinchonine (corresponding to 0.12 mg of the gluconate) in diluted alcohol

Sample solution: 6 mg/mL of Quinidine Gluconate in diluted alcohol

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform, acetone, and diethylamine (50:40:10)

Analysis

Samples: *Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*

Proceed as directed in the chapter. The solvent chamber is used without previous equilibration. When the solvent front has moved about 15 cm, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray with glacial acetic acid. Locate the spots on the plate by examination under long-wavelength UV light. Then spray the plate with potassium iodoplatinate TS.

Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution C* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spots appearing at the R_F value of Quinidine Gluconate and dihydroquinidine gluconate (the two spots most evident from *Standard solution A*), any additional fluorescent spot is not greater in size or intensity than the principal spot of *Standard solution B*. After treatment with potassium iodoplatinate TS, any spot produced by the *Sample solution* is not greater in size or intensity than a corresponding spot from *Standard solution C*.

• **LIMIT OF DIHYDROQUINIDINE GLUCONATE**

Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.

Solution B: Dissolve 10.0 mL of diethylamine in water to prepare a 100-mL solution.

Mobile phase: Acetonitrile, *Solution A, Solution B,* and water (100:20:20:860). Adjust with *Solution B* to a pH of 2.6.

System suitability solution: 0.2 mg/mL each of quinidine gluconate and dihydroquinidine hydrochloride prepared as follows. Dissolve a suitable quantity of each quinidine gluconate and dihydroquinidine hydrochloride in about 10% of total volume of methanol, and dilute with *Mobile phase* to volume.

Sample solution: 0.26 mg/mL of Quinidine Gluconate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 30-cm; packing L1

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for quinidine and dihydroquinidine are 1 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2.5 between quinidine and dihydroquinidine

Relative standard deviation: NMT 2.0%

Analysis

Sample: *Sample solution*

Acceptance criteria: 20%; the response of the dihydroquinidine peak is NMT 0.25 that of the quinidine peak.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 1 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Quinidine Gluconate RS

USP Quininone RS

$C_{20}H_{22}N_2O_2$ 322.40

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Quinidine Gluconate Injection

DEFINITION

Quinidine Gluconate Injection is a sterile solution of Quinidine Gluconate in Water for Injection. Each mL contains amounts of quinidine gluconate and dihydroquinidine gluconate totaling to NLT 76 mg and NMT 84 mg of quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$).

IDENTIFICATION

• **A.**

Sample solution: 1-in-150 solution of Injection in dilute sulfuric acid (1 in 350)

Acceptance criteria: The *Sample solution* exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid, the fluorescence disappears.

• **B.**

Sample solution: 1-in-4 solution of Injection

Acceptance criteria: The *Sample solution* is dextrorotatory.

• **C.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Organic Impurities*.

ASSAY

• **PROCEDURE**

Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.

Solution B: Dissolve 10.0 mL of diethylamine in water to prepare a 100-mL solution.

Mobile phase: Acetonitrile, *Solution A*, *Solution B*, and water (100:20:20:860). Adjust with *Solution B* to a pH of 2.6.

System suitability solution: 0.2 mg/mL each of quinidine gluconate and dihydroquinidine hydrochloride prepared as follows. Dissolve a suitable quantity of each quinidine gluconate and dihydroquinidine hydrochloride in about 10% of the total volume of methanol, and dilute with *Mobile phase* to volume.

Standard solution: 0.26 mg/mL of USP Quinidine Gluconate RS in *Mobile phase*

Sample stock solution: Transfer a volume of Injection, equivalent to 400 mg of quinidine gluconate, to a 50-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Transfer 3 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 30-cm column; packing L1

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for quinidine and dihydroquinidine are 1 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2.5 between quinidine and dihydroquinidine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity in mg/mL of the sum of quinidine gluconate and dihydroquinidine gluconate in the portion of Injection taken:

$$\text{Result} = (r_{B,U} + r_{D,U})/(r_{B,S} + r_{D,S}) \times (C_S/V) \times F$$

$r_{B,U}$ = peak response of quinidine from the *Sample solution*

$r_{D,U}$ = peak response of dihydroquinidine from the *Sample solution*

$r_{B,S}$ = peak response of quinidine from the *Standard solution*

$r_{D,S}$ = peak response of dihydroquinidine from the *Standard solution*

C_S = concentration of USP Quinidine Gluconate RS in the *Standard solution* (mg/mL)

V = volume of the Injection taken (mL)

F = dilution factor, 5000/3

Acceptance criteria: Each mL contains amounts of quinidine gluconate and dihydroquinidine gluconate totaling to NLT 76 mg and NMT 84 mg of quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$).

IMPURITIES

• **ORGANIC IMPURITIES**

Standard solution A: 6 mg/mL of USP Quinidine Gluconate RS in diluted alcohol

Standard solution B: 0.06 mg/mL of USP Quinidine Gluconate RS in diluted alcohol from the *Standard solution A*

Standard solution C: 0.04 mg/mL of USP Quininone RS (corresponding to 0.06 mg of the gluconate) and 0.08 mg/mL of cinchonine (corresponding to 0.12 mg of the gluconate) in diluted alcohol

Sample solution: Mix, equivalent to 80 mg of quinidine gluconate from a volume of Injection, with 25 mL of water. Add 2 drops of 2 N sulfuric acid, and extract with 50 mL of ether, discarding the ether extract. To the aqueous solution add 2 mL of 1 N sodium hydroxide, extract with 50 mL of ether, wash the extract with 25 mL of water, and discard the aqueous solutions. Evaporate the ether extract just to dryness, and dissolve the residue in 10 mL of alcohol.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform, acetone, and diethylamine (5:4:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Proceed as directed in the chapter. The solvent chamber is used without previous equilibration. When the solvent front has moved about 15 cm, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray with glacial acetic acid. Locate the spots on the plate by examination under long-wavelength UV light. Then spray the plate with potassium iodoplatinate TS.

Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution C* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spots appearing at the R_F value of quinidine gluconate and dihydroquinidine gluconate (the two spots most evident from *Standard solution A*), any additional fluorescent spot is not greater in size or intensity than the principal spot of *Standard solution B*. After the treatment with potassium iodoplatinate TS, any spot produced by the *Sample solution* is not greater in size or intensity than a corresponding spot from *Standard solution C*.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.6 USP Endotoxin Unit/mg of quinidine gluconate

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• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Quinidine Gluconate RS
USP Quininone RS
Cinchonan-9-one, 6'-methoxy-, (8alpha)-.
$C_{20}H_{22}N_2O_2$ 322.40

Quinidine Gluconate Extended-Release Tablets

DEFINITION

Quinidine Gluconate Extended-Release Tablets contain amounts of quinidine gluconate and dihydroquinidine gluconate totaling NLT 90.0% and NMT 110.0% of the labeled amount of quinidine gluconate, calculated as quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$).

IDENTIFICATION

• **A.**
Sample solution: Shake an amount, equivalent to 50 mg of quinidine gluconate from powdered Tablets, with 100 mL of dilute sulfuric acid (1 in 350), and filter.
Acceptance criteria: The filtrate so obtained exhibits a vivid blue fluorescence when viewed under long-wavelength UV light. On the addition of hydrochloric acid, the fluorescence disappears.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Organic Impurities*.

ASSAY

• **PROCEDURE**
Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.
Solution B: Dissolve 10.0 mL of diethylamine in water to prepare a 100-mL solution.
Mobile phase: Acetonitrile, *Solution A*, *Solution B*, and water (100:20:20:860). Adjust with *Solution B* to a pH of 2.6, if found to be lower.
System suitability solution: Transfer 10 mg each of quinidine gluconate and dihydroquinidine hydrochloride to a 50-mL volumetric flask. Dissolve in 5 mL of methanol, and dilute with *Mobile phase* to volume.
Standard solution: 0.2 mg/mL of USP Quinidine Gluconate RS in *Mobile phase*
Sample stock solution: To an amount equivalent to 160 mg of quinidine gluconate from NLT 20 finely powdered Tablets in a 100-mL volumetric flask add 80 mL of a mixture of methanol and water (1:1), and sonicate until evenly dispersed. Cool to room temperature, dilute with a mixture of methanol and water (1:1) to volume, and filter, discarding the first 20 mL of the filtrate.
Sample solution: 0.19 mg/mL of quinidine gluconate in *Mobile phase* prepared as follows. Transfer 3.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 3- to 5-mm × 25- to 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for quinidine and dihydroquinidine are 1 and 1.5, respectively, for the *System suitability solution*.]
Suitability requirements
Resolution: NLT 1.2 between quinidine and dihydroquinidine, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the sum of the percentages of quinidine gluconate and dihydroquinidine gluconate in the Tablets taken:

$$\text{Result} = [(r_{B,U} + r_{D,U})/(r_{B,S} + r_{D,S})] \times (C_S/C_U) \times 100$$

$r_{B,U}$ = peak response of quinidine from the *Sample solution*
$r_{D,U}$ = peak response of dihydroquinidine from the *Sample solution*
$r_{B,S}$ = peak response of quinidine from the *Standard solution*
$r_{D,S}$ = peak response of dihydroquinidine from the *Standard solution*
C_S = concentration of USP Quinidine Gluconate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinidine gluconate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
Medium: Add 6.9 g of anhydrous sodium acetate and 0.525 mL of glacial acetic acid to 1 L of water. Adjust with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 5.4; 900 mL.
Apparatus 2: 75 rpm
Times: 1, 2, 4, and 8 h
Standard solution: A known concentration of USP Quinidine Gluconate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* if necessary, in comparison with the *Standard solution* concentration.
Instrumental conditions
Mode: UV
Analytical wavelength: 235 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$) dissolved from UV absorbances of the *Sample solution* and *Standard solution*.
Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved
1	30%–50%

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Table 1 *(continued)*

Time (h)	Amount Dissolved
2	45%–65%
4	60%–85%
8	NLT 85%

The percentages of the labeled amount of quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.1 N hydrochloric acid; 600 mL
Apparatus 2: 75 rpm
Times: Proceed as directed for *Test 1*.
Standard solution: A known concentration of USP Quinidine Gluconate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* if necessary, in comparison with the *Standard solution* concentration.
Instrumental conditions
Mode: UV
Analytical wavelength: 235 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$) dissolved from UV absorbances of the *Sample solution* and *Standard solution*.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved
1	30%–45%
2	45%–60%
4	60%–80%
8	NLT 85%

The percentages of the labeled amount of quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: Proceed as directed for *Test 1*.
Apparatus: Proceed as directed for *Test 1*, using 8-mesh sinker baskets.[1]
Times: 1, 2, and 4 h
Standard solution: USP Quinidine Gluconate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, in comparison with the *Standard solution* concentration.
Instrumental conditions
Mode: UV
Analytical wavelength: 235 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$) dissolved from the UV absorbances of the *Sample solution* and the *Standard solution*.

[1] A suitable sinker is available from Van Kel, www.varianinc.com, catalog number 12-3062.

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved
1	20%–50%
2	40%–70%
4	NLT 75%

The percentages of the labeled amount of quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Standard solution: 0.0525 mg/mL of USP Quinidine Gluconate RS in 0.1 N hydrochloric acid
Sample solution: Transfer 1 intact or powdered Tablet to a 250-mL volumetric flask, and add 125 mL of 0.1 N hydrochloric acid. Heat the sample with frequent agitation just to boiling, and cool to room temperature. Dilute with 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 20 mL of filtrate. If necessary, further dilute quantitatively with 0.1 N hydrochloric acid.
Instrumental conditions
Mode: UV
Cell: 1 cm
Analytical wavelength: 347 nm
Blank: 0.1 N hydrochloric acid
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Concomitantly determine the absorbances of the *Samples*.
Calculate the percentage of the labeled amounts of active ingredients, calculated as quinidine gluconate ($C_{20}H_{24}N_2O_2 \cdot C_6H_{12}O_7$), in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Quinidine Gluconate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinidine gluconate in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Standard solution A: 6 mg/mL of USP Quinidine Gluconate RS in diluted alcohol
Standard solution B: 0.06 mg/mL of USP Quinidine Gluconate RS in diluted alcohol from *Standard solution A*
Standard solution C: 0.04 mg/mL of USP Quininone RS (corresponding to 0.06 mg of the gluconate) in diluted alcohol
Sample solution: Nominally equivalent to 6 mg/mL of quinidine gluconate prepared as follows. Shake a quantity of powdered Tablets, equivalent to about 150 mg of quinidine gluconate, with 25 mL of diluted alcohol for 10 min, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, acetone, and diethylamine (50:40:10)

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Analysis

Samples: *Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*

Proceed as directed in the chapter. The solvent chamber is used without previous equilibration. When the solvent front has moved 15 cm, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray with glacial acetic acid. Locate the spots on the plate by examination under long-wavelength UV light.

Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution C* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spots appearing at the R_F value of quinidine gluconate and dihydroquinidine gluconate (the two spots most evident from *Standard solution A*), any additional fluorescent spot is not greater in size or intensity than the principal spot of *Standard solution B.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **LABELING:** The labeling indicates the *Dissolution Test* with which the product complies.
- **USP REFERENCE STANDARDS** ⟨11⟩

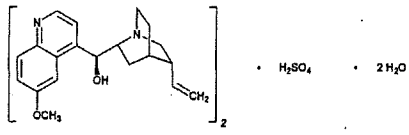
USP Quinidine Gluconate RS

USP Quininone RS

Cinchonan-9-one, 6′-methoxy-, (8alpha)-.

$C_{20}H_{22}N_2O_2$ 322.40

Quinidine Sulfate



$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ 782.94

$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$ 746.93

Cinchonan-9-ol, 6′-methoxy-, (9*S*)-, sulfate (2:1) (salt), dihydrate;

Quinidine sulfate (2:1) (salt) dihydrate [6591-63-5].

Anhydrous [50-54-4].

DEFINITION

Quinidine Sulfate is the sulfate of an alkaloid obtained from various species of *Cinchona* and their hybrids and from *Remijia pedunculata* Flückiger (Fam. Rubiaceae), or prepared from quinine. It contains NLT 99.0% and NMT 101.0% of total alkaloid salt, calculated as Quinidine Sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4]$, on the anhydrous basis.

IDENTIFICATION

- **A.**

Sample solution: 1-in-2000 solution of Quinidine Sulfate in dilute sulfuric acid (1 in 350)

Acceptance criteria: The *Sample solution* exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid, the fluorescence disappears.

- **B.** The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution,* as obtained in *Organic Impurities.*
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩

Sample solution: 20-mg/mL solution made with the aid of a few drops of hydrochloric acid

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Sample solution: Dissolve 200 mg of Quinidine Sulfate in 20 mL of glacial acetic acid, heating if necessary, and cool the solution.

Titrimetric system

Mode: Titrimetry

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: To the *Sample solution* add 20 mL of acetic anhydride and 4 drops of *p*-naphtholbenzein TS, and titrate with *Titrant* from a 10-mL microburet to a green endpoint. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 24.90 mg of total alkaloid salt, calculated as quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4]$.

Acceptance criteria: 99.0%–101.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Standard solution A: 6 mg/mL of USP Quinidine Sulfate RS in diluted alcohol

Standard solution B: 0.06 mg/mL of USP Quinidine Sulfate RS in diluted alcohol from *Standard solution A*

Standard solution C: 0.05 mg/mL of USP Quininone RS (corresponding to 0.06 mg of the sulfate) and 0.10 mg/mL of cinchonine (corresponding to 0.12 mg of the sulfate) in diluted alcohol

Sample solution: 6 mg/mL of Quinidine Sulfate in diluted alcohol

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform, acetone, and diethylamine (50:40:10)

Analysis

Samples: *Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*

Proceed as directed in the chapter. The solvent chamber is used without previous equilibration. When the solvent front has moved 15 cm, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray with glacial acetic acid. Locate the spots on the plate by examination under long-wavelength UV light. Then spray the plate with potassium iodoplatinate TS.

Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution C* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spots appearing at the R_F value of quinidine sulfate and dihydroquinidine sulfate (the two spots most evident from *Standard solution A*), any additional fluorescent spot is not greater in size or intensity than the principal spot of *Standard solution B.* After the treatment with potassium iodoplatinate TS, any spot produced by the *Sample solution* is not greater in size or intensity than a corresponding spot from *Standard solution C.*

- **LIMIT OF DIHYDROQUINIDINE SULFATE**

Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.

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Solution B: Dilute 10.0 mL of diethylamine with water to 100 mL.
Mobile phase: Acetonitrile, *Solution B*, *Solution A*, and water (100:20:20:860). Adjust with diethylamine to a pH of 2.6.
System suitability solution: 0.2 mg/mL each of USP Quinidine Sulfate RS and dihydroquinidine hydrochloride prepared as follows. Dissolve a suitable quantitiy of each USP Quinidine Sulfate RS and dihydroquinidine hydrochloride in about 10% of total volume of methanol, and dilute with *Mobile phase* to volume.
Sample solution: 0.2 mg/mL of Quinidine Sulfate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 3.9-mm × 30-cm; packing L1
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for quinidine and dihydroquinidine are 1 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 2.5 between quinidine and dihydroquinidine
Relative standard deviation: NMT 2.0%
Analysis
Sample: *Sample solution*
Acceptance criteria: 20.0%; the response of the dihydroquinidine peak is NMT 0.25 that of the quinidine peak.

SPECIFIC TESTS
- **CHLOROFORM–ALCOHOL-INSOLUBLE SUBSTANCES**
Sample solution: Warm 2 g of Quinidine Sulfate with 15 mL of chloroform and dehydrated alcohol mixture (2:1) at 50° for 10 min.
Analysis: Pass the *Sample solution* through a tared, sintered-glass filter, using gentle suction. Wash the filter with five 10-mL portions of the chloroform–alcohol mixture (2:1), dry at 105° for 1 h, and weigh.
Acceptance criteria: NMT 0.1%; the weight of the residue does not exceed 2 mg.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL of Quinidine Sulfate in 0.1 N hydrochloric acid
Acceptance criteria: +275° to +288° on the anhydrous basis
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 4.0%–5.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Quinidine Sulfate RS
USP Quininone RS
Cinchonan-9-one, 6′-methoxy-, (8-alpha)-.
$C_{20}H_{22}N_2O_2$ 322.40

Quinidine Sulfate Capsules

» Quinidine Sulfate Capsules contain amounts of quinidine sulfate and dihydroquinidine sulfate totaling not less than 90.0 percent and not more than 110.0 percent of the labeled amount of quinidine sulfate, calculated as $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Quinidine Sulfate RS
USP Quininone RS
$C_{20}H_{22}N_2O_2$ 322.40

Identification—
A: Shake a quantity of the contents of Capsules, equivalent to about 100 mg of quinidine sulfate, with 10 mL of dilute sulfuric acid (1 in 350), and filter: an appropriate dilution of the filtrate exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid the fluorescence disappears.
B: In the test for *Chromatographic purity*, the R_F value of the principal spot obtained from the *Test preparation* corresponds to that from the *Standard preparation*.
C: Shake a quantity of the contents of Capsules, equivalent to about 100 mg of quinidine sulfate, with 10 mL of dilute hydrochloric acid (1 in 100), and filter: the filtrate responds to the tests for *Sulfate* ⟨191⟩.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 248 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Quinidine Sulfate RS in the same *Medium*.
Tolerances—Not less than 85% (*Q*) of the labeled amount of $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Transfer the contents of 1 Capsule to a 250-mL volumetric flask, add about 175 mL of dilute hydrochloric acid (1 in 100), and shake by mechanical means for 30 minutes. Add dilute hydrochloric acid (1 in 100) to volume, and mix. Filter a portion of the mixture, discarding the first 20 mL of the filtrate. Concomitantly determine the absorbances of this solution, quantitatively diluted, if necessary, and a Standard solution of USP Quinidine Sulfate RS in dilute hydrochloric acid (1 in 100) having a known concentration of about 40 µg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 345 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of active ingredients, calculated as quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$, in the Capsule taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which T is the labeled quantity, in mg, of quinidine sulfate in the Capsule, D is the concentration, in µg per mL, of quinidine sulfate in the solution from the Capsule, based on the labeled quantity per Capsule and the extent of dilution, C is the concentration, in µg per mL, of USP Quinidine Sulfate RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Capsule and the Standard solution, respectively.
Chromatographic purity—Shake a quantity of the contents of Capsules, equivalent to about 150 mg of quinidine sulfate, with 25 mL of diluted alcohol for 10 minutes, and filter.

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Using this as the *Test preparation,* proceed as directed in the test for *Chromatographic purity* under *Quinidine Sulfate.*

Assay—

Methanesulfonic acid solution, Diethylamine solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed for *Limit of dihydroquinidine sulfate* under *Quinidine Sulfate.*

Standard preparation—Transfer about 20 mg of USP Quinidine Sulfate RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer the contents of not fewer than 20 Capsules to a container, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 160 mg of quinidine sulfate, to a 100-mL volumetric flask, add 80 mL of methanol, and shake the flask by mechanical means for 30 minutes. Dilute with methanol to volume, mix, and filter, discarding the first 10 mL of the filtrate. Transfer 3.0 mL of the filtrate to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure —Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into a chromatograph. Calculate the quantity, in mg, of the sum of quinidine sulfate and dihydroquinidine sulfate in the portion of Capsules taken by the formula:

$$(2500/3)C(r_{b,U} + r_{d,U})/(r_{b,S} + r_{d,S})$$

in which C is the concentration, in mg per mL, of USP Quinidine Sulfate RS in the *Standard preparation;* $r_{b,U}$ and $r_{b,S}$ are the peak responses of quinidine obtained from the *Assay preparation* and the *Standard preparation,* respectively; and $r_{d,U}$ and $r_{d,S}$ are the peak responses of dihydroquinidine obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Quinidine Sulfate Compounded Oral Suspension

DEFINITION

Quinidine Sulfate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of quinidine sulfate [$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$]. Prepare Quinidine Sulfate Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Quinidine Sulfate	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF,* and Vehicle for Oral Suspension, *NF,* a sufficient quantity to make	100 mL

If using *Quinidine Sulfate* tablets, place in a suitable mortar, and comminute into a fine powder, or add *Quinidine Sulfate* powder to the mortar. Add 15 mL of the *Vehicle,* and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar to the calibrated bottle. Add sufficient *Vehicle* to volume, and mix well.

ASSAY

• **PROCEDURE**

Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.

Solution B: Dissolve 10.0 mL of diethylamine in water to obtain 100 mL of solution.

Mobile phase: Acetonitrile, *Solution A, Solution B,* and water (10:1:1:40)

Standard solution: 100 µg/mL of USP Quinidine Sulfate RS in *Mobile phase*

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the *Sample solution* into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for quinidine sulfate is about 8.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of quinidine sulfate [$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$] in the portion of Oral Suspension taken:

$$\text{Result} = [(r_{B,U} + r_{D,U})/(r_{B,S} + r_{D,S})] \times (C_S/V) \times 100$$

$r_{B,U}$ = peak response of quinidine from the *Sample solution*

$r_{D,U}$ = peak response of dihydroquinidine from the *Sample solution*

$r_{B,S}$ = peak response of quinidine from the *Standard solution*

$r_{D,S}$ = peak response of dihydroquinidine from the *Standard solution*

C_S = concentration of USP Quinidine Sulfate RS in the *Standard solution* (mg/mL)

V = volume of Oral Suspension taken (mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.4–4.4

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at room temperature, or in a refrigerator.

• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at room temperature, or in a refrigerator

• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date.*

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Quinidine Sulfate RS

Quinidine Sulfate Tablets

DEFINITION

Quinidine Sulfate Tablets contain amounts of quinidine sulfate and dihydroquinidine sulfate totaling NLT 90.0% and NMT

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110.0% of the labeled amount of quinidine sulfate, calculated as quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$.

IDENTIFICATION

• **A.**

Analysis: Shake the equivalent to 100 mg of quinidine sulfate from powdered Tablets, with 10 mL of dilute sulfuric acid (1 in 350), and filter.

Acceptance criteria: An appropriate dilution of the filtrate exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid, the fluorescence disappears.

• **B.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Organic Impurities*.

• **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩

Analysis: Shake the equivalent to 100 mg of quinidine sulfate from powdered Tablets, with 10 mL of dilute hydrochloric acid (1 in 100), and filter.

Acceptance criteria: The filtrate meets the requirements.

ASSAY

• **PROCEDURE**

Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.

Solution B: Dilute 10.0 mL of diethylamine with water to 100 mL.

Mobile phase: Acetonitrile, *Solution B*, *Solution A*, and water (100:20:20:860). Adjust with diethylamine to a pH of 2.6.

System suitability solution: 0.2 mg/mL each of USP Quinidine Sulfate RS and dihydroquinidine hydrochloride prepared as follows. Dissolve a suitable quantitiy of each USP Quinidine Sulfate RS and dihydroquinidine hydrochloride in about 10% of total volume of methanol, and dilute with *Mobile phase* to volume.

Standard solution: 0.2 mg/mL of USP Quinidine Sulfate RS in *Mobile phase*

Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 160 mg of quinidine sulfate, to a 100-mL volumetric flask. Add 80 mL of methanol, and shake the flask by mechanical means for 30 min. Dilute with methanol to volume, and filter, discarding the first 10 mL of filtrate.

Sample solution: Nominally equivalent to 0.192 mg/mL of quinidine sulfate in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 30-cm; packing L1

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for quinidine and dihydroquinidine are 1 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2.5 between quinidine and dihydroquinidine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of quinidine sulfate (quinidine sulfate and dihydroquinidine sulfate) in the portion of the Tablets taken:

$$\text{Result} = (r_{B,U} + r_{D,U})/(r_{B,S} + r_{D,S}) \times (C_S/C_U) \times 100$$

$r_{B,U}$ = peak response of quinidine from the *Sample solution*

$r_{D,U}$ = peak response of dihydroquinidine from the *Sample solution*

$r_{B,S}$ = peak response of quinidine from the *Standard solution*

$r_{D,S}$ = peak response of dihydroquinidine from the *Standard solution*

C_S = concentration of USP Quinidine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of quinidine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Quinidine Sulfate RS in *Medium*

Sample solution: Dilute with *Medium* to a concentration that is similar to the *Standard solution*. Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

Mode: UV

Analytical wavelength: 248 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 85% (*Q*) of the labeled amount of quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O)]$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Standard solution: 40 µg/mL of USP Quinidine Sulfate RS in dilute hydrochloric acid (1 in 100)

Sample solution: Transfer a crushed Tablet to a 250-mL volumetric flask, add 175 mL of dilute hydrochloric acid (1 in 100), and shake by mechanical means for 30 min. Add dilute hydrochloric acid (1 in 100) to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate. Dilute quantitatively, if necessary.

Instrumental conditions

Mode: UV

Cell: 1 cm

Analytical wavelength: 345 nm

Blank: Water

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of quinidine sulfate and dihydroquinidine sulfate, calculated as quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$, in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Quinidine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of quinidine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Standard solution A: 6 mg/mL of USP Quinidine Sulfate RS in diluted alcohol

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Standard solution B: 0.06 mg/mL of USP Quinidine Sulfate RS in diluted alcohol from *Standard solution A*
Standard solution C: 0.05 mg/mL of USP Quininone RS (corresponding to 0.06 mg of the sulfate) and 0.10 mg/mL of cinchonine (corresponding to 0.12 mg of the sulfate) in diluted alcohol
Sample solution: Equivalent to 6 mg/mL of quinidine sulfate in diluted alcohol prepared as follows. Shake an amount equivalent to 150 mg of quinidine sulfate from a suitable amount of powdered Tablets in 25 mL of diluted alcohol for 10 min, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, acetone, and diethylamine (50:40:10)
Analysis
Samples: *Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*
Proceed as directed in the chapter. The solvent chamber is used without previous equilibration. When the solvent front has moved 15 cm, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray with glacial acetic acid. Locate the spots on the plate by examination under long-wavelength UV light. Then spray the plate with potassium iodoplatinate TS.
Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution C* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spots appearing at the R_F value of quinidine sulfate and dihydroquinidine sulfate (the two spots most evident from *Standard solution A*), any additional fluorescent spot is not greater in size or intensity than the principal spot of *Standard solution B*. After the treatment with potassium iodoplatinate TS, any spot produced by the *Sample solution* is not greater in size or intensity than a corresponding spot from *Standard solution C*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Quinidine Sulfate RS
USP Quininone RS
Cinchonan-9-one, 6'-methoxy-, (8alpha)-.
$C_{20}H_{22}N_2O_2$ 322.40

Quinidine Sulfate Extended-Release Tablets

DEFINITION

Quinidine Sulfate Extended-Release Tablets contain amounts of quinidine sulfate and dihydroquinidine sulfate totaling to NLT 90.0% and NMT 110.0% of the labeled amount of quinidine sulfate, calculated as $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$.

IDENTIFICATION

• **A.**
Sample: 50 mg of quinidine sulfate from powdered Tablets
Analysis: Shake with 100 mL of dilute sulfuric acid (1 in 350), and filter.
Acceptance criteria: The filtrate so obtained exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid, the fluorescence disappears.
• **B.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Organic Impurities*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩
Sample: 100 mg of quinidine sulfate from powdered Tablets
Analysis: Shake with 10 mL of dilute hydrochloric acid (1 in 100), and filter.
Acceptance criteria: The filtrate meets the requirements.

ASSAY

• **PROCEDURE**
Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.
Solution B: Dilute 10.0 mL of diethylamine with water to 100 mL.
Mobile phase: Acetonitrile, *Solution A*, *Solution B*, and water (100:20:20:860). Adjust with diethylamine to a pH of 2.6.
System suitability solution: 0.2 mg/mL each of USP Quinidine Sulfate RS and dihydroquinidine hydrochloride prepared as follows. Dissolve a suitable quantity of each USP Quinidine Sulfate RS and dihydroquinidine hydrochloride in about 10% of total volume of methanol, and dilute with *Mobile phase* to volume.
Standard solution: 0.2 mg/mL of USP Quinidine Sulfate RS in *Mobile phase*
Sample stock solution: Transfer 160 mg of quinidine sulfate from the contents of NLT 20 Tablets to a 100-mL volumetric flask. Add 80 mL of methanol, and shake the flask by mechanical means for 30 min. Dilute with methanol to volume and filter, discarding the first 10 mL of the filtrate.
Sample solution: Transfer 3.0 mL of the filtrate from the *Sample stock solution* to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 3.9-mm × 30-cm; packing L1
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for quinidine and dihydroquinidine are 1 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 2.5 between quinidine and dihydroquinidine
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quinidine sulfate (quinidine sulfate and dihydroquinidine sulfate) in the portion of Tablets taken:

$$\text{Result} = [(r_{B,U} + r_{D,U})/(r_{B,S} + r_{D,S})] \times (C_S/C_U) \times 100$$

$r_{B,U}$ = peak response of quinidine from the *Sample solution*
$r_{D,U}$ = peak response of dihydroquinidine from the *Sample solution*
$r_{B,S}$ = peak response of quinidine from the *Standard solution*
$r_{D,S}$ = peak response of dihydroquinidine from the *Standard solution*

USP Monographs

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C_S = concentration of USP Quinidine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinidine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–110%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Times: 1, 4, and 12 h
Detector: UV 248 nm
Standard solution: A known concentration of USP Quinidine Sulfate RS in *Medium*
Sample solution: Pass a portion of solution under test through a suitable filter. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Analysis: Calculate the percentage of the labeled amount of quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ dissolved:
Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved
1	20%–50%
4	43%–73%
12	NLT 70%

The percentages of the labeled amount of quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Apparatus, Time, Detector, Standard solution, Sample solution, and **Analysis:** Proceed as directed in *Test 1*
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved
1	10%–35%
4	30%–55%
12	NLT 75%

The percentages of the labeled amount of quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

• **Uniformity of Dosage Units** ⟨905⟩

Procedure for content uniformity
Standard solution: 40 µg/mL of USP Quinidine Sulfate RS in dilute hydrochloric acid (1 in 100)
Sample solution: Transfer the contents of 1 Tablet to a 250-mL volumetric flask. Add about 175 mL of dilute hydrochloric acid (1 in 100), and shake by mechanical means for 30 min. Add dilute hydrochloric acid (1 in 100) to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate. Determine the absorbances of this solution, diluted, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Spectrophotometry
Cell: 1 cm
Analytical wavelength: 345 nm
Blank: Water
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of active ingredients, calculated as quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$, in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Quinidine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinidine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **Organic Impurities**

Standard solution A: 6 mg/mL of USP Quinidine Sulfate RS in diluted alcohol
Standard solution B: 0.06 mg/mL of USP Quinidine Sulfate RS in diluted alcohol from *Standard solution A*
Standard solution C: 0.05 mg/mL of USP Quininone RS (corresponding to 0.06 mg of the sulfate) and 0.10 mg/mL of cinchonine (corresponding to 0.12 mg of the sulfate) in diluted alcohol
Sample solution: Equivalent to 6 mg/mL of quinidine sulfate in diluted alcohol prepared as follows. Shake an amount equivalent to 150 mg of quinidine sulfate from a suitable amount of powdered Tablets in 25 mL of diluted alcohol for 10 min, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, acetone, and diethylamine (50:40:10)
Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*
Proceed as directed in the chapter. The solvent chamber is used without previous equilibration. When the solvent front has moved 15 cm, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray with glacial acetic acid. Locate the spots on the plate by examination under long-wavelength UV light. Then spray the plate with potassium iodoplatinate TS.
Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution C* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spots appearing at the R_F value of quinidine sulfate and dihydroquinidine sulfate (the two spots most evident from *Standard solution A*), any additional fluorescent spot is not greater in size or intensity than the principal spot of *Standard solution B*. After the treatment with potassium iodoplatinate TS, any spot produced by the *Sample solution* is not greater in size or intensity than a corresponding spot from *Standard solution C*.

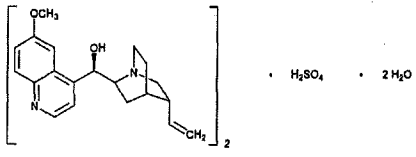
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

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• **LABELING:** The labeling indicates the *Dissolution Test* with which the product complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Quinidine Sulfate RS
USP Quininone RS
Cinchonan-9-one, 6′-methoxy-, (8alpha)-.
$C_{20}H_{22}N_2O_2$ 322.40

Quinine Sulfate



$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ 782.94
Cinchonan-9-ol, 6′-methoxy-, (8α,9*R*)-, sulfate (2:1) (salt), dihydrate;
Quinine sulfate (2:1) (salt) dihydrate [207671-44-1].
Anhydrous 746.93
[804-63-7].

DEFINITION
Quinine Sulfate is the sulfate of an alkaloid obtained from the bark of species of *Cinchona*. It contains NLT 99.0% and NMT 101.0% of total alkaloid salt, calculated as $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$, on the anhydrous basis.

IDENTIFICATION
• **A.** A 0.5-mg/mL solution in dilute sulfuric acid (1 in 350) exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid, the fluorescence disappears.
• **B.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard stock solution*, as obtained in the test for *Organic Impurities*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩
Sample solution: 20-mg/mL solution made with the aid of a few drops of hydrochloric acid
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Sample: 200 mg of Quinine Sulfate
Analysis: Dissolve the *Sample* in 20 mL of acetic anhydride, and add 4 drops of *p*-naphtholbenzein TS. Titrate with 0.1 N perchloric acid VS from a 10-mL microburet to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 24.90 mg of total alkaloid salt, calculated as quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4]$.
Acceptance criteria: 99.0%–101.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **CHLOROFORM–ALCOHOL-INSOLUBLE SUBSTANCES**
Sample: 2 g
Analysis: Warm the *Sample* with 15 mL of a mixture of chloroform and dehydrated alcohol (2:1) at 50° for 10 min. Pass through a tared, sintered-glass filter, using gentle suction. Wash the filter with five 10-mL portions of the chloroform–alcohol mixture, dry at 105° for 1 h, and weigh.
Acceptance criteria: The weight of the residue is NMT 2 mg (0.1%).

• **ORGANIC IMPURITIES**
Standard stock solution: 6 mg/mL of USP Quinine Sulfate RS, in diluted alcohol
Standard solution A: 0.06 mg/mL of USP Quinine Sulfate RS from the *Standard stock solution*, in diluted alcohol
Standard solution B: 0.05 mg/mL of USP Quininone RS (corresponding to 0.06 mg/mL of the sulfate), and 0.10 mg/mL of cinchonidine (corresponding to 0.12 mg/mL of the sulfate), in diluted alcohol
Sample solution: 6 mg/mL of Quinine Sulfate, in diluted alcohol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, acetone, and diethylamine (5:4:1). [NOTE—The solvent chamber being used without previous equilibration.]
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Allow the spots to dry, and develop the chromatogram in a solvent system until the solvent front has moved 15 cm. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with glacial acetic acid, and examine under long-wavelength UV light.
Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution B* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spot appearing at the R_F value of quinine, any additional fluorescent spot is not greater in size or intensity than the spot of *Standard solution A*. Spray the plate with potassium iodoplatinate TS. Any spot produced by the *Sample solution* is not greater in size or intensity than a corresponding spot from *Standard solution B*.
• **DIHYDROQUININE SULFATE**
Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.
Solution B: Dissolve 10.0 mL of diethylamine in water to obtain 100 mL of solution.
Mobile phase: Acetonitrile, *Solution A*, *Solution B*, and water (100:20:20:860). Adjust with *Solution B* to a pH of 2.6 if found to be lower.
System suitability solution: 0.2 mg/mL of USP Quinine Sulfate RS and 0.2 mg/mL dihydroquinine prepared as follows. Dissolve in methanol using 5% of the final volume, and dilute with *Mobile phase* to volume.
Sample solution: 0.2 mg/mL of Quinine Sulfate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 235 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection size: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for quinine and dihydroquinine are 1 and 1.5, respectively.]

USP Monographs

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Suitability requirements
Resolution: NLT 1.2 between quinine and dihydroquinine
Relative standard deviation: NMT 2.0% for the quinine peak
Analysis
Sample: *Sample solution*
Calculate the percentage of dihydroquinine in the portion of Quinine Sulfate taken:

$$\text{Result} = r_U/(r_U + r_S) \times 100$$

r_U = peak response of dihydroquinine from the *Sample solution*
r_S = peak response of quinine from the *Sample solution*

Acceptance criteria: NMT 10.0% of dihydroquinine

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL, in 0.1 N hydrochloric acid
Acceptance criteria: −235° to −245°
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 4.0%–5.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Quinine Sulfate RS
USP Quininone RS
Cinchonan-9-one, 6′-methoxy-, (8α)-.
$C_{20}H_{22}N_2O_2$ 322.40

Quinine Sulfate Capsules

DEFINITION
Quinine Sulfate Capsules contain amounts of quinine sulfate and dihydroquinine sulfate totaling NLT 90.0% and NMT 110.0% of the labeled amount of quinine sulfate, calculated as $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$.

IDENTIFICATION
• **A.**
Sample: Nominally 100 mg of quinine sulfate from the contents of Capsules
Analysis: Shake the *Sample* with 100 mL of dilute sulfuric acid (1 in 350), and filter.
Acceptance criteria: An appropriate dilution of the filtrate exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid, the fluorescence disappears.
• **B.** The R_F value of the principal spot from the *Sample solution* corresponds to that from *Standard solution A*, as obtained in the test for *Organic Impurities*.
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sulfate*
Sample: Nominally 20 mg of quinine sulfate from the contents of Capsules
Analysis: Shake the *Sample* with 10 mL of dilute hydrochloric acid (1 in 100), and filter.
Acceptance criteria: The filtrate meets the requirements.
• **D.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.
Solution B: Dissolve 10.0 mL of diethylamine in water to obtain 100 mL of solution.
Mobile phase: Acetonitrile, *Solution A*, *Solution B*, and water (100:20:20:860). Adjust with *Solution B* to a pH of 2.6 if the pH is found to be lower.
System suitability solution: 0.2 mg/mL each of USP Quinine Sulfate RS and dihydroquinine, dissolved in 10% of the final volume of methanol. Dilute with *Mobile phase* to volume.
Standard solution: 0.2 mg/mL of USP Quinine Sulfate RS in *Mobile phase*
Sample stock solution: Nominally 1.6 mg/mL of quinine sulfate in methanol prepared as follows. Transfer an amount, equivalent to 160 mg of quinine sulfate from the contents of NLT 20 Capsules, to a 100-mL volumetric flask, add 80 mL of methanol, and shake the flask by mechanical means for 30 min. Dilute with methanol to volume, and filter, discarding the first 10 mL of the filtrate.
Sample solution: Nominally 0.2 mg/mL of quinine sulfate in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for quinine and dihydroquinine are 1 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 1.2 between quinine and dihydroquinine
Relative standard deviation: NMT 2.0% for the quinine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ calculated as the sum of quinine sulfate and dihydroquinine sulfate in the portion of Capsules taken:

$$\text{Result} = [(r_{b,U} + r_{d,U})/(r_{b,S} + r_{d,S})] \times (C_S/C_U) \times 100$$

$r_{b,U}$ = peak area response of quinine from the *Sample solution*
$r_{d,U}$ = peak area response of dihydroquinine from the *Sample solution*
$r_{b,S}$ = peak area response of quinine from the *Standard solution*
$r_{d,S}$ = peak area response of dihydroquinine from the *Standard solution*
C_S = concentration of USP Quinine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm

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Time: 45 min
Detection: UV maximum at about 248 nm
Standard solution: Prepare a solution of known concentration of USP Quinine Sulfate RS in *Medium*.
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Solution A: Add 7.0 mL of methanesulfonic acid to 4.0 mL of glacial acetic acid, and dilute with water to 100 mL.
Solution B: Dissolve 10.0 mL of diethylamine in water to obtain 100 mL of solution.
Mobile phase: Water, acetonitrile, *Solution A*, and *Solution B* (81:15:2:2). Adjust with *Solution B* to a pH of 3.0.
Standard solution: Prepare a solution of known concentration of USP Quinine Sulfate RS in *Medium*.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and suitably dilute with *Medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the quinine peak
Relative standard deviation: NMT 2.0% for the sum of quinine and dihydroquinine peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ dissolved.

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times D \times V \times 100$$

r_U = sum of the peak responses of quinine and dihydroquinine from the *Sample solution*
r_S = sum of the peak responses of quinine and dihydroquinine from the *Standard solution*
C_S = concentration of USP Quinine Sulfate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
M_{r1} = molecular weight of quinine sulfate, 782.94
M_{r2} = molecular weight of anhydrous quinine sulfate, 746.92
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Diluent: Hydrochloric acid (1 in 100)
Standard solution: 40 µg/mL of USP Quinine Sulfate RS in *Diluent*
Sample solution: Transfer the contents of one Capsule to a 250-mL volumetric flask, add 175 mL of *Diluent*, and shake by mechanical means for 30 min. Add *Diluent* to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Cell: 1 cm
Analytical wavelength: Maximum at about 345 nm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Quinine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **Organic Impurities**
Standard stock solution: 6 mg/mL of USP Quinine Sulfate RS in diluted alcohol
Standard solution A: 0.06 mg/mL of USP Quinine Sulfate RS from the *Standard stock solution* in diluted alcohol
Standard solution B: 0.05 mg/mL of USP Quininone RS (corresponding to 0.06 mg/mL of the sulfate) and 0.10 mg/mL of cinchonidine (corresponding to 0.12 mg/mL of the sulfate) in diluted alcohol
Sample solution: Nominally 6 mg/mL of quinine sulfate in diluted alcohol prepared as follows. Shake the equivalent of 150 mg of quinine sulfate from the contents of Capsules with 25 mL of diluted alcohol for 10 min, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, acetone, and diethylamine (50:40:10). [Note—The solvent chamber being used without previous equilibration.]
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Proceed as directed in *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*. Allow the spots to dry, and develop the chromatogram using a solvent chamber without previous equilibration. When the solvent front has moved about 15 cm, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with glacial acetic acid, and examine under long-wavelength UV light.
Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution B* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spot appearing at the R_F value of quinine sulfate, any

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additional fluorescent spot is not greater in size or intensity than the spot from *Standard solution A*. Spray the plate with potassium iodoplatinate TS. Any spot produced by the *Sample solution* is not greater in size or intensity than a corresponding spot from *Standard solution B*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Quinine Sulfate RS
  USP Quininone RS
  Cinchonan-9-one, 6′-methoxy-, (8α)-.
  $C_{20}H_{22}N_2O_2$ 322.40

Quinine Sulfate Tablets

DEFINITION

Quinine Sulfate Tablets contain amounts of quinine sulfate and dihydroquinine sulfate totaling NLT 90.0% and NMT 110.0% of the labeled amount of quinine sulfate, calculated as $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$.

IDENTIFICATION

- **A.**
  Sample: Nominally 100 mg of quinine sulfate from powdered Tablets
  Analysis: Shake the *Sample* well with 100 mL of dilute sulfuric acid (1 in 350), and filter.
  Acceptance criteria: An appropriate dilution of the filtrate exhibits a vivid blue fluorescence. On the addition of a few drops of hydrochloric acid, the fluorescence disappears.
- **B.** The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution A*, as obtained in the test for *Organic Impurities*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩
  Sample: Nominally 20 mg of quinine sulfate from powdered Tablets
  Analysis: Shake *Sample* with 10 mL of dilute hydrochloric acid (1 in 100), and filter
  Acceptance criteria: The filtrate meets the requirements.
- **D.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Solution A: Add 35.0 mL of methanesulfonic acid to 20.0 mL of glacial acetic acid, and dilute with water to 500 mL.
  Solution B: Dissolve 10.0 mL of diethylamine in water to obtain 100 mL of solution.
  Mobile phase: Acetonitrile, *Solution B*, *Solution A*, and water (10:2:2:86). Adjust with *Solution B* to a pH of 2.6 if the pH is found to be lower.
  System suitability solution: 0.2 mg/mL each of USP Quinine Sulfate RS and dihydroquinine, dissolved in 10% of the final volume of methanol. Dilute with *Mobile phase* to volume.
  Standard solution: 0.2 mg/mL of USP Quinine Sulfate RS in *Mobile phase*
  Sample stock solution: Nominally 1.6 mg/mL of quinine sulfate prepared as follows. Transfer an equivalent to 160 mg of quinine sulfate from NLT 20 powdered Tablets to a 100-mL volumetric flask, add 80 mL of methanol, and shake by mechanical means for 30 min. Dilute with methanol to volume, and filter, discarding the first 10 mL of the filtrate.
  Sample solution: Nominally 0.2 mg/mL of quinine sulfate in *Mobile phase* from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 235 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 50 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for quinine and dihydroquinine are 1 and 1.5, respectively.]
  Suitability requirements
  Resolution: NLT 1.2 between quinine and dihydroquinine
  Relative standard deviation: NMT 2.0% for the quinine peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of quinine sulfate and dihydroquinine sulfate in the portion of Tablets taken:

$$\text{Result} = [(r_{B,U} + r_{D,U})/(r_{B,S} + r_{D,S})] \times (C_S/C_U) \times 100$$

$r_{B,U}$	= peak area response of quinine from the *Sample solution*
$r_{D,U}$	= peak area response of dihydroquinine from the *Sample solution*
$r_{B,S}$	= peak area response of quinine from the *Standard solution*
$r_{D,S}$	= peak area response of dihydroquinine from the *Standard solution*
C_S	= concentration of USP Quinine Sulfate RS in the *Standard solution* (mg/mL)
C_U	= nominal concentration of quinine sulfate in the *Sample solution* (mg/mL)

  Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: 0.01 N hydrochloric acid; 900 mL
  Apparatus 1: 100 rpm
  Time: 45 min
  Detection: UV maximum at about 248 nm
  Standard solution: Prepare a solution of known concentration of USP Quinine Sulfate RS in *Medium*.
  Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*
  Analysis: Determine the percentage of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot H_2O]$ dissolved.
  Tolerances: NLT 75% (*Q*) of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  Procedure for content uniformity
  Diluent: Hydrochloric acid (1 in 100)
  Standard solution: 40 µg/mL of USP Quinine Sulfate RS in *Diluent*
  Sample solution: Transfer the contents of one powdered Tablet to a 250-mL volumetric flask, add 175 mL of *Diluent*, and shake by mechanical means for 30 min. Add *Diluent* to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate.
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

USP Monographs

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Mode: UV
Cell: 1 cm
Analytical wavelength: Maximum at about 345 nm
Blank: Water

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O]$, in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Quinine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of quinine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

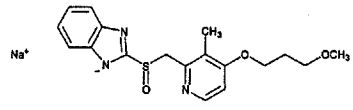
• **ORGANIC IMPURITIES**
Standard stock solution: 6 mg/mL of USP Quinine Sulfate RS in diluted alcohol
Standard solution A: 0.06 mg/mL of USP Quinine Sulfate RS from *Standard stock solution* in diluted alcohol
Standard solution B: 0.05 mg/mL of USP Quininone RS (corresponding to 0.06 mg/mL of the sulfate), and 0.10 mg/mL of cinchonidine (corresponding to 0.12 mg/mL of the sulfate) in diluted alcohol
Sample solution: Nominally 6 mg/mL of quinine sulfate prepared as follows. Shake the equivalent of 150 mg of quinine sulfate from powdered Tablets with 25 mL of diluted alcohol for 10 min, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, acetone, and diethylamine (50:40:10). [NOTE—The solvent chamber being used without previous equilibration.]
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Allow the spots to dry, and develop the chromatogram in a solvent system until the solvent front has moved 15 cm. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with glacial acetic acid, and examine under long-wavelength UV light.
Acceptance criteria: Any spot produced by the *Sample solution* at the R_F value of a spot produced by *Standard solution B* is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spot appearing at the R_F value of quinine, any additional fluorescent spot is not greater in size or intensity than the spot from *Standard solution A*. Spray the plate with potassium iodoplatinate TS. Any spot produced by the *Sample solution* is not greater in size or intensity than a corresponding spot from *Standard solution B*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Quinine Sulfate RS
USP Quininone RS
Cinchonan-9-one, 6′-methoxy-, (8α)-.
$C_{20}H_{22}N_2O_2$ 322.40

Rabeprazole Sodium



$C_{18}H_{20}N_3NaO_3S$ (anhydrous) 381.42

$C_{18}H_{20}N_3NaO_3S \cdot xH_2O$ (hydrated form)
1*H*-Benzimidazole, 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]methyl]sulfinyl]-, sodium salt;
2-[[[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl]methyl] sulfinyl]benzimidazole sodium salt [117976-90-6].

DEFINITION

Rabeprazole Sodium contains NLT 98.0% and NMT 102.0% of rabeprazole sodium ($C_{18}H_{20}N_3NaO_3S$), calculated on the dried basis. The hydrated form contains NLT 98.0% and NMT 102.0% of rabeprazole sodium ($C_{18}H_{20}N_3NaO_3S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
[NOTE—If the spectra obtained in the solid state shows differences, dissolve the substance to be examined and the reference substance separately in methanol, evaporate to dryness, and record new spectra using the residues.]
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

ASSAY

• **PROCEDURE**
[NOTE—Protect solutions containing rabeprazole from light.]
Buffer 1: 4.35 g/L of dibasic potassium phosphate. Adjust with phosphoric acid to a pH of 7.0.
Solution A: Acetonitrile and *Buffer 1* (5:95)
Solution B: Methanol
Solution C: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
2	100	0	0
7	85	0	15
27	30	40	30
32	15	55	30

USP Monographs

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Return to original conditions and re-equilibrate the system for 10 min.
Buffer 2: Dissolve 17.4 g of dibasic potassium phosphate in 950 mL of water, adjust using a 2 N solution of potassium hydroxide to a pH of 11.3, and dilute with water to 1000 mL. Pass through a suitable filter of 0.45-µm pore size.
Diluent: Methanol and *Buffer 2* (20:80)
Standard solution: 0.1 mg/mL of USP Rabeprazole Sodium RS in *Diluent*
Sample solution: 0.1 mg/mL of Rabeprazole Sodium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 6°
Column: 45°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of rabeprazole sodium ($C_{18}H_{20}N_3NaO_3S$) in the portion of Rabeprazole Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rabeprazole Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Rabeprazole Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis for the anhydrous form or on the anhydrous basis for the hydrated form

IMPURITIES

• **ORGANIC IMPURITIES**
[NOTE—Protect solutions containing rabeprazole from light.]
Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1.0 mg/mL of USP Rabeprazole Sodium RS and 0.002 mg/mL each of USP Rabeprazole Related Compound A RS, USP Rabeprazole Related Compound B RS, USP Rabeprazole Related Compound C RS, USP Rabeprazole Related Compound D RS, USP Rabeprazole Related Compound E RS, and USP Rabeprazole Related Compound F RS in *Diluent*
Sensitivity solution: 0.5 µg/mL of USP Rabeprazole Sodium RS in *Diluent*
Standard solution: 0.001 mg/mL of USP Rabeprazole Sodium RS in *Diluent*
Sample solution: 1.0 mg/mL of Rabeprazole Sodium in *Diluent*
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Relative standard deviation: NMT 5.0%, *Standard solution*
Peak-to-valley ratio: NLT 1.5 for rabeprazole related compound F and rabeprazole, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Rabeprazole Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of rabeprazole from the *Standard solution*
C_S = concentration of USP Rabeprazole Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Rabeprazole Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyridinone analog (rabeprazole related compound A)	0.32	0.15
Benzimidazolol[a]	0.47	0.10
Mercaptobenzimidazole (rabeprazole related compound C)	0.50	0.10
Rabeprazole sulfone *N*-oxide[b]	0.74	0.10
Rabeprazole *N*-oxide (rabeprazole related compound B)	0.76	0.15
Methoxy analog[c]	0.82	0.15
Rabeprazole sulfone (rabeprazole related compound D)	0.90	0.8
Chloro analog (rabeprazole related compound F)	0.98	0.10
Rabeprazole	1.0	—
Methoxy sulfide analog[d]	1.04	0.10
Rabeprazole sulfide (rabeprazole related compound E)	1.24	0.15
Any other individual impurity	—	0.10
Total impurities	—	1.0

[a] 1*H*-Benzimidazol-2-ol.
[b] 2-{[(1*H*-Benzimidazol-2-yl)sulfonyl]methyl}-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide.
[c] 2-{[(4-Methoxy-3-methylpyridin-2-yl)methyl]sulfinyl}benzimidazole.
[d] 2-{[(4-Methoxy-3-methylpyridin-2-yl)methyl]thio}benzimidazole.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
[NOTE—Perform this test for the anhydrous form.]
Analysis: Dry under vacuum over phosphorus pentoxide for 24 h.
Acceptance criteria: NMT 1.0%

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- **WATER DETERMINATION,** *Method I* ⟨921⟩
  [NOTE—Perform this test where it is labeled as a hydrated form.]
  [NOTE—Hydranal composite 5 is a suitable titrant.]
  Sample: 0.2 g
  Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

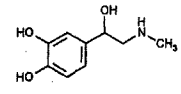
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **LABELING:** Where it is the hydrated form, the label so indicates.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Rabeprazole Sodium RS
  USP Rabeprazole Related Compound A RS
  Sodium 1-(1*H*-benzimidazol-2-yl)-3-methyl-4-oxo-1,4-dihydropyridine-2-carboxylate.
  $C_{14}H_{10}N_3NaO_3$ 291.24
  USP Rabeprazole Related Compound B RS
  2-{[(1*H*-Benzimidazol-2-yl)sulfinyl]methyl}-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide.
  $C_{18}H_{21}N_3O_4S$ 375.44
  USP Rabeprazole Related Compound C RS
  1*H*-Benzimidazole-2-thiol.
  $C_7H_6N_2S$ 150.20
  USP Rabeprazole Related Compound D RS
  2-({[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl]methyl}sulfonyl)benzimidazole.
  $C_{18}H_{21}N_3O_4S$ 375.44
  USP Rabeprazole Related Compound E RS
  2-{[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl]methylthio}benzimidazole.
  $C_{18}H_{21}N_3O_2S$ 343.44
  USP Rabeprazole Related Compound F RS
  2-{[(4-Chloro-3-methyl-2-pyridyl)methyl]sulfinyl}benzimidazole.
  $C_{14}H_{12}ClN_3OS$ 305.78

Rabies Immune Globulin

» Rabies Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic, slightly opalescent solution consisting of globulins derived from blood plasma or serum that has been tested for the absence of hepatitis B surface antigen, derived from selected adult human donors who have been immunized with rabies vaccine and have developed high titers of rabies antibody. It has a potency such that when labeled as 150 International Units (IU) per mL, it has a geometric mean lower limit (95% confidence) potency value of not less than 110 IU per mL, and proportionate lower limit potency values for other labeled potencies, based on the U.S. Standard Rabies Immune Globulin and using the CVS Virus challenge, by neutralization test in mice or tissue culture. It contains not less than 10 g and not more than 18 g of protein per 100 mL, of which not less than 80 percent is monomeric immunoglobulin G, having a sedimentation coefficient in the range of 6.0 to 7.5S, with no fragments having a sedimentation coefficient less than 6S and no aggregates having a sedimentation coefficient greater than 12S. It contains 0.3 M glycine as a stabilizing agent, and it contains a suitable preservative. It has a pH between 6.4 and 7.2, measured in a solution diluted to contain 1 percent of protein with 0.15 M sodium chloride. It meets the requirements of the test for heat stability.

Packaging and storage—Preserve at a temperature between 2° and 8°.
Expiration date—The expiration date is not later than 1 year after date of issue from manufacturer's cold storage (5°, 1 year).
Labeling—Label it to state that it is not for intravenous injection.

Racepinephrine



$C_9H_{13}NO_3$ 183.20
1,2-Benzenediol, 4-[1-hydroxy-2-(methylamino)ethyl]-, (±)-.
(±)-3,4-Dihydroxy-α-[(methylamino)methyl]benzyl alcohol [329-65-7].

» Racepinephrine is a racemic mixture of the enantiomorphs of epinephrine. It contains not less than 97.0 percent and not more than 102.0 percent of $C_9H_{13}NO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Change to read:

USP Reference standards ⟨11⟩—
▲▲ (CN 1-May-2018)
▲USP Epinephrine Bitartrate RS▲ (ERR 1-Aug-2018)
USP Norepinephrine Bitartrate RS
Identification—To 5 mL of pH 4.0 acid phthalate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) add 0.5 mL of a solution of Racepinephrine (1 in 1000) and 1.0 mL of 0.1 N iodine. Mix, and allow to stand for 5 minutes. Add 2 mL of sodium thiosulfate solution (1 in 40): a deep red color is produced.
Specific rotation ⟨781S⟩: between −1° and +1°.
Test solution: 10 mg per mL, in 0.6 N hydrochloric acid.
Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 18 hours: it loses not more than 2.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.5%.
Limit of adrenalone—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 310 nm, determined in a solution in dilute hydrochloric acid (1 in 200) containing 2 mg per mL, is not more than 0.2.

Limit of norepinephrine—
Epinephrine standard solution—Dilute with methanol an accurately measured volume of a solution of USP Epinephrine Bitartrate RS in formic acid containing about 364 mg per mL

USP Monographs

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to obtain a solution having a concentration of about 20 mg per mL.

Norepinephrine standard solution—Dilute with methanol an accurately measured volume of a solution of USP Norepinephrine Bitartrate RS in formic acid containing 16 mg per mL to obtain a solution having a known concentration of 1.6 mg per mL.

Test solution—Dissolve 200 mg of Racepinephrine in 1.0 mL of formic acid, dilute with methanol to 10.0 mL, and mix.

Procedure—Apply 5-µL portions of *Epinephrine standard solution*, *Norepinephrine standard solution*, and *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow to dry, and develop the chromatogram in an unsaturated tank using a solvent system consisting of a mixture of *n*-butanol, water, and formic acid (7:2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm circulating air. Spray with Folin-Ciocalteu Phenol TS, followed by sodium carbonate solution (1 in 10): the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Epinephrine standard solution*. Any spot obtained from the *Test solution* is not larger nor more intense than the spot with the same R_F value obtained from the *Norepinephrine standard solution*, corresponding to not more than 4.0% of norepinephrine.

Assay—

Ferro-citrate solution, *Buffer solution*, and *Standard preparation*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Assay preparation—Transfer about 10 mg of Racepinephrine, accurately weighed, to a 1-liter volumetric flask. Dilute with sodium bisulfite solution (1 in 500) to volume, and mix.

Procedure—Proceed as directed for *Procedure* under *Epinephrine Assay* ⟨391⟩. Calculate the quantity, in mg, of $C_9H_{13}NO_3$ in the portion of Racepinephrine taken by the formula:

$$(183.20/333.29)C(A_U/A_S)$$

in which 183.20 and 333.29 are the molecular weights of racepinephrine and epinephrine bitartrate, respectively, C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Racepinephrine Inhalation Solution

DEFINITION

Racepinephrine Inhalation Solution is a sterile solution of Racepinephrine in Purified Water prepared with the aid of Hydrochloric Acid, or of Racepinephrine Hydrochloride in Purified Water. It contains NLT 90.0% and NMT 110.0% of the labeled amount of racepinephrine ($C_9H_{13}NO_3$).

IDENTIFICATION

• **A.**

Sample solution: 0.5 mL of Inhalation Solution

Analysis: To 5 mL of pH 4.0 acid phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*) add the *Sample solution* and 1.0 mL of 0.1 N iodine. Mix, and allow to stand for 5 min. Add 2 mL of sodium thiosulfate solution (1 in 40).

Acceptance criteria: A deep red color is produced.

ASSAY

• **PROCEDURE**

Solution A: 0.05 M monobasic sodium phosphate and methanol (85:15)

Mobile phase: 0.005 M sodium 1-octanesulfonate in *Solution A*

System suitability solution: 0.04 mg/mL of dopamine hydrochloride and 0.01 mg/mL of USP Epinephrine Bitartrate RS in *Solution A*

Standard solution: 0.1 mg/mL of USP Epinephrine Bitartrate RS in *Solution A*

Sample solution: Nominally 0.055 mg/mL of racepinephrine prepared as follows. Transfer a suitable volume of Inhalation Solution equivalent to 11 mg of racepinephrine to a suitable volumetric flask, and dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between dopamine and racepinephrine, *System suitability solution*

Tailing factor: NMT 1.2 for racepinephrine, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of racepinephrine ($C_9H_{13}NO_3$) in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Epinephrine Bitartrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of racepinephrine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of racepinephrine, 183.20

M_{r2} = molecular weight of epinephrine bitartrate, 333.29

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **PH** ⟨791⟩: 2.0–3.5

• **COLOR AND CLARITY**

Standard solution: Dilute 2.0 mL of 0.100 N iodine VS with water to 500 mL.

Sample solution: A portion of Inhalation Solution

Analysis: Visually examine the *Sample solution* in a suitable clear glass test tube against a white background.

Acceptance criteria: The *Sample solution* is not pinkish, and it contains no precipitate.

If any yellow color is observed in the *Sample solution*, concomitantly determine the absorbances of the *Sample*

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solution and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm.
The absorbance of the *Sample solution* does not exceed that of the *Standard solution*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Do not freeze.
- **LABELING:** The label indicates that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

Add the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP Epinephrine Bitartrate RS▲ (ERR 1-Aug-2018)

Racepinephrine Hydrochloride

$C_9H_{13}NO_3 \cdot HCl$ 219.67

» Racepinephrine Hydrochloride is a racemic mixture of the hydrochlorides of the enantiomorphs of epinephrine. It contains not less than 97.0 percent and not more than 102.0 percent of $C_9H_{13}NO_3 \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Change to read:

USP Reference standards ⟨11⟩—
▲▲ (CN 1-May-2018)
▲USP Epinephrine Bitartrate RS▲ (ERR 1-Aug-2018)
USP Norepinephrine Bitartrate RS

Identification—

Change to read:

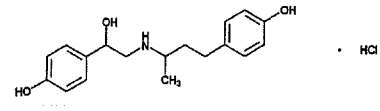
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—*Solution:* 50 µg per mL. [NOTE—Use USP Epinephrine Bitartrate RS in the preparation of the *Standard solution*.] *Medium:* water.
B: A solution (1 in 100) meets the requirements of the tests for *Chloride* ⟨191⟩.
Specific rotation ⟨781S⟩: between −1° and +1°.
Test solution: 10 mg per mL, in 0.12 N hydrochloric acid.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Residue on ignition ⟨281⟩: not more than 0.5%.
Other requirements—It meets the requirements for *Limit of adrenalone* and *Limit of norepinephrine* under *Racepinephrine*.

Assay—
Mobile phase—Prepare a filtered and degassed solution of 0.17 N acetic acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in *Mobile phase* to obtain a solution having a known concentration of about 150 µg per mL.
Assay preparation—Transfer about 25 mg of Racepinephrine Hydrochloride, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 4.0 mL of this solution to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution containing about 100 µg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_3 \cdot HCl$ in the portion of Racepinephrine Hydrochloride taken by the formula:

$$0.25C(219.67/333.30)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation:* 219.67 and 333.30 are the molecular weights of racepinephrine hydrochloride and epinephrine bitartrate, respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ractopamine Hydrochloride Suspension



$C_{18}H_{23}NO_3 \cdot HCl$ 337.84
Benzenemethanol, 4-hydroxy-α-[[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]methyl]-, hydrochloride;
(±)-all-*rac*-*p*-Hydroxy-α-[[[3-(*p*-hydroxyphenyl)-1-methylpropyl]amino]methyl]benzyl alcohol, hydrochloride [90274-24-1].

DEFINITION
Ractopamine Hydrochloride Suspension contains NLT 10% and NMT 20%, by weight, of ractopamine hydrochloride ($C_{18}H_{23}NO_3 \cdot HCl$) in water. [NOTE—The material partially precipitates out at room temperature to form a slurry, and redissolves when heated to 50°–60°.]

IDENTIFICATION

Change to read:

• ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
Sample: Dry a portion of Ractopamine Hydrochloride Suspension under vacuum for 3 h at 60°.

ASSAY
• **Procedure**
Solution A: 5.75 mg/mL solution of monobasic ammonium phosphate adjusted with 10% phosphoric acid to a pH of 4.0 ± 0.1
Solution B: 1.1 mg/mL solution of 1-heptanesulfonic acid sodium salt in *Solution A*
Mobile phase: Stabilizer-free tetrahydrofuran and *Solution B* (3:17)

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Diluent: Stabilizer-free tetrahydrofuran and water (3:17). [NOTE—The *Standard solutions* and *System suitability solution* are stable for up to 72 h at room temperature. The *Sample solution* is stable for up to 90 h at room temperature.]
System suitability solution: 100 µg/mL of USP Ractopamine Hydrochloride RS and 10 µg/mL of USP Raspberry Alcohol RS in *Diluent*
Standard solution A: 0.08 mg/mL of USP Ractopamine Hydrochloride RS in *Diluent*
Standard solution B: 0.1 mg/mL of USP Ractopamine Hydrochloride RS in *Diluent*
Standard solution C: 0.12 mg/mL of USP Ractopamine Hydrochloride RS in *Diluent*
Sample stock solution: Stir Ractopamine Hydrochloride Suspension in a 60° water bath for up to 1 h, to ensure complete dissolution. While hot, transfer 700 mg of the Ractopamine Hydrochloride Suspension dropwise to a 100-mL volumetric flask, and dilute with *Diluent* to volume.
Sample solution: Dilute a portion of the *Sample stock solution* with *Diluent* (1:10).
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution B*
Suitability requirements
Resolution: NLT 1.5 between raspberry alcohol and ractopamine, *System suitability solution*
Tailing factor: NLT 0.7 and NMT 2.0 for the ractopamine peak, *Standard solution B*
Relative standard deviation: NMT 2.0% for three replicate injections, *Standard solution B*
Analysis
Samples: *Standard solutions* and *Sample solution*
Prepare a calibration curve using the three ractopamine peak responses from *Standard solutions A, B,* and *C* and their corresponding concentrations. From the graph determine the concentration, C, in mg/mL, of ractopamine hydrochloride in the *Sample solution*.
Calculate the percentage (w/w) of $C_{18}H_{23}NO_3 \cdot HCl$ in the portion of Ractopamine Hydrochloride Suspension taken:

$$\text{Result} = (V/W) \times C_S \times D \times 100$$

V = volume of the *Sample stock solution*, 100 mL
W = weight of Ractopamine Hydrochloride Suspension taken (mg)
C_S = concentration of ractopamine hydrochloride from the *Sample solution*
D = dilution factor to prepare the *Sample solution*, 10

Acceptance criteria: 10%–20% of $C_{18}H_{23}NO_3 \cdot HCl$

IMPURITIES

ORGANIC IMPURITIES

• Procedure
Solution A: 5.75 mg/mL of monobasic ammonium phosphate in water; pH NLT 4.4
Solution B: 1.1 mg/mL of 1-heptanesulfonic acid sodium salt in *Solution A*
Solution C: Acetonitrile and *Solution B* (1:9)
Solution D: Acetonitrile and *Solution B* (17:33)
Mobile phase: See the gradient table below.

Time (min)	Solution C (%)	Solution D (%)
0	100	0
22	0	100
32	0	100
37	100	0
55	100	0

Diluent: Acetonitrile and water (1:4)
System suitability solution: 9 µg/mL each of USP Raspberry Ketone RS and USP Ractopamine Hydrochloride RS in *Diluent*
Blank: *Diluent*
Sample solution A: Stir Ractopamine Hydrochloride Suspension in a 60° water bath for up to 1 h, to ensure complete dissolution. While hot, transfer 200 mg of the Ractopamine Hydrochloride Suspension dropwise into a 50-mL volumetric flask, and dilute with *Diluent* to volume.
Sample solution B: Dilute a portion of *Sample solution A* with *Diluent* (1:100). [NOTE—The *Sample solutions* are stable for up to 48 h if stored at 5°.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between raspberry ketone and ractopamine
Analysis
Samples: *Blank, Sample solution A,* and *Sample solution B*
[NOTE—Disregard any peaks that correspond to those in the *Blank*. Correct the response of the ractopamine peak in *Sample solution B* by subtracting the peak response at the retention time of ractopamine in the *Blank*.]
Calculate the percentage of each individual impurity in the portion of Ractopamine Hydrochloride Suspension taken:

$$\text{Result} = (r_A/r_B) \times 100/D$$

r_A = peak response of each individual impurity from *Sample solution A*
r_B = corrected peak response for ractopamine from *Sample solution B*
D = dilution factor to prepare *Sample solution B*, 100

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 3.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Octopamine[a]	0.37	0.5
Tyramine[b]	0.55	0.5
N-Isopropyloctopamine[c]	0.63	0.5
Piperazinediphenol[d]	0.74	0.5
Aminobutylphenol[e]	0.76	0.5
Raspberry alcohol[f]	0.85	0.5

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Impurity Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Raspberry ketone[g]	0.96	1.0
Ractopamine	1.0	—
Deoxyractopamine[h]	1.1	0.5
Ractopamine *O*-methyl[i]	1.2	1.0
Ractopamine *N*-hydroxybenzyl[j]	1.26	1.0
Ractopamine cyclohexyl analog[k]	1.29	0.5
Ractopamine dimer[l]	1.4	1.0
Any individual unspecified impurity	—	0.2

[a] 4-(2-Amino-1-hydroxyethyl)phenol.
[b] 4-(2-Aminoethyl)phenol.
[c] 4-[1-Hydroxy-2-(isopropylamino)ethyl]phenol.
[d] 4,4′-(Piperazine-2, 5-diyl)diphenol.
[e] 4-(3-Aminobutyl)phenol.
[f] 4-(3-Hydroxybutyl)phenol.
[g] 4-(4-Hydroxyphenyl)butan-2-one.
[h] 4-[3-(4-Hydroxyphenethylamino)butyl]phenol.
[i] 4-{3-[2-(4-Hydroxyphenyl)-2-methoxyethylamino]butyl}phenol.
[j] 4-(1-Hydroxy-2-{(4-hydroxybenzyl)[4-(4-hydroxyphenyl)butan-2-yl]amino}ethyl)phenol.
[k] 4-{1-Hydroxy-2-[3-(4-hydoxyphenyl)-5-methylcyclohexylamino]ethyl}phenol).
[l] 4,4′-(1,1′-Oxybis{2-[4-(4-hydroxyphenyl)butan-2-ylamino]ethane-1,1-diyl}) diphenol.

SPECIFIC TESTS

- **DIASTEREOMER RATIO**

Solution A: 5.75 mg/mL of monobasic ammonium phosphate in water
Solution B: Add 10 mL of triethylamine to 950 mL of *Solution A*, dilute with *Solution A* to 1000 mL, and adjust with phosphoric acid to a pH of 4.5.
Mobile phase: Acetonitrile and *Solution B* (3:22)
Diluent: Acetonitrile and *Solution A* (1:4)
System suitability solution: 0.4 mg/mL of USP Ractopamine Hydrochloride RS in *Diluent*
Sample solution: Stir Ractopamine Hydrochloride Suspension in a 60° water bath for up to 1 h to ensure complete dissolution. While hot, transfer 275 mg of it dropwise into a 100-mL volumetric flask, and dilute with *Diluent* to volume.
[NOTE—The *Sample solution* is stable for up to 36 h when stored at ambient conditions.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The elution order is *RS,SR* diastereoisomer followed by *RR,SS* diastereoisomer.]
Suitability requirements
Resolution: NLT 1.25 between the diastereomers
Analysis
Sample: *Sample solution*
Calculate the *RS,SR* diastereomer content, in percentage:

$$\text{Result} = r_A/(r_A + r_B) \times 100$$

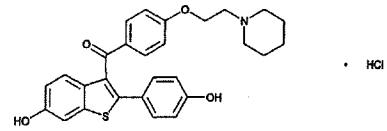
r_A = peak response of the *RS,SR* diastereoisomer from the *Sample solution*
r_B = peak response of the *RR,SS* diastereoisomer from the *Sample solution*

Acceptance criteria: 45%–49%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store at a temperature not exceeding 70°.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ractopamine Hydrochloride RS
USP Raspberry Alcohol RS
4-(3-Hydroxybutyl)phenol.
$C_{10}H_{14}O_2$ 166.22
USP Raspberry Ketone RS
4-(4-Hydroxyphenyl)butan-2-one.
$C_{10}H_{12}O_2$ 164.20

Raloxifene Hydrochloride



$C_{28}H_{27}NO_4S \cdot HCl$ 510.04
Methanone, [6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]-, hydrochloride; 6-Hydroxy-2-(*p*-hydroxyphenyl)benzo[*b*]thien-3-yl-*p*-(2-piperidinoethoxy)phenyl ketone, hydrochloride [82640-04-8].

DEFINITION
Raloxifene Hydrochloride contains NLT 97.5% and NMT 102.0% of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: It meets the requirements, the sample being dissolved in methanol.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 7.2 g of monobasic potassium phosphate in 1000 mL of water. Add 1.5 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 2.5 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (33:67)
System suitability solution: Prepare as directed in the test for *Organic Impurities*.
Standard solution: 0.05 mg/mL of USP Raloxifene Hydrochloride RS in *Mobile phase*
Sample solution: 0.05 mg/mL of Raloxifene Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm

USP Monographs

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Column: 4.6-mm × 15-cm; 3.5-µm base-deactivated packing L7
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between raloxifene and raloxifene related compound C
Tailing factor: NMT 2.0 for raloxifene
Relative standard deviation: NMT 0.7% for raloxifene
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) in the portion of Raloxifene Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Raloxifene Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Solution A: Dissolve 9.0 g of monobasic potassium phosphate in 1000 mL of water. Add 0.6 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 3.0 ± 0.1.
Solution B: Acetonitrile
Mobile phase: See *Table 1*. [NOTE—Adjust the start time of the gradient step on the basis of the instrument's dwell volume.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.00	75	25
9.00	75	25
40.25	50	50
42.25	75	25
49.00	75	25

Diluent A: *Solution A* and acetonitrile (70:30)
Diluent B: Tetrahydrofuran and methanol (70:30)
Raloxifene related compound C solution: 0.15 mg/mL of USP Raloxifene Related Compound C RS in *Diluent B*
System suitability solution: Transfer 15 mg of USP Raloxifene Hydrochloride RS to a 50-mL volumetric flask, add 1.0 mL of *Raloxifene related compound C solution*, and dilute with *Diluent A* to volume.
Standard solution: 0.003 mg/mL of USP Raloxifene Hydrochloride RS in *Diluent A*
Sample solution: 3 mg/mL of Raloxifene Hydrochloride in *Diluent A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L7
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between raloxifene and raloxifene related compound C
Tailing factor: NMT 2.0 for raloxifene
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for NLT two times the retention time of the raloxifene peak, and measure all of the peak responses.
Calculate the percentage of each impurity in the portion of Raloxifene Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity in the *Sample solution*
r_S = peak response of raloxifene in the *Standard solution*
C_S = concentration of USP Raloxifene Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Raloxifene 3,7-diketone[a]	0.74	0.20
Raloxifene	1.00	—
Any unspecified individual impurity	—	0.10
Total impurities	—	0.5

[a] Methanone, [6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thien-3,7-diyl]bis[4-[2-(1-piperidinyl)ethoxy]phenyl].

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Raloxifene Hydrochloride RS
USP Raloxifene Related Compound C RS
1-(2-{4-[6-Hydroxy-2-(4-hydroxyphenyl) benzothiophene-3-carbonyl]phenoxy}ethyl)piperidine 1-oxide monohydrate.
$C_{28}H_{27}NO_5S \cdot H_2O$ 507.60

USP Monographs

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Raloxifene Hydrochloride Tablets

DEFINITION

Raloxifene Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Transfer a quantity of powdered Tablets, equivalent to 120 mg of raloxifene hydrochloride, to a suitable container. Add 20 mL of water, and shake to form a uniform slurry. Centrifuge, and discard the supernatant. Add 5 mL of isopropyl alcohol, shake to form a slurry, filter, and rinse the residue with isopropyl alcohol. Dry the residue at 105° for 30 min. Alternatively, add 40 mL of water to the powdered Tablets and vortex to form a uniform slurry. Centrifuge, and discard the supernatant. Repeat the washing process. Add 40 mL of methanol to the residue, vortex to form a uniform slurry, and centrifuge. Transfer the clear liquid to an appropriate container and evaporate to dryness.

Analysis: Prepare a potassium bromide dispersion with the *Sample*. Similarly prepare the Standard, starting with a slurry containing 12 mg/mL of USP Raloxifene Hydrochloride RS in water.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 7.2 g of monobasic potassium phosphate in 1000 mL of water. Add 1.3 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 2.5 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (33:67)

Diluent: Acetonitrile and *Buffer* (60:40)

System suitability solution: Prepare as directed in the test for *Organic Impurities*.

Standard solution: 0.06 mg/mL of USP Raloxifene Hydrochloride RS in *Diluent*

Sample solution: Transfer a sufficient quantity of Tablets to a volumetric flask of suitable size, add *Diluent*, and shake to disintegrate the Tablets. Sonicate if necessary. Dilute with *Diluent* to obtain a solution having a concentration of 0.06 mg/mL of raloxifene hydrochloride, based on the label claim. Filter, and use the clear solution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 3.5-µm base-deactivated packing L7

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between raloxifene and raloxifene related compound C, *System suitability solution*

Tailing factor: NMT 2.0 for raloxifene, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Raloxifene Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of raloxifene hydrochloride from the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.1% polysorbate 80; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Mobile phase: Acetonitrile, water, and triethylamine (500:500:2). Adjust with phosphoric acid to a pH of 4.0.

Triethylamine phosphate suspension: Add 2.0 mL of triethylamine to 500 mL of acetonitrile, and adjust with phosphoric acid to a pH of 4.0. [NOTE—Triethylamine phosphate will precipitate; keep the suspension well mixed.]

Standard solution: Prepare a solution having a known concentration equivalent to the expected concentration of the *Sample solution* by dissolving USP Raloxifene Hydrochloride RS in a small volume (NMT 10% of the final volume) of methanol. Dilute with *Medium* to volume, and mix the resulting solution with *Triethylamine phosphate suspension* (1:1).

Sample solution: Pass a portion of the solution under test through an appropriate filter of 0.45-µm pore size. Mix the resulting solution and *Triethylamine phosphate suspension* (1:1).

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 15-cm; 3.5-µm base-deactivated packing L10. If the analyte peak splits, use a guard column containing packing L3.

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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C_S = concentration of USP Raloxifene Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
F = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of raloxifene hydrochloride is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 1% sodium dodecyl sulfate in 0.05 M phosphate buffer prepared as follows. 1.7 g/L of sodium hydroxide and 7 g/L of monobasic sodium phosphate monohydrate. Adjust to a pH of 7.5, if necessary. Add 10 g of sodium dodecyl sulfate per L; 900 mL.
Apparatus 2: 75 rpm
Time: 45 min
Buffer: 2.8 g/L of sodium dodecyl sulfate. Adjust with glacial acetic acid to a pH of 4.0.
Diluent: Acetonitrile and water (50:50)
Mobile phase: Acetonitrile and *Buffer* (55:45)
Standard stock solution: 0.48 mg/mL of USP Raloxifene Hydrochloride RS in *Diluent*
Standard solution: 0.072 mg/mL of USP Raloxifene Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 286 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.3 times the retention time of raloxifene
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Raloxifene Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 2.2 phosphate buffer prepared as follows. Dissolve 3.7 g of monobasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 2.2; 1000 mL.
Apparatus 2: 50 rpm
Time: 20 min
Mobile phase: Acetonitrile, water, and triethylamine (400:600:2). Adjust with diluted phosphoric acid to a pH of 4.0.
Standard stock solution: 0.30 mg/mL of USP Raloxifene Hydrochloride RS prepared as follows. Transfer an appropriate amount of USP Raloxifene Hydrochloride RS to a suitable volumetric flask and add 50% of the flask volume of methanol. Sonicate for 20 min with occasional shaking and then dilute with *Medium* to volume.
Standard solution: 0.06 mg/mL of USP Raloxifene Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: Centrifuge a portion of the solution under test and use the clear supernatant. [NOTE—The use of a centrifuge speed of 2000 rpm for 10 min may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.7 times the retention time of raloxifene
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Raloxifene Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: Dissolve 9.0 g of monobasic potassium phosphate in 1000 mL of water. Add 0.5 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 3.0 ± 0.1.
Solution A: *Buffer* and acetonitrile (75:25)
Solution B: *Buffer* and acetonitrile (50:50)
Mobile phase: See *Table 1*. Adjust the start time of the gradient step on the basis of the instrument's dwell volume.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.00	100	0
5.00	100	0
36.25	0	100
38.25	100	0

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
48.00	100	0

Diluent A: Acetonitrile and *Buffer* (60:40)
Diluent B: Tetrahydrofuran and methanol (70:30)
Raloxifene related compound C solution: 0.15 mg/mL of USP Raloxifene Related Compound C RS in *Diluent B*
System suitability solution: Transfer 15 mg of USP Raloxifene Hydrochloride RS to a 50-mL volumetric flask, add 1.0 mL of *Raloxifene related compound C solution*, and dilute with *Diluent A* to volume.
Standard stock solution: 0.06 mg/mL of USP Raloxifene Hydrochloride RS in *Diluent A*
Standard solution: Mix 5 mL of the *Standard stock solution* and 45 mL of *Diluent A*, and dilute with *Solution A* to 100.0 mL (0.003 mg/mL).
Sample solution: Transfer a sufficient quantity of Tablets to a volumetric flask of a suitable size to obtain a solution of raloxifene hydrochloride having a concentration of 6 mg/mL, based on the label claim. Add *Diluent A*, and shake to disintegrate the Tablets. Sonicate, if necessary, and add *Diluent A* to volume. Transfer 5 mL of this solution to a 10-mL volumetric flask, and dilute with *Solution A* to volume to obtain a solution having a concentration of 3 mg/mL of raloxifene hydrochloride, based on the label claim. Filter, and use the clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L7
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between raloxifene and raloxifene related compound C
Tailing factor: NMT 2.0 for the raloxifene peak
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for NLT 2 times the retention time of the raloxifene peak, and measure all of the peak responses.
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of raloxifene from the *Standard solution*
C_S = concentration of USP Raloxifene Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of raloxifene hydrochloride from the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

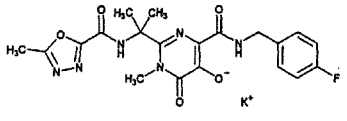
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Raloxifene	1.00	—
Raloxifene related compound C[a]	1.17	0.3
Any unspecified individual impurity	—	0.2
Total impurities	—	1.0

[a] 1-(2-{4-[6-Hydroxy-2-(4-hydroxyphenyl)benzothiophene-3-carbonyl] phenoxy}ethyl)piperidine 1-oxide.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Raloxifene Hydrochloride RS
  USP Raloxifene Related Compound C RS
  1-(2-{4-[6-Hydroxy-2-(4-hydroxyphenyl) benzothiophene-3-carbonyl]phenoxy}ethyl)piperidine 1-oxide monohydrate.
  $C_{28}H_{27}NO_5S \cdot H_2O$ 507.60

Raltegravir Potassium



$C_{20}H_{20}FKN_6O_5$ 482.51
4-Pyrimidinecarboxamide, *N*-[(4-fluorophenyl)methyl]-1,6-dihydro-5-hydroxy-1-methyl-2-[1-methyl-1-[[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino]ethyl]-6-oxo-, monopotassium salt;
Potassium 4-[(4-fluorobenzyl)carbamoyl]-1-methyl-2-(1-methyl-1-{[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino} ethyl)-6-oxo-1,6-dihydropyrimidin-5-olate [871038-72-1].

DEFINITION
Raltegravir Potassium contains NLT 98.0% and NMT 102.0% of raltegravir potassium ($C_{20}H_{20}FKN_6O_5$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
  [NOTE—If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in methanol, evaporate to dryness, and record new spectra using the residues.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**
  Solution A: 100 g/L of sodium cobaltinitrite in water
  Sample solution: 40 mg/mL of Raltegravir Potassium in water

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Analysis: Combine 1 mL of *Sample solution* with 1 mL of dilute acetic acid and 1 mL of *Solution A*.
Acceptance criteria: A yellow or orange yellow precipitate is formed immediately.

ASSAY

• **PROCEDURE**

Diluent: Acetonitrile and water (25:75)
Solution A: Phosphoric acid and water (1:1000)
Solution B: Acetonitrile
Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
2	75	25
5	60	40
10	55	45
19	10	90
22	5	95

Standard solution: 0.1 mg/mL of USP Raltegravir Potassium RS in *Diluent* prepared as follows. Transfer a suitable amount of USP Raltegravir Potassium RS to a suitable volumetric flask and add about 40% of the flask volume of *Diluent*. Sonicate for 5 min, add the *Diluent* to about 90% of the final volume, and allow to stand for 30 min. Dilute with *Diluent* to volume.
Sample solution: 0.1 mg/mL of Raltegravir Potassium in *Diluent* prepared as follows. Transfer a suitable amount of the sample to a suitable volumetric flask and add about 40% of the flask volume of *Diluent*. Sonicate for 5 min, add the *Diluent* to about 90% of the final volume, and allow to stand for 30 min. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L11
Column temperature: 15°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of raltegravir potassium ($C_{20}H_{20}FKN_6O_5$) in the portion of Raltegravir Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of raltegravir from the *Sample solution*
r_S = peak response of raltegravir from the *Standard solution*
C_S = concentration of USP Raltegravir Potassium RS in the *Standard solution* (mg/mL)
C_U = concentration of Raltegravir Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Peak identification solution: Prepare a solution containing 2 mg/mL of USP Raltegravir Potassium RS in 1 N sodium hydroxide solution. Stir the solution for 2 h at room temperature. Transfer 5 mL of this solution to a 50-mL volumetric flask and add 5 mL of 1 N hydrochloric acid. Dilute with *Diluent* to volume. [NOTE—In situ degradation generates raltegravir amine and raltegravir oxalylacetohydrazide analog.]
System suitability solution: 0.1 mg/mL of USP Raltegravir Potassium RS and 0.2 µg/mL of USP Raltegravir Related Compound E RS in *Diluent*
Standard stock solution: Use the *Standard solution* prepared in the *Assay*.
Standard solution: 0.1 µg/mL of USP Raltegravir Potassium RS in *Diluent* from *Standard stock solution*
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between raltegravir related compound E and raltegravir
Analysis
Samples: *Sample solution, Peak identification solution,* and *Standard solution*
Calculate the percentage of any individual impurity in the portion of Raltegravir Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of raltegravir from the *Standard solution*
C_S = concentration of USP Raltegravir Potassium RS in the *Standard solution* (mg/mL)
C_U = concentration of Raltegravir Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Raltegravir amine[a]	0.43	0.15
Raltegravir formididyl analog[b]	0.60	0.15
Raltegravir oxalylacetohydrazide analog[c]	0.72	0.20
Raltegravir related compound E	0.94	0.15
Raltegravir	1.0	—
Raltegravir *E*-ethoxyacethydrazone analog[d]	1.1	0.20
Raltegravir *Z*-ethoxyacethydrazone analog[e]	1.2	0.15
Any individual, unspecified impurity	—	0.10

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Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	0.7

[a] 2-(2-Aminopropan-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.
[b] (*E*)-2-(2-{[(Dimethylamino)methylidene]amino}propan-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.
[c] 2-{2-[2-(2-Acetylhydrazinyl)-2-oxoacetamido]propan-2-yl}-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.
[d] Ethyl (*E*)-*N*-{2-[(2-{4-[(4-fluorobenzyl)carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl}propan-2-yl)amino]-2-oxoacetyl}acetohydrazonate.
[e] Ethyl (*Z*)-*N*-{2-[(2-{4-[(4-fluorobenzyl)carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl}propan-2-yl)amino]-2-oxoacetyl}acetohydrazonate.

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩
Sample: 0.5 g
[NOTE—A mixture of equal volumes of methanol and formamide is suitable as solvent. Hydranal-Composite 2 is suitable as reagent.]
Acceptance criteria: NMT 0.6%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Raltegravir Potassium RS
USP Raltegravir Related Compound E RS
N-{2-[4-(Benzylcarbamoyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl}-5-methyl-1,3,4-oxadiazole-2-carboxamide.
$C_{20}H_{22}N_6O_5$ 426.43

Raltegravir Tablets

DEFINITION

Raltegravir Tablets contain an amount of Raltegravir Potassium equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of raltegravir ($C_{20}H_{20}FN_6O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A OR 197K▲** (CN 1-May-2020)
Sample: Grind a Tablet, and use a suitable amount of the powdered Tablet to prepare a specimen.
Acceptance criteria: The spectrum obtained from the *Sample* shows bands at approximately 1633, 1515, 1188, 810, and 728 cm^{-1}, similar to the spectrum from the Standard similarly obtained. [NOTE—Peak positions may vary slightly between instruments (within ±10 cm^{-1}). Other peaks may be present in the spectra that do not appear in this list.]
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Buffer* (29:71)
Solution A: Acetonitrile and water (50:50)
Diluent: Acetonitrile and water (30:70)
Standard solution: 0.22 mg/mL of USP Raltegravir Potassium RS in *Diluent*
Sample stock solution: Nominally equivalent to 8 mg/mL of raltegravir from Tablets prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask and dilute with *Solution A* to volume. Stir the flask vigorously for 1 h. Centrifuge a portion of the solution and use the supernatant for *Sample solution* preparation.
Sample solution: Nominally 0.2 mg/mL of raltegravir in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing *L1*
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 30 µL
Run time: NLT 1.5 times the retention time of raltegravir
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of raltegravir ($C_{20}H_{20}FN_6O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of raltegravir from the *Sample solution*
r_S = peak response of raltegravir from the *Standard solution*
C_S = concentration of USP Raltegravir Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of raltegravir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of raltegravir, 444.44
M_{r2} = molecular weight of raltegravir potassium, 482.51

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: *Water*; 900 mL, deaerated
Apparatus 2: 100 rpm with sinker
Times: 15 and 60 min
Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (38:62)
Diluent: Acetonitrile and water (20:80)
Standard solution: 0.48 mg/mL of USP Raltegravir Potassium RS in *Diluent*. Sonicate, if necessary, to dissolve prior to final dilution.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 303 nm
Column: 4.6-mm × 10-cm; packing L1
Column temperature: 40°
Flow rate: 5 mL/min
Injection volume: 10 µL
Run time: 1 min

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System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of raltegravir ($C_{20}H_{20}FN_6O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (1/L) \times V \times D \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the USP Raltegravir Potassium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, if needed
M_{r1} = molecular weight of raltegravir, 444.44
M_{r2} = molecular weight of raltegravir potassium, 482.51

Tolerances: 15%–45% of the labeled amount of raltegravir is dissolved in 15 min, and NLT 70% (Q) of the labeled amount of raltegravir is dissolved in 60 min.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Solution A, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Peak identification solution: Prepare a solution containing 2 mg/mL of USP Raltegravir Potassium RS in 1 N sodium hydroxide solution. Stir the solution for 2 h at room temperature. Transfer 5 mL of this solution to a 50-mL volumetric flask and add 5 mL of 1 N hydrochloric acid. Dilute with *Diluent* to volume. [NOTE—In situ degradation generates the raltegravir amine and raltegravir oxalylacetohydrazide analog peaks along with a small peak for raltegravir oxalyl analog impurity.]
System suitability solution: 0.1 mg/mL of USP Raltegravir Potassium RS and 0.2 µg/mL of USP Raltegravir Related Compound E RS in *Diluent*
Standard stock solution: Use the *Standard solution* prepared in the *Assay*.
Standard solution: 0.44 µg/mL of USP Raltegravir Potassium RS in *Diluent* from *Standard stock solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between raltegravir related compound E and raltegravir, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Sample solution*, *Peak identification solution*, and *Standard solution*
Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of raltegravir from the *Standard solution*
C_S = concentration of USP Raltegravir Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of raltegravir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of raltegravir, 444.44
M_{r2} = molecular weight of raltegravir potassium, 482.51
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Reporting threshold is 0.1%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Raltegravir amine[a]	0.14	1.0	—[b]
Raltegravir formididyl analog[c]	0.29	1.0	—[b]
Raltegravir oxalyl analog[d]	0.33	1.0	0.3
Raltegravir oxalylacetohydrazide analog[e]	0.48	0.75	0.5
Raltegravir related compound E	0.83	1.0	—[b]
Raltegravir	1.0	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.8

[a] 2-(2-Aminopropan-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.
[b] This is a process impurity controlled in the drug substance and not monitored in the drug product.
[c] (*E*)-2-(2-{[(Dimethylamino)methylidene]amino}propan-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.
[d] 2-[(2-(4-[(4-Fluorobenzyl)carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl}propan-2-yl)amino]-2-oxoacetic acid.
[e] 2-(2-[2-(2-Acetylhydrazinyl)-2-oxoacetamido]propan-2-yl}-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Raltegravir Potassium RS
USP Raltegravir Related Compound E RS
N-{2-[4-(Benzylcarbamoyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl}-5-methyl-1,3,4-oxadiazole-2-carboxamide.
$C_{20}H_{22}N_6O_5$ 426.43

Raltegravir Chewable Tablets

DEFINITION

Raltegravir Chewable Tablets contain an amount of Raltegravir Potassium equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of raltegravir ($C_{20}H_{21}FN_6O_5$).

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A OR 197K▲** (CN 1-May-2020)

Sample: Grind a Tablet, and use a suitable amount of the powdered Chewable Tablet to prepare a specimen.

Acceptance criteria: The spectrum obtained from the *Sample* shows bands at approximately 1633, 1515, 1188, 810, and 728 cm^{-1}, similar to the spectrum from the Standard similarly obtained. [NOTE—Peak positions may vary slightly between instruments (within ±10 cm^{-1}). Other peaks may be present in the spectra that do not appear in this list.]

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Solution A: Acetonitrile and *Buffer* (20:80)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	50	50
25.1	100	0
30	100	0

Diluent: Acetonitrile and water (30:70)
Standard solution: 0.11 mg/mL of USP Raltegravir Potassium RS in *Diluent*
Sample stock solution: Nominally equivalent to 1 mg/mL of raltegravir from Chewable Tablets prepared as follows. Transfer NLT 10 Chewable Tablets to a suitable volumetric flask and dilute with *Diluent* to 20% of the flask volume. Stir the contents of the flask for about 10 min to break apart the Chewable Tablets. Dilute with *Diluent* to volume and stir the contents of the flask for about 1 h. Centrifuge a portion of the solution and use the supernatant for *Sample solution* preparation.
Sample solution: Nominally 0.1 mg/mL of raltegravir from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing *L1*
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of raltegravir ($C_{20}H_{21}FN_6O_5$) in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of raltegravir from the *Sample solution*
r_S = peak response of raltegravir from the *Standard solution*
C_S = concentration of USP Raltegravir Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of raltegravir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of raltegravir, 444.44
M_{r2} = molecular weight of raltegravir potassium, 482.51

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: *Water*; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 15 min
Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (38:62)
Diluent: Acetonitrile and water (30:70)
Standard solution: (*L*/900) mg/mL of raltegravir from USP Raltegravir Potassium RS in *Diluent*, where *L* is the label claim in mg/Chewable Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 303 nm
Column: 4.6-mm × 10-cm; packing *L1*
Column temperature: 40°
Flow rate: 5 mL/min
Injection volume: 30 µL
Run time: 1 min
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of raltegravir ($C_{20}H_{21}FN_6O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (1/L) \times V \times D \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of raltegravir from the *Sample solution*
r_S = peak response of raltegravir from the *Standard solution*
C_S = concentration of the USP Raltegravir Potassium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Chewable Tablet)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, if applicable
M_{r1} = molecular weight of raltegravir, 444.44
M_{r2} = molecular weight of raltegravir potassium, 482.51

Tolerances: NLT 85% (*Q*) of the labeled amount of raltegravir is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

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IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Peak identification solution: Prepare a solution containing 2 mg/mL of USP Raltegravir Potassium RS in 1 N sodium hydroxide solution. Stir the solution for 2 h at room temperature. Transfer 5 mL of this solution to a 50-mL volumetric flask and add 5 mL of 1 N hydrochloric acid. Dilute with *Diluent* to volume. [NOTE—In situ degradation generates the raltegravir amine and raltegravir oxalylacetohydrazide analog peaks along with a small peak for raltegravir oxalyl analog impurity.]

System suitability solution: 0.1 mg/mL of USP Raltegravir Potassium RS and 0.2 µg/mL of USP Raltegravir Related Compound E RS in *Diluent*

Standard stock solution: Use the *Standard solution* prepared in the *Assay*.

Standard solution: 0.22 µg/mL of USP Raltegravir Potassium RS in *Diluent* from *Standard stock solution*

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between raltegravir related compound E and raltegravir, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Sample solution*, *Peak identification solution*, and *Standard solution*

Calculate the percentage of any individual impurity in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of raltegravir from the *Standard solution*

C_S = concentration of USP Raltegravir Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of raltegravir in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of raltegravir, 444.44

M_{r2} = molecular weight of raltegravir potassium, 482.51

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Raltegravir amine[a]	0.42	1.0	—[b]
Raltegravir formididyl analog[c]	0.53	1.0	—[b]
Raltegravir oxalyl analog[d]	0.69	0.70	0.2
Raltegravir oxalylacetohydrazide analog[e]	0.81	0.63	0.3
Raltegravir related compound E	0.96	1.0	—[b]
Raltegravir	1.0	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.8

[a] 2-(2-Aminopropan-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.

[b] This is a process impurity controlled in the drug substance and not included in total impurities.

[c] (*E*)-2-(2-{[(Dimethylamino)methylidene]amino}propan-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.

[d] 2-[(2-{4-[(4-Fluorobenzyl)carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl}propan-2-yl)amino]-2-oxoacetic acid.

[e] 2-{2-[2-(2-Acetylhydrazinyl)-2-oxoacetamido]propan-2-yl}-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

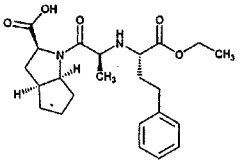
USP Raltegravir Potassium RS

USP Raltegravir Related Compound E RS

N-{2-[4-(Benzylcarbamoyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl}-5-methyl-1,3,4-oxadiazole-2-carboxamide.

$C_{20}H_{22}N_6O_5$ 426.43

Ramipril



$C_{23}H_{32}N_2O_5$ 416.51

Cyclopenta[*b*]pyrrole-2-carboxylic acid, 1-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl] octahydro-, [2*S*-[1[*R**(*R**)],2α,3aβ,6aβ]]-;
(2*S*,3a*S*,6a*S*)-1-[(*S*)-*N*-[(*S*)-1-Carboxy-3-phenylpropyl]alanyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid, 1-ethyl ester [87333-19-5].

DEFINITION

Ramipril contains NLT 98.0% and NMT 102.0% of ramipril ($C_{23}H_{32}N_2O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

• **PROCEDURE**

Buffer: 0.1% solution of sodium dodecyl sulfate. Adjust with phosphoric acid to a pH of 2.4 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (450:550). Adjust with phosphoric acid to a pH of 2.75 ± 0.1.

System suitability solution: 0.2 mg/mL of USP Ramipril RS and 0.01 mg/mL of USP Ramipril Related Compound A RS, in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Ramipril RS in *Mobile phase*

Sample stock solution: 1 mg/mL of Ramipril prepared as follows. Dissolve 100 mg of Ramipril in 10 mL of acetonitrile in a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: 0.2 mg/mL of Ramipril in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between ramipril and ramipril related compound A

Column efficiency: NLT 4000 theoretical plates for the ramipril peak

Relative standard deviation: NMT 1.0% for ramipril peak

Analysis

Samples: *Standard solution* and *Sample solution*

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for ramipril from the *Sample solution*

r_S = peak response for ramipril from the *Standard solution*

C_S = concentration of USP Ramipril RS in the *Standard solution* (mg/mL)

C_U = concentration of Ramipril in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **LIMIT OF PALLADIUM**

Diluent: Water and nitric acid (997:3)

Standard stock solution: Dissolve 50 mg of palladium metal taken in a 100-mL volumetric flask, in 9 mL of hydrochloric acid, and dilute with water to volume.

Standard solutions: 0.02, 0.03, and 0.05 µg/mL of palladium in *Diluent* from *Standard stock solution*

Sample solution: 2 mg/mL of Ramipril in *Diluent*

Blank solution: 1.5 mg/mL of magnesium nitrate in *Diluent*

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 247.6 nm palladium emission line

Lamp: Palladium hollow-cathode

Flame: Air–acetylene

Injection volume: 20 µL each for the *Standard solutions* and *Sample solution*, and 10 µL for the *Blank solution*

Analysis: Concomitantly determine the absorbances of the *Standard solutions*, *Sample solution*, and the *Blank solution*.

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of palladium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C_P, in µg/mL, of palladium in the *Sample solution*.

Calculate the percentage of palladium in the portion of Ramipril taken:

$$\text{Result} = (C_P/C_R) \times 100$$

C_P = concentration of palladium in the *Sample solution* (µg/mL)

C_R = concentration of Ramipril in the *Sample solution* (µg/mL)

Acceptance criteria: 20 ppm

• **ORGANIC IMPURITIES**

Solution A: 2.0 g of sodium perchlorate in a mixture of 800 mL of water and 0.5 mL of triethylamine. Adjust with phosphoric acid to a pH of about 3.6 ± 0.1, and add 200 mL of acetonitrile.

Solution B: 2.0 g of sodium perchlorate in a mixture of 300 mL of water and 0.5 mL of triethylamine. Adjust with phosphoric acid to a pH of about 2.6 ± 0.1, and add 700 mL of acetonitrile.

System suitability solution: 0.5 mg/mL each of USP Ramipril RS, USP Ramipril Related Compound A RS, USP Ramipril Related Compound B RS, USP Ramipril Related Compound C RS, and USP Ramipril Related Compound D RS in *Solution B*

Standard solution: 5 µg/mL of USP Ramipril RS in *Solution B*

Sample solution: 1 mg/mL of Ramipril in *Solution A*. Keep the *Sample solution* cold until injected.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
6	90	10
7	75	25
20	65	35
30	25	75
40	25	75
45	90	10
55	90	10

[NOTE—Make adjustments at the 75:25 ratio stage, if necessary, to achieve elution of ramipril 16–19 min after injection of the *Standard solution*.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 25-cm; 3-µm packing L1

Column temperature: 65°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between ramipril related compound A and ramipril, *System suitability solution*

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Tailing factor: 0.8–2.0 for ramipril, *Sample solution*
Relative standard deviation: NMT 5.0% for ramipril, *Standard solution*
Retention time: 16–19 min, *Sample solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified and any other impurity in the portion of Ramipril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = response of each individual peak from the *Sample solution*
r_S = peak response for ramipril from the *Standard solution*
C_S = concentration of USP Ramipril RS in the *Standard solution* (mg/mL)
C_U = concentration of Ramipril in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ramipril related compound A	0.8	1.0	0.5
Ramipril	1.0	—	—
Ramipril related compound B	1.3	1.0	0.5
Ramipril related compound C	1.5	0.42	0.5
Ramipril related compound D	1.6	1.0	0.5
Any other individual impurity	—	—	0.1
Total impurities	—	—	1.0

SPECIFIC TESTS

• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL, in 0.1 M methanolic hydrochloric acid
Acceptance criteria: +32.0° to +38.0°, at 20°
• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 6 h.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Ramipril RS
USP Ramipril Related Compound A RS
((2*S*,3a*S*,6a*S*)-1-[(*S*)2-[[(*S*)-1-(Methoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid).
$C_{22}H_{30}N_2O_5$ 402.48
USP Ramipril Related Compound B RS
((2*S*,3a*S*,6a*S*)-1-[(*S*)2-[[(*S*)-1-(Methylethoxy)carbonyl-3-phenylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid).
$C_{24}H_{34}N_2O_5$ 430.54
USP Ramipril Related Compound C RS
(2*S*,3a*S*,6a*S*)-1-[(*S*)2-[[(*S*)1-Ethoxycarbonyl-3-cyclohexyl propyl]amino]-1-oxopropyl]-octahydrocyclopenta[*b*] pyrrole-2-carboxylic acid.
$C_{23}H_{38}N_2O_5$ 422.56
USP Ramipril Related Compound D RS
Ethyl (2*S*)2-[(3*S*,5a*S*,8a*S*, 9a*S*)-3-methyl-1,4-dioxodecahydro-1*H*-cyclopenta[*e*]pyrrolo[1,2-a] pyrazin-2-yl]-4-phenyl-butanoate.
$C_{23}H_{30}N_2O_4$ 398.50

Ramipril Capsules

DEFINITION

Ramipril Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{23}H_{32}N_2O_5$.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Phosphoric acid solution: 30 mL/L of phosphoric acid in water
Diluent: Acetonitrile and *Phosphoric acid solution* (2:3)
Standard solution: 0.2 mg/mL of USP Ramipril RS in *Diluent*. Sonicate for 1 min, if necessary, for complete dissolution.
Sample solution: Use the *Sample solution* prepared as directed in the *Assay*.
Wavelength range: 200–400 nm
Path length: 0.1-cm cell
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Buffer: Dissolve 17 g of monobasic potassium phosphate and 11.2 g of sodium perchlorate in 750 mL of water in a 1-L flask. Dilute with water to volume. Adjust with phosphoric acid to a pH of 2.3.
Solution A: Acetonitrile, *Buffer*, and water (1:2:2). [Note—Do not filter *Solution A*.]
Solution B: Acetonitrile, *Buffer*, and water (9:10:6). [Note—Do not filter *Solution B*.]
Phosphoric acid solution and **Diluent:** Prepare as directed in *Identification* test *A*.
Mobile phase: Use the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
50	0	100
51	0	100
51.1	100	0
60	100	0

Standard solution: 0.2 mg/mL of USP Ramipril RS and 0.002 mg/mL of USP Ramipril Related Compound A RS in *Diluent*

USP Monographs

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Sample stock solution: Transfer the contents of 8 Capsules into each of the flasks as described in *Table 1*. Add Capsule shells into the flasks. Add acetonitrile per *Table 1*, and swirl to agitate the contents. Sonicate for 15 min, and mechanically shake for 10 min. Dilute with acetonitrile to volume for Capsule strengths 5.0 and 10 mg only. For 1.25- and 2.5-mg Capsules, use the solution as is without further dilution. [NOTE—Extracts from the vial cap may result in extraneous peaks.]

Table 1

Strength of Capsule (mg)	Volumetric Flask Size (mL)	Acetonitrile (mL)
1.25	50	25
2.5	100	50
5.0	100	70
10	200	140

Sample solution: Nominally 0.2 mg/mL of ramipril in *Phosphoric acid solution* from the *Sample stock solution*. Pass through a nylon filter of 0.20-µm pore size, and discard the first 2 mL of filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1 with a guard column, packing L1
Temperature: 60°
Flow rate: 1.5 mL/min
Injection size: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between ramipril and ramipril related compound A
Tailing factor: NMT 2.5 for the ramipril peak
Relative standard deviation: NMT 2.0% for the ramipril peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{23}H_{32}N_2O_5$, based on the label claim, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ramipril from the *Sample solution*
r_S = peak response of ramipril from the *Standard solution*
C_S = concentration of ramipril in the *Standard solution* (mg/mL)
C_U = nominal concentration of ramipril in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm, with sinkers. [NOTE—A suitable sinker is catalog number CAPWHT-02 available from www.QLA-LLC.com.]
Time: 30 min
Standard solution: 0.01 mg/mL of USP Ramipril RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Phosphoric acid solution: Prepare as directed in *Identification* test *A*.
Mobile phase: Acetonitrile and *Phosphoric acid solution* (2:3)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperature: 30°
Flow rate: 1 mL/min
Injection size: 25 µL
Suitability requirements
Sample: *Standard solution*
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ramipril dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of ramipril in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of ramipril is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
Procedure for content uniformity
Phosphoric acid solution: Prepare as directed in *Identification* test *A*.
Mobile phase: Acetonitrile and *Phosphoric acid solution* (2:3). Pass through a nylon filter of 0.45-µm pore size.
Standard solution: 0.03 mg/mL of USP Ramipril RS in *Mobile phase*. Sonicate for 1 min, if not dissolved completely.
Sample solution: Transfer the contents of 1 Capsule into a suitable flask as described in *Table 2*. Add *Mobile phase* (about 50% of total volume), and sonicate for 25 min. Mechanically shake for 10 min, and dilute with *Mobile phase* to volume. Further dilute the solution from the 10-mg strength Capsule with *Mobile phase*, as shown in *Table 2*. Pass through a nylon filter of 0.20-µm pore size, and discard the first 2 mL of filtrate.

Table 2

Strength of Capsule (mg)	Volumetric Flask Size (mL)	Dilution Volume (mL)	Volumetric Flask (mL)
1.25	50	—	—
2.5	100	—	—
5.0	200	—	—
10	50	6.0	50

Chromatographic system: Proceed as directed in the test for *Dissolution*.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{23}H_{32}N_2O_5$, based on the label claim, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of ramipril from the *Sample solution*
r_S = peak response of ramipril from the *Standard solution*
C_S = concentration of ramipril in the *Standard solution* (mg/mL)
C_U = concentration of ramipril in the *Sample solution* (mg/mL)

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Buffer, Solution A, Solution B, Phosphoric acid solution, Diluent, Standard solution, and **Sample solution:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.1 µg/mL of ramipril in *Diluent* from the *Standard solution*

Chromatographic system: Prepare as directed in the *Assay*.

Suitability requirements

Samples: *Standard solution* and *Sensitivity solution*

Resolution: NLT 2.5 between ramipril and ramipril related compound A, *Standard solution*

Tailing factor: NMT 2.5 for the ramipril peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the ramipril peak, *Standard solution*

Signal-to-noise ratio: NLT 10 for the ramipril peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100 \times (1/F)$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of ramipril from the *Standard solution*
C_U = nominal concentration of ramipril in the *Sample solution* (mg/mL)
C_S = concentration of ramipril in the *Standard solution* (mg/mL)
F = relative response factor (see *Impurity Table*)

Acceptance criteria

Individual impurities: See *Impurity Table*.

Total impurities: NMT 8.0% for Capsule strength 1.25 mg, NMT 7.0% for Capsule strength 2.5 mg, and NMT 6.0% for Capsule strengths 5 mg and 10 mg. [NOTE—Total impurities include the sum of individual specified and unspecified degradants. Disregard any peak below 0.1%.]

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ramipril RS

USP Ramipril Related Compound A RS
(2*S*,3a*S*,6a*S*)-1-[(*S*)2-[[(*S*)1-(Methoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid.
$C_{22}H_{30}N_2O_5$ 402.48

Ramipril Tablets

DEFINITION

Ramipril Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of ramipril ($C_{23}H_{32}N_2O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

Sample: Finely powder a suitable number of Tablets and transfer a portion of the powder, equivalent to 100 mg of ramipril, to a suitable beaker. Add 25 mL of methanol, shake to dissolve, and pass through the filter. Evaporate the solution in air and heat to complete dryness at 105° for 1 h.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Impurity Table (Ramipril Capsules)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%) for 1.25-mg Capsules	Acceptance Criteria, NMT (%) for 2.5-mg Capsules	Acceptance Criteria, NMT (%) for 5-mg and 10-mg Capsules
Ramipril diacid	0.24	0.41	1.0	1.0	1.0
Ramipril related compound A[a]	0.72	—	—	—	—
Ramipril diacid impurity[a]	0.85	—	—	—	—
Ramipril	1	—	—	—	—
Ramipril related compound B[a]	1.31	—	—	—	—
Ramipril related compound C[a]	1.68	—	—	—	—
Ramipril related compound D[b]	1.84	1	8.0	5.5	5.0
Any other individual unspecifed degradant	—	—	0.2	0.2	0.2

[a] Disregard this impurity as it is process related and is controlled in the drug substance.
[b] Ethyl (2*S*)-2-[(3*S*,5a*S*,8a*S*,9a*S*)-3-methyl-1,4-dioxodecahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-a]pyrazin-2-yl]-4-phenylbutanoate (Ramipril diketopiperazine).

USP Monographs

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ASSAY

• **PROCEDURE**

Solution A: 2 g of sodium perchlorate in a mixture of 0.5 mL of triethylamine and 800 mL of water. Adjust with phosphoric acid to a pH of 3.6, and add 200 mL of acetonitrile.

Solution B: 2 g of sodium perchlorate in a mixture of 0.5 mL of triethylamine and 300 mL of water. Adjust with phosphoric acid to a pH of 2.6, and add 700 mL of acetonitrile.

Mobile phase: *Solution A* and *Solution B* (60:40)

Diluent: 6.8 g of monobasic sodium phosphate in 500 mL of water. Add 1000 mL each of methanol and acetonitrile. Adjust with phosphoric acid to a pH of 5.5.

Standard solution: 0.1 mg/mL of USP Ramipril RS in *Diluent*

Sample stock solution: Nominally 0.25 mg/mL of ramipril from NLT 10 Tablets prepared as follows. Add 50% of the final volume of *Diluent* to the flask and sonicate to disperse the Tablets. Sonicate for an additional 30 min and dilute with *Diluent* to volume.

Sample solution: Nominally 0.1 mg/mL of ramipril from the *Sample stock solution* in *Diluent*. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 15 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ramipril ($C_{23}H_{32}N_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ramipril from the *Sample solution*

r_S = peak response of ramipril from the *Standard solution*

C_S = concentration of USP Ramipril RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ramipril in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: 0.25 mg/mL of USP Ramipril RS in methanol

Standard solution: (*L*/500) mg/mL of USP Ramipril RS in *Medium* from the *Standard stock solution* where *L* is the label claim of ramipril in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Solution A, Solution B, Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* except for the *Injection volume*.

Injection volume: 100 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ramipril ($C_{23}H_{32}N_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ramipril RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim for ramipril (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of ramipril ($C_{23}H_{32}N_2O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A and **Solution B:** Prepare as directed in the *Assay*.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
6	70	30
30	30	70
40	30	70
45	70	30
50	70	30

System suitability solution: 50 µg/mL each of USP Ramipril RS, USP Ramipril Related Compound A RS, and USP Ramipril Related Compound D RS in *Solution B*

Sensitivity solution: 1 µg/mL of USP Ramipril RS in *Solution B*

Standard solution: 0.005 mg/mL of USP Ramipril RS in *Solution B*

Sample solution: Nominally 1 mg/mL of ramipril prepared as follows. Finely powder NLT 20 Tablets and transfer a portion of the powder to an appropriate volumetric flask. Add about 60% of the flask volume of *Solution A*, and sonicate for 30 min with intermittent shaking to dissolve. Dilute with *Solution A* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 25-cm; 3-µm packing L1

Column temperature: 65°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between ramipril related compound A and ramipril, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

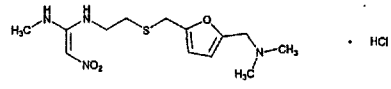
r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of ramipril from the *Standard solution*
C_S = concentration of USP Ramipril RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ramipril in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.04%.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ramipril RS
  USP Ramipril Related Compound A RS
  (2*S*,3a*S*,6a*S*)-1-[(*S*)2-[[(*S*)-1-(Methoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid.
  $C_{22}H_{30}N_2O_5$ 402.48
  USP Ramipril Related Compound D RS
  Ethyl (2*S*)2-[(3*S*,5a*S*,8a*S*,9a*S*)-3-methyl-1,4-dioxodecahydro-1*H*-cyclopenta[*e*]pyrrolo[1,2-*a*]pyrazin-2-yl]-4-phenyl-butanoate.
  $C_{23}H_{30}N_2O_4$ 398.50

Ranitidine Hydrochloride



$C_{13}H_{22}N_4O_3S \cdot HCl$ 350.86

1,1-Ethenediamine, *N*-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]-methyl]thio]ethyl]-*N*′-methyl-2-nitro-, monohydrochloride.
N-[2-[[[5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio] ethyl]-*N*′-methyl-2-nitro-1,1-ethenediamine, hydrochloride [66357-59-3].

» Ranitidine Hydrochloride contains not less than 97.5 percent and not more than 102.0 percent of $C_{13}H_{22}N_4O_3S \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Ranitidine Hydrochloride RS
USP Ranitidine Resolution Mixture RS
It is a mixture of ranitidine hydrochloride and four related impurities: ranitidine-*N*-oxide, ranitidine complex nitroacetamide, ranitidine diamine hemifumarate, and ranitidine amino alcohol hemifumarate.
Ranitidine-N-oxide: *N*,*N*-dimethyl[5-[[[2-[[1-(methylamino)-2-nitroethenyl]amino]ethyl]sulphanyl] meth yl]furan-2-yl]methanamine *N*-oxide.
Ranitidine complex nitroacetamide: *N*-[2-[[[5-[(dimethyl amino)methyl]furan-2-yl]methyl]sulphanyl]ethyl]-2-nitroacetamide.
Ranitidine diamine hemifumarate (related compound A): 5-[[(2-aminoethyl)thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine, hemifumarate salt.
Ranitidine amino alcohol hemifumarate: [5-[(dimethylamino)methyl]furan-2-yl]methanol.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: water.
Absorptivities at 229 nm and 315 nm, calculated on the dried basis, do not differ by more than 3.0%.

Table 2 (Ramipril Tablets)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria for 1.25-mg Tablets, NMT (%)	Acceptance Criteria for 2.5-, 5-, and 10-mg Tablets, NMT (%)
Ramipril related compound A[a]	0.71	—	—	—
Ramipril	1.0	—	—	—
Ramipril isopropyl ester[a, b]	1.31	—	—	—
Hexahydroramipril[a, c]	1.63	—	—	—
Ramipril related compound D[d]	1.92	0.91	8.0	6.0
Any unspecified degradation product	—	1.0	0.2	0.2
Total degradation products	—	—	1.0	1.0

[a] This is a process impurity monitored in the drug substance that is for identification only. It is not included in the total degradation products.
[b] (2*S*,3a*S*,6a*S*)-1-[(*S*)2-[[(*S*)-1-(Methylethoxy)carbonyl-3-phenylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid.
[c] Ethyl (2*S*)2-[(3*S*,5a*S*,8a*S*,9a*S*)-3-methyl-1,4-dioxodecahydro-1*H*-cyclopenta[*e*]pyrrolo[1,2-*a*]pyrazin-2-yl]-4-phenyl-butanoate.
[d] Not included in the total degradation products.

USP Monographs

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C: A solution of it meets the requirements of the tests for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 4.5 and 6.0, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 0.75% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Diluent, Mobile phase, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Prepare as directed for *Standard preparation* in the *Assay.*
Test solution—Prepare as directed for *Assay preparation* in the *Assay.*
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and identify the ranitidine peak and the peaks due to impurities and degradation products listed in the table below.

Name	Relative Retention Time
Ranitidine simple nitroacetamide[1]	0.14
Ranitidine oxime[2]	0.21
Ranitidine amino alcohol[3]	0.45
Ranitidine diamine[4]	0.57
Ranitidine *S*-oxide[5]	0.64
Ranitidine *N*-oxide[6]	0.72
Ranitidine complex nitroacetamide[7]	0.84
Ranitidine formaldehyde adduct[8]	1.36
Ranitidine *bis*-compound[9]	1.75

[1] *N*-Methyl-2-nitroacetamide.
[2] 3-(Methylamino)-5,6-dihydro-2*H*-1,4-thiazin-2-one oxime.
[3] {5-[(Dimethylamino)methyl]furan-2-yl}methanol.
[4] 5-{[(2-Aminoethyl)thio]methyl}-*N*,*N*-dimethyl-2-furanmethanamine (ranitidine related compound A).
[5] *N*-{2-[({5-[(Dimethylamino)methyl]-2-furanyl}methyl)sulfinyl]ethyl}-*N'*-methyl-2-nitro-1,1-ethenediamine (ranitidine related compound C).
[6] *N*,*N*-Dimethyl(5-{[(2-{[1-(methylamino)-2-nitroethenyl]amino}ethyl) sulphanyl]methyl}furan-2-yl)methanamine *N*-oxide.
[7] *N*-{2-[({5-[(Dimethylamino)methyl]furan-2-yl}methyl)sulphanyl]ethyl}-2-nitroacetamide.
[8] 2,2'-Methylenebis(*N*-{2-[({5-[(dimethylamino)methyl]furan-2-yl}methyl)-sulphanyl]ethyl}-*N'*-methyl-2-nitroethene-1,1-diamine).
[9] *N*,*N'*-bis{2-[({5-[(Dimethylamino)methyl]-2-furanyl}methyl)thio]ethyl}-2-nitro-1,1-ethenediamine (ranitidine related compound B).

Measure the responses for the major peaks, and calculate the percentage of each impurity in the portion of Ranitidine Hydrochloride taken by the formula:

$$100CV/W(r_i/r_S)$$

in which *C* is the concentration, in mg per mL, of ranitidine hydrochloride in the *Standard solution; V* is the volume, in mL, of the *Test solution; W* is the weight, in mg, of Ranitidine Hydrochloride taken to prepare the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the ranitidine peak response obtained from the *Standard solution:* not more than 0.3% of ranitidine bis-compound is found, not more than 0.1% of any other single impurity is found, and not more than 0.5% of total impurities is found. The reporting level for impurities is 0.05%.

Assay—
Phosphate buffer—Place approximately 1900 mL of water in a 2.0-L volumetric flask, accurately add 6.8 mL of phosphoric acid, and mix. Accurately add 8.6 mL of 50% sodium hydroxide solution, and dilute with water to volume. If necessary, adjust with 50% sodium hydroxide solution or phosphoric acid to a pH of 7.1, and filter.
Solution A—Prepare a mixture of *Phosphate buffer* and acetonitrile (98:2).
Solution B—Prepare a mixture of *Phosphate buffer* and acetonitrile (78:22).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Use *Solution A.*
Standard preparation—Dissolve an accurately weighed quantity of USP Ranitidine Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 0.125 mg of ranitidine hydrochloride per mL.
Resolution solution—Transfer about 1.3 mg of USP Ranitidine Resolution Mixture RS to a 10-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume.
[NOTE—USP Ranitidine Resolution Mixture RS contains ranitidine hydrochloride and four related impurities: ranitidine amino alcohol hemifumarate, ranitidine diamine hemifumarate, ranitidine *N*-oxide, and ranitidine complex nitroacetamide.]
Assay preparation—Transfer about 25 mg of Ranitidine Hydrochloride, accurately weighed, to a 200-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 10-cm column containing 3.5-µm packing L1 that is stable from pH 1 to 12. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 35°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–10	100→0	0→100	linear gradient
10–15	0	100	isocratic
15–16	0→100	100→0	linear gradient
16–20	100	0	re-equilibration

Chromatograph the *Resolution solution,* and identify the peaks using the table of impurities and degradation products (found above): the resolution, *R,* between the peaks for ranitidine *N*-oxide and ranitidine complex nitroacetamide is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of $C_{13}H_{22}N_4O_3S \cdot HCl$ in the portion of Ranitidine Hydrochloride taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of ranitidine hydrochloride in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

USP Monographs

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Ranitidine Injection

» Ranitidine Injection is a sterile solution of Ranitidine Hydrochloride in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ranitidine ($C_{13}H_{22}N_4O_3S$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers of Type I glass, protected from light. Store below 30°. Do not freeze.
Labeling—Label Injection to state both the content of the active moiety and the content of the salt used in formulating the article.
USP Reference standards ⟨11⟩—
USP Ranitidine Hydrochloride RS
USP Ranitidine Related Compound A RS
5-[[(2-Aminoethyl)thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine, hemifumarate salt.
USP Ranitidine Related Compound C RS
N-{2-[({5-[(Dimethylamino)methyl]-2-furanyl}methyl) sulfinyl]ethyl}-*N*′-methyl-2-nitro-1,1-ethenediamine.

Identification—
A: The R_F value of the principal spot observed in the chromatogram of the *Test preparation* obtained as directed in the *Chromatographic purity* test corresponds to that obtained from the *Standard preparation.*
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 7.00 USP Endotoxin Units per mg of ranitidine.
pH ⟨791⟩: between 6.7 and 7.3.
Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.

Chromatographic purity—
Test preparation—Dilute Injection quantitatively with water, if necessary, to obtain a solution containing 25 mg of ranitidine per mL. [NOTE—Use Injection of lower concentration without dilution as directed under *Procedure.*]
Standard preparation—Dissolve USP Ranitidine Hydrochloride RS in water to obtain a solution having a known concentration of 560 µg per mL. Dilute portions of this *Standard preparation* quantitatively with water to obtain solutions having concentrations of 280 µg per mL (*Diluted standard preparation A*), 140 µg per mL (*Diluted standard preparation B*), 84 µg per mL (*Diluted standard preparation C*), 28 µg per mL (*Diluted standard preparation D*), and 14 µg per mL (*Diluted standard preparation E*), respectively.
Resolution preparation—Dissolve USP Ranitidine Related Compound A RS in methanol to obtain a solution having a known concentration of 1.27 mg per mL.
Procedure—Apply separately 10 µL of the *Standard preparation, Diluted standard preparations A, B, C, D* and *E,* and the required volume of the *Test preparation,* equivalent to 250 µg of ranitidine, to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. In addition, apply separately a further loading of the same volume of the *Test preparation* to the same plate, and on top of this application, apply 10 µL of the *Resolution preparation.* Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate, isopropyl alcohol, ammonium hydroxide, and water (25:15:5:1) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Expose the plate to iodine vapors in a closed chamber until the chromatogram is fully revealed. Examine the plate and compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparation* and *Diluted standard preparations* (*A, B, C, D,* and *E*): the system suitability requirements are met when there is complete resolution between the primary spots of the *Test preparation,* and the *Resolution preparation* and if a spot is observed in the chromatogram of *Diluted standard preparation E.* The major secondary spot is not greater in size or intensity than the principal spot produced by the *Standard preparation* (2.0%), and no other secondary spot is greater in size or intensity than the principal spot produced by *Diluted standard preparation A* (1.0%). The sum of the intensities of all secondary spots obtained from the *Test preparation,* corresponds to not more than 5.0%.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of methanol and 0.1 M aqueous ammonium acetate (85:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Ranitidine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.112 mg (equivalent to 0.100 mg of ranitidine base) per mL.
System suitability solution—Dissolve accurately weighed quantities of USP Ranitidine Hydrochloride RS and USP Ranitidine Related Compound C RS in *Mobile phase* to obtain a solution having known concentrations of about 0.112 mg per mL and 0.01 mg per mL, respectively.
Assay preparation—Dilute an accurately measured volume of Injection, quantitatively and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of 0.1 mg of ranitidine per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 322-nm detector and a 4.6-mm × 20- to 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between ranitidine hydrochloride and *N*-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]sulfinyl]ethyl]-*N*′-methyl-2-nitro-1,1-ethenediamine (ranitidine related compound C) is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the ranitidine hydrochloride peak is not more than 2.0; the column efficiency determined from the ranitidine hydrochloride peak is not less than 700 theoretical plates; and the relative standard deviation for replicate injections is not more than 2%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{22}N_4O_3S$ in the portion of Injection taken by the formula:

$$(314.40/350.87)(L/D)(C)(r_U/r_S)$$

in which 314.40 and 350.87 are the molecular weights of ranitidine and ranitidine hydrochloride, respectively; *L* is the labeled quantity of ranitidine in the Injection taken; *D* is the concentration, in mg per mL, of ranitidine in the *Assay preparation* on the basis of the labeled quantity and the extent

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of dilution; *C* is the concentration, in mg per mL, of USP Ranitidine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ranitidine Oral Solution

» Ranitidine Oral Solution is a solution of Ranitidine Hydrochloride in water. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ranitidine ($C_{13}H_{22}N_4O_3S$).

Packaging and storage—Preserve in tight, light-resistant containers. Store below 25°. Do not freeze.

USP Reference standards ⟨11⟩—
USP Ranitidine Hydrochloride RS
USP Ranitidine Related Compound A RS
5-[[(2-Aminoethyl)thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine, hemifumarate salt.
USP Ranitidine Related Compound C RS
N-{2-[({5-[(Dimethylamino)methyl]-2-furanyl}methyl) sulfinyl]ethyl}-*N'*-methyl-2-nitro-1,1-ethenediamine.

Identification—

A: The R_F value of the principal spot observed in the chromatogram of the *Test preparation* obtained as directed in the *Chromatographic purity* test corresponds to that obtained from the *Standard preparation.*

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli;* and the total aerobic microbial count does not exceed 100 cfu per mL.

pH ⟨791⟩: between 6.7 and 7.5.

Chromatographic purity—

Test preparation— [NOTE—Apply a quantity of extractives from Oral Solution to the chromatographic plate so as to achieve a nominal loading of 200 µg of ranitidine.] Transfer a weighed quantity of Oral Solution, equivalent to 10 mg of ranitidine, to a suitable syringe. Attach the tip of the syringe to the top of a cartridge (11 mm × 12 mm) of volume 0.5 mL containing 0.4 g of an L1 packing for high-pressure liquid chromatography that has been previously prepared by passage of 10 mL of methanol followed by passage of 20 mL of 0.5 M ammonia solution. Add 2.0 mL of 0.5 M ammonia solution to the syringe and force the mixture slowly through the cartridge. Repeat with 2 further 3-mL portions of 0.5 M ammonia solution. Discard all the liquid that has traversed the cartridge. Pass 5 mL of a mixture of 0.1 M hydrochloric acid and methanol (3:1) through the cartridge, and collect the eluant in a clean round-bottom, 25-mL flask. Repeat this with another 5-mL portion of the same eluting mixture and collect the eluant in the same flask. Evaporate the contents of the flask to dryness at a temperature not exceeding 30°. Redissolve the residue in 1.0 mL of a mixture of methanol and water (50:50).

Standard preparation—Dissolve USP Ranitidine Hydrochloride RS in a mixture of methanol and water (50:50) to obtain a solution having a known concentration of 448 µg (equivalent to 400 µg of ranitidine) per mL. Dilute portions of this *Standard preparation* quantitatively with the mixture of methanol and water (50:50) to obtain solutions having concentrations of 224 µg per mL *(Diluted standard preparation A),* 112 µg per mL *(Diluted standard preparation B),* 56 µg per mL *(Diluted standard preparation C),* 22 µg per mL *(Diluted standard preparation D),* and 11 µg per mL *(Diluted standard preparation E),* respectively.

Resolution preparation—Dissolve USP Ranitidine Related Compound A RS in methanol to obtain a solution having a known concentration of 1.27 mg per mL.

Procedure—Apply separately 10 µL of the *Standard preparation,* the *Diluted standard preparations (A, B, C, D,* and *E),* and 20 µL (superposition of 2 × 10 µL) of the *Test preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. In addition, apply separately a further loading of 10 µL of the *Test preparation* to the same plate, and on top of this application, apply 10 µL of the *Resolution preparation.* Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate, isopropyl alcohol, ammonium hydroxide, and water (25:15:5:1) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Expose the plate to iodine vapors in a closed chamber until the chromatogram is fully revealed. Examine the plate and compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparation* and *Diluted standard preparations (A, B, C, D,* and *E):* the system suitability requirements are met when there is complete resolution between the primary spots of the *Test preparation* and the *Resolution preparation* and if a spot is observed in the chro matogram of *Diluted standard preparation E.* The major secondary spot is not greater in size or intensity than the principal spot produced by the *Standard preparation* (2.0%), and no other secondary spot is greater in size or intensity than the principal spot produced by *Diluted standard preparation A* (1.0%). The sum of the intensities of all secondary spots obtained from the *Test preparation* corresponds to not more than 5.0%. [NOTE—Spots established as arising from other components in the formulation are to be ignored.]

Assay—

Mobile phase, Standard preparation, System suitability solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Ranitidine Injection,* the chromatographic column being fitted with a suitable pre-column also containing packing L1.

Assay preparation—Dilute an accurately measured quantity of Oral Solution, quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of 0.1 mg of ranitidine per mL.

Procedure—Separately inject an equal quantity (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of ranitidine ($C_{13}H_{22}N_4O_3S$) in the portion of Oral Solution taken by the formula:

$$(314.40/350.87)(L/D)(C)(r_U/r_S)$$

in which 314.40 and 350.87 are the molecular weights of ranitidine and ranitidine hydrochloride respectively; *L* is the labeled quantity of ranitidine in the Oral Solution taken; *D* is the concentration, in mg per mL, of ranitidine in the *Assay preparation,* on the basis of the labeled quantity and the extent of dilution; *C* is the concentration, in mg per mL, of USP Ranitidine Hydrochloride RS in the *Standard preparation;* and

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r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ranitidine Tablets

» Ranitidine Tablets contain an amount of ranitidine hydrochloride ($C_{13}H_{22}N_4O_3S \cdot HCl$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ranitidine ($C_{13}H_{22}N_4O_3S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Ranitidine Hydrochloride RS
USP Ranitidine Related Compound A RS
5-[[(2-Aminoethyl)thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine, hemifumarate salt.
USP Ranitidine Related Compound C RS
N-{2-[({5-[(Dimethylamino)methyl]-2-furanyl}methyl)sulfinyl]ethyl}-*N'*-methyl-2-nitro-1,1-ethenediamine.

Identification—
A: The R_F value of the principal spot observed in the chromatogram of the *Test preparation* obtained as directed in the *Chromatographic purity* test corresponds to that obtained from the *Standard preparation*.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.
C: Shake a quantity of crushed Tablets, equivalent to about 100 mg of ranitidine, with 2 mL of water, and filter: the filtrate responds to the tests for *Chloride* ⟨191⟩.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{13}H_{22}N_4O_3S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 314 nm using filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Ranitidine Hydrochloride RS in the same medium.
Tolerances—Not less than 80% (*Q*) of the labeled amount of $C_{13}H_{22}N_4O_3S$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity—
Test preparation—Prepare a filtered solution in methanol containing 20 mg of ranitidine per mL (equivalent to 22.4 mg of ranitidine hydrochloride per mL) by shaking an appropriate number of Tablets in a suitable volume of methanol until the tablets have disintegrated completely.
Standard preparations—Dissolve USP Ranitidine Hydrochloride RS in methanol to obtain a solution having a known concentration of 0.22 mg per mL. Dilute portions of this *Standard preparation* quantitatively with methanol to obtain solutions having concentrations of 110 µg per mL (*Diluted standard preparation A*), 66 µg per mL (*Diluted standard preparation B*), 22 µg per mL (*Diluted standard preparation C*), and 11 µg per mL (*Diluted standard preparation D*), respectively.
Resolution preparation—Dissolve USP Ranitidine Related Compound A RS, 5-[[(2-aminoethyl)thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine, hemifumarate salt, in methanol to obtain a solution having a known concentration of 1.27 mg per mL.
Procedure—Apply separately 10 µL of the *Test preparation*, the *Standard preparation*, and *Diluted standard preparations A*, *B*, *C*, and *D* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. In addition, apply separately 10 µL of the *Test preparation* to the same plate, and on top of this application, apply 10 µL of the *Resolution preparation*. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate, isopropyl alcohol, ammonium hydroxide, and water (25:15:5:1) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and air-dry. Expose the plate to iodine vapor in a closed chamber until the chromatogram is fully revealed. Examine the plate, and compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparation* and *Diluted standard preparations A*, *B*, *C*, and *D*: the system suitability requirements are met if there is complete resolution between the primary spots in the chromatogram of the combined *Test preparation* and the *Resolution preparation*, and if a spot is observed in the chromatogram of *Diluted standard preparation D*. No single secondary spot exhibits an intensity greater than that of *Diluted standard preparation A* (0.5%), and no other secondary spot exhibits an intensity greater than that of *Diluted standard preparation B* (0.3%). The sum of the intensities of all secondary spots obtained from the *Test preparation* correspond to not more than 2.0%.

Assay—
Mobile phase, *Standard preparation*, *System suitability solution*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Ranitidine Injection*.
Assay preparation—Transfer 10 Tablets to a minimum of 250 mL of *Mobile phase*, accurately measured. Shake the mixture until the Tablets have disintegrated completely, and filter. Dilute the filtrate quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of ranitidine similar to that of the *Standard preparation*.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{22}N_4O_3S$ in the portion of Tablets taken by the formula:

$$(314.40/350.87)(L/D)(C)(r_U/r_S)$$

in which 314.40 and 350.87 are the molecular weights of ranitidine and ranitidine hydrochloride, respectively; *L* is the labeled amount, in mg, of ranitidine in each tablet; *D* is the concentration, in mg per mL, of ranitidine in the *Assay preparation*, based on the labeled quantity per Tablet and the extent of dilution; C is the concentration, in mg per mL, of USP Ranitidine Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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Ranitidine in Sodium Chloride Injection

» Ranitidine in Sodium Chloride Injection is a sterile solution of Ranitidine Hydrochloride and Sodium Chloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of both ranitidine ($C_{13}H_{22}N_4O_3S$) and sodium chloride.

Packaging and storage—Preserve in glass containers, preferably of Type I or Type II glass, or in containers of suitable plastic, protected from light. Store at a temperature between 2° and 25°. Do not freeze.

USP Reference standards ⟨11⟩—
USP Ranitidine Hydrochloride RS
USP Ranitidine Related Compound A RS
5-[[(2-Aminoethyl)thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine, hemifumarate salt.
USP Ranitidine Related Compound C RS
N-{2-[({5-[(Dimethylamino)methyl]-2-furanyl}methyl) sulfinyl]ethyl}-*N'*-methyl-2-nitro-1,1-ethenediamine.

Identification—
A: The R_F value of the principal spot observed in the chromatogram of the *Test preparation* obtained as directed in the *Chromatographic purity* test corresponds to that obtained from the *Standard preparation.*
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay.*
C: Meets the requirements of the tests for *Sodium* ⟨191⟩ and for *Chloride* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 7.0 USP Endotoxin Units per mg of ranitidine.

pH ⟨791⟩: between 6.7 and 7.3.

Chromatographic purity—
Test preparation— [NOTE—Apply a quantity of extractives from Injection to the chromatographic plate to achieve a nominal loading of 200 µg of ranitidine.] Transfer an accurately measured volume of Injection, equivalent to 10 mg of ranitidine, to a suitable flask, add about 5 times this volume of alcohol, and evaporate to dryness at a temperature not exceeding 30°. Redissolve the residue in 1.0 mL of a mixture of methanol and water (50:50).
Standard preparation—Dissolve USP Ranitidine Hydrochloride RS in a mixture of methanol and water (50:50) to obtain a *Standard preparation* having a known concentration of 672 µg (equivalent to 600 µg of ranitidine base) per mL. Dilute portions of this *Standard preparation* quantitatively, and stepwise if necessary, with the mixture of methanol and water (50:50) to obtain solutions having concentrations of 448 µg per mL (*Diluted standard preparation A*), 224 µg per mL (*Diluted standard preparation B*), 112 µg per mL (*Diluted standard preparation C*), 56 µg per mL (*Diluted standard preparation D*), and 11 µg per mL (*Diluted standard preparation E*), respectively.
Resolution preparation—Dissolve USP Ranitidine Related Compound A RS in methanol to obtain a solution having a known concentration of 1.27 mg per mL.
Procedure—Apply separately 10 µL of the *Standard preparation*, the *Diluted standard preparations* (*A*, *B*, *C*, *D*, and *E*) and 20 µL (superposition of 2 × 10 µL) of the *Test preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. In addition, apply separately a further loading of 10 µL of the *Test preparation* to the same plate, and on top of this application, apply 10 µL of the *Resolution preparation.* Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate, isopropyl alcohol, ammonium hydroxide, and water (25:15:5:1) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Expose the plate to iodine vapors in a closed chamber until the chromatogram is fully revealed. Examine the plate and compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparation* and *Diluted standard preparations* (*A*, *B*, *C*, *D*, and *E*): the system suitability requirements are met when there is complete resolution between the primary spots of the *Test preparation* and the *Resolution preparation* and if a spot is observed in the chromatogram of *Diluted standard preparation E*. The major secondary spot is not greater in size or in intensity than the principal spot produced by the *Standard preparation* (3.0%), and no other secondary spot is greater in size or intensity than the principal spot produced by *Diluted standard preparation A* (2.0%). The sum of the intensities of all secondary spots obtained from the *Test preparation* corresponds to not more than 6.0%.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for ranitidine—
Mobile phase, Standard preparation, System suitability solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Ranitidine Injection.*
Assay preparation—Dilute an accurately measured volume of Injection, quantitatively and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of 0.1 mg of ranitidine per mL.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{22}N_4O_3S$ in the portion of Injection taken by the formula:

$$(314.40/350.87)(L/D)(C)(r_U/r_S)$$

in which 314.40 and 350.87 are the molecular weights of ranitidine and ranitidine hydrochloride, respectively; *L* is the labeled quantity of ranitidine in the Injection taken; *D* is the concentration, in mg per mL, of ranitidine in the *Assay preparation*, on the basis of the labeled quantity and the extent of dilution; *C* is the concentration, in mg per mL, of USP Ranitidine Hydrochloride RS in the *Standard preparation* and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for sodium chloride—Dilute an accurately measured volume of Injection, quantitatively and stepwise if necessary, with water to obtain a suitable volume of a solution having a concentration of about 0.5 mg of sodium chloride per mL. Titrate with 0.1 N silver nitrate VS using a silver-silver chloride electrode. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride. From the determined concentration per mL, subtract the quantity (35.453/314.40)*W* so as to correct for the chloride present as ranitidine hydrochloride where *W* is the quantity, in mg per mL, of ranitidine as determined in the *Assay for ranitidine.* Multiplication of the answer by 1.648 gives the amount of sodium chloride per mL.

USP Monographs

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Rauwolfia Serpentina

DEFINITION

Rauwolfia Serpentina is the dried root of *Rauwolfia* (L.) Bentham ex Kurz (Fam. Apocynaceae), sometimes having fragments of rhizome and aerial stem bases attached. It contains NLT 0.15% of reserpine–rescinnamine group alkaloids, calculated as reserpine.

IDENTIFICATION

• **A. Chemical Identification**

[Note—In these analyses, use formamide treated as directed if it has an ammoniacal odor.]

Immobile solvent: Formamide and acetone (30:70)

Mobile solvent A: Isooctane, carbon tetrachloride, piperidine, and tertiary butyl alcohol (90:60:4:2)

Mobile solvent B: Chloroform, isooctane, and tertiary butyl alcohol (75:75:2)

Spray solution: 250 mg/mL of trichloroacetic acid in methanol

Standard solution: 200 mg/mL of USP Rauwolfia Serpentina RS in alcohol. [Note—Warm USP Rauwolfia Serpentina RS with alcohol at 55°–65° for 30 min, with occasional mixing. Cool, and filter.]

Sample solution: Reduce 10 g of Rauwolfia Serpentina root to a fine powder. Treat a 1-g portion as in the preparation of the *Standard solution*.

Analysis A: Line the sides of a chromatographic chamber suitable for ascending chromatography (see *Chromatography* ⟨621⟩) with blotting paper. Transfer *Mobile solvent A* to the bottom of the container, and cover the chamber. Immerse a 20-cm × 20-cm sheet of filter paper (Whatman No. 1 or equivalent) in the *Immobile solvent*, and blot between paper toweling. Allow the acetone solvent to evaporate completely. Apply 1-µL portions of the *Sample solution* and the *Standard solution* to a line 2.5 cm from the bottom of the filter paper. Allow to dry. Apply a 2-µL portion of the *Immobile solvent* to each spot, allow to dry, and suspend the paper so that it dips into the *Mobile solvent*. Cover the chamber and after 1 h, when the *Mobile solvent* has risen approximately seven-eighths of the height of the paper, remove the chromatogram and dry at 90° in a current of air. Spray the paper lightly and evenly with the *Spray solution*, and dry at 90° for 10 min.

Analysis B: Use the apparatus described in *Analysis A*, but containing a glass trough with 2 mL of ammonium hydroxide to saturate the atmosphere of the tank with ammonia (NH_3). Transfer *Mobile solvent B* to the bottom of the tank outside the trough. Complete the test as described in *Analysis A*, omitting the *Spray solution*. Examine both chromatograms under UV light, and note the fluorescent spots.

Acceptance criteria: In both chromatograms, the *Sample solution* yields spots corresponding in position and color to those from the *Standard solution*.

COMPOSITION

• **Procedure**

Apparatus: A medium-sized continuous-extraction apparatus provided with a 250-mL flask and a 35-mm × 80-mm thimble is convenient, although a smaller apparatus may be used.

[Note—Protect the flask and thimble and all solutions of Rauwolfia Serpentina alkaloids from direct or strong light.]

Solvents: Alcohol, chloroform, and 1,1,1-trichloroethane. [Note—Use 1,1,1-trichloroethane with a boiling range between 73° and 76°.]

Standard stock solution: Dissolve 20.0 mg of USP Reserpine RS in 25 mL of hot alcohol, cool, dilute with alcohol to 50.0 mL, and mix. When stored in a tightly-stoppered, light-resistant bottle in the dark, this solution is chromogenically stable for several weeks.

Standard solution: Dilute 5.0 mL of the *Standard stock solution* with alcohol to 100.0 mL, and mix before using.

Analysis: Extract 2.5 g of finely powdered Rauwolfia Serpentina, accurately weighed, in a continuous-extraction apparatus for 4 h. Use 100 mL of vigorously boiling alcohol as solvent, and a few boiling chips to prevent bumping. Wash the extract into a 100-mL volumetric flask with alcohol, cool, dilute with alcohol to volume, and mix. Transfer 20.0 mL to a separator containing 200 mL of 0.5 N sulfuric acid, mix, and extract with three 25-mL portions of 1,1,1-trichloroethane. Lubricate stopcocks only with lubricants insoluble in trichloroethane or chloroform (polytef stopcocks are satisfactory). Drain the lower phase as completely as possible. Wash each of the 1,1,1-trichloroethane extracts in a second separator containing 50 mL of 0.5 N sulfuric acid, and discard the trichloroethane extracts.

Extract the weakly basic alkaloids from the first acid solution with 25-, 15-, 15-, 10-, 10-, and 10-mL portions of chloroform. Wash each chloroform extract with the acid in the second separator, then with two 10-mL portions of sodium bicarbonate solution (1 in 50) in two additional separators. Filter the chloroform extracts through chloroform-washed cotton into a 100-mL volumetric flask containing 10 mL of alcohol. Dilute with alcohol to volume, and mix. Transfer duplicate 10.0-mL aliquots to glass-stoppered, 25-mL conical flasks, and mix with 4 mL of alcohol. Evaporate with gentle heating almost to dryness, place in a vacuum desiccator, and evaporate to dryness. Dissolve the residues by agitating with 5.0 mL of alcohol.

Transfer duplicate 5.0-mL aliquots of the *Standard solution* to flasks. Add 2.0 mL of 0.5 N sulfuric acid to one of the sample flasks and to one of the standard flasks (the blanks). Add to the other flasks 1.0 mL of 0.5 N sulfuric acid and 1.0 mL of sodium nitrite solution (3 in 1000). Mix the contents of each flask, and warm in a water bath at 50°–60° for 20 min. Cool, add to each flask 500 µL of sulfamic acid solution (1 in 20), and mix. After stabilization of the solution colors, determine their absorbances in 1-cm cells at 390 nm, relative to a blank consisting of a mixture of alcohol and water (2:1).

Calculate the quantity of reserpine–rescinnamine group alkaloids, in mg, in the sample taken:

$$\text{Result} = 5(A - A_0)/(S - S_0)$$

A = absorbance of the nitrite-treated sample
A_0 = absorbance of the sample blank
S = corresponding absorbance of the solution from the respective *Standard solution* aliquot
S_0 = corresponding absorbance of the blank from the respective *Standard solution* aliquot

Acceptance criteria: NLT 0.15% of reserpine–rescinnamine group alkaloids, calculated as reserpine

CONTAMINANTS

• **Articles of Botanical Origin** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: Rauwolfia Serpentina (as a ground root) meets the requirements of the tests for absence of *Salmonella* species.

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- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*: NMT 2.0% of stems and NMT 3.0% of other foreign organic matter
- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*: NMT 2.0%
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 100° to constant weight.

Acceptance criteria: It loses NMT 12.0% of its weight.

- **BOTANICAL CHARACTERISTICS**

Macroscopic

Unground Rauwolfia Serpentina root: This occurs as segments usually 5–15 cm in length (pieces sometimes shorter) and 3–20 mm in diameter. The pieces are subcylindrical to tapering, rather tortuous or curved, rarely branched, but bearing occasional twisted rootlets, which are larger, more abundant, and more rigid and woody on the thicker parts of the roots. Externally: light brown to grayish yellow to grayish brown; dull, rough, or slightly wrinkled longitudinally yet peculiarly smooth to the touch; occasionally showing small circular rootlet scars in the larger pieces, with some exfoliation of the bark in small areas to reveal the paler wood beneath. When scraped, the bark separates readily from the wood. Fracture short, but irregular, the longer pieces readily breaking with a snap, slightly fibrous marginally. The freshly fractured surfaces show a rather thin layer of grayish yellow bark, with the pale yellowish white wood constituting 80% of the radius. The smoothed transverse surface of larger pieces shows a finely radiate stele with three or more clearly marked growth rings; a small knoblike protuberance is frequently noticeable at the center. The wood is hard and of relatively low density. The odor is indistinct, earthy, and reminiscent of stored white potatoes.

Microscopic

Rauwolfia Serpentina root: A transverse section shows externally 2–8 alternating strata of cork cells, the strata with larger cells alternating with strata made up of markedly smaller cells (distinction from *R. canescens*). Each stratum composed of smaller cells includes 3–5 tangentially arranged cell layers; each stratum made up of larger cells includes 1–6 tangential layers. In a cross-sectional view, the largest central cells of the larger cell group measure 40–90 µm radially and up to 75 µm tangentially (although usually smaller); the cells of the smaller cell groups measure 5–20 µm radially and up to 75 µm tangentially. The walls are thin and suberized. The secondary cortex consists of several rows of tangentially elongated to isodiametric parenchyma cells, most being densely filled with starch grains; others (the short latex cells) occur singly or in short series and contain brown resin masses. The secondary phloem is relatively narrow and is made up of phloem parenchyma (bearing starch grains and less commonly tabular to angular calcium oxalate crystals up to 20 µm in length; also, occasionally, with some brown resin masses in outer cells and phloem rays) interlaid with scattered sieve tissue and traversed by phloem rays 2–4 cells in width. Sclerenchyma cells (stone cells and fibers) are absent in root (distinction from other species of *Rauwolfia*). Cambium is indistinct, narrow, dark, and wavering. The secondary xylem represents the large bulk of the root and shows one or more prominent annual rings with a denser core of wood 500 µm across at the center. The xylem is composed of many wood wedges separated by xylem rays, and on closer examination reveals vessels in interrupted radial rows, much xylem parenchyma, many large-celled xylem rays, few wood fibers, and tracheids, all lignified-walled. The xylem fibers occur in both tangential and radial rows. The xylem rays are 1–12, occasionally up to 16, cells in width.

Rauwolfia Serpentina rhizome: This is similar to that of the root except for the presence of a prominent cortex, pericycle fibers, bicollateral vascular bundles, and a small central pith. The pericycle fibers occur singly or in groups of 2–5, and have thick, nonlignified walls and tapering, often lobed ends, with subterminal enlargements having thin walls and broad lumina. Vessel elements up to 485 µm are found. The xylem rays are 1–4 cells in width, with lignified and pitted walls. Internal phloem strands occur embedded in the outer region of the pith. The xylem fibers are somewhat less wavy than those of the root. The pith consists of starch parenchyma cells, among which are scattered short latex cells with yellowish contents stained brown with iodine TS.

Ground Rauwolfia Serpentina root: This is brownish to reddish gray in color. Present are very numerous starch grains (mostly simple, two- to three-compound, occasionally four-compound); simple grains spheroid, ovate, muller-shaped, plano- to angular-convex, or irregular; hilum simple, Y-shaped, stellate, or irregularly cleft; unaltered grains 6–34 µm (average 20 µm) in diameter, mostly in the lower range (maximum sizes larger than in *R. canescens* and *R. micrantha*); altered grains up to 50 µm in diameter; large unaltered grains show polarization cross clearly; calcium oxalate prisms and cluster crystals scattered, 10–15 µm in size; brown resin masses and yellowish granular secretion masses occur occasionally; isolated cork cells elongated, up to 90 µm in length; phelloderm and phloem parenchyma cells similar in appearance; vessels subcylindrical, up to 360 µm in length and 20–57 µm in diameter (narrower than in *R. canescens*) (the wall markings generally consist of simple pits, with bordered pits adjacent to xylem ray cells), the vessel end walls oblique to transverse, generally with openings in the end walls, some vessels showing tyloses; tracheids pitted, with moderately thick, tapering, beaded walls, with relatively broad lumina, polygonal in cross-section; xylem parenchyma cells with moderately thick walls with simple circular pits, cells polygonal in cross-section, bearing considerable starch; phloem and xylem-ray cells with pitted walls bearing much starch, sometimes with brown resin masses, xylem fibers with thick, heavily lignified walls showing small transverse and oblique linear pits and pointed simple to bifurcate ends, measuring 200–750 µm in length (shorter than in *R. micrantha* and *R. canescens*). No phloem fibers or sclereids are present in root (colorless nonlignified pericycle or primary phloem fibers, single or in small groups, may be present from rhizome or stem tissues).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature, in a dry place, secure against insect attack.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Rauwolfia Serpentina RS
USP Reserpine RS

Powdered Rauwolfia Serpentina

» Powdered Rauwolfia Serpentina is Rauwolfia Serpentina reduced to a fine or a very fine powder, and adjusted, if necessary, to conform to the

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requirements for reserpine-rescinnamine group alkaloids by admixture with lactose or starch or with a powdered rauwolfia serpentina containing a higher or lower content of these alkaloids. It contains not less than 0.15 percent and not more than 0.20 percent of reserpine-rescinnamine group alkaloids, calculated as reserpine.

Packaging and storage—Preserve in well-closed containers, and store at controlled room temperature, in a dry place, secure against insect attack (see *Preservation* under *Vegetable and Animal Drugs*, and the *General Notices*).
USP Reference standards ⟨11⟩—
USP Rauwolfia Serpentina RS
USP Reserpine RS
Identification—It conforms to the requirements for *Ground Rauwolfia Serpentina root* under *Botanic characteristics* and meets the requirements of the test for *Chemical identification* under *Rauwolfia Serpentina*.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the test for absence of *Salmonella* species.
Acid-insoluble ash ⟨561⟩: not more than 2.0%.
Assay—Proceed with Powdered Rauwolfia Serpentina as directed in the *Assay* under *Rauwolfia Serpentina*.

Rauwolfia Serpentina Tablets

» Rauwolfia Serpentina Tablets contain an amount of reserpine-rescinnamine group alkaloids, calculated as reserpine, equivalent to not less than 0.15 percent and not more than 0.20 percent of the labeled amount of powdered rauwolfia serpentina.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Rauwolfia Serpentina RS
USP Reserpine RS
Identification—The powdered rauwolfia serpentina in the Tablets conforms to the requirements for *Ground Rauwolfia Serpentina root* under *Botanic characteristics* and meets the requirements of the test for *Chemical identification* under *Rauwolfia Serpentina*.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—Tablets meet the requirements of the test for absence of *Salmonella* species.
Disintegration ⟨701⟩: 1 hour, with disks using simulated gastric fluid TS, without enzyme.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of this powder, equivalent to 2.5 g of powdered rauwolfia serpentina, and proceed as directed for *Procedure* in the *Assay* under *Rauwolfia Serpentina*.

Purified Rayon

» Purified Rayon is a fibrous form of bleached, regenerated cellulose.

Alkalinity or acidity—Immerse about 10 g in 100 mL of recently boiled and cooled water, and decant 25-mL portions of the water, with the aid of a glass rod, into each of two dishes. To one portion add 3 drops of phenolphthalein TS, and to the other portion add 1 drop of methyl orange TS: neither portion appears pink when viewed against a white background.
Residue on ignition ⟨281⟩: not more than 1.50%, determined on a 5.0-g test specimen.
Acid-insoluble ash—To the residue obtained in the test for *Residue on ignition*, add 25 mL of 3 N hydrochloric acid, and boil for 5 minutes. Collect the insoluble matter on a tared filtering crucible, wash with hot water, ignite, and weigh: the residue weighs not more than 63.0 mg (1.25%).
Water-soluble substances—Proceed as directed in the test for *Water-soluble substances* under *Purified Cotton:* the residue weighs not more than 100 mg (1.0%).
Fiber length and absorbency—Remove it from its wrappings, and condition it for not less than 4 hours in a standard atmosphere of 65 ± 2% relative humidity at 21 ± 1.1°. Determine the *Fiber length* and *Absorbency* as follows.
Fiber length—Determine the fiber length of Purified Rayon as directed for *Fiber Length* under *Cotton* ⟨691⟩: not less than 70.0%, by weight, of the fibers are 19 mm or greater in length, and not more than 5.0%, by weight, of the fibers are 6.3 mm or less in length.
Absorbency—Proceed as directed for the *Absorbency Test* under *Cotton* ⟨691⟩: submersion is complete in 5 seconds, and the rayon retains not less than 16 times its weight of water.
Other requirements—It meets the requirements of the tests for *Dyes* and *Other foreign matter* under *Purified Cotton*.

Oral Rehydration Salts

DEFINITION
Oral Rehydration Salts is a dry mixture of Sodium Chloride, Potassium Chloride, Sodium Bicarbonate, and Dextrose (anhydrous). Alternatively, it may contain Sodium Citrate (anhydrous or dihydrate) instead of Sodium Bicarbonate. It may contain Dextrose (monohydrate) instead of Dextrose (anhydrous), provided that the Sodium Bicarbonate or Sodium Citrate is packaged in a separate, accompanying container. It contains the equivalent of NLT 90.0% and NMT 110.0% of the amounts of sodium (Na), potassium (K), chloride (Cl), and bicarbonate (HCO_3) or citrate ($C_6H_5O_7$) calculated from the labeled amounts of Sodium Chloride, Potassium Chloride, and Sodium Bicarbonate [or Sodium Citrate (anhydrous or dihydrate)]. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of anhydrous dextrose ($C_6H_{12}O_6$) or dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$). It may contain suitable flavors.

IDENTIFICATION
- **A. Sodium:** The sample imparts an intense yellow color to a nonluminous flame.
- **B. Potassium:** The sample imparts a violet color to a nonluminous flame. Since the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent

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to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements

• **D. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Bicarbonate*

Analysis: Collect the gas that evolves upon dissolution.

Acceptance criteria: Where it contains Sodium Bicarbonate, it dissolves with effervescence, and the collected gas so obtained meets the requirements in test *A*.

• **E. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Citrate*

Sample solution: Constitute the Oral Rehydration Salts as directed in the labeling.

Analysis: Add 3–5 drops of the *Sample solution* to 20 mL of the mixture of pyridine and acetic anhydride.

Acceptance criteria: Where it contains Sodium Citrate, it meets the requirements.

• **F.**

Sample solution: Constitute the Oral Rehydration Salts as directed in the labeling.

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: Where it contains Dextrose, a copious red precipitate of cuprous oxide is formed (presence of dextrose).

ASSAY

• **DEXTROSE**

Sample stock solution: Nominally 200 mg/mL of dextrose from Oral Rehydration Salts prepared as follows. Transfer the contents of 1 or more unit-dose containers of Oral Rehydration Salts, or a portion of the contents of 1 multiple-unit container equivalent to 20 g of dextrose, to a 100-mL volumetric flask. Dilute with water to volume.

Sample solution: Nominally 100 mg/mL of dextrose from the *Sample stock solution* prepared as follows. Transfer 50.0 mL of the *Sample stock solution* to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

[NOTE—Reserve the remaining *Sample stock solution* for the *Assay* procedures for *Sodium and Potassium*, *Chloride*, *Bicarbonate*, and *Citrate*.]

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781A⟩, *Angular Rotation*).

Sample: *Sample solution*

Where Oral Rehydration Salts is labeled to contain anhydrous dextrose, calculate the observed concentration (g/100 mL) of anhydrous dextrose ($C_6H_{12}O_6$) in the portion of Oral Rehydration Salts taken:

$$\text{Result} = (100 \times a)/(l \times \alpha)$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

Where Oral Rehydration Salts is labeled to contain dextrose monohydrate, calculate the observed concentration (g/100 mL) of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Oral Rehydration Salts taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (M_{r1}/M_{r2})$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Calculate the percentage of the labeled amount of anhydrous dextrose ($C_6H_{12}O_6$) or dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Oral Rehydration Salts taken:

$$\text{Result} = (C_{U1}/C_{U2}) \times 100$$

C_{U1} = observed concentration of the *Sample solution* (g/100 mL)
C_{U2} = nominal concentration of the *Sample solution* (g/100 mL)

Acceptance criteria: 90.0%–110.0%

• **SODIUM AND POTASSIUM**

Sodium stock solution: 58.44 mg/mL of sodium chloride prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Diluent: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, then add water to volume.

Standard stock solution: 0.5844 mg/mL of sodium chloride and 0.7456 mg/mL of potassium chloride in water from the *Sodium stock solution* and the *Potassium stock solution*, respectively

Standard solution: 0.01150 mg/mL of sodium and 0.01955 mg/mL of potassium from the *Standard stock solution* in *Diluent*

Sample stock solution A: Nominally 0.23 mg/mL of sodium from the *Sample stock solution* remaining from the *Assay* for *Dextrose* in water

Sample solution A: Nominally 0.0115 mg/mL of sodium from *Sample stock solution A* in *Diluent*

Sample stock solution B: Nominally 0.39 mg/mL of potassium from the *Sample stock solution* remaining from the *Assay* for *Dextrose* in water

Sample solution B: Nominally 0.01955 mg/mL of potassium from *Sample stock solution B* in *Diluent*

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: 766 nm

Sodium: 589 nm

Blank: *Diluent*

Analysis

Samples: *Standard solution*, *Sample solution A*, *Sample solution B*, and *Blank*

Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution*, *Sample solution A*, and *Sample solution B*.

Calculate the percentage of the labeled amount of sodium (Na) in the unit-dose container or containers taken or in the portion of Oral Rehydration Salts taken from the multiple-unit container:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = photometer reading of sodium from *Sample solution A*
r_S = photometer reading of sodium from the *Standard solution*
C_S = concentration of sodium in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium in *Sample solution A* (mg/mL)

Calculate the percentage of the labeled amount of potassium (K) in the unit-dose container or containers taken or in the portion of Oral Rehydration Salts taken from the multiple-unit container:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = photometer reading of potassium from *Sample solution B*
r_S = photometer reading of potassium from the *Standard solution*
C_S = concentration of potassium in the *Standard solution* (mg/mL)
C_U = nominal concentration of potassium in *Sample solution B* (mg/mL)

Acceptance criteria
Potassium: 90.0%–110.0%
Sodium: 90.0%–110.0%

• **CHLORIDE**
Sample solution: Transfer a volume of the *Sample stock solution* remaining from the *Assay* for *Dextrose*, containing nominally 55 mg of chloride (Cl), to a suitable container.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Titrate with *Titrant* until the silver chloride flocculates and the mixture acquires a faint pink color, using potassium chromate TS as the indicator.
Calculate the percentage of the labeled amount of chloride (Cl) in the unit-dose container or containers taken or in the portion of Oral Rehydration Salts taken from the multiple-unit container:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = volume of *Titrant* consumed (mL)
N = normality of *Titrant* (mEq/mL)
F = equivalent weight of chloride, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

• **BICARBONATE** (if present)
Sample solution: Transfer a volume of the *Sample stock solution* remaining from the *Assay* for *Dextrose*, containing nominally 100 mg of bicarbonate, to a suitable beaker.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N hydrochloric acid VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Add 25 mL of water and 3 drops of methyl orange TS to the *Sample solution*. Titrate the resulting solution with *Titrant*.
Calculate the percentage of the labeled amount of bicarbonate (HCO_3) in the unit-dose container or containers taken or in the portion of Oral Rehydration Salts taken from the multiple-unit container:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = volume of *Titrant* consumed (mL)
N = normality of *Titrant* (mEq/mL)
F = equivalent weight of bicarbonate, 61.02 mg/mEq
W = nominal amount of bicarbonate in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

• **CITRATE** (if present)
Mobile phase, Standard solution 1, and **Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.
Sample solution: Transfer a volume of the *Sample stock solution* remaining from the *Assay* for *Dextrose*, containing nominally 180 mg of citrate, to a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure*.
Analysis
Samples: *Standard solution 1* and *Sample solution*
Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure*.
Calculate the percentage of the labeled amount of citrate ($C_6H_5O_7$) in the portion of Oral Rehydration Salts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of citrate from the *Sample solution*
r_S = peak response of citrate from *Standard solution 1*
C_S = concentration of citrate in *Standard solution 1* (µg/mL)
C_U = nominal concentration of citrate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩: Proceed as directed, except the average net weight of the contents of the 10 containers is NLT the labeled amount, and the net weight of the contents of any single container is NLT 95% and NMT 105% of the labeled amount. If the contents of NMT 1 container are less than 95% but NLT 90% of the labeled amount, or more than 105% but NMT 110% of the labeled amount, determine the net weight of the contents of 20 additional containers. The average net weight of the contents of 30 containers is NLT the labeled amount, and the net weight of the contents of NMT 1 of the 30 containers is less than 95% but NLT 90% of the labeled amount, or more than 105% but NMT 110% of the labeled amount.
[NOTE—In performing the *Assay* procedures for *Sodium and Potassium*, *Chloride*, and *Bicarbonate* or *Citrate*, calculate from the labeled amounts of sodium chloride, potassium chloride, and sodium bicarbonate or sodium citrate the total equivalent amounts of sodium (Na), potassium (K), chloride (Cl), and bicarbonate (HCO_3) or citrate ($C_6H_5O_7$) contained therein (see *Table 1*).]

Table 1. mg Equivalent of Each g of Component

Component	Na	K	Cl	HCO_3	$C_6H_5O_7$
Sodium chloride	393.4	—	606.6	—	—
Potassium chloride	—	524.4	475.6	—	—

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Table 1. mg Equivalent of Each g of Component *(continued)*

Component	Na	K	Cl	HCO_3	$C_6H_5O_7$
Sodium bicarbonate	273.6	—	—	726.4	—
Anhydrous sodium citrate	267.2	—	—	—	732.8
Sodium citrate dihydrate	234.5	—	—	—	643.0

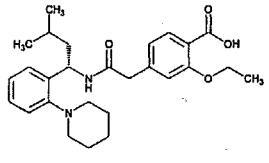
SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: Constitute the Oral Rehydration Salts as directed in the labeling.
  Acceptance criteria: 7.0–8.8
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 50° to constant weight.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and avoid exposure to temperatures in excess of 30°. The Sodium Bicarbonate or Sodium Citrate component may be omitted from the mixture and packaged in a separate, accompanying container.
- **Labeling:** The label indicates prominently whether Sodium Bicarbonate or Sodium Citrate is a component by the placement of the word "Bicarbonate" or "Citrate", as appropriate, in juxtaposition to the official title. The label states the name and quantity, in g, of each component in each unit-dose container, or in a stated quantity, in g, of Oral Rehydration Salts in a multiple-unit container. The label states the net weight in each container and provides directions for constitution. Where packaged in individual unit-dose pouches, the label instructs the user not to open the pouch until the time of use. The label also states that any solution that remains unused 24 h after constitution is to be discarded.

Repaglinide



$C_{27}H_{36}N_2O_4$ 452.59

(*S*)-2-Ethoxy-4-[2-{methyl-1-[2-(1-piperidinyl)phenyl] butylamino}-2-oxoethyl]-benzoic acid;
(+)-2-Ethoxy-α-[[(*S*)-α-isobutyl-*o*-piperidinobenzyl] carbamoyl]-*p*-toluic acid [135062-02-1].

DEFINITION

Repaglinide contains NLT 98.0% and NMT 102.0% of repaglinide ($C_{27}H_{36}N_2O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Enantiomeric Purity*.

ASSAY

- **Procedure**
  Buffer: 1 g/L of monobasic potassium phosphate solution, adjusted with phosphoric acid to a pH of 2.5
  Mobile phase: Methanol and *Buffer* (8:2)
  Standard solution: 0.5 mg/mL of USP Repaglinide RS in methanol
  Sample solution: 0.5 mg/mL of Repaglinide in methanol
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 12.5-cm; 5-µm packing L1
  Column temperature: 45°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 0.73%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of repaglinide ($C_{27}H_{36}N_2O_4$) in the portion of Repaglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩
  Ignition temperature: 600° ± 25°
  Acceptance criteria: NMT 0.1%
- **Organic Impurities**
  Solution A: 3 mg/mL of monobasic potassium phosphate solution, adjusted with 1 N sodium hydroxide to a pH of 7.0
  Solution B: Methanol
  Identification solution: 6 mg/mL of USP Repaglinide RS, 60 µg/mL of USP Repaglinide Related Compound A RS, 60 µg/mL of USP Repaglinide Related Compound B RS, and 60 µg/mL of USP Repaglinide Related Compound C RS in methanol
  Sample solution: 6 mg/mL of Repaglinide in methanol
  Standard solution: 0.06 mg/mL of repaglinide in methanol, from the *Sample solution*
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
2	30	70
8	30	70
12	5	95
15	5	95

Return to original conditions and re-equilibrate the system.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 240 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10% of repaglinide, *Standard solution*
Analysis
Samples: *Identification solution*, *Sample solution*, and *Standard solution*
Chromatograph the *Identification solution*, and identify the components on the basis of their relative retention times, given in *Table 2*.
Calculate the percentage of each impurity in the portion of Repaglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of repaglinide from the *Standard solution*
C_S = concentration of repaglinide in the *Standard solution* (mg/mL)
C_U = concentration of Repaglinide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-(Carboxymethyl)-2-ethoxybenzoic acid	0.15	1.4	0.1
Repaglinide related compound B	0.31	1.7	0.1
Repaglinide related compound C	0.96	1.0	0.1
Repaglinide	1.0	—	—
Repaglinide related compound A	1.6	0.5	0.1
Repaglinide ethyl ester[a]	1.9	1.3	0.1
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] (*S*)-Ethyl 2-ethoxy-4-[2-({3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl}amino)-2-oxoethyl]benzoate.

• **ENANTIOMERIC PURITY**
[NOTE—Protect all solutions containing repaglinide from light.]
Buffer: Dissolve 1 g of monobasic potassium phosphate in 1 L of water. If needed, adjust with 2 N sodium hydroxide or with dilute phosphoric acid to a pH of 4.7.
Solution A: *Buffer*
Solution B: Acetonitrile
Mobile phase: See *Table 3*. [NOTE—Equilibrate after installation of the column as follows. Using water, slowly increase the flow rate from 0.2 mL/min to 0.5 mL/min. Maintain the flow rate at 0.5 mL/min for 5 min. The column must be washed with water for 1 h at a flow rate of 1 mL/min, and for 1 h with *Mobile phase* at the initial composition prior to the first analysis.]

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
4	60	40
6	60	40

Return to the original conditions and re-equilibrate the system.
System suitability solution: 1.0 mg/mL of USP Repaglinide RS and 0.02 mg/mL of USP Repaglinide Related Compound E RS in methanol
Standard solution: 2.0 µg/mL of USP Repaglinide Related Compound E RS in methanol
Sample solution: 1.0 mg/mL of Repaglinide in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.0-mm × 10-cm; 5-µm packing L41
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The typical relative retention times for repaglinide and repaglinide related compound E are 1.0 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 1.5 between repaglinide and repaglinide related compound E, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of repaglinide related compound E in the portion of Repaglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of repaglinide related compound E in the *Sample solution*
r_S = peak response of repaglinide related compound E in the *Standard solution*
C_S = concentration of USP Repaglinide Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Repaglinide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Repaglinide RS

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USP Repaglinide Related Compound A RS
(*S*)-3-Methyl-1-[2-(1-piperidinyl)phenyl]butylamine, *N*-acetyl-L-glutamate salt.
$C_{16}H_{26}N_2 \cdot C_7H_{11}NO_5$ 435.6

USP Repaglinide Related Compound B RS
3-Ethoxy-4-ethoxycarbonylphenylacetic acid.
$C_{13}H_{16}O_5$ 252.27

USP Repaglinide Related Compound C RS
(*S*)-2-Ethoxy-4-[2-[[2-phenyl-1-[2-(1-piperidinyl)phenyl]ethyl]amino]-2-oxoethyl]benzoic acid.
$C_{30}H_{34}N_2O_4$ 486.61

USP Repaglinide Related Compound E RS
(*R*)-2-Ethoxy-4-[2-({3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl}amino)-2-oxoethyl]benzoic acid.
$C_{27}H_{36}N_2O_4$ 452.59

Repaglinide Tablets

DEFINITION
Repaglinide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of repaglinide ($C_{27}H_{36}N_2O_4$).

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Sample solution: To a quantity of powdered Tablets, equivalent to 10 mg of repaglinide, add 10 mL of a mixture of methanol and methylene chloride (1:1), shake for 15 min, and centrifuge.
Developing solvent system: Toluene, methylene chloride, and methanol (2:2:1)
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer pH 4.0: 2 g/L of monobasic ammonium phosphate solution. Adjust with phosphoric acid to a pH of 4.0.
Buffer pH 2.5: 2 g/L of monobasic ammonium phosphate solution. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Methanol and *Buffer pH 2.5* (7:3)
Diluent: Methanol and *Buffer pH 4.0* (7:3)
Standard solution 1: 800 µg/mL of USP Repaglinide RS in methanol
Standard solution 2: 80 µg/mL of USP Repaglinide RS, prepared by diluting 5.0 mL of *Standard solution 1* with *Diluent* to 50.0 mL
System suitability stock solution: 80 µg/mL of USP Repaglinide Related Compound A RS in methanol
System suitability solution: 80 µg/mL of USP Repaglinide RS and 1.6 µg/mL of USP Repaglinide Related Compound A RS prepared as follows. Transfer 1.0 mL of *System suitability stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Standard solution 1*, and dilute with *Diluent* to volume.
Sample solution: Transfer 8 whole Tablets to a suitable volumetric flask, and dissolve in and dilute with *Diluent* to volume to obtain a solution containing 80 µg/mL. Stir for 20 min, and filter or centrifuge a portion of the solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm or diode array. [Note—Use diode array detector to perform *Identification* test C.]
Column: 4.0-mm × 6-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution 2* and *System suitability solution*
[Note—The typical relative retention times for repaglinide related compound A and repaglinide are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 7.0 between repaglinide related compound A and repaglinide, *System suitability solution*
Tailing factor: 0.8–2.0 for the repaglinide peak, *System suitability solution*
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution 2*
Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentage of the labeled amount of repaglinide ($C_{27}H_{36}N_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution 2*
C_S = concentration of USP Repaglinide RS in *Standard solution 2* (µg/mL)
C_U = nominal concentration of repaglinide in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: pH 5.0 buffer, prepared by mixing 10.2 g of citric acid monohydrate and 18.16 g of dibasic sodium phosphate dihydrate with 1 L of water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: 1.5 g/L of monobasic potassium phosphate in water, adjusted with phosphoric acid to a pH of 2.3
Mobile phase: Acetonitrile, *Buffer*, and methanol (49:40:11)
Standard stock solution: 44 µg/mL of USP Repaglinide RS in methanol
Sample solution: Pass a portion of the solution under test through a suitable filter.
Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 25 mL of methanol, and dilute with *Medium* to volume. Further dilute with *Medium*, if needed, to a concentration that is similar to that of the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorometric detector; excitation wavelength of 244 nm and emission wavelength of 348 nm
Column: 4.0-mm × 12.5-cm; 10-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: Between 0.5 and 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution* and *Standard solution*

USP Monographs

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Calculate the percentage of the labeled amount of repaglinide ($C_{27}H_{36}N_2O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Repaglinide RS in the *Standard solution* (mg/mL)
L = label claim of repaglinide (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (Q) of the labeled amount of repaglinide ($C_{27}H_{36}N_2O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer pH 4.0, Buffer pH 2.5, Mobile phase, Diluent, Standard solution 1, Standard solution 2, System suitability stock solution, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.

Standard solution 3: 0.2 µg/mL of USP Repaglinide RS, prepared by diluting 2.5 mL of *Standard solution 2* with *Diluent* to 1000 mL

Chromatographic system

Mode: LC
Detector: UV 210 nm
Column: 4.0-mm × 6-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution 3*

[NOTE—The typical relative retention times for repaglinide related compound A and repaglinide are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 7.0 between repaglinide related compound A and repaglinide, *System suitability solution*
Tailing factor: 0.8–2.0 for the repaglinide peak, *System suitability solution*
Relative standard deviation: NMT 10% for replicate injections, *Standard solution 3*

Analysis

Samples: *Sample solution* and *Standard solution 3*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of repaglinide from *Standard solution 3*
C_S = concentration of USP Repaglinide RS in *Standard solution 3* (µg/mL)
C_U = nominal concentration of repaglinide in the *Sample solution* (µg/mL)

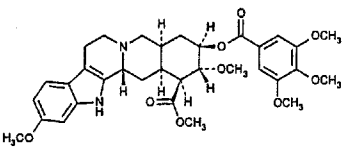
Acceptance criteria: NMT 0.5% of total impurities

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Repaglinide RS
USP Repaglinide Related Compound A RS
(*S*)-3-Methyl-1-[2-(1-piperidinyl)phenyl]butylamine, *N*-acetyl-L-glutamate salt.
$C_{16}H_{26}N_2 \cdot C_7H_{11}NO_5$ 435.6

Reserpine



$C_{33}H_{40}N_2O_9$ 608.68

Yohimban-16-carboxylic acid, 11,17-dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]-, methyl ester, (3β,16β,17α,18β,20α)-.

Methyl 18β-hydroxy-11,17α-dimethoxy-3β,20α-yohimban-16β-carboxylate 3,4,5-trimethoxybenzoate (ester) [50-55-5].

» Reserpine contains not less than 97.0 percent and not more than 101.0 percent of $C_{33}H_{40}N_2O_9$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Reserpine RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: [NOTE—Conduct this test promptly, with a minimum exposure to light.] Dissolve 25.0 mg of it, previously dried, in 0.25 mL of chloroform; mix with about 30 mL of methanol previously warmed to 50°; transfer the mixture with the aid of warm methanol to a 250-mL volumetric flask; cool the solution to room temperature; dilute with methanol to volume; and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, add 36 mL of chloroform, dilute with methanol to volume, and mix: the UV absorption spectrum of a 1 in 50,000 solution so obtained exhibits the same maxima in the range of 255 nm to 350 nm as that of a similar solution of USP Reserpine RS, concomitantly measured; and the respective absorptivities, determined with reference to a mixture of 36 volumes of chloroform and 14 volumes of methanol as the blank, at the wavelength of maximum absorbance at about 268 nm, do not differ by more than 3.0%.

Loss on drying ⟨731⟩—Dry it at 60° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Assay—

Mobile phase—Prepare a filtered and degassed 1:1 mixture of acetonitrile and ammonium chloride solution (1 in 100). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). The pH is about 5.6.

Standard preparation—Dissolve an accurately weighed quantity of USP Reserpine RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to

USP Monographs

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obtain a solution having a known concentration of about 10 µg per mL.

Assay preparation—Transfer about 10 mg of Reserpine, accurately weighed, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Dilute 1.0 mL of this solution with 9.0 mL of *Mobile phase*, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 268-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{33}H_{40}N_2O_9$ in the portion of Reserpine taken by the formula:

$$C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Reserpine RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Reserpine Tablets

DEFINITION

Reserpine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$).

IDENTIFICATION

• **A.**

Sample solution: Evaporate about 2 mL of *Sample solution 2*, obtained from the test for *Other Alkaloids*, in a test tube to dryness. Add to the residue 0.5 mL of glacial acetic acid, and swirl for 1–2 min.

Analysis: Add 1 mL of a 20 mg/mL of vanillin in hydrochloric acid.

Acceptance criteria: A pink color is produced that turns to deep violet-red within a few min or as a result of warming the solution for 10–20 s.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and ammonium chloride solution (10 mg/mL) (1:1). The pH of the resulting solution is about 5.6.

Standard solution: 10 µg/mL of USP Reserpine RS in *Mobile phase*

Sample solution: 10 µg/mL of reserpine in *Mobile phase* from powdered Tablets (NLT 20). Pass through a suitable filter of 0.8-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 268 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of reserpine from the *Sample solution*

r_S = peak response of reserpine from the *Standard solution*

C_S = concentration of USP Reserpine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of reserpine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

[NOTE—Do not substitute membrane filters for filter paper where the filtration of reserpine-containing solutions is indicated. Reserpine has been shown to be adsorbed onto membranes.]

Test 1

Medium: 0.1 N acetic acid; 500 mL

Apparatus 1: 100 rpm

Time: 45 min

***p*-Toluenesulfonic acid solution:** 10 mg/mL of *p*-toluenesulfonic acid in glacial acetic acid

Standard solution: 0.1 µg/mL of USP Reserpine RS in glacial acetic acid

Sample solution: Pipet an aliquot of the filtered solution under test, containing about 11 µg of reserpine, into a 125-mL separatory funnel. Extract with three 10-mL portions of chloroform, collecting the extracts in a 100-mL volumetric flask, dilute with glacial acetic acid to volume, and mix.

Analysis: Pipet 10 mL each of the *Standard solution*, the *Sample solution*, and glacial acetic acid into three individual 50-mL test tubes. To each tube, add 10 mL of *p-Toluenesulfonic acid solution*, insert a stopper, and mix gently. Place in a steam bath for 10 min. Remove from the steam bath, and cool. Determine the amount of reserpine ($C_{33}H_{40}N_2O_9$) dissolved from the fluorescences of the *Standard solution* and *Sample solution* using a suitable spectrophotometer arranged to deliver activation radiation at 390 nm and to measure the resultant fluorescence at the emission wavelength of about 480 nm.

Tolerances: NLT 75% (*Q*) of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*

Medium, Apparatus 1, *p*-Toluenesulfonic acid solution, Standard solution, Sample solution, and Analysis: Proceed as directed for *Test 1.*

Time: 30 min

Tolerances: NLT 80% (*Q*) of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

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SPECIFIC TESTS

• **OTHER ALKALOIDS**

Adsorbent: Use acid-washed chromatographic siliceous earth.

Chromatographic tube: Chromatographic tube of 20 cm length and 22 mm internal diameter with an outlet at the bottom. Insert at the constriction a small pledget of glass wool previously washed with chloroform and air dried.

Chromatographic column: Mix 1 g of *Adsorbent* with 0.5 mL of freshly prepared sodium bicarbonate solution (20 mg/mL) in a 100-mL beaker until the mixture appears fluffy and uniformly moistened, transfer to the *Chromatographic tube*, and tamp lightly with a packing rod to a thickness of about 7–9 mm. Mix uniformly 1 g of *Adsorbent* with 0.5 mL of freshly prepared citric acid solution (5 mg/mL), transfer to the *Chromatographic tube*, and tamp lightly with a packing rod. Mix uniformly 1 g of *Adsorbent* with 0.5 mL of water, transfer to the *Chromatographic tube*, and tamp lightly with a packing rod.

Blank mixture: 1 mL of dimethyl sulfoxide and 2 g of *Adsorbent* in a suitable container. Stir until the mass is uniformly wetted and free from lumps.

Blank solution: Transfer the *Blank mixture* through a powder funnel to a prepared *Chromatographic column*. Scrub the beaker with about 1 g of *Adsorbent*, and add it through the funnel to the tube. Wipe the spatula, beaker, and funnel with a tuft of glass wool, previously washed with chloroform and air-dried. Place the glass wool in the tube, and press it down on the column with the packing rod, so that the overall height of the column is between 55 mm and 65 mm. Rinse the spatula, beaker, and funnel with the first portion of the chloroform used to elute the specimen. Elute the reserpine with 45 mL of chloroform. [NOTE—A properly packed column elutes in 4–8 min.] Collect the eluate in a 50-mL volumetric flask containing 14 mL of methanol. Rinse the tip of the column with chloroform, add chloroform to volume, and mix.

Standard stock solution: [NOTE—Use actinic glassware for this solution.] 0.1 mg/mL of USP Reserpine RS in methanol prepared as follows: Dissolve USP Reserpine RS in chloroform (0.01 mL/mg of USP Reserpine RS), and mix with previously warmed to 50° methanol (1.2 mL/mg of USP Reserpine RS). Transfer the mixture to a suitable volumetric flask with the aid of warm methanol, cool the solution to room temperature, dilute with methanol to volume, and mix.

Standard solution: [NOTE—Use actinic glassware for this solution.] 0.02 mg/mL of USP Reserpine RS from the *Standard stock solution* in chloroform and methanol. Initially add chloroform to about 72% of the volume of the flask, and then dilute with methanol to volume.

Sample solution 1: Weigh a portion of the finely powdered (powder should be able to pass through a 60-mesh sieve) Tablets (NLT 20), equivalent to about 1 mg of reserpine, but NMT 1 g of the powder, and transfer to a 150-mL beaker. Dry-mix the powder with about 500 mg of *Adsorbent*, then mix with 1 mL of dimethyl sulfoxide (immobile solvent). Stir thoroughly until the mass is uniformly wetted and free from lumps, and allow the mixture to stand for 5 min. Add another 500 mg of *Adsorbent*, and thoroughly work it into the mass. Again add an amount of *Adsorbent* so that the total amount added is 2 g, and disperse it completely in the mass.

Sample solution 2: Transfer *Sample solution 1* through a powder funnel to a prepared *Chromatographic column*. Proceed as directed for *Blank solution* beginning with "Scrub the beaker with about 1 g of *Adsorbent*."

Analysis: Determine the UV absorption spectrum of *Sample solution 2*, between 255 and 350 nm, using the *Blank solution* in the reference cell. Similarly, determine the UV absorption spectrum of the *Standard solution*, using a solution of 3.6 volumes of chloroform and 1.4 volumes of methanol as the blank. The two spectra are similar, and the ratio, A_{268}/A_{295}, for *Sample solution 2* does not differ by more than 4.0% from the corresponding ratio for the *Standard solution*.

Calculate the percentage of reserpine ($C_{33}H_{40}N_2O_9$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times 100$$

A_U = absorbance of *Sample solution 2* at the absorption maximum at about 268 nm

A_S = absorbance of the *Standard solution* at the absorption maximum at about 268 nm

Acceptance criteria: The result does not differ more than 6.0% from that obtained in the *Assay*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light resistant containers.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Reserpine RS

Reserpine and Chlorothiazide Tablets

» Reserpine and Chlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$) and not less than 93.0 percent and not more than 107.0 percent of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Reserpine RS

USP Chlorothiazide RS

Identification—

A: Transfer a quantity of powdered Tablets, equivalent to about 1 mg of reserpine, to a stoppered, 50-mL centrifuge tube. Add 20 mL of citric acid solution (1 in 50), and shake until the powder is suspended. Extract the mixture with two 20-mL portions of chloroform, centrifuge, and withdraw the chloroform, filtering each extract through a pledget of cotton into a 50-mL volumetric flask. Dilute with chloroform to volume, and mix: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Reserpine RS, concomitantly measured (*presence of reserpine*).

B: Transfer a quantity of powdered Tablets, equivalent to about 50 mg of chlorothiazide, to a test tube containing 10 mL of acetone, agitate for 5 minutes, and centrifuge. Use the clear supernatant as the Test solution. Separately apply 10 µL each of the Test solution and a Standard solution of USP Chlorothiazide RS in acetone containing 5 mg per mL to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously washed with methanol. Develop the chromatogram in a solvent system consisting of a mixture of

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ethyl acetate and isopropyl alcohol (17:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and examine under short-wavelength UV light: the R_F value of the principal spot in the chromatogram of the Test solution corresponds to that obtained from the Standard solution (*presence of chlorothiazide*).

Dissolution ⟨711⟩—

Medium: mixture of pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) and *n*-propyl alcohol (3:2); 900 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Determination of dissolved reserpine—

STANDARD PREPARATION—Dissolve about 34 mg of USP Reserpine RS, accurately weighed, in a 50-mL volumetric flask containing 5 mL of chloroform, dilute with *n*-propyl alcohol to volume (*Solution 1*), and mix. Pipet 1 mL of *Solution 1* into a 50-mL volumetric flask, dilute with *n*-propyl alcohol to volume (*Solution 2*), and mix. Pipet 1 mL of *Solution 2* into a 100-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix to obtain the *Standard preparation*.

PROCEDURE—Filter a portion of the solution under test through paper, and transfer 5.0 mL of the clear filtrate to a 25-mL volumetric flask. Pipet 5 mL of the *Standard preparation* into a separate 25-mL volumetric flask. Treat each flask as follows: Add 5 drops of hydrochloric acid and 5 mL of a mixture of water, alcohol, and sulfuric acid (29:20:1), and mix. Add by pipet 5 mL of sodium nitrite solution (3 in 1000), dilute with alcohol to volume, mix, and allow to stand for 30 minutes. Concomitantly determine the fluorescences of the solution under test and the *Standard preparation* in a suitable spectrophotometer arranged to deliver activation radiation at 405 nm and to measure the resultant fluorescence at the emission wavelength of about 500 nm. Calculate the amount of reserpine ($C_{33}H_{40}N_2O_9$) dissolved.

Determination of dissolved chlorothiazide—

STANDARD PREPARATION—Transfer about 27 mg of USP Chlorothiazide RS, accurately weighed, to a 50-mL volumetric flask containing 5 mL of methanol, swirl to dissolve, dilute with *Dissolution Medium* to volume, and mix. Pipet 2 mL of this solution into a 100-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix.

PROCEDURE—Determine the amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 292 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with the *Standard preparation*.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{33}H_{40}N_2O_9$ is dissolved in 60 minutes, and not less than 75% *(Q)* of the labeled amount of $C_7H_6ClN_3O_4S_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements with respect to chlorothiazide and to reserpine.

Procedure for content uniformity for reserpine—

Standard preparation—Prepare as directed for *Standard preparation* in the *Assay for reserpine*.

Test preparation—Weigh 1 Tablet, grind to a fine powder, and transfer to a stoppered, 50-mL centrifuge tube. Add 25.0 mL of chloroform and methanol solution (1:1), shake by mechanical means for 15 minutes, and centrifuge. Pipet 4 mL of the clear supernatant into a 100-mL volumetric flask, dilute with the chloroform and methanol solution to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay for reserpine*. Calculate the quantity, in mg, of $C_{33}H_{40}N_2O_9$, in the Tablet taken by the formula:

$$(W_t/W_U)(TC/D)(I_U/I_S)$$

in which W_t is the weight, in mg, of the Tablet, W_U is the weight, in mg, of the portion of Tablet taken, T is the labeled quantity, in mg, of reserpine in the Tablet, C is the concentration, in µg per mL, of USP Reserpine RS in the *Standard preparation*, D is the concentration, in µg per mL, of reserpine in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution, and I_U and I_S are the fluorescence intensities of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Procedure for content uniformity for chlorothiazide—

Standard preparation—Prepare as directed for *Standard preparation* under *Assay for chlorothiazide*.

Test preparation—Transfer 1 Tablet to a 500-mL volumetric flask, add 300 mL of 0.1 N sodium hydroxide, and sonicate, swirling the flask intermittently, until the Tablet is dissolved. Dilute with 0.1 N sodium hydroxide to volume, mix, and filter, discarding the first 15 mL of the filtrate. Dilute a portion of the clear filtrate quantitatively with 0.1 N sodium hydroxide to obtain a solution having a concentration of about 10 µg of chlorothiazide per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay for chlorothiazide*. Calculate the quantity, in mg, of $C_7H_6ClN_3O_4S_2$, in the Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which T is the labeled quantity, in mg, of chlorothiazide in the Tablet, C is the concentration, in µg per mL, of USP Chlorothiazide RS in the *Standard preparation*, D is the concentration, in µg per mL, of chlorothiazide in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution, and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Assay for reserpine—

Standard preparation—Dissolve about 25 mg of USP Reserpine RS, accurately weighed, in 1 mL of chloroform contained in a 50-mL volumetric flask, dilute with methanol to volume, and mix. Dilute a portion of this solution quantitatively and stepwise with chloroform and methanol solution (1:1) to obtain a solution having a known concentration of about 0.2 µg of reserpine per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 mg of reserpine, to a stoppered, 50-mL centrifuge tube, add 25.0 mL of chloroform and methanol solution (1:1), shake by mechanical means for 15 minutes, and centrifuge. Dilute a portion of the clear supernatant quantitatively and stepwise with chloroform and methanol solution (1:1) to obtain a solution having a concentration of about 0.2 µg of reserpine per mL.

Procedure—Separately transfer 5.0 mL of the *Assay preparation*, 5.0 mL of the *Standard preparation*, and 5.0 mL of chloroform and methanol solution (1:1) to provide the reagent blank, respectively, to three 25-mL volumetric flasks. To each flask add 0.5 mL of hydrochloric acid, 1.0 mL of a 3 in 1000 solution of sodium nitrite in dilute methanol (1 in 2), mix, and allow to stand for 30 minutes. Add 1 mL of ammonium sulfamate solution (1 in 20) to each flask, add chloroform and methanol solution (1:1) to volume, mix, and allow to stand for 10 minutes. Concomitantly determine the fluorescence intensities of the solutions in a suitable spectrophotometer arranged to deliver activation radiation at 405 nm and to measure the resultant fluorescence at the emission wavelength of about 500 nm. Calculate the quantity,

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in mg, of $C_{33}H_{40}N_2O_9$ in the portion of Tablets taken by the formula:

$$5C(I_U/I_S)$$

in which C is the concentration, in µg per mL, of USP Reserpine RS in the *Standard preparation*, and I_U and I_S are the fluorescence intensities of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for chlorothiazide—

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorothiazide RS in 0.1 N sodium hydroxide, and dilute quantitatively and stepwise with 0.1 N sodium hydroxide to obtain a solution having a known concentration of about 10 µg per mL. Use a freshly prepared solution.

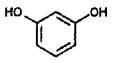
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of chlorothiazide, to a 500-mL volumetric flask, add about 300 mL of 0.1 N sodium hydroxide, and shake by mechanical means for 15 minutes. Dilute with 0.1 N sodium hydroxide to volume, and mix. Filter through paper, discarding the first 15 mL of the filtrate. Pipet 2 mL of the clear filtrate into a 100-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using 0.1 N sodium hydroxide as the blank. Calculate the quantity, in mg, of $C_7H_6ClN_3O_4S_2$, in the portion of Tablets taken by the formula:

$$25C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Chlorothiazide RS in the *Standard preparation*, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Resorcinol



$C_6H_6O_2$ 110.11
1,3-Benzenediol
Resorcinol [108-46-3].

» Resorcinol contains not less than 99.0 percent and not more than 100.5 percent of $C_6H_6O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Resorcinol RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)— [NOTE—If necessary to recrystallize, dissolve in dehydrated alcohol.]

B: Dissolve 100 mg in 2 mL of 1 N sodium hydroxide, add 1 drop of chloroform, and heat the mixture: an intense crimson color is produced. Then add a slight excess of hydrochloric acid: the color changes to pale yellow.

C: To 10 mL of a solution (1 in 100) add 1 drop of ferric chloride TS: a blue-violet color is produced, and it fades slowly.

Melting range ⟨741⟩: between 109° and 111°.

Loss on drying ⟨731⟩—Dry it over silica gel for 4 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.05%.

Phenol—Heat gently a solution (1 in 20): the odor of phenol is not perceptible.

Catechol—To 10 mL of a solution (1 in 20) previously mixed with 2 drops of 1 N acetic acid add 0.5 mL of lead acetate TS: no turbidity is produced.

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Eluant: a mixture of hexanes and ethyl acetate (70:30).
Visualization: 17 and then 1.
Application volume: 10 µL.
Limits: not more than 1.0%.

Assay—Dissolve about 1.5 g of Resorcinol, accurately weighed, in water to make 500.0 mL. Transfer to an iodine flask 25.0 mL of the resulting solution, add 50.0 mL of 0.1 N bromine VS, dilute with 50 mL of water, add 5 mL of hydrochloric acid, and at once insert the stopper in the flask. Shake for 1 minute, allow to stand for 2 minutes, and add 10 mL of potassium iodide TS while slightly loosening the stopper. Shake thoroughly, allow to stand for 5 minutes, remove the stopper, and rinse it and the neck of the flask with 20 mL of water into the flask. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding starch TS as the endpoint is approached. Perform a blank determination. From the volume of 0.1 N sodium thiosulfate VS used, calculate the volume, in mL, of 0.1 N bromine VS consumed by the resorcinol. Each mL of 0.1 N bromine is equivalent to 1.835 mg of $C_6H_6O_2$.

Compound Resorcinol Ointment

DEFINITION
Prepare Compound Resorcinol Ointment as follows.

Resorcinol	60 g
Zinc Oxide	60 g
Bismuth Subnitrate	60 g
Juniper Tar	20 g
Yellow Wax	100 g
Petrolatum	290 g
Lanolin	280 g
Glycerin	130 g
To make	1000 g

Melt the *Yellow Wax* and the *Lanolin* in a dish on a steam bath. Triturate the *Zinc Oxide* and the *Bismuth Subnitrate* with the *Petrolatum* until smooth, and add it to the melted mixture. Dissolve the *Resorcinol* in the *Glycerin*, incorporate the solution with the warm mixture just prepared, then add the *Juniper Tar*, and stir the Ointment until it congeals.

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers, and avoid prolonged exposure to temperatures exceeding 30°.

Resorcinol and Sulfur Topical Suspension

» Resorcinol and Sulfur Topical Suspension is Resorcinol and Sulfur in a suitable hydroalcoholic vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of resorcinol ($C_6H_6O_2$) and not less than 95.0 percent and not more than 110.0 percent of the labeled amount of sulfur (S).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Resorcinol RS

Identification—

A: Transfer a quantity of Topical Suspension, equivalent to about 20 mg of resorcinol, to a 15-mL centrifuge tube, add 5 mL of 5 N sodium hydroxide, mix, and centrifuge the mixture for 5 minutes. Decant the supernatant into a test tube, and retain the residue for *Identification* test *B*. Add 0.5 mL of chloroform, mix, and heat on a steam bath: an intense crimson color is produced. Add a slight excess of hydrochloric acid: the color changes to pale yellow *(presence of resorcinol).*

B: Place a small portion of the residue from the centrifuge tube in *Identification* test *A* on the tip of a spatula, and burn it: sulfur dioxide, which turns moistened starch-iodate paper blue, is formed *(presence of sulfur).*

Alcohol Determination ⟨611⟩ —Determine by the gas-liquid chromatographic method, acetone being used as the internal standard: it contains between 90.0% and 110.0% of the labeled amount of C_2H_5OH.

Assay for resorcinol—

Mobile phase—Prepare a suitable degassed solution of water, acetonitrile, and methanol (about 55:7:6) such that the retention times of resorcinol and caffeine are about 3 minutes and 4 minutes, respectively.

Internal standard solution—Dissolve about 140 mg of caffeine in 2 mL of chloroform, add methanol to make 100 mL, and mix.

Standard preparation—Transfer 50 mg of USP Resorcinol RS, accurately weighed, to a 25-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Assay preparation—Transfer an accurately weighed portion of Topical Suspension, equivalent to about 20 mg of resorcinol, to a 150-mL beaker. Add 40 mL of methanol and 5.0 mL of *Internal standard solution,* and heat on a steam bath for 5 minutes. Cool the mixture to room temperature, and decant the liquid into a 100-mL volumetric flask. Wash the residue in the beaker by adding 20 mL of methanol to the beaker. Heat on a steam bath for 5 minutes, cool the mixture to room temperature, and decant the liquid into the volumetric flask. Repeat the washing, heating, cooling, and decanting. Dilute the contents of the volumetric flask with methanol to volume, and mix.

Procedure—Introduce equal volumes (about 10 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩), operated at room temperature, by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters such that the peak obtained from the *Standard preparation* is about 0.6 full scale. Typically, the apparatus is fitted with a 4-mm × 30-cm column containing packing L1 and is equipped with an UV detector capable of monitoring absorption at 280 nm, and a suitable recorder. In a suitable chromatogram the coefficient of variation for five replicate injections of the *Standard preparation* is not more than 3.0%. Measure the peak responses at equivalent retention times, obtained from the *Assay preparation* and the *Standard preparation,* and calculate the quantity, in mg, of resorcinol ($C_6H_6O_2$) in the portion of Topical Suspension taken by the formula:

$$(100C)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Resorcinol RS in the *Standard preparation;* and R_U and R_S are the ratios of the responses of the resorcinol and caffeine peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for sulfur—Transfer an accurately weighed portion of Topical Suspension, equivalent to about 85 mg of sulfur, to a suitable flask, add 40 mL of sodium sulfite solution (1 in 20), a few drops of antifoam, and a few boiling chips, and boil under a reflux condenser for 1 hour. Cool to room temperature, add 10 mL of formaldehyde solution and 6 mL of 6 N acetic acid, and dilute with water to 150 mL. Add 3 mL of starch TS, and titrate with 0.1 N iodine VS until a permanent blue color is produced. Each mL of 0.1 N iodine is equivalent to 3.206 mg of sulfur (S).

Resorcinol Monoacetate

$C_8H_8O_3$ 152.15
1,3-Benzenediol, monoacetate.
Resorcinol monoacetate [102-29-4].
Packaging and storage—Preserve in tight, light-resistant containers.

Identification—

A: Fuse 3 drops with about 300 mg of phthalic anhydride and about 50 mg of zinc chloride: a small portion of the fused mixture when dissolved in 10 mL of 1 N sodium hydroxide produces an intense yellow-green fluorescence typical of fluorescein.

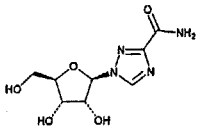
B: Dissolve 0.5 mL in 3 mL of alcohol, add 3 drops of sulfuric acid, and boil: ethyl acetate, recognizable by its odor, is evolved.

Specific gravity ⟨841⟩: between 1.203 and 1.207.
Acidity—A solution of 10 mL of Resorcinol Monoacetate in 20 mL of benzene, when shaken with 100 mL of water, requires not more than 0.50 mL of 0.10 N sodium hydroxide for neutralization, methyl orange TS being used as the indicator.
Loss on drying ⟨731⟩—Dry it on a steam bath for 1 hour: it loses not more than 2.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

USP Monographs

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Ribavirin



$C_8H_{12}N_4O_5$ 244.20

1*H*-1,2,4-Triazole-3-carboxamide, 1-β-D-ribofuranosyl-;
1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide
[36791-04-5].

DEFINITION

Ribavirin contains NLT 98.9% and NMT 101.5% of ribavirin ($C_8H_{12}N_4O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: 10 mg/mL
Developing solvent system: Acetonitrile and 0.1 M ammonium chloride (9:2)
Spray reagent: Anisaldehyde, sulfuric acid, glacial acetic acid, and alcohol (5:5:1:90)
Analysis: Proceed as directed in the chapter. Allow the plate to air-dry for 15 min, spray with *Spray reagent*, heat the plate at 110° for 30 min, and locate the spots on the plate by examining the plate in daylight.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Adjust water with sulfuric acid to a pH of 2.5 ± 0.1. Pass through a suitable filter of 0.5-µm or finer pore size.
Standard solution: 0.025 mg/mL of USP Ribavirin RS in *Mobile phase*
Sample solution: 0.025 mg/mL of Ribavirin in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 207 nm
Column: 7.8-mm × 10-cm; packing L17
Column temperature: 65 ± 0.5°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.7–1.5
Relative standard deviation: NMT 0.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ribavirin ($C_8H_{12}N_4O_5$) in the portion of Ribavirin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = concentration of Ribavirin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.9%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.25%
- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Sample stock solution: 0.5 mg/mL of Ribavirin in *Mobile phase*
Analysis
Sample: *Sample stock solution*
Calculate the percentage of each peak, other than the solvent peak and the ribavirin peak, in the portion of Ribavirin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of the individual peak
r_T = sum of the responses of all the peaks

Acceptance criteria
Any individual impurity: NMT 0.25%
Total impurities: NMT 1.0%

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL
Acceptance criteria: −33.5° to −37.0° (t = 20°)

- **pH** ⟨791⟩

Sample solution: 20 mg/mL
Analysis: Add 0.2 mL of a saturated potassium chloride solution to 50 mL of *Sample solution*.
Acceptance criteria: 4.0–6.5

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 5 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ribavirin RS

Ribavirin Capsules

DEFINITION

Ribavirin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Water. Adjust with sulfuric acid to a pH of 2.5.
Standard solution: 0.025 mg/mL of USP Ribavirin RS in *Mobile phase*
Sample stock solution: Transfer an equivalent to 50 mg of ribavirin, from contents of Capsules (NLT 20), to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and sonicate with occasional shaking for about 20 min. Cool to room temperature, and dilute with *Mobile phase* to volume.
Sample solution: Nominally 0.025 mg/mL of ribavirin in *Mobile phase* from *Sample stock solution*. Pass the solution through a suitable filter of 0.45-µm pore size.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 207 nm
Column: 7.8-mm × 15-cm; 7-µm packing L17
Column temperature: 65°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.7–1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Determine the percentage of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) dissolved by using one of the following procedures.
Procedure 1
Mobile phase: Proceed as directed in the *Assay.*
Standard solution: 22.5 µg/mL of USP Ribavirin RS in *Medium*
Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size. Transfer 5.0 mL of the filtrate to a 50.0-mL volumetric flask, and dilute with *Medium* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 207 nm
Column: 7.8-mm × 30-cm; 9-µm packing L17
Column temperature: 65°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
D = dilution factor of the solution under test

Procedure 2
Sulfuric acid solution: 3% sulfuric acid
Mobile phase: Water. Adjust with *Sulfuric acid solution* to a pH of 2.5.
Standard solution: 0.02 mg/mL of USP Ribavirin RS in *Medium*
Sample solution: Pass the solution through a suitable filter of 0.8-µm pore size. Transfer 5.0 mL of the filtrate to a 50.0-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 207 nm
Column: 7.8-mm × 10-cm; 9-µm packing L17
Column temperature: 40 ± 2°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
D = dilution factor of the solution under test

Tolerances: NLT 80% (Q) of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2.*
Medium: Water; 900 mL, deaerated
Apparatus 1: 100 rpm
Time: 15 min
Buffer: 4 g/L of sodium dihydrogen orthophosphate dihydrate in water. Adjust with 5% (v/v) sodium hydroxide solution to a pH of 5.0. Pass through a suitable filter of 0.45-µm or finer pore size.
Mobile phase: Acetonitrile and *Buffer* (2:98)
Standard solution: 0.22 mg/mL of USP Ribavirin RS in *Medium.* Sonicate, if necessary, to dissolve.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.9 times the retention time of ribavirin
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: 0.22 mg/mL of USP Ribavirin RS in *Medium*. Sonicate, if necessary, to dissolve.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm or finer pore size.
Capsule blank solution: Dissolve 6 empty Capsule shells in 900 mL of *Medium*. Pass through a suitable filter of 0.45-µm or finer pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: UV 225 nm
Cell: 0.1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Capsule blank solution*
Calculate the percentage of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) dissolved:

$$\text{Result} = \{[A_U - (A_B/6)]/A_S\} \times C_S \times (1/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_B = absorbance of the *Capsule blank solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Standard solution, and Chromatographic system: Proceed as directed in the *Assay*.
Sample solution: Nominally 0.5 mg/mL of ribavirin in *Mobile phase* prepared as follows. Transfer an amount equivalent to 50 mg of ribavirin, from contents of Capsules (NLT 20), to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and sonicate with occasional shaking for about 20 min. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Pass the solution through a suitable filter of 0.45-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ribose triazolole carboxylic acid and any other unknown impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of ribose triazolole carboxylic acid or any other unknown impurity from the *Sample solution*
r_S = peak response of ribavirin from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ribose triazolole carboxylic acid[a]	0.7	0.7	0.25
Ribavirin	1.0	—	—
Any individual unknown impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store between 15° and 30°.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ribavirin RS

Ribavirin for Inhalation Solution

DEFINITION
Ribavirin for Inhalation Solution is a sterile, freeze-dried form of ribavirin. When constituted as directed in the labeling, the inhalation solution so obtained contains NLT 95.0% and NMT 105.0% of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Sample solution: 10 mg/mL
Chromatographic system
Developing solvent system: Acetonitrile and 0.1 M ammonium chloride (9:2)
Spray reagent: Anisaldehyde, alcohol, glacial acetic acid, and sulfuric acid (5:90:1:5)
Analysis: Proceed as directed in the chapter. Allow the plate to air-dry for about 15 min, spray with *Spray reagent*, heat

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the plate at 110° for 30 min, and locate the spots on the plate by examining the plate in daylight.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Adjust water with sulfuric acid to a pH of 2.5 ± 0.1. Filter through a suitable filter of 0.5-µm or finer pore size.
Standard solution: 0.025 mg/mL of USP Ribavirin RS in *Mobile phase*
Sample stock solution: Constitute Ribavirin for Inhalation Solution as directed in the labeling, using a suitable volume of diluent. Transfer an aliquot of constituted solution, equivalent to 100 mg of ribavirin, to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Nominally 0.025 mg/mL of ribavirin in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 207 nm
Column: 7.8-mm × 10-cm; packing L17
Column temperature: 65 ± 0.5°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.7–1.5
Relative standard deviation: NMT 0.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) in the portion of Ribavirin for Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.25%
• **ORGANIC IMPURITIES**
Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Prepare as directed for the *Sample stock solution* in the *Assay*.
Analysis
Sample: *Sample stock solution*
Calculate the percentage of each peak, other than that of the solvent peak and the ribavirin peak, in the portion of Ribavirin for Inhalation Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of the individual peak
r_T = sum of all the peak responses

Acceptance criteria
Individual impurity: NMT 0.25%
Total impurities: NMT 1.0%

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL
Acceptance criteria: −33.5° to −37.0° (t = 20°)
• **pH** ⟨791⟩
Sample solution: A solution constituted as directed in the labeling. To each 50 mL of reconstituted solution add 0.2 mL of a saturated potassium chloride solution.
Acceptance criteria: 4.0–6.5
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 5 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, in a dry place at controlled room temperature.
• **LABELING:** The labeling indicates that Ribavirin for Inhalation Solution must be constituted with a measured volume of Sterile Water for Injection or with Sterile Water for Inhalation containing no preservatives, and that the constituted solution is to be administered only by a small-particle aerosol generator.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ribavirin RS

Ribavirin Tablets

DEFINITION
Ribavirin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$).

IDENTIFICATION
• **A.** The retention time of the ribavirin peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 4.0 g/L of sodium dihydrogen phosphate dihydrate in water. Adjust with 5% sodium hydroxide solution to a pH of 5.0 ± 0.05. Pass the solution through a suitable filter of 0.45-µm pore size.
Mobile phase: Acetonitrile and *Buffer* (1:49)
Diluent: Acetonitrile and water (3:7)
Standard stock solution: 0.6 mg/mL of USP Ribavirin RS in *Diluent*
Standard solution: 0.03 mg/mL of USP Ribavirin RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: Transfer a portion of ribavirin, equivalent to 1000 mg of ribavirin from finely powdered Tablets (NLT 10), to a 1000-mL volumetric flask. Add about 750 mL of *Diluent*, and sonicate with occasional shaking for 30 min. Cool to room temperature, dilute with *Diluent* to volume, and mix. Centrifuge, and decant the supernatant.
Sample solution: 0.03 mg/mL of ribavirin in *Mobile phase* from the *Sample stock solution*. Pass the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 207 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: 10 min

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System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ribavirin ($C_8H_{12}N_4O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ribavirin from the *Sample solution*
r_S = peak response of ribavirin from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: 0.22 mg/mL of USP Ribavirin RS in *Medium*
Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Calculate the percentage of ribavirin ($C_8H_{12}N_4O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of ribavirin from the *Sample solution*
r_S = peak response of ribavirin from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES: PROCEDURE 1**
If uracil and/or uridine are known impurities, *Procedure 2* is recommended.
Solution A: 3.4 g/L of potassium dihydrogen phosphate in water. Adjust with 5% potassium hydroxide solution to a pH of 5.00 ± 0.05. Pass the solution through a suitable filter of 0.45-µm pore size.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	90	10
40	75	25
50	50	50
55	50	50
56	100	0
70	100	0

Standard stock solution: 0.4 mg/mL of USP Ribavirin RS in *Solution A*
Standard solution: 1 µg/mL of USP Ribavirin RS in *Solution A* from the *Standard stock solution*
Sample solution: Transfer a portion of ribavirin, equivalent to 100 mg of ribavirin from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask. Add about 150 mL of *Solution A*, and sonicate with occasional shaking for 15 min. Cool to room temperature, dilute with *Solution A* to volume, and mix. Pass the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: 70 min. [NOTE—Data collection is only for the first 55 min. The remaining gradient steps re-equilibrate the column.]
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Impurities are listed in *Table 2*.]
Calculate the percentage of any unknown impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unknown impurity from the *Sample solution*
r_S = peak response of ribavirin from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak area less than 0.05%.

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Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Triazole acid[a, b]	0.35	—
Ribavirin acid[a, c]	0.40	—
Triazole amide[a, d]	0.64	—
Ribavirin	1.0	—
Ribavirin 5-isomer[a, e]	1.37	—
Ribavirin methyl ester[a, f]	2.09	—
Ribavirin 5′-acetyl[a, g]	2.43	—
Ribavirin 5′-benzoyl[a, h]	4.83	—
Individual unknown impurity	—	0.10
Total impurities	—	0.30

[a] These are process impurities listed for information only.
[b] 1*H*-1,2,4-Triazole-3-carboxylic acid.
[c] 1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid.
[d] 1*H*-1,2,4-Triazole-3-carboxamide.
[e] 1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-5-carboxamide.
[f] Methyl 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylate.
[g] 1-(5-*O*-Acetyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide.
[h] 1-(5-*O*-Benzoyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide.

• **ORGANIC IMPURITIES: PROCEDURE 2**

Buffer: 3.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0 ± 0.1. Pass the solution through a suitable filter of 0.45-µm pore size.

Mobile phase: Methanol and *Buffer* (1:39)

Standard stock solution: 1 mg/mL each of USP Ribavirin RS, USP Ribavirin Related Compound A RS, USP Ribavirin Related Compound D RS, USP Uracil RS, and USP Uridine RS in water. Sonicate with occasional shaking to dissolve the solids.

Standard solution: 0.01 mg/mL each of USP Ribavirin RS, USP Ribavirin Related Compound A RS, USP Ribavirin Related Compound D RS, USP Uracil RS, and USP Uridine RS in water from the *Standard stock solution*

Sensitivity solution: 0.5 µg/mL of USP Ribavirin RS from the *Standard solution* in water

Sample solution: 1.0 mg/mL. Transfer a portion of ribavirin, equivalent to 1000 mg of ribavirin from finely powdered Tablets (NLT 20), to a 1000-mL volumetric flask. Add about 500 mL of water, and sonicate with occasional shaking for 15 min. Shake the solution for 15 min, and cool to room temperature. Dilute with water to volume, and centrifuge the solution for 10 min.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 207 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: NLT 4.3 times the retention time of the ribavirin peak

System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*

[NOTE—Impurities are listed in *Table 3*.]
Calculate the percentage of any impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of corresponding reference standard from the *Standard solution* (use ribavirin for any other individual impurity)
C_S = concentration of corresponding reference standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any peak area less than 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ribavirin related compound A[a]	0.55	0.25
Ribavirin related compound D[b]	0.73	0.25
Uracil[c]	0.89	0.25
Ribavirin	1.00	—
Uridine[d]	1.71	0.25
Any other individual impurity	—	0.17
Total impurities	—	1.0

[a] 1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid.
[b] 1*H*-1,2,4-Triazole-3-carboxamide.
[c] Pyrimidine-2,4(1*H*,3*H*)-dione.
[d] 1-β-D-Ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15°–30°.

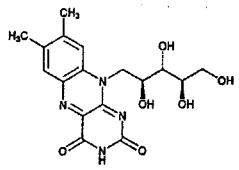
• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states with which *Organic Impurities* test the article complies.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ribavirin RS
USP Ribavirin Related Compound A RS
1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid.
$C_8H_{11}N_3O_6$ 245.19
USP Ribavirin Related Compound D RS
1*H*-1,2,4-Triazole-3-carboxamide.
$C_3H_4N_4O$ 112.09
USP Uracil RS
Pyrimidine-2,4(1*H*,3*H*)-dione.
$C_4H_4N_2O_2$ 112.09
USP Uridine RS
1-β-D-Ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione.
$C_9H_{12}N_2O_6$ 244.20

USP Monographs

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Riboflavin



$C_{17}H_{20}N_4O_6$ 376.36
Riboflavine [83-88-5].

DEFINITION
Riboflavin contains NLT 98.0% and NMT 102.0% of riboflavin ($C_{17}H_{20}N_4O_6$), calculated on the dried basis.

IDENTIFICATION
• **A. Color and Fluorescence of Solution**
Sample solution: 0.01 mg/mL in water
Analysis: Alternately expose to transmitted light and long-wavelength UV light.
Acceptance criteria: The *Sample solution* is pale greenish yellow by transmitted light. By reflected light, it exhibits an intense yellowish-green fluorescence that disappears upon the addition of mineral acids or alkalies.

ASSAY
• **Procedure**
[Note—Conduct the entire *Analysis* without exposure to direct sunlight.]
Standard solution: Transfer 50 mg of USP Riboflavin RS to a 1000-mL volumetric flask containing 50 mL of water. Add 5 mL of 6 N acetic acid and sufficient water to make 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to 25°, and dilute with water to volume. Dilute this solution with water to bring it within the operating sensitivity of the fluorometer used.
Sample solution: Transfer 50 mg of Riboflavin to a 1000-mL volumetric flask containing 50 mL of water. Add 5 mL of 6 N acetic acid and sufficient water to make 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to 25°, and dilute with water to volume. Dilute this solution with water to bring it to the same concentration as that of the *Standard solution*.
Blank: Prepare as directed for the *Sample solution*, except omit the test specimen.
Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)
Mode: Fluorescence
Excitation wavelength: 444 nm
Emission wavelength: 530 nm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Measure the fluorescence intensity of the *Standard solution*. Immediately after the reading, add to the solution 10 mg of sodium hydrosulfite, stirring with a glass rod until dissolved, and at once measure the fluorescence again. [Note—Depending on the final concentration of riboflavin in the solution, it may be necessary to increase the amount of sodium hydrosulfite to suppress the fluorescence activity completely.] The difference between the two readings represents the fluorescence intensity (I_S) due to the *Standard solution*. Similarly, measure the fluorescence intensity (I_U) due to the *Sample solution*. Perform the *Blank* determination, and make any necessary correction.
Calculate the percentage of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of Riboflavin taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence of the *Sample solution*
I_S = fluorescence of the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)
C_U = concentration of Riboflavin in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.3%
• **Limit of Lumiflavin**
Alcohol-free chloroform: Shake 20 mL of chloroform gently but thoroughly with 20 mL of water for 3 min, draw off the chloroform layer, and wash twice more with 20-mL portions of water. Finally, pass the chloroform through a dry filter paper, and shake it for 5 min with 5 g of powdered anhydrous sodium sulfate. Allow the mixture to stand for 2 h, and decant or filter the clear chloroform.
Sample solution: Shake 25 mg of Riboflavin with 10 mL of *Alcohol-free chloroform* for 5 min, and filter.
Blank: Alcohol-free chloroform
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Analytical wavelength: 440 nm
Cell: 1 cm
Analysis
Samples: *Sample solution* and *Blank*
Measure the absorbances of the *Sample solution* and *Blank*. Correct the absorbance of the *Sample solution* with that of the *Blank*.
Acceptance criteria: Absorbance is NMT 0.025.

SPECIFIC TESTS
• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Sample solution: 5 mg/mL in 0.05 M carbonate-free sodium hydroxide
Analysis: Measure the specific rotation within 30 min of preparation.
Acceptance criteria: −115° to −135°
• **Loss on Drying** ⟨731⟩: Dry 500 mg at 105° for 2 h. It loses NMT 1.5% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Riboflavin RS

Riboflavin Injection

» Riboflavin Injection is a sterile solution of Riboflavin in Water for Injection. It contains not less than 95.0 percent and not more than 120.0 percent of the labeled amount of $C_{17}H_{20}N_4O_6$. It may contain niacinamide or other suitable solubilizers.

Packaging and storage—Preserve in light-resistant, in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Riboflavin RS

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Identification—It responds to the *Identification* test under *Riboflavin*.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 7.1 USP Endotoxin Units per mg of riboflavin.
pH ⟨791⟩: between 4.5 and 7.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Dilute an accurately measured volume of not less than 1 mL of Injection to make a solution containing approximately 0.1 µg of riboflavin per mL. Using this as the *Assay Preparation*, proceed as directed under *Riboflavin Assay* ⟨481⟩, *Chemical Methods, Procedure 1*. Calculate the quantity, in mg, of $C_{17}H_{20}N_4O_6$ in each mL of the Injection taken by the formula:

$$C(A/B)$$

in which C is the concentration, in mg per mL, of $C_{17}H_{20}N_4O_6$ obtained for the *Assay Preparation*, A is the test specimen dilution volume, in mL, and B is the volume, in mL, of Injection taken.

Riboflavin Tablets

DEFINITION
Riboflavin Tablets contain NLT 95.0% and NMT 115.0% of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$).

ASSAY
• **Riboflavin Assay** ⟨481⟩, *Chemical Methods, Procedure 1*
Sample solution: Transfer a portion of the powder from NLT 20 finely powdered Tablets, equivalent to 20 mg of riboflavin, to a 250-mL flask, and add 150 mL of 0.1 N hydrochloric acid. Shake vigorously, and wash down the sides of the flask with sufficient 0.1 N hydrochloric acid to ensure that the pH remains below 1.5 during the subsequent period of heating. Heat the mixture on a steam bath, with frequent agitation, until the riboflavin has dissolved, or in an autoclave at 121° for 30 min. Cool, and with vigorous agitation, adjust the mixture with 1 N sodium hydroxide to a pH of 5–6. Transfer to a 1000-mL volumetric flask, and dilute with water to volume. If the solution is not clear, filter through paper known not to adsorb riboflavin. Dilute an aliquot of the clear solution with water to a final volume that contains 0.1 µg/mL of riboflavin.
Analysis: Proceed as directed in the chapter.
Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of Tablets taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = corrected fluorescence value from the *Sample solution*
I_S = corrected fluorescence value from the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of riboflavin in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–115.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: Known concentration of USP Riboflavin RS in *Medium*
Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary
Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 1.40 mg/mL of sodium 1-hexanesulfonate
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) dissolved:

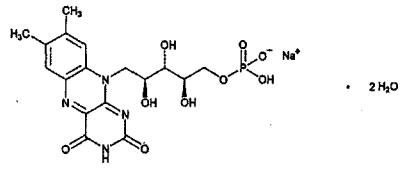
$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak area of riboflavin from the *Sample solution*
r_S = peak area of riboflavin from the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Riboflavin RS

Riboflavin 5′-Phosphate Sodium



$C_{17}H_{20}N_4NaO_9P \cdot 2H_2O$ 514.36
$C_{17}H_{20}N_4NaO_9P$ 478.33
Riboflavin 5′-(dihydrogen phosphate), monosodium salt, dihydrate;
Riboflavine 5′-(sodium hydrogen phosphate), dihydrate
Anhydrous [130-40-5].

DEFINITION
Riboflavin 5′-Phosphate Sodium contains NLT the equivalent of 73.0% and NMT the equivalent of 79.0% of riboflavin ($C_{17}H_{20}N_4O_6$), calculated on the dried basis.

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IDENTIFICATION

• **A. Color and Fluorescence of Solution**

Sample solution: 0.01 mg/mL in water

Analysis: Alternately expose to transmitted light and long-wavelength UV light.

Acceptance criteria: The solution is pale greenish yellow by transmitted light, and it exhibits an intense yellowish green fluorescence by reflected light under long-wavelength UV light that disappears upon the addition of mineral acids or alkalies.

• **B. Identification Tests—General,** *Sodium* ⟨191⟩ and *Phosphate* ⟨191⟩

Sample solution: To 0.5 g add 10 mL of nitric acid. Evaporate the mixture on a water bath to dryness, and ignite the residue until the carbon is removed. Dissolve the residue in 5 mL of water, and filter.

Acceptance criteria: The *Sample solution* meets the requirements.

ASSAY

• **Procedure**

[Note—Conduct the assay so that all solutions are protected from actinic light at all stages, preferably by using low-actinic glassware.]

Standard solution: 0.35 µg/mL prepared as follows. Transfer 35 mg of USP Riboflavin RS to a 250-mL conical flask. Add 20 mL of pyridine and 75 mL of water, and dissolve the riboflavin by frequent shaking. Transfer the solution to a 1000-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of this solution to a second 1000-mL volumetric flask, add sufficient 0.1 N sulfuric acid (about 4 mL) so that the final pH of the solution is between 5.9 and 6.1, and dilute with water to volume.

Sample solution: Transfer 50 mg of Riboflavin 5′-Phosphate Sodium to a 250-mL conical flask. Add 20 mL of pyridine and 75 mL of water, and dissolve the riboflavin by frequent shaking. Transfer the solution to a 1000-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of this solution to a second 1000-mL volumetric flask, add sufficient 0.1 N sulfuric acid (about 4 mL) so that the final pH of the solution is between 5.9 and 6.1, and dilute with water to volume.

Blank: Proceed as directed in the *Analysis*, omitting the test specimen.

Instrumental conditions

(See *Fluorescence Spectroscopy* ⟨853⟩.)

Mode: Fluorescence

Excitation wavelength: 440 nm

Emission wavelength: 530 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Determine the maximum fluorescence intensities of the solutions against the *Blank*.

Calculate the percentage of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of Riboflavin 5′-Phosphate Sodium taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence intensity from the *Sample solution*

I_S = fluorescence intensity from the *Standard solution*

C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)

C_U = concentration of Riboflavin 5′-Phosphate Sodium in the *Sample solution* (µg/mL)

Acceptance criteria: 73.0%–79.0% on the dried basis

IMPURITIES

• **Free Phosphate**

Acid molybdate solution: Prepare a 70-mg/mL solution of ammonium molybdate in water. Dilute 25 mL of this solution with water to 200 mL. To this dilution slowly add 25 mL of 7.5 N sulfuric acid.

Ferrous sulfate solution: 100 mg/mL of ferrous sulfate in 0.15 N sulfuric acid, prepared just before use

Standard solution: 44.0 µg/mL of monobasic potassium phosphate in water.

Sample solution: 3 mg/mL of Riboflavin 5′-Phosphate Sodium in water

Blank: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 700 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer 10.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 50-mL conical flasks. Add 10.0 mL of *Acid molybdate solution* and 5.0 mL of *Ferrous sulfate solution* to each flask, and mix. Determine the absorbances of the solutions against that of the *Blank*.

Acceptance criteria: NMT 1% as PO_4. The absorbance of the *Sample solution* is NMT that of the *Standard solution*.

• **Free Riboflavin and Riboflavin Diphosphates**

[Note—Conduct this test so that all solutions are protected from actinic light at all stages, preferably by using low-actinic glassware.]

Mobile phase: Methanol and 0.054 M monobasic potassium phosphate (15:85)

System suitability solution: Prepare a 2-mg/mL solution of USP Phosphated Riboflavin RS in water. Dilute this solution with *Mobile phase* to 160 µg/mL.

Standard solution: Transfer 60 mg of USP Riboflavin RS to a 250-mL volumetric flask. Dissolve carefully in 1 mL of hydrochloric acid, and dilute with water to volume. Dilute with *Mobile phase* to 9.6 µg/mL.

Sample solution: Prepare a 2-mg/mL solution of Riboflavin 5′-Phosphate Sodium in water. Dilute this solution with *Mobile phase* to 160 µg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Fluorometer

Excitation wavelength: 440 nm

Emission wavelength: 530 nm (monochromator-based detector) or 470 nm (filtered-type detector)

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection size: 100 µL

System suitability

Sample: *System suitability solution*

The retention time of riboflavin 5′-monophosphate is 20–25 min. The approximate relative retention times for the components are listed in *Table 1*.

Table 1

Name	Relative Retention Time
Riboflavin 3′4′-diphosphate	0.23
Riboflavin 3′5′-diphosphate	0.39
Riboflavin 4′5′-diphosphate	0.58
Riboflavin 3′-monophosphate	0.70

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Table 1 *(continued)*

Name	Relative Retention Time
Riboflavin 4′-monophosphate	0.87
Riboflavin 5′-monophosphate	1.00
Riboflavin	1.63

Suitability requirements
Resolution: NLT 1.0 between riboflavin 4′-monophosphate and riboflavin 5′-monophosphate
Relative standard deviation: NMT 1.5% for riboflavin 5′-monophosphate
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the peak areas of the solutions. Identify the peaks to be measured in the *Sample solution* by comparison with the chromatogram obtained from the *System suitability solution*.
Calculate the percentage of free riboflavin in the portion of Riboflavin 5′-Phosphate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of free riboflavin from the *Sample solution*
r_S = peak area of riboflavin from the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)
C_U = concentration of Riboflavin 5′-Phosphate Sodium in the *Sample solution* (µg/mL)

Calculate the percentage of riboflavin diphosphates in the portion of Riboflavin 5′-Phosphate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak areas for any of the 3 riboflavin diphosphates from the *Sample solution*
r_S = peak area of riboflavin from the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)
C_U = concentration of Riboflavin 5′-Phosphate Sodium in the *Sample solution* (µg/mL)

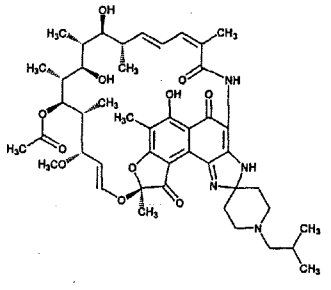
Acceptance criteria
Free riboflavin: NMT 6.0% on the dried basis
Riboflavin diphosphates, as riboflavin: NMT 6.0% on the dried basis

• **LIMIT OF LUMIFLAVIN**
Alcohol-free chloroform: Shake 20 mL of chloroform gently but thoroughly with 20 mL of water for 3 min, draw off the chloroform layer, and wash twice more with 20-mL portions of water. Finally, pass the chloroform through a dry filter paper, shake it for 5 min with 5 g of powdered anhydrous sodium sulfate, allow the mixture to stand for 2 h, and decant or filter the clear chloroform.
Sample solution: Shake 35 mg of Riboflavin 5′-Phosphate Sodium with 10 mL of *Alcohol-free chloroform* for 5 min, and filter.
Blank: Alcohol-free chloroform
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Analytical wavelength: 440 nm
Cell: 1 cm
Analysis
Samples: *Sample solution* and *Blank*
Measure the absorbances of the *Sample solution* and *Blank*. Correct the absorbance of the *Sample solution* with the *Blank*.
Acceptance criteria: The absorbance is NMT 0.025.

SPECIFIC TESTS
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 25.0%
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 15 mg/mL in 5 N hydrochloric acid. Use the solution within 15 min.
Acceptance criteria: +37.0° to +42.0°
• **pH** ⟨791⟩
Sample solution: 10 mg/mL solution
Acceptance criteria: 5.0–6.5
• **LOSS ON DRYING** ⟨731⟩: Dry a sample under vacuum over phosphorus pentoxide at 100° for 5 h: it loses NMT 7.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Phosphated Riboflavin RS
USP Riboflavin RS

Rifabutin



$C_{46}H_{62}N_4O_{11}$ 847.00
(9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,16,18,20-Tetrahydroxy-1′-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo [2′,3′:7,8] naphth[1,2-*d*]imidazole-2,4′-piperidine]-5,10,26-(3*H*,9*H*)-trione-16-acetate [72559-06-9].

» Rifabutin contains not less than 950 µg and not more than 1020 µg of $C_{46}H_{62}N_4O_{11}$ per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, protected from light and from excessive heat.
USP Reference standards ⟨11⟩—
USP Rifabutin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

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Water Determination, *Method I* ⟨921⟩: not more than 2.5%.

Limit of *N*-isobutylpiperidone—Prepare a test solution of Rifabutin in a mixture of chloroform and methanol (1:1) containing 10 mg per mL. Prepare a series of Standard solutions of *N*-isobutylpiperidone in a mixture of chloroform and methanol (1:1) containing 0.005, 0.01, 0.02, 0.05, and 0.1 mg per mL, respectively. Separately apply 10-µL spots of the test solution and the Standard solutions to the starting line of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Develop the chromatograms in a solvent system consisting of a mixture of hexanes and acetone (100:30) in an equilibrated unlined chromatographic chamber until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, allow the plate to air-dry, and place it in an iodine vapor chamber until the spots are visible (about 5 minutes). Remove the plate from the chamber, spray the plate with starch TS, and examine the plate: no spot in the chromatogram of the test solution at an R_F value corresponding to that of *N*-isobutylpiperidone is more intense than that of the principal spot observed in the chromatogram obtained from the Standard solution containing 0.05 mg of *N*-isobutylpiperidone per mL (0.5%).

Chromatographic purity—Using the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of impurities by the formula:

$$100(r_i/r_S)$$

in which r_i is the response of an individual impurity and r_S is the sum of the responses of all peaks: any impurity peak detected at a retention time of about 0.5, 0.6, 0.8, or 1.4 relative to the retention time of the rifabutin peak does not exceed 1.0%, not more than 0.5% of any other impurity is detected, and the total of all impurity peaks is not more than 3.0%.

Assay—

0.1 M Monobasic potassium phosphate—Prepare a solution containing 13.6 g of monobasic potassium phosphate per L.

Mobile phase—Prepare a mixture of acetonitrile and *0.1 M Monobasic potassium phosphate* (50:50). Adjust by dropwise addition of 2 N sodium hydroxide to a pH of 6.5 ± 0.1. Filter through a 0.5-µm or finer porosity filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 25 mg of USP Rifabutin RS, accurately weighed, to a 50-mL volumetric flask. Add 5 mL of acetonitrile, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer about 25 mg of Rifabutin, accurately weighed, to a 50-mL volumetric flask. Add 5 mL of acetonitrile, dilute with *Mobile phase* to volume, and mix.

Resolution solution—Dissolve about 10 mg of Rifabutin and 2 mL of methanol, add 1 mL of 2 N sodium hydroxide, and allow to stand for about 4 minutes. Add 1 mL of 2 N hydrochloric acid, and dilute with *Mobile phase* to 50 mL. [NOTE—Portions of this solution may be stored in the frozen state for future use.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 12.5-cm column that contains 5-µm diameter packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the chromatogram exhibits a major peak for a degradant, two minor peaks for degradants, and a major peak for rifabutin at relative retention times of about 0.5, 0.6, 0.8, and 1.0, respectively. The resolution, *R*, between the rifabutin peak and the degradant peak eluting at a relative retention time of about 0.8 is not less than 1.3. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for a period of time that is twice the retention time of the major rifabutin peak, and measure the area responses for the major peaks. Calculate the quantity, in µg, of $C_{46}H_{62}N_4O_{11}$ in each mg of Rifabutin taken by the formula:

$$50(CP/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Rifabutin RS in the *Standard preparation*, *P* is the designated potency, in µg per mg, of USP Rifabutin RS, *W* is the weight, in mg, of Rifabutin taken to prepare the *Assay preparation*, and r_U and r_S are the rifabutin peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Rifabutin Capsules

» Rifabutin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{46}H_{62}N_4O_{11}$.

Packaging and storage—Preserve in well-closed containers, protected from light and from excessive heat.

USP Reference standards ⟨11⟩—
USP Rifabutin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution—Prepare the test solution as follows. Suspend a quantity of Capsule contents, equivalent to about 200 mg of rifabutin, in 20 mL of methanol, sonicate for about 5 minutes, and filter through a 0.5-µm or finer porosity filter. Dilute a portion of the filtrate quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of about 20 µg per mL. Prepare a Standard solution by dissolving a quantity of USP Rifabutin RS in methanol, with the aid of sonication, to obtain a solution having a concentration of about 20 µg per mL, and filter through a 0.5-µm or finer porosity filter.

Medium: methanol.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Dissolution—

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{46}H_{62}N_4O_{11}$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 280 nm of filtered portions of the solution under test, suitably diluted with *Dissolution*

USP Monographs

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Medium, in comparison with a Standard solution having a known concentration of USP Rifabutin RS in the same medium, taking into account its designated potency.

Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{46}H_{62}N_4O_{11}$ is dissolved in 45 minutes.

Chromatographic purity—Using the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of impurities by the formula:

$$100(r_i/r_S)$$

in which r_i is the response of an individual impurity and r_S is the sum of the responses of all peaks: any impurity peak detected at a retention time of about 0.5, 0.6, 0.8, or 1.4 relative to the retention time of the rifabutin peak does not exceed 1.0%, not more than 0.5% of any other impurity is detected, and the total of all impurity peaks is not more than 4.5%.

Assay—

0.1 M Monobasic potassium phosphate, *Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Rifabutin*.

Assay preparation—Remove as completely as possible the contents of not less than 20 Capsules, weigh accurately, and determine the average weight of the Capsule contents. Transfer an accurately weighed portion of the powder, equivalent to about 25 mg of rifabutin, to a 50-mL volumetric flask. Add 5 mL of acetonitrile, dilute with *Mobile phase* to volume, and mix. Filter through a 0.5-µm or finer porosity filter, and use the filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Rifabutin*. Calculate the quantity, in mg, of $C_{46}H_{62}N_4O_{11}$ in the portion of Capsules taken by the formula:

$$0.05CP(r_U/r_S)$$

in which the terms are as defined therein.

Rifabutin Compounded Oral Suspension

DEFINITION

Rifabutin Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of rifabutin ($C_{46}H_{62}N_4O_{11}$).

Prepare Rifabutin Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Rifabutin capsules[a] equivalent to	3 g of rifabutin
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus,[b] a sufficient quantity to make	150 mL

[a] Mycobutin 150-mg capsules, Pfizer Inc., New York, NY.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Empty the required number of *Rifabutin capsules* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a rifabutin liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Solution A: 100 mM monobasic potassium phosphate buffer, adjusted with 2 N sodium hydroxide to a pH of 6.5

Mobile phase: Acetonitrile and *Solution A* (50:50). Filter and degas.

Standard stock solution: 2.0 mg/mL of USP Rifabutin RS in *Mobile phase*

Standard solution: Transfer 10 mL of *Standard stock solution* to a 100-mL volumetric flask. Immediately rinse the volumetric apparatus with 10 mL of acetonitrile and 10 mL of *Mobile phase*. Dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 0.2 mg/mL of rifabutin. Pass through a filter of 0.45-µm pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension into a 100-mL volumetric flask. Immediately rinse the pipette with 10 mL of acetonitrile and 10 mL of *Mobile phase*. Dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 0.2 mg/mL of rifabutin. Pass through a filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for the rifabutin is about 14.0 min.]
Suitability requirements
Column efficiency: NLT 4500 theoretical plates
Tailing factor: NMT 3.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rifabutin ($C_{46}H_{62}N_4O_{11}$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rifabutin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of rifabutin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.5–5.5

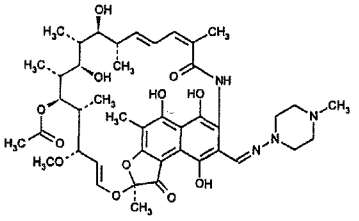
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
• **BEYOND-USE DATE:** NMT 84 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Rifabutin RS

USP Monographs

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Rifampin



$C_{43}H_{58}N_4O_{12}$ 822.94

Rifamycin, 3-[[(4-methyl-1-piperazinyl)imino]methyl]-. 5,6,9,17,19,21-Hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[*N*-(4-methyl-1-piperazinyl)formimidoyl]-2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-*b*]furan-1,11-(2*H*)-dione 21-acetate [13292-46-1].

» Rifampin contains not less than 95.0 percent and not more than 103.0 percent of $C_{43}H_{58}N_4O_{12}$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, protected from excessive heat.

USP Reference standards ⟨11⟩—
USP Rifampin RS
USP Rifampin Quinone RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197M▲ (CN 1-May-2020)

Crystallinity ⟨695⟩: meets the requirements.

pH ⟨791⟩: between 4.5 and 6.5, in a suspension (1 in 100).

Loss on drying ⟨731⟩—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 4 hours: it loses not more than 2.0% of its weight.

Related substances—

Phosphate buffer , *Mobile phase*, *Solvent mixture*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Stock test preparation—Transfer about 200 mg of Rifampin to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Sonicate for about 30 seconds, if necessary, to ensure dissolution. [NOTE—Use this solution within 2 hours.]

Test preparation—Transfer 5.0 mL of *Stock test* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. [NOTE—Prepare this solution immediately prior to injection into the chromatograph.]

Dilute test preparation—Transfer 10.0 mL of *Stock test preparation* to a 100-mL volumetric flask, dilute with acetonitrile to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. Transfer 5.0 mL of this solution to another 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. [NOTE—Prepare this final dilution immediately prior to injection into the chromatograph.]

Procedure—Separately inject equal volumes (about 50 µL) of the *Test preparation* and the *Dilute test preparation* into the chromatograph, record the chromatograms, and measure the responses for all of the peaks. Calculate the percentage of each related substance by the formula:

$$r_{Ti}/(r_D + 0.01\Sigma r_{Ti})$$

in which r_{Ti} is the area of the peak of the individual related substance in the chromatogram obtained from the *Test preparation*, r_D is the area of the rifampin peak in the chromatogram obtained from the *Dilute test preparation*, and Σr_{Ti} is the sum of the areas of all of the peaks of the related substances obtained in the chromatogram of the *Test preparation:* not more than 1.5% of rifampin quinone is found, not more than 1.0% of any other individual related substance is found, and a total of not more than 3.5% of all individual related substances, other than rifampin quinone, having retention times of up to 3 in relation to the retention time of rifampin is found.

Assay—

Phosphate buffer—Dissolve 136.1 g of monobasic potassium phosphate in about 500 mL of water, add 6.3 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a suitable mixture of water, acetonitrile, *Phosphate buffer*, 1.0 M citric acid, and 0.5 M sodium perchlorate (510:350:100:20:20), filter through a suitable filter of 0.7 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Solvent mixture—Prepare a mixture of water, acetonitrile, 1.0 M dibasic potassium phosphate, 1.0 M monobasic potassium phosphate, and 1.0 M citric acid (640:250:77:23:10).

Standard preparation—Transfer about 40 mg of USP Rifampin RS, accurately weighed, to a 200-mL volumetric flask. Dissolve in and dilute with acetonitrile to volume, and mix. Sonicate for about 30 seconds, if necessary, to ensure dissolution. [NOTE—Use this solution within 5 hours.] Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. [NOTE—Prepare this final dilution immediately prior to injection into the chromatograph.]

Assay preparation—Using Rifampin, proceed as directed for *Standard preparation*.

Resolution solution—Dissolve suitable quantities of USP Rifampin RS and USP Rifampin Quinone RS in acetonitrile to obtain a solution containing about 0.1 mg of each per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the rifampin quinone and rifampin peaks is not less than 4.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the rifampin peak is not less than 1000 theoretical plates, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. The relative retention times are about 0.6 for rifampin quinone and 1.0 for rifampin. Calculate the quantity, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$) in the portion of Rifampin taken to prepare the *Assay preparation* by the formula:

$$2000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, calculated on the dried basis, of USP Rifampin RS in the *Standard preparation*, and r_U and r_S are the area responses of the

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rifampin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Rifampin Capsules

» Rifampin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{43}H_{58}N_4O_{12}$.

Packaging and storage—Preserve in tight, light-resistant containers, protected from excessive heat.
USP Reference standards ⟨11⟩—
USP Rifampin RS
USP Rifampin Quinone RS

Identification—
A: Triturate a quantity of Capsule contents, equivalent to about 50 mg of rifampin, with 5 mL of chloroform, and filter. Apply 3 µL each of the filtrate (test solution) and of a solution of USP Rifampin RS in chloroform containing 10 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in an equilibrated chromatographic chamber containing a solvent system consisting of a mixture of chloroform and methanol (90:10) until the solvent front has moved about one-half of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the red spots on the plate: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
B: The chromatogram of the *Assay preparation* exhibits a major peak for rifampin, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{43}H_{58}N_4O_{12}$ dissolved from absorbances at the wavelength of maximum absorbance at about 475 nm on filtered portions of the solution under test, suitably diluted, if necessary, with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Rifampin RS, calculated on the dried basis, in the same *Medium*, prepared concomitantly and held in the water bath for the *Time* specified.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{43}H_{58}N_4O_{12}$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—
Phosphate buffer, Mobile phase, Solvent mixture, Diluent, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay*.
Test preparation—Transfer the contents of 1 Capsule to a suitable volumetric flask so that when diluted to volume as directed below, each mL of solution contains about 1.5 mg of rifampin. Rinse the Capsule shell with a small quantity of *Solvent mixture*, and add the washing to the volumetric flask. Add *Solvent mixture* until the flask is about four-fifths full. Proceed as directed for *Assay preparation* in the *Assay*, beginning with "Sonicate for about 5 minutes."
Procedure—Proceed as directed for *Procedure* in the *Assay*. Calculate the quantity, in mg, of $C_{43}H_{58}N_4O_{12}$ in the Capsule content by the formula:

$$(LC/D)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of rifampin in the Capsule, C is the concentration, in mg per mL, of USP Rifampin RS, calculated on the dried basis, in the *Standard preparation*, D is the concentration, in mg per mL, of rifampin in the *Test preparation*, based on the labeled quantity per Capsule and the extent of dilution, and r_U and r_S are the rifampin peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.
Loss on drying ⟨731⟩—Dry about 100 mg of Capsule contents in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 3.0% of its weight.

Assay—
Phosphate buffer—Dissolve 136.1 g of monobasic potassium phosphate in about 500 mL of water, add 6.3 mL of phosphoric acid, dilute with water to 1000 mL, and mix (pH 3.1 ± 0.1).
Mobile phase—Prepare a suitable mixture of water, acetonitrile, *Phosphate buffer*, 1.0 M citric acid, and 0.5 M sodium perchlorate (510:350:100:20:20), filter through a suitable filter of 0.7 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Solvent mixture—Prepare a mixture of acetonitrile and methanol (1:1).
Diluent—Prepare a suitable mixture of water, acetonitrile, 1.0 M dibasic sodium phosphate, 1.0 M monobasic potassium phosphate, and 1.0 M citric acid (640:250:77:23:10).
Standard preparation—Dissolve an accurately weighed quantity of USP Rifampin RS in *Solvent mixture* to obtain a solution having a known concentration of about 1.5 mg per mL, sonicating for about 30 seconds, if necessary, to ensure dissolution. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. [NOTE—Use this working solution within 5 hours.] Transfer 5.0 mL of the working solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. Each mL of this solution contains about 0.03 mg of USP Rifampin RS. [NOTE—Inject this *Standard preparation* into the chromatograph within 30 to 60 seconds after preparation.]
Assay preparation—Remove, as completely as possible, the contents of not less than 20 Capsules, and weigh accurately. Mix the Capsule contents, and transfer an accurately weighed portion of the powder, equivalent to about 300 mg of rifampin, to a 200-mL volumetric flask, and add about 180 mL of *Solvent mixture*. Sonicate for about 5 minutes, allow to equilibrate to room temperature, dilute with *Solvent mixture* to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. [NOTE—Use this solution within 5 hours.] Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. [NOTE—Inject this *Assay preparation* into the chromatograph within 30 to 60 seconds after preparation.]
Resolution solution—Dissolve USP Rifampin Quinone RS in *Solvent mixture* to obtain a solution containing about 0.1 mg per mL. Transfer 1.5 mL of this solution and 5.0 mL of the working solution used to prepare the *Standard preparation* to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak

USP Monographs

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responses as directed for *Procedure:* the relative retention times are about 0.6 for rifampin quinone and 1.0 for rifampin, and the resolution, *R*, between the rifampin quinone and rifampin peaks is not less than 4.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{43}H_{58}N_4O_{12}$ in the portion of Capsules taken by the formula:

$$10{,}000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Rifampin RS, calculated on the dried basis, in the *Standard preparation*, and r_U and r_S are the rifampin peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Rifampin for Injection

» Rifampin for Injection contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of rifampin ($C_{43}H_{58}N_4O_{12}$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

USP Reference standards ⟨11⟩—
USP Rifampin RS
USP Rifampin Quinone RS

Identification—

A: It responds to *Identification* test *A* under *Rifampin Capsules*, the test solution being prepared by dissolving the contents of a container in chloroform to obtain a solution containing about 10 mg of rifampin per mL.

B: The retention time of the rifampin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —Dissolve Rifampin for Injection in endotoxin-free water to obtain a stock solution containing 10 mg of rifampin per mL. Dilute the stock solution quantitatively, and stepwise if necessary, with endotoxin-free water to obtain a solution containing 0.12 mg of rifampin per mL: it contains not more than 0.5 USP Endotoxin Unit per mg of rifampin.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 7.8 and 8.8, in a solution containing 60 mg of rifampin per mL.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—

Phosphate buffer, Mobile phase, Solvent mixture, Standard preparation, Resolution solution, and *Chromatographic system* —Prepare as directed in the *Assay* under *Rifampin.*

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute a container of Rifampin for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. [NOTE—Use this solution within 2 hours.] Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and transfer to a suitable volumetric flask of such capacity that when diluted with acetonitrile to volume, a solution is obtained containing about 6 mg of rifampin ($C_{43}H_{58}N_4O_{12}$) per mL. [NOTE—Use this stock solution within 5 hours.] Dilute an accurately measured volume of this stock solution quantitatively and stepwise with *Solvent mixture* to obtain a solution having a concentration of about 0.02 mg of rifampin per mL. [NOTE—Prepare this final dilution immediately prior to injection into the chromatograph.]

Assay preparation 2 (where the label states the quantity of rifampin in a given volume of constituted solution)—Constitute a container of Rifampin for Injection in a volume of water, accurately measured, equivalent to the volume of diluent specified in the labeling. [NOTE—Use this solution within 2 hours.] Dilute an accurately measured volume of the constituted solution quantitatively and stepwise with acetonitrile to obtain a solution having a concentration of about 0.2 mg of rifampin ($C_{43}H_{58}N_4O_{12}$) per mL. [NOTE—Use this stock solution within 5 hours.] Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. [NOTE—Prepare this final dilution immediately prior to the injection into the chromatogram.]

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Rifampin.* Calculate the quantity, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$) withdrawn from the container of constituted Rifampin for Injection, or in the volume of constituted Rifampin for Injection taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of rifampin in the container, or in the volume of constituted solution taken, D is the concentration, in mg per mL, of rifampin in *Assay preparation 1* or in *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the volume of constituted solution taken, and the extent of dilution, C is the concentration, in mg per mL, calculated on the dried basis, of USP Rifampin RS in the *Standard preparation,* and r_U and r_S are the rifampin peak responses obtained from *Assay preparation 1*, or *Assay preparation 2*, and the *Standard preparation,* respectively.

Rifampin Compounded Oral Suspension

DEFINITION

Rifampin Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of rifampin ($C_{43}H_{58}N_4O_{12}$).

Prepare Rifampin Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Rifampin	1.20 g
Citric Acid or Sodium Citrate	To adjust pH
Syrup, a sufficient quantity to make	120 mL

Empty the required number of capsules into a suitable mortar, or use *Rifampin* powder. If necessary, gently crush the capsule contents with a pestle to produce a fine powder. Add 2 mL of *Syrup* to the mortar, and triturate until a smooth paste is formed. Add 10 mL of *Syrup*, and triturate to form a

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suspension. Continue to add *Syrup* until 80 mL has been added. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add *Syrup* in portions to rinse the mortar, and add the rinses to the bottle. If necessary, add *Citric Acid* or *Sodium Citrate* to adjust to a pH of 5.0. Add a suitable flavor if desired. Add sufficient *Syrup* to bring the preparation to final volume. Shake vigorously.

ASSAY

• **PROCEDURE**

Solution A: 1.0 M monobasic potassium phosphate containing 6.3 mL/L of phosphoric acid

Solution B: Acetonitrile, 1.0 M dibasic potassium phosphate, 1.0 M monobasic potassium phosphate, 1.0 M citric acid, and water (25: 7.7: 2.3: 1: 64)

Mobile phase: Acetonitrile, *Solution A*, 1.0 M citric acid, 0.5 M sodium perchlorate, and water (36:10:2:2:50). Pass through a suitable filter of 0.7-µm or finer pore size, and degas.

Diluent: Acetonitrile and water (50:50)

System suitability solution: 0.1 mg/mL of USP Rifampin RS and 0.1 mg/mL of USP Rifampin Quinone RS in acetonitrile. Transfer 1.0 mL of the solution into a 10-mL volumetric flask, and dilute with *Solution B* to volume.

Standard solution: 0.5 mg/mL of USP Rifampin RS in *Diluent*. If necessary, sonicate for 30 s to dissolve. Transfer 5.0 mL of the solution to a 50-mL, low-actinic volumetric flask, and dilute with *Diluent* to volume. Use the solution within 1 h.

Sample solution: Transfer 5.0 mL of Oral Suspension, freshly mixed and free of air bubbles, to a 100-mL, low-actinic volumetric flask, and dissolve in and dilute with *Diluent* to volume. Transfer 5.0 mL of the resulting solution to a 50-mL, low-actinic volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 5-µm packing L7

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for rifampin quinone and rifampin are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between rifampin quinone and rifampin, *System suitability solution*

Relative standard deviation: NMT 1.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of rifampin ($C_{43}H_{58}N_4O_{12}$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Rifampin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of rifampin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **PH** ⟨791⟩: 4.5–5.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in a tight, light-resistant container. Store at controlled room temperature.

• **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded when stored at controlled room temperature

• **LABELING:** Label it to state that it is to be well shaken and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Rifampin RS

USP Rifampin Quinone RS

Rifampin and Isoniazid Capsules

» Rifampin and Isoniazid Capsules contain not less than 90.0 percent and not more than 130.0 percent of the labeled amount of rifampin ($C_{43}H_{58}N_4O_{12}$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isoniazid ($C_6H_7N_3O$).

[NOTE—Where Rifampin and Isoniazid Capsules are prescribed without reference to the quantity of rifampin or isoniazid contained therein, a product containing 300 mg of rifampin and 150 mg of isoniazid shall be dispensed.]

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩—

USP Rifampin RS

USP Isoniazid RS

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—*Test solution*—Transfer a portion of Capsule contents, equivalent to about 120 mg of rifampin, to a suitable flask, add 20 mL of methanol, and shake for several minutes. Pass this suspension through a filter having a 1-µm or finer porosity, discarding the first few mL of the filtrate. Dilute a volume of the filtrate with an equal volume of acetone, and mix. *Standard solutions*—Dissolve a quantity of USP Rifampin RS in methanol to obtain a solution containing 6 mg per mL. Add an equal volume of acetone, and mix. Dissolve a quantity of USP Isoniazid RS in methanol to obtain a solution containing 2.5 mg per mL. Add an equal volume of acetone, and mix. *Application volume:* 2 µL.

Developing solvent solution: a mixture of acetone and glacial acetic acid (100:1).

B: The retention times of the rifampin and isoniazid peaks in the chromatogram of the *Assay preparation* correspond to those of rifampin and isoniazid in the chromatogram of the *Standard preparation*, as obtained in the *Assay for rifampin and isoniazid*.

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Determine the amount of rifampin ($C_{43}H_{58}N_4O_{12}$) dissolved by employing the following method.

Phosphate buffer solution—Dissolve 15.3 g of dibasic potassium phosphate and 80.0 g of monobasic potassium phosphate into a 1-L volumetric flask, mix, dilute with water to volume, and mix.

USP Monographs

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Isoniazid standard solution—Accurately weigh about 66 mg of USP Isoniazid RS into a 100-mL volumetric flask. Dissolve in and dilute with 0.1 N hydrochloric acid to volume, and mix.

Standard stock solution—Accurately weigh about 66 mg of USP Rifampin RS into a 200-mL volumetric flask, dissolve in 10 mL of 0.1 N hydrochloric acid, and mix. Add 50.0 mL of *Isoniazid standard solution,* dilute with 0.1 N hydrochloric acid to volume, and mix. [NOTE—Prepare this solution immediately before the test is performed, and place in the dissolution bath at the start of the test.]

Standard solution—At the end of the test run, transfer a 5.0-mL aliquot of the *Standard stock solution* and 10.0 mL of *Phosphate buffer solution* to a 50-mL volumetric flask. Dilute with water to volume, and mix. [NOTE—Analyze the solution immediately, if possible, and if not, within 3 hours after final dilution.]

Test solution—At the end of the test run, withdraw a 25-mL aliquot, and filter, discarding the first 10 mL of the filtrate. Allow to cool for about 10 minutes, and transfer 5.0 mL of the filtrate and 10.0 mL of the *Phosphate buffer solution* to a 50-mL volumetric flask. Dilute with water to volume, and mix. [NOTE—Analyze the solution immediately, if possible, and if not, within 3 hours after final dilution.]

Determine the amount of rifampin ($C_{43}H_{58}N_4O_{12}$) dissolved from absorbances at the wavelength of maximum absorbance at about 475 nm of the *Standard solution* and the *Test solution.*

Determine the amount of isoniazid ($C_6H_7N_3O$) dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of water, *Phosphate buffer solution,* and methanol (850:100:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 1.5 mL per minute.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the isoniazid peaks.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{43}H_{58}N_4O_{12}$ and not less than 80% *(Q)* of the labeled amount of $C_6H_7N_3O$ are dissolved in 45 minutes.

Loss on drying ⟨731⟩—Dry about 100 mg of Capsule contents in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 3.0% of its weight.

Assay for rifampin and isoniazid—

Buffer solution—Dissolve 1.4 g of dibasic sodium phosphate in 1 L of water, and adjust with phosphoric acid to a pH of 6.8.

Solution A—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (96:4).

Solution B—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (55:45).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Rifampin RS and USP Isoniazid RS in a mixture of *Buffer solution* and methanol (96:4) to obtain a solution having known concentrations of about 0.16 mg per mL and 0.08 mg per mL, respectively.

[NOTE—Use this solution within 10 minutes.]

Assay preparation—Weigh the contents of not fewer than 10 Capsules, mix, and transfer an accurately weighed portion of the powder, equivalent to about 8 mg of isoniazid, to a 100-mL volumetric flask, and add about 90 mL of *Buffer solution.* Sonicate for about 10 minutes, allow to equilibrate to room temperature, dilute with *Buffer solution* to volume, and mix.

[NOTE—Use this solution within 2 hours.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 25-cm column that contains 5-µm base-deactivated packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–5	100	0	isocratic
5–6	100→0	0→100	linear gradient
6–15	0	100	isocratic

Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 2.6 and 1.0 for rifampin and isoniazid, respectively; the column efficiency is not less than 50,000 and not less than 6,000 theoretical plates for rifampin and isoniazid, respectively; the tailing factors are not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$) and isoniazid ($C_6H_7N_3O$) in the portion of Capsules taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Rifampin RS, calculated on the dried basis, or of USP Isoniazid RS, as appropriate, in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the corresponding analytes obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Rifampin, Isoniazid, and Pyrazinamide Tablets

» Rifampin, Isoniazid, and Pyrazinamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of rifampin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$), and pyrazinamide ($C_5H_5N_3O$).

Packaging and storage—Preserve in tight, light-resistant containers at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Isoniazid RS
USP Pyrazinamide RS
USP Rifampin RS

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—*Test solution*—Transfer an accurately weighed portion of ground Tablets, equivalent to about 120 mg of rifampin, to a suitable flask, add 20 mL of methanol, and shake for several minutes. Pass this suspension through a filter having a 1-µm

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or finer porosity, discarding the first few mL of the filtrate. Dilute a volume of the filtrate with an equal volume of acetone, and mix.

Standard solutions—Dissolve a quantity of USP Rifampin RS in methanol to obtain a solution containing 6 mg per mL. Add an equal volume of acetone, and mix. Dissolve a quantity of USP Isoniazid RS in methanol to obtain a solution containing 2.5 mg per mL. Add an equal volume of acetone, and mix. Dissolve a quantity of USP Pyrazinamide RS in methanol to obtain a solution containing 15 mg per mL. Add an equal volume of acetone, and mix.

Application volume: 2 µL.

Developing solvent system: a mixture of acetone and glacial acetic acid (100:1).

B: The retention times of the rifampin, isoniazid, and pyrazinamide peaks in the chromatogram of the *Assay preparation* correspond to those of rifampin, isoniazid, and pyrazinamide in the chromatogram of the *Standard preparation,* as obtained in the *Assay for rifampin, isoniazid, and pyrazinamide.*

Dissolution ⟨711⟩—

Medium: simulated gastric fluid TS, without pepsin; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Standard stock solution—Prepare a solution in *Medium* having known concentrations of about 0.22 mg of USP Isoniazid RS and 1.3 mg of USP Pyrazinamide RS per mL. Use this solution on the day prepared.

Intermediate standard solution—Transfer about 27 mg of USP Rifampin RS, accurately weighed, to a 200-mL volumetric flask, add 50.0 mL of the *Standard stock solution,* and swirl to dissolve. Dilute with *Medium* to volume, and mix. Place this flask into the dissolution bath immediately prior to starting the tablet dissolution. Withdraw the flask from the dissolution bath at the same time that the solutions under test are withdrawn.

Determine the amount of $C_{43}H_{58}N_4O_{12}$ dissolved by employing the following method.

Standard solution—Transfer 10.0 mL of the *Intermediate standard solution* to a 50-mL volumetric flask, dilute with *Medium* to volume, and mix.

Procedure—Transfer 10.0 mL of the filtered solution under test to a separate 50-mL volumetric flask, dilute with *Medium* to volume, and mix. Concomitantly determine the UV absorbances at 475 nm of the solution obtained and the *Standard solution,* using the *Medium* as the blank. Calculate the quantity, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$) dissolved by the formula:

$$4500C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Rifampin RS in the *Standard solution;* and A_U and A_S are the absorbances of the solution under test and the *Standard solution,* respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of rifampin ($C_{43}H_{58}N_4O_{12}$) is dissolved in 30 minutes.

Determine the amount of $C_6H_7N_3O$ and $C_5H_5N_3O$ dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of water, 1 M monobasic potassium phosphate, and acetonitrile (860:100:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution of isonicotinic acid in *Medium* containing about 0.125 mg per mL. Transfer 10 mL of this solution and 4 mL of the *Standard stock solution* to a 100-mL volumetric flask containing 15 mL of 1 M dibasic potassium phosphate and 30 mL of *Mobile phase.* Dilute with *Mobile phase* to volume, and mix.

Standard solution—Transfer 15.0 mL of the *Intermediate standard solution* to a 100-mL volumetric flask containing 15 mL of 1 M dibasic potassium phosphate and 30 mL of *Mobile phase.* Dilute with *Mobile phase* to volume, and mix. This solution may be used for 20 hours.

Test solution—Withdraw 60 mL of the solution under test, and filter, discarding the first 20 mL of the filtrate. Centrifuge the filtrate for 5 minutes. Transfer 15.0 mL of this solution to a 100-mL volumetric flask containing 15 mL of 1 M dibasic potassium phosphate and 30 mL of *Mobile phase.* Dilute with *Mobile phase* to volume, and mix. This solution may be used for 20 hours.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L44. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for isonicotinic acid, 1.0 for pyrazinamide, and 1.8 for isoniazid; and the resolution, *R,* between isonicotinic acid and pyrazinamide is not less than 2.5 and between pyrazinamide and isoniazid not less than 4.0. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviations determined from the pyrazinamide and isoniazid responses for replicate injections are not more than 1.5%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of isoniazid ($C_6H_7N_3O$) dissolved by the formula:

$$6000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Isoniazid RS in the *Standard solution;* and r_U and r_S are the isoniazid peak areas obtained from the *Test solution* and the *Standard solution,* respectively. Calculate the quantity, in mg, of pyrazinamide ($C_5H_5N_3O$) dissolved by the same formula, except to read "USP Pyrazinamide RS" where "USP Isoniazid RS" is specified, and "pyrazinamide" where "isoniazid" is specified.

Tolerances—Not less than 80% *(Q)* of the labeled amount of isoniazid ($C_6H_7N_3O$) and not less than 75% of the labeled amount of pyrazinamide ($C_5H_5N_3O$) are dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Loss on drying—Dry about 100 mg of powdered Tablets, accurately weighed, in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 3.0% of its weight.

Assay for rifampin, isoniazid, and pyrazinamide—

Buffer solution—Dissolve 1.4 g of dibasic sodium phosphate in 1 L of water, and adjust with phosphoric acid to a pH of 6.8.

Solution A—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (96:4).

Solution B—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (55:45).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Rifampin RS, USP Isoniazid RS, and USP Pyrazinamide RS in a mixture of *Buffer solution* and methanol (96:4) to obtain a solution having known concentrations of

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about 0.16 mg per mL, 0.08 mg per mL, and 0.43 mg per mL, respectively.

[NOTE—Use this solution within 10 minutes.]

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 8 mg of isoniazid, to a 100-mL volumetric flask, and add about 90 mL of *Buffer solution*. Sonicate for about 10 minutes, allow to equilibrate to room temperature, dilute with *Buffer solution* to volume, and mix.

[NOTE—Use this solution within 2 hours.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 25-cm column that contains 5-µm base-deactivated packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–5	100	0	isocratic
5–6	100→0	0→100	linear gradient
6–15	0	100	isocratic

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 1.8, 0.7, and 1.0 for rifampin, isoniazid, and pyrazinamide, respectively; the resolution, *R*, between isoniazid and pyrazinamide is not less than 4; the column efficiency is not less than 50,000, not less than 6,000, and not less than 10,000 theoretical plates for rifampin, isoniazid, and pyrazinamide, respectively; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantities, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$), and pyrazinamide ($C_5H_5N_3O$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Rifampin RS, calculated on the dried basis, or of USP Isoniazid RS or of USP Pyrazinamide RS, as appropriate, in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the corresponding analytes obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Rifampin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride Tablets

» Rifampin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of rifampin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$), pyrazinamide ($C_5H_5N_3O$), and ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$).

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Ethambutol Hydrochloride RS
USP Isoniazid RS
USP Pyrazinamide RS
USP Rifampin RS

Identification—

A: The retention times of the rifampin, isoniazid, and pyrazinamide peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay for rifampin, isoniazid, and pyrazinamide.*

B: The retention time of the ethambutol peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for ethambutol hydrochloride.*

Dissolution ⟨711⟩—

Medium: 10 mM pH 6.8 sodium phosphate buffer, prepared by dissolving 7 g of anhydrous dibasic sodium phosphate in 5 L of water, and adjusting with phosphoric acid to a pH of 6.8; 900 mL.

Apparatus 2: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amounts of rifampin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$), pyrazinamide ($C_5H_5N_3O$), and ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) dissolved using filtered portions of the solution under test and by employing the procedures set forth in the *Assay for rifampin, isoniazid, and pyrazinamide* and the *Assay for ethambutol hydrochloride.*

Tolerances—Not less than 75% (*Q*) of the labeled amounts of $C_{43}H_{58}N_4O_{12}$, $C_6H_7N_3O$, $C_5H_5N_3O$, and $C_{10}H_{24}N_2O_2 \cdot 2HCl$ is dissolved in 45 minutes.

Loss on drying—Dry about 100 mg of powdered Tablets in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 3.0% of its weight.

Assay for rifampin, isoniazid, and pyrazinamide—

Buffer solution—Dissolve 1.4 g of anhydrous dibasic sodium phosphate in 1 L of water, and adjust with phosphoric acid to a pH of 6.8.

Solution A—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (96:4).

Solution B—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (55:45).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Rifampin RS, USP Isoniazid RS, and USP Pyrazinamide RS in a mixture of *Buffer solution* and methanol (96:4) to obtain a solution having known concentrations of about 0.16 mg per mL, 0.08 mg per mL, and 0.43 mg per mL, respectively. [NOTE—Use this solution within 10 minutes.]

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 8 mg of isoniazid, to a 100-mL volumetric flask, and add about 90 mL of *Buffer solution.* Sonicate for about 10 minutes, allow to equilibrate to room temperature, dilute with *Buffer solution* to volume, and mix. [NOTE—Use this solution within 2 hours.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 25-cm column that contains a 5-µm base-deactivated packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

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Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–5	100	0	isocratic
5–6	100→0	0→100	linear gradient
6–15	0	100	isocratic

Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times for rifampin, isoniazid, and pyrazinamide are about 1.8, 0.7, and 1.0, respectively; the resolution, *R,* between isoniazid and pyrazinamide is not less than 4; the column efficiencies, determined from the rifampin, isoniazid, and pyrazinamide peaks are not less than 50,000 theoretical plates, 6000 theoretical plates, and 10,000 theoretical plates, respectively; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantities, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$), and pyrazinamide ($C_5H_5N_3O$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation;* and r_U and r_S are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for ethambutol hydrochloride—

Diluent—Dissolve 1.4 g of anhydrous dibasic sodium phosphate in 1 L of water, and adjust with phosphoric acid to a pH of 6.8.

Buffer solution—Mix 1.0 mL of triethylamine and 1 L of water, and adjust with phosphoric acid to a pH of 7.0.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ethambutol Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 30 mg of ethambutol hydrochloride, to a 100-mL volumetric flask, and add about 90 mL of *Diluent.* Sonicate for about 10 minutes, allow to equilibrate to room temperature, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter, discarding the first 10 mL of the filtrate.

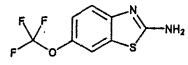
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 15-cm column that contains a 5-µm base-deactivated packing L10. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ethambutol Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the ethambutol peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Riluzole



$C_8H_5F_3N_2OS$ 234.20

2-Benzothiazolamine, 6-(trifluoromethoxy)-;
2-Amino-6-(trifluoromethoxy)benzothiazole [1744-22-5].

DEFINITION

Riluzole contains NLT 98.0% and NMT 102.0% of the labeled amount of $C_8H_5F_3N_2OS$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (9:11)

Standard solution: 0.05 mg/mL of USP Riluzole RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Riluzole in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 221 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 2.0 mL/min

Injection size: 20 µL. [NOTE—Monitor for 3 times the retention time of riluzole.]

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_8H_5F_3N_2OS$ in the portion of Riluzole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Riluzole RS in the *Standard solution* (mg/mL)

C_U = concentration of Riluzole in the *Sample solution* (mg/mL)

USP Monographs

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Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%, a 1.5-g of sample used

ORGANIC IMPURITIES

• **Procedure**

Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: 500 µg/mL of USP Riluzole RS and 0.5 µg/mL USP Riluzole Related Compound A RS in *Mobile phase*

Standard solution: 0.5 µg/mL of USP Riluzole RS in *Mobile phase*

Sample solution: 500 µg/mL of Riluzole in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, and use an injection volume of 100 µL. [NOTE—Monitor for 11 times the retention time of riluzole.]

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

Resolution: NLT 1.5 between Riluzole and riluzole related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the impurities using the relative retention times shown in *Impurity Table 1*. Calculate the percentage of each impurity in the portion of Riluzole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution*

r_S = peak response of Riluzole from the *Standard solution*

C_S = concentration of USP Riluzole RS in the *Standard solution* (µg/mL)

C_U = concentration of Riluzole in the *Sample solution* (µg/mL)

F = relative response factor relative to riluzole

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Riluzole	1.0	—	—
Riluzole related compound A[a]	1.3	0.16	0.1
Bromoriluzole[b]	1.9	0.54	0.2
Dibromotrifluoromethoxyaniline[c]	7.4	0.28	0.1
Any unspecified impurity	—	1.0	0.1

[a] 4-(Trifluoromethoxy)aniline.
[b] 4-Bromo-6-(trifluoromethoxy)benzothiazol-2-amine.
[c] 2,6-Dibromo-4-(trifluoromethoxy)aniline.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Riluzole RS

USP Riluzole Related Compound A RS

4-Trifluoromethoxyphenylamine; 4-trifluoromethoxyanaline.

$C_7H_6F_3NO$ 177.12

Riluzole Tablets

DEFINITION

Riluzole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of riluzole ($C_8H_5F_3N_2OS$).

IDENTIFICATION

• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (45:55)

Standard solution: 0.05 mg/mL of USP Riluzole RS in *Mobile phase*

Sample stock solution: Nominally 0.5 mg/mL of riluzole prepared as follows. Transfer an equivalent to 50 mg of riluzole, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, sonicate for about 10 min, and stir for another 10 min. Dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.05 mg/mL of riluzole in *Mobile phase*, prepared from the *Sample stock solution*. Pass this solution through a filter of 0.45-µm pore size, and discard the first 5 mL of filtrate. Use the filtrate for analysis. [NOTE—A PVDF or equivalent filter may be suitable.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 221 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of the riluzole peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of riluzole ($C_8H_5F_3N_2OS$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Riluzole RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of riluzole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid VS; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: 0.05 mg/mL of USP Riluzole RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: 254 nm
Cell: 0.5 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of riluzole ($C_8H_5F_3N_2OS$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S \times V/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Riluzole RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of riluzole ($C_8H_5F_3N_2OS$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Sample stock solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 500 µg/mL of USP Riluzole RS and 0.5 µg/mL of USP Riluzole Related Compound A RS in *Mobile phase*
Standard solution: 2.5 µg/mL of USP Riluzole RS in *Mobile phase*, prepared from the *Standard solution* in the *Assay*
Sample solution: Prepare as directed in the *Sample stock solution*.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between riluzole and riluzole related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of riluzole from the *Standard solution*
C_S = concentration of USP Riluzole RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of riluzole in the *Sample solution* (µg/mL)

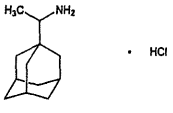
Acceptance criteria
Any individual degradation product: NMT 0.2%
Total degradation products: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Riluzole RS
USP Riluzole Related Compound A RS
4-Trifluoromethoxyaniline.
$C_7H_6F_3NO$ 177.12

Rimantadine Hydrochloride



$C_{12}H_{21}N \cdot HCl$ 215.76
Tricyclo[3.3.1.1^{3,7}]-decane-1-methanamine, α-methyl-, hydrochloride.
α-Methyl-1-adamantanemethylamine hydrochloride [1501-84-4].

» Rimantadine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{12}H_{21}N \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, and store between 15° to 30°.
USP Reference standards ⟨11⟩—
USP Rimantadine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the rimantadine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

X-ray diffraction ⟨941⟩—The X-ray diffraction pattern conforms to that of USP Rimantadine Hydrochloride RS, similarly determined.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.2%.

Ordinary impurities ⟨466⟩—
Test solution—Transfer 100 mg of Rimantadine Hydrochloride to a 10-mL centrifuge tube, add 2 mL of 1 N sodium hydroxide, and mix. Add 2 mL of chloroform, and mix on a vortex mixer for 1 minute. Allow the layers to separate, and apply 10 µL of the organic layer.

USP Monographs

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Standard solution—Proceed as directed for the *Test solution,* using USP Rimantadine Hydrochloride RS in place of the test specimen.

Eluant: a mixture of ethyl acetate, methanol, and ammonium hydroxide (80:10:4).

Procedure—Use a low-actinic glass tank. Dry the plate in a stream of hot air, then heat in an oven at 105° for 30 minutes. Allow the plate to cool to room temperature.

Visualization—Place the plate in an atmosphere of chlorine, prepared by mixing 1.5% potassium permanganate solution and diluted hydrochloric acid (1:1), for about 90 minutes. Allow to air-dry for 60 minutes, and follow with visualization technique 20.

Limit of toluene—

Standard solution—Transfer 10 µL of toluene to a 100-mL volumetric flask, dilute with chloroform to volume, and mix.

Test solution—Transfer about 750 mg of Rimantadine Hydrochloride, accurately weighed, to a 10-mL volumetric flask, dilute with chloroform to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 2-m column that contains 80- to 100-mesh support S1A. The column temperature is maintained at about 200°, and nitrogen is used as the carrier gas. The injection port and detector temperatures are maintained at about 250°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5 for toluene; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for 9 minutes, and measure the responses for the toluene peaks. Calculate the percentage of toluene in the portion of Rimantadine Hydrochloride taken by the formula:

$$0.867(100/W_U)(r_U/r_S)$$

in which 0.867 is the specific gravity of toluene; W_U is the weight, in mg, of Rimantadine Hydrochloride taken to prepare the *Test solution;* and r_U and r_S are the toluene peak responses obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.1% is found.

Assay—

Internal standard solution—Transfer about 400 mg of *n*-eicosane to a 250-mL volumetric flask, dilute with hexane to volume, and mix.

Standard preparation—Transfer about 40 mg of USP Rimantadine Hydrochloride RS, accurately weighed, to a 50-mL centrifuge tube, add 15 mL of 1 N sodium hydroxide, and mix. Add 25.0 mL of *Internal standard solution,* and shake by mechanical means for about 15 minutes. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear filtrate.

Assay preparation—Using about 40 mg of Rimantadine Hydrochloride, accurately weighed, proceed as directed for *Standard preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 1.8-m glass column that is packed with 3% phase G19 on 100- to 200-mesh support S1A. The column temperature is maintained at about 160°, and the injection port and detector temperatures are maintained at about 250°. Nitrogen is used as the carrier gas. Adjust the carrier flow rate and temperature so that the *n*-eicosane elutes at about 8 minutes. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0 for rimantadine; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{12}H_{21}N \cdot HCl$ in the portion of Rimantadine Hydrochloride taken by the formula:

$$25C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Rimantadine Hydrochloride in the *Standard preparation;* and R_U and R_S are the ratios of the rimantadine peak response to the *n*-eicosane peak response obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Rimantadine Hydrochloride Tablets

» Rimantadine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of rimantadine hydrochloride ($C_{12}H_{21}N \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers, and store between 15° to 30°.

USP Reference standards ⟨11⟩—
USP Rimantadine Hydrochloride RS

Identification—

A: The retention time of the rimantadine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

B: [**CAUTION**—Avoid contact with o-tolidine when performing this test, and conduct the test in a well-ventilated hood.] Weigh and finely powder not fewer than 5 Tablets. Transfer a portion of the powder, equivalent to 100 mg of riman tadine hydrochloride, to a 10-mL centrifuge tube, add 2 mL of 1 N sodium hydroxide, and mix. Add 2 mL of chloroform, and mix on a vortex mixer for 1 minute. Allow the layers to separate, and use the organic layer as the test solution. Separately apply 10 µL of the test solution and 10 µL of a Standard solution of USP Rimantadine Hydrochloride RS, similarly prepared, to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in a low-actinic glass chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (80:10:4) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, dry it in a stream of hot air, and then heat in an oven at 105° for 30 minutes. Allow the plate to cool to room temperature. Place the dried plate in an atmosphere of chlorine, prepared from a mixture of 1.5% potassium permanganate solution and 3 N hydrochloric acid (1:1), for about 90 minutes. Remove the plate, and allow it to air-dry for 60 minutes. Prepare a spray reagent as follows. Dissolve 160 mg of *o*-tolidine in 30 mL of glacial acetic acid, dilute with water to 500 mL, add 1 g of potassium iodide, and mix until the potassium iodide is dissolved. Locate the spots on the plate by spraying with the spray reagent: the R_F value of the principal

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spot in the chromatogram of the test solution corresponds to that of the principal spot obtained from the Standard solution.
Uniformity of dosage units ⟨905⟩: meet the requirements.

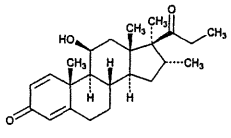
Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{12}H_{21}N \cdot HCl$ dissolved, employing the procedure set forth in the *Assay*.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{12}H_{21}N \cdot HCl$ is dissolved in 30 minutes.

Assay—
Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Rimantadine Hydrochloride.*
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of rimantadine hydrochloride, to a 50-mL centrifuge tube, add 15 mL of 1 N sodium hydroxide, and mix. Add 25.0 mL of *Internal standard solution*, and shake by mechanical means for about 15 minutes. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear filtrate as the *Assay preparation.*
Procedure—Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of rimantadine hydrochloride ($C_{12}H_{21}N \cdot HCl$) in the portion of Tablets taken by the formula:

$$25C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Rimantadine Hydrochloride RS in the *Standard preparation;* and R_U and R_S are the ratios of the rimantadine peak response to the *n*-eicosane peak response obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Rimexolone



$C_{24}H_{34}O_3$ 370.52
Androsta-1,4-diene-3-one, 11-hydroxy-16,17-dimethyl-17-(1-oxopropyl)-, (11β,16α,17β)-;
11β-Hydroxy-16α,17α-dimethyl-17-propionylandrosta-1,4-diene-3-one [49697-38-3].

DEFINITION
Rimexolone contains NLT 97.0% and NMT 102.0% of rimexolone ($C_{24}H_{34}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. THIN-LAYER CHROMATOGRAPHY**
  Standard solution: 10 mg/mL of USP Rimexolone RS in chloroform
  Sample solution: 10 mg/mL of rimexolone in chloroform
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
  Adsorbent: 0.25-mm layer of silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Chloroform and methanol (19:1)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Apply the samples to the thin-layer chromatographic plate. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Observe the plate under short-wavelength UV light.
  Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the principal spot of the *Standard solution.*

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile and water (6:4)
  Standard solution: 0.2 mg/mL of USP Rimexolone RS, prepared by dissolving a suitable quantity in methanol and diluting with *Mobile phase* to volume
  Sample stock solution: 1 mg/mL of Rimexolone in methanol
  Sample solution: 0.2 mg/mL of Rimexolone diluted with *Mobile phase* from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 242 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Capacity factor, *k'*: NLT 1.5
  Column efficiency: NLT 3000 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of rimexolone ($C_{24}H_{34}O_3$) in the portion of Rimexolone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rimexolone RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Mobile phase: Prepare as directed in the *Assay.*
  Standard solution: Use the *Standard solution* prepared in the *Assay.*
  Sample stock solution: 1 mg/mL of Rimexolone in methanol
  Sample solution: 0.2 mg/mL of Rimexolone in *Mobile phase* from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC

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Detector: UV 242 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, *k'*: NLT 1.5
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Rimexolone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria
Individual impurities: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL in chloroform
Acceptance criteria: +47° to +54°
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample under vacuum at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Rimexolone RS

Rimexolone Ophthalmic Suspension

DEFINITION
Rimexolone Ophthalmic Suspension is a sterile suspension of Rimexolone in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of rimexolone ($C_{24}H_{34}O_3$). It may contain suitable stabilizers, buffers, and antimicrobial agents.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Mobile phase: Acetonitrile and water (60:40)
Standard stock solution: 1 mg/mL of USP Rimexolone RS in methanol
Standard solution: 0.2 mg/mL of USP Rimexolone RS in *Mobile phase* from *Standard stock solution*
Sample stock solution: Nominally 1 mg/mL of rimexolone, prepared as follows. Transfer an amount nominally equivalent to 25 mg of rimexolone from a portion of Ophthalmic Suspension to a 25-mL volumetric flask. Dilute with methanol to volume and sonicate for 2 min.
Sample solution: Nominally 0.2 mg/mL of rimexolone from the *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, *k'*: NLT 1.5
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rimexolone ($C_{24}H_{34}O_3$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rimexolone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of rimexolone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **pH** ⟨791⟩**:** 6.0–8.0
- **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩
Analysis: Equip a cone-and-plate rheometer[1] following *Method III*. The shear rate under the test condition is 11.5 s^{-1} and temperature is 25°.
Acceptance criteria: 15–200 mPa · s

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Rimexolone RS

Ringer's Injection

DEFINITION
Ringer's Injection is a sterile solution of Sodium Chloride, Potassium Chloride, and Calcium Chloride in Water for Injection. It contains, in each 100 mL, NLT 323.0 mg and NMT 354.0 mg of sodium (Na) [equivalent to NLT 820.0 mg and NMT 900.0 mg of sodium chloride (NaCl)]; NLT 14.9 mg and NMT 16.5 mg of potassium (K) [equivalent to NLT 28.5 mg and NMT 31.5 mg of potassium chloride (KCl)] ; NLT 8.20 mg and NMT 9.80 mg of calcium (Ca) [equivalent to NLT 30.0 mg and NMT 36.0 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)]; and NLT 523.0 mg and NMT 580.0 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)]. It contains no antimicrobial agents.

[1] Brookfield cone-and-plate rheometer, with spindle CP-42 (#42), is operated at 3 rpm. Any other equivalent rheometer is suitable as well.

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[NOTE—The calcium, chloride, potassium, and sodium ion contents of Ringer's Injection are approximately 4.5, 156, 4, and 147.5 mEq/L, respectively.]

Sodium Chloride	8.6 g
Potassium Chloride	0.3 g
Calcium Chloride	0.33 g
Water for Injection, a sufficient quantity to make	1000 mL

Dissolve the three salts in the *Water for Injection*, filter until clear, place in suitable containers, and sterilize.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests*, *Calcium* and *Chloride*: Meets the requirements of test *A* for *Calcium*, and test *A* for *Chloride*
- **B. SODIUM:** A sample imparts an intense yellow color to a nonluminous flame.
- **C. POTASSIUM:** A sample imparts a violet color to a nonluminous flame. The presence of small quantities of sodium masks the color unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks the emission at 589 nm (sodium). It is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

ASSAY

- **CALCIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Lanthanum chloride solution: Transfer 17.69 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium (Ca) prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of diluted hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 20.0 mL of Injection, equivalent to 1.8 mg of calcium (Ca), to a 100-mL volumetric flask containing 5.0 mL of *Lanthanum chloride solution*. Dilute with water to volume.

Blank: Transfer 5.0 mL of *Lanthanum chloride solution* to a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Emission wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration (*C*), in mg/mL, of calcium in the *Sample solution*.

Calculate the quantity (mg) of calcium (Ca) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution*, as determined from the graph (mg/mL)
D = dilution factor of the *Sample solution*, 5
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 8.20–9.80 mg of calcium (Ca) in each 100 mL

- **POTASSIUM**

Solution A: Suitable nonionic wetting agent (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride in water

Standard stock solution: 100 µg/mL of potassium prepared as follows. Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 h, in 50 mL of water, transfer to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.01, 0.015, and 0.020 mg/mL of potassium prepared as follows. Transfer 10 mL of *Solution B* to each of four 100-mL volumetric flasks containing 10.0 mL of *Solution A*. To each flask add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, respectively, and dilute with water to volume.

Sample solution: Transfer 10 mL of Injection to a 100-mL volumetric flask. Add 10.0 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution B* to a 100-mL volumetric flask containing 10.0 mL of *Solution A*. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 766 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance at a wavelength of 766 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium. Read the percentage transmittance of the *Sample solution*. Calculate the quantity (mg) of potassium (K) in each 100 mL of Injection.

Acceptance criteria: 14.9–16.5 mg of potassium (K) in each 100 mL

- **SODIUM**

Solution A: Suitable nonionic wetting agent (1 in 500)

Standard stock solution: 100 µg/mL of sodium in water prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium prepared as follows. Transfer 10 mL of *Solution A* to each of four 100-mL volumetric flasks. To each flask add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, respectively, and dilute with water to volume.

Sample solution: Transfer 5 mL of Injection into a 1-L volumetric flask containing 100 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

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Instrumental conditions
Mode: Atomic emission spectrophotometry
Emission wavelength: 589 nm
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Set the flame photometer for maximum transmittance at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium. Read the percentage transmittance of the *Sample solution*. Calculate the quantity (mg) of sodium (Na) in each 100 mL of Injection.
Acceptance criteria: 323.0–354.0 mg of sodium (Na) in each 100 mL

- **CHLORIDE**

Sample solution: Transfer 10 mL of Injection into a conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Titrate, with shaking, with *Titrant* to a pink endpoint. Each mL of *Titrant* is equivalent to 3.545 mg of chloride (Cl). Calculate the quantity (mg) of chloride (Cl) in each 100 mL of Injection.
Acceptance criteria: 523.0–580.0 mg of chloride (Cl) in each 100 mL

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.5 USP Endotoxin Units/mL
- **pH** ⟨791⟩: 5.0–7.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Ringer's and Dextrose Injection

DEFINITION

Ringer's and Dextrose Injection is a sterile solution of Sodium Chloride, Potassium Chloride, Calcium Chloride, and Dextrose in Water for Injection. It contains, in each 100 mL, NLT 323.0 mg and NMT 354.0 mg of sodium (Na) [equivalent to NLT 820.0 mg and NMT 900.0 mg of sodium chloride (NaCl)], NLT 14.9 mg and NMT 16.5 mg of potassium (K) [equivalent to NLT 28.5 mg and NMT 31.5 mg of potassium chloride (KCl)], NLT 8.20 mg and NMT 9.80 mg of calcium (Ca) [equivalent to NLT 30.0 mg and NMT 36.0 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)], and NLT 523.0 mg and NMT 608.5 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)]. It contains NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.

[NOTE—The potassium, calcium, sodium, and chloride ion contents of Ringer's and Dextrose Injection are approximately 4, 4.5, 147.5, and 156 mEq/L, respectively.]

IDENTIFICATION

- **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water
Analysis: Add a few drops of *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩: Meets the requirements of test *A* under *Calcium*, and test *A* under *Chloride*.
- **C. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **D. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium).
[NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

ASSAY

- **CALCIUM**

[NOTE—Concentrations of the *Standard solution* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]
Lanthanum chloride solution: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.
Calcium stock solution: 1 mg/mL of calcium (Ca) prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of diluted hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.
Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.
Sample solution: Transfer 20.0 mL of Injection to a 100-mL volumetric flask containing 5.0 mL of *Lanthanum chloride solution*. Dilute with water to volume.
Blank: Transfer 5.0 mL of *Lanthanum chloride solution* to a 100-mL volumetric flask and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Calcium emission line at 422.7 nm
Lamp: Calcium hollow-cathode
Flame: Air–acetylene
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (*C*), in mg/mL, of calcium in the *Sample solution*.

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Calculate the quantity (mg) of calcium (Ca) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution*, as determined from the graph (mg/mL)
D = dilution factor of the *Sample solution*, 5
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 8.20–9.80 mg of calcium (Ca) in each 100 mL

• **CHLORIDE**

Sample solution: Transfer 10 mL of Injection into a conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint. Calculate the amount, in mg, of chloride (Cl) in each 100 mL of Injection taken:

$$\text{Result} = V \times N \times D \times F$$

V = volume of *Titrant* consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
D = dilution factor of the *Sample solution*, 10
F = equivalency factor, 35.45 mg/mEq

Acceptance criteria: 523.0–608.5 mg of chloride (Cl) in each 100 mL

• **DEXTROSE**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

• **POTASSIUM**

Solution A: Suitable nonionic wetting agent (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride in water

Standard stock solution: 100 µg/mL of potassium in water prepared as follows. Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium prepared as follows. Transfer 10 mL of *Solution B* to each of four 100-mL volumetric flasks containing 10.0 mL of *Solution A*. To each flask add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, respectively, and dilute with water to volume.

Sample solution: Transfer 10 mL of Injection to a 100-mL volumetric flask. Add 10.0 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution B* to a 100-mL volumetric flask containing 10.0 mL of *Solution A*. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 766 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set a suitable flame photometer for maximum transmittance at a wavelength of 766 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium. From the graph so obtained, read the percentage transmittance of the *Sample solution*.

Calculate the quantity (mg) of potassium (K) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of potassium in the *Sample solution*, as determined from the graph (mg/mL)
D = dilution factor of the *Sample solution*, 10
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 14.9–16.5 mg of potassium (K) in each 100 mL

• **SODIUM**

Solution A: Suitable nonionic wetting agent (1 in 500)

Standard stock solution: 100 µg/mL of sodium in water prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium prepared as follows. Transfer 10 mL of *Solution A* to each of four 100-mL volumetric flasks. To each flask add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, respectively, and dilute with water to volume.

Sample solution: Transfer 5 mL of Injection to a 1-L volumetric flask containing 100 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Atomic emission spectrophotometry

Emission wavelength: 589 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set a suitable flame photometer for maximum transmittance at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the

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percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium. From the graph so obtained, read the percentage transmittance of the *Sample solution*.

Calculate the quantity (mg) of sodium (Na) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of sodium in the *Sample solution*, as determined from the graph (mg/mL)
D = dilution factor of the *Sample solution*, 200
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 323.0–354.0 mg of sodium (Na) in each 100 mL

IMPURITIES

- **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from Injection in water

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Determine the absorbance of the *Sample solution* with a suitable spectrophotometer.

Acceptance criteria: The absorbance is NMT 0.25

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
- **pH** ⟨791⟩: 3.5–6.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Lactated Ringer's Injection

DEFINITION

Lactated Ringer's Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, and Sodium Lactate in Water for Injection. It contains, in each 100 mL, NLT 285.0 and NMT 315.0 mg of sodium [as sodium chloride (NaCl) and sodium lactate ($C_3H_5NaO_3$)], NLT 14.2 and NMT 17.3 mg of potassium (K) [equivalent to NLT 27.0 and NMT 33.0 mg of potassium chloride (KCl)], NLT 4.90 and NMT 6.00 mg of calcium (Ca) [equivalent to NLT 18.0 and NMT 22.0 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)], NLT 368.0 and NMT 428.0 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)], and NLT 231.0 and NMT 261.0 mg of lactate ($C_3H_5O_3$) [equivalent to NLT 290.0 and NMT 330.0 mg of sodium lactate ($C_3H_5NaO_3$)]. It contains no antimicrobial agents.

[NOTE—The calcium, potassium, and sodium contents of Lactated Ringer's Injection are approximately 2.7, 4, and 130 mEq/L, respectively.]

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩: Meets the requirements of test *A* under *Chloride* and test *A* under *Calcium*
- **B. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **C. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **D.** The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.

ASSAY

- **CALCIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: Transfer 17.69 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, carefully add 50 mL of hydrochloric acid, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask and add 10 mL of water. Carefully add 5 mL of dilute hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca), prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Dilute with water, 20.0 mL of Injection, equivalent to 1 mg of calcium (Ca), in a 100-mL volumetric flask containing 5.0 mL of *Solution A* to volume, and mix.

Blank: 5.0 mL of *Solution A* diluted with water to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometer

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Concomitantly determine the absorbance of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus concentration in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From this graph, determine the concentration (C) in mg/mL, of calcium in the *Sample solution*.

Calculate the quantity of calcium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

USP Monographs

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C = concentration of calcium in the *Sample solution* (mg/mL)
D = dilution factor of the *Sample solution*, 5
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 4.90–6.00 mg of calcium per 100 mL of Injection

• **POTASSIUM**

Solution A: Suitable nonionic wetting solution (1 in 500)
Solution B: 10.93 mg/mL of sodium chloride in water
Standard stock solution: 0.100 mg/mL of potassium, prepared as follows. Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1-L volumetric flask and dilute with water to volume.
Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* and 10 mL of *Solution B*, add 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with water to volume.
Sample solution: Pipet 10 mL of Injection to a 100-mL volumetric flask containing 10 mL of *Solution A*, dilute with water to volume, and mix.
Blank: Transfer 10.0 mL of *Solution A* and 10 mL of *Solution B* to a 100-mL volumetric flask. Dilute with water to volume.
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: Maximum transmittance at 766 nm
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of potassium. Draw the straight line best fitting the four plotted points. From this graph, calculate the concentration of potassium in the *Sample solution*.
Calculate the quantity, in mg, of potassium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of potassium in the *Sample solution* (mg/mL)
D = *Sample solution* dilution factor, 10
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 14.2–17.3 mg of potassium per 100 mL of Injection

• **SODIUM**

Solution A: Suitable nonionic wetting solution (1 in 500)
Standard stock solution: 0.100 mg/mL of sodium, prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer this solution to a 1-L volumetric flask and dilute with water to volume.
Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.
Sample solution: Transfer 5 mL of Injection in a 1-L volumetric flask containing 100.0 mL of *Solution A* and dilute with water to volume.
Blank: Transfer 10.0 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: Maximum transmittance at 589 nm
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus concentration, in mg/mL, of sodium, and draw the straight line best fitting the four plotted points. From this graph, calculate the concentration of sodium in the *Sample solution*.
Calculate the quantity of sodium in each 100 mL of Injection:

$$\text{Result} = C \times D \times F$$

C = concentration of sodium in the *Sample solution* (mg/mL)
D = dilution factor of the *Sample solution*, 200
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 285.0–315.0 mg of sodium per 100 mL of Injection

• **CHLORIDE**

Sample solution: Transfer 10 mL of Injection into a conical flask, and add 10 mL of glacial acetic acid and 75 mL of methanol.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Titrate the *Sample solution*, with shaking, with 0.1 N silver nitrate VS to a pink endpoint using 3 drops of eosin Y TS, as an indicator.
Calculate the labeled amount of chloride (Cl) in 100 mL of Injection:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

Analysis: Titrate the *Sample solution*, with shaking, with 0.1 N silver nitrate VS to a pink endpoint using 3 drops of eosin Y TS, as an indicator. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).
Acceptance criteria: 368.0–428.0 mg of chloride per 100 mL of Injection

• **LACTATE**

Mobile phase: Dicyclohexylamine, formic acid, and water (1:1:998)

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System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water
Standard solution: 3 mg/mL of USP Sodium Lactate RS in water
Sample solution: Use undiluted Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between the acetate and lactate peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of lactate ($C_3H_5O_3$) in each 100 mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of lactate, 89.07
M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

Acceptance criteria: 231.0–261.0 mg of lactate ($C_3H_5O_3$) per 100 mL of Injection

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
- **pH** ⟨791⟩: 6.0–7.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label also includes the warning: "Not for use in the treatment of lactic acidosis".
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Sodium Lactate RS

Lactated Ringer's and Dextrose Injection

DEFINITION
Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, Sodium Lactate, and Dextrose in Water for Injection. It contains, in each 100 mL, NLT 285.0 and NMT 315.0 mg of sodium (Na) [as sodium chloride (NaCl) and sodium lactate ($C_3H_5NaO_3$)], NLT 14.2 and NMT 17.3 mg of potassium (K) [equivalent to NLT 27.0 and NMT 33.0 mg of potassium chloride (KCl)], NLT 4.90 and NMT 6.00 mg of calcium (Ca) [equivalent to NLT 18.0 and NMT 22.0 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)], NLT 368.0 and NMT 428.0 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)], and NLT 231.0 and NMT 261.0 mg of lactate ($C_3H_5O_3$) [equivalent to NLT 290.0 mg and NMT 330.0 mg of sodium lactate ($C_3H_5NaO_3$)]. It contains NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.
[NOTE—The calcium, potassium, and sodium contents of Lactated Ringer's and Dextrose Injection are approximately 2.7, 4, and 130 mEq/L, respectively.]

IDENTIFICATION
- **A.**
Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection
Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A copious red precipitate of cuprous oxide is formed.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩: Meets the requirements of the test *A under Chloride* and the test *A under Calcium*
- **C. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium).
[NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **D. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **E.** The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.

ASSAY
- **CALCIUM**
[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]
Solution A: Transfer 17.69 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, carefully add 50 mL of hydrochloric acid, and allow to cool. Dilute with water to volume.
Calcium stock solution: 1 mg/mL of calcium, prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask and add 10 mL of water. Carefully add 5 mL of dilute hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.
Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca), prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.
Sample solution: Transfer 25.0 mL of Injection (equivalent to about 1.4 mg of calcium, to a 100-mL volumetric flask containing 5.0 mL of *Solution A*. Dilute with water to volume and mix.
Blank: 5.0 mL of *Solution A* diluted with water to 100.0 mL
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

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Mode: Atomic absorption spectrophotometer
Analytical wavelength: Calcium emission line at 422.7 nm
Lamp: Calcium hollow-cathode
Flame: Air–acetylene
Blank: *Blank*

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From this graph, calculate the concentration of calcium in the *Sample solution*.

Calculate the quantity of calcium in each 100 mL of the Injection:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution* (mg/mL)
D = dilution factor of the *Sample solution*, 4
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 4.90–6.00 mg of calcium per 100 mL of Injection

• **POTASSIUM**

Solution A: Suitable nonionic wetting solution (1 in 500)
Solution B: 10.93 mg/mL of sodium chloride in water
Standard stock solution: 0.100 mg/mL of potassium, prepared as follows. Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1-L volumetric flask and dilute with water to volume.
Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* and 10 mL of *Solution B*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.
Sample solution: Pipet 10 mL of Injection to a 100-mL volumetric flask containing 10 mL of *Solution A*, dilute with water to volume, and mix.
Blank: Transfer 10.0 mL of *Solution A* and 10 mL of *Solution B* to a 100-mL volumetric flask. Dilute with water to volume.
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: Maximum transmittance at 766 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of potassium. Draw the straight line best fitting the four plotted points. From this graph, calculate the concentration of potassium in the *Sample solution*.

Calculate the quantity of potassium, in mg, in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of potassium in the *Sample solution* (mg/mL)
D = dilution factor of the *Sample solution*, 10
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 14.2–17.3 mg of potassium per 100 mL of Injection

• **SODIUM**

Solution A: Suitable nonionic wetting solution (1 in 500)
Standard stock solution: 0.100 mg/mL of sodium, prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer this solution to a 1-L volumetric flask and dilute with water to volume.
Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.
Sample solution: Pipet 5 mL of Injection into a 1-L volumetric flask containing 100 mL of *Solution A* and dilute with water to volume.
Blank: Transfer 10.0 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: Maximum transmittance at 589 nm

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus concentration, in mg/mL, of sodium, and draw the straight line best fitting the four plotted points. From this graph, calculate the concentration of sodium in the *Sample solution*.

Calculate the quantity of sodium in each 100 mL of Injection:

$$\text{Result} = C \times D \times F$$

C = concentration of sodium in the *Sample solution* (mg/mL)
D = *Sample solution* dilution factor, 200
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 285.0–315.0 mg of sodium per 100 mL of Injection

• **CHLORIDE**

Sample solution: Transfer 10 mL of Injection into a conical flask, and add 10 mL of glacial acetic acid and 75 mL of methanol.

Analysis

Sample: *Sample solution*

Titrate the *Sample solution*, with shaking, with 0.1 N silver nitrate VS to a pink endpoint, using 3 drops of eosin Y TS as an indicator.

Calculate the labeled amount of chloride (Cl) in 100 mL of Injection:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

USP Monographs

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Acceptance criteria: 368.0–428.0 mg of chloride per 100 mL of Injection

• **LACTATE**

Mobile phase: Dicyclohexylamine, formic acid, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS, in water

Standard solution: 3 mg/mL of USP Sodium Lactate RS in water

Sample solution: Use undiluted Injection.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between the acetate and lactate peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of lactate ($C_3H_5O_3$) in each 100 mL of Injection:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of lactate, 89.07

M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

Acceptance criteria: 231.0–261.0 mg of lactate ($C_3H_5O_3$) per 100 mL of Injection

• **DEXTROSE**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times \alpha)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

α = observed angular rotation of the *Sample solution* (°)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

IMPURITIES

• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally equivalent to 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from a suitable volume of Injection, in water

Instrumental conditions

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.5 USP Endotoxin Units/mL

• **pH** ⟨791⟩**:** 4.0–6.5

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label also includes the warning: "Not for use in the treatment of lactic acidosis".

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Lactate RS

Half-Strength Lactated Ringer's and Dextrose Injection

DEFINITION

Half-Strength Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, Sodium Lactate, and Dextrose in Water for Injection. It contains, in each 100 mL, NLT 142.5 and NMT 157.5 mg of sodium (Na) [as sodium chloride (NaCl) and sodium lactate ($C_3H_5NaO_3$)], NLT 7.08 and NMT 8.65 mg of potassium (K) [equivalent to NLT 13.5 and NMT 16.5 mg of potassium chloride (KCl)], NLT 2.45 and NMT 3.00 mg of calcium (Ca) [equivalent to NLT 9.0 and NMT 11.0 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)], NLT 184.0 and NMT 214.0 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)], and NLT 115.5 and NMT 130.5 mg of lactate ($C_3H_5O_3$) [equivalent to NLT 145.0 and NMT 165.0 mg of sodium lactate ($C_3H_5NaO_3$)]. It contains NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.

[NOTE—The calcium, potassium, and sodium contents of Half-Strength Lactated Ringer's and Dextrose Injection are approximately 1.4, 2, and 65 mEq/L, respectively.]

IDENTIFICATION

• **A.**

Sample solution: Nominally 50 mg/mL of dextrose from Injection in water

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

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- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩: Meets the requirements of the test *A* under *Chloride* and the test *A* under *Calcium*
- **C. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **D. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **E.** The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.

ASSAY

- **CALCIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1000 µg/mL of calcium, prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask and add 10 mL of water. Carefully add 5 mL of dilute hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca), prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Nominally 0.014 mg/mL of calcium, prepared as follows. Transfer 50.0 mL of Injection, equivalent to 1.4 mg of calcium, to a 100-mL volumetric flask containing 5.0 mL of *Solution A*, and dilute with water to volume.

Blank: 5.0 mL of *Solution A* diluted with water to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometer

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Concomitantly determine the absorbance of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points.

From this graph, calculate the concentration of calcium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution* (mg/mL)

D = dilution factor of the *Sample solution*, 2

F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 2.45–3.00 mg of calcium (Ca) in each 100 mL

- **POTASSIUM**

Solution A: Suitable nonionic wetting solution (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride

Standard stock solution: 0.100 mg/mL of potassium, prepared as follows. Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* and 10 mL of *Solution B*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 20.0 mL of Injection to a 100-mL volumetric flask, add 10.0 mL of *Solution A*, and dilute with water to volume.

Blank: Transfer 10.0 mL of *Solution A* and 10 mL of *Solution B* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 766 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of potassium. Draw the straight line best fitting the four plotted points.

From this graph, calculate the concentration of potassium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of potassium in the *Sample solution* (mg/mL)

D = dilution factor of the *Sample solution*, 5

F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 7.08–8.65 mg of potassium (K) in each 100 mL

- **SODIUM**

Solution A: Suitable nonionic wetting solution (1 in 500)

Standard stock solution: 0.100 mg/mL of sodium, prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer this solution to a 100-mL volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* add 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 10 mL of Injection to a 1-L volumetric flask containing 100.0 mL of *Solution A*. Dilute with water to volume.

Blank: Transfer 10.0 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

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Instrumental conditions
Mode: Flame photometry
Analytical wavelength: Maximum transmittance at 589 nm
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of sodium, and draw the straight line best fitting the four plotted points.
From this graph, calculate the concentration of sodium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of sodium in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*, 100
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 142.5–157.5 mg of sodium (Na) in each 100 mL

• **CHLORIDE**
Sample solution: Transfer 10 mL of Injection into a conical flask, and add 10 mL of glacial acetic acid and 75 mL of methanol.
Analysis: Titrate the *Sample solution*, with shaking, with 0.1 N silver nitrate VS to a pink endpoint using 3 drops of eosin Y TS as an indicator. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).
Acceptance criteria: 184.0–214.0 mg of chloride (Cl) in each 100 mL of Injection

• **LACTATE**
Mobile phase: Dicyclohexylamine, formic acid, and water (1:1:998)
System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS, in water
Standard solution: 1.6 mg/mL of USP Sodium Lactate RS, in water
Sample solution: Use undiluted Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between the acetate and lactate peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of lactate ($C_3H_5O_3$) in each 100 mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of lactate, 89.07
M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

Acceptance criteria: 115.5–130.5 mg of lactate ($C_3H_5O_3$) in each 100 mL

• **DEXTROSE**
Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.
Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).
Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

IMPURITIES

• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**
Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from Injection, in water
Instrumental conditions
Mode: UV
Analytical wavelength: 284 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Sample solution* and *Blank*
Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
• **PH** ⟨791⟩: 4.0–6.5
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label also includes the warning: "Not for use in the treatment of lactic acidosis".
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sodium Lactate RS

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Modified Lactated Ringer's and Dextrose Injection

DEFINITION

Modified Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, Sodium Lactate, and Dextrose in Water for Injection. It contains, in each 100 mL, NLT 57.0 and NMT 63.0 mg of sodium (Na) [as sodium chloride (NaCl) and sodium lactate, ($C_3H_5NaO_3$)], NLT 2.82 and NMT 3.46 mg of potassium (K) [equivalent to NLT 5.4 and NMT 6.6 mg of potassium chloride (KCl)], NLT 0.98 and NMT 1.20 mg of calcium (Ca) [equivalent to NLT 3.6 and NMT 4.4 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)], NLT 73.6 and NMT 85.6 mg of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)], NLT 46.2 and NMT 52.20 mg of lactate ($C_3H_5O_3$) [equivalent to NLT 58.0 and NMT 66.0 mg of sodium lactate ($C_3H_5NaO_3$)]. It contains NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.

[NOTE—The calcium, potassium, and sodium contents of Modified Lactated Ringer's and Dextrose Injection are approximately 0.5, 0.8, and 26 mEq/L, respectively.]

IDENTIFICATION

- **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩**:** Meets the requirements of the test *A* under *Chloride* and the test *A* under *Calcium*
- **C. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **D. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **E.** The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.

ASSAY

- **CALCIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium, prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask and add 10 mL of water. Carefully add 5 mL of dilute hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium (Ca), prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Add 5.0 mL of *Solution A* to a 100-mL volumetric flask, and dilute with Injection to volume.

Blank: 5.0 mL of *Solution A* diluted with water to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometer

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene

Blank: *Blank*

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Concomitantly determine the absorbance of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus the concentration of calcium, in mg/mL, and draw the straight line best fitting the three plotted points. From this graph, calculate the concentration of calcium in the *Sample solution*.

Calculate the quantity, in mg, of calcium in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution* (mg/mL)
D = dilution factor of the *Sample solution*, 1.055
F = conversion factor for each 100 mL of Injection, 100

Acceptance criteria: 0.98 mg–1.20 mg of calcium per 100 mL of Injection

- **POTASSIUM**

Solution A: Suitable nonionic wetting solution (1 in 500)

Solution B: 10.93 mg/mL of sodium chloride in water

Standard stock solution: 0.100 mg/mL of potassium, prepared as follows. Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A* and 10 mL of *Solution B*, add 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 50.0 mL of Injection to a 100-mL volumetric flask containing 10 mL of *Solution A*, dilute with water to volume, and mix.

Blank: Transfer 10.0 mL of *Solution A* and 10 mL of *Solution B* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 766 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus the concentration, in mg/mL, of potassium. Draw the straight line best fitting the four plotted points. From this

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graph, calculate the concentration of potassium in the *Sample solution*.

Calculate the quantity of potassium in each 100 mL of Injection:

$$\text{Result} = C \times D \times F$$

C = concentration of potassium in the *Sample solution* (mg/mL)
D = dilution factor of the *Sample solution*, 2
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 2.82–3.46 mg of potassium per 100 mL of Injection

• **SODIUM**

Solution A: Suitable nonionic wetting solution (1 in 500)

Standard stock solution: 0.100 mg/mL of sodium, prepared as follows. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer this solution to a 1-L volumetric flask and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium, prepared as follows. To four separate 100-mL volumetric flasks, each containing 10.0 mL of *Solution A*, add 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 25.0 mL of Injection in a 1-L volumetric flask containing 100.0 mL of *Solution A* and dilute with water to volume.

Blank: Transfer 10.0 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Maximum transmittance at 589 nm

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Set the flame photometer for maximum transmittance. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances of the *Standard solutions* versus concentration, in mg/mL, of sodium, and draw the straight line best fitting the four plotted points. From the graph, calculate the concentration of sodium in the *Sample solution*.

Calculate the quantity of sodium in each 100 mL of Injection:

$$\text{Result} = C \times D \times F$$

C = concentration of sodium in the *Sample solution* (mg/mL)
D = dilution factor of the *Sample solution*, 40
F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 57.0–63.0 mg of sodium per 100 mL of Injection

• **CHLORIDE**

Sample solution: Transfer a volume of Injection equivalent to 55 mg of chloride (1.55 mEq) to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate the *Sample solution*, with shaking, with *Titrant* to a pink endpoint, using 3 drops of eosin Y TS.

Calculate the labeled amount of chloride (Cl) in 100 mL of Injection:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 73.6–85.6 mg of chloride per 100 mL

• **LACTATE**

Mobile phase: Dicyclohexylamine, formic acid, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 0.6 mg/mL of USP Sodium Lactate RS, in water

Sample solution: Use undiluted Injection.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between the acetate and lactate peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of lactate ($C_3H_5O_3$) in each 100 mL of Injection:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of lactate, 89.07
M_{r2} = molecular weight of anhydrous sodium lactate, 112.06

Acceptance criteria: 46.2–52.20 mg of lactate ($C_3H_5O_3$) per 100 mL of Injection

• **DEXTROSE**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

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$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

IMPURITIES

- **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from a suitable volume of Injection, in water
Instrumental conditions
Analytical wavelength: Maximum transmittance at 284 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Sample solution* and *Blank*
Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
- **pH** ⟨791⟩: 4.0–6.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

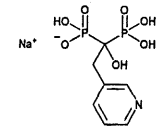
- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label also includes the warning: "Not for use in the treatment of lactic acidosis".
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Sodium Lactate RS

Ringer's Irrigation

» Ringer's Irrigation is Ringer's Injection that has been suitably packaged, and it contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass. The container may be designed to empty rapidly and may contain a volume of more than 1 L.
Labeling—The designation "not for injection" appears prominently on the label.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.5 USP Endotoxin Unit per mL.
Sterility Tests ⟨71⟩: meets the requirements.
Other requirements—It responds to the *Identification* tests, and meets the requirements for *pH*, *Assay for calcium*, *Assay for potassium*, *Assay for sodium*, and *Assay for chloride* under *Ringer's Injection*.

Risedronate Sodium



$C_7H_{10}NNaO_7P_2$ 305.09
$C_7H_{10}NNaO_7P_2 \cdot H_2O$ 323.12
$C_7H_{10}NNaO_7P_2 \cdot 2.5\ H_2O$ 350.13
Phosphonic acid, [1-hydroxy-2-(3-pyridinyl)ethylidene]bis-, monosodium salt;
Sodium trihydrogen [1-hydroxy-2-(3-pyridyl) ethylidene] diphosphonate;
Hemi-pentahydrate [329003-65-8].
Monohydrate [353228-19-0].

DEFINITION

Risedronate Sodium contains one or two-and-one-half molecules of hydration. The monohydrate form contains NLT 98.0% and NMT 102.0% of risedronate sodium ($C_7H_{10}NNaO_7P_2$), calculated on the dried basis. The hemi-pentahydrate form contains NLT 98.0% and NMT 102.0% of risedronate sodium ($C_7H_{10}NNaO_7P_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K or 197A▲ (USP 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements of test *A*. [NOTE—Complete dissolution of the sample is achieved only after the addition of the 15% potassium carbonate.]

Add the following:

▲• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: 1.8 g/L of edetate disodium in water. Adjust with 1 N sodium hydroxide to a pH of 9.5 ± 0.1.
Standard solution: ▲1.0 mg/mL of USP Risedronate Sodium RS and 0.1 mg/mL of USP Risedronate Related Compound A RS in *Mobile phase*▲ (USP 1-May-2020)
Sample solution: 1.1 mg/mL of Risedronate Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 263 nm
Column: 4.0-mm × 25-cm; 10-µm packing L48
Flow rate: 0.8 mL/min
Injection volume: 20 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.3 between risedronate related compound A and risedronate
Tailing factor: NMT 1.6 for the risedronate peak
Relative standard deviation: NMT 1.0% for the risedronate peak ▲(USP 1-May-2020)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of risedronate sodium ($C_7H_{10}NNaO_7P_2$) in the portion of Risedronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response ▲of risedronate▲ (USP 1-May-2020) from the *Sample solution*
r_S = peak response ▲of risedronate▲ (USP 1-May-2020) from the *Standard solution*
C_S = concentration of USP Risedronate Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Risedronate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria
Monohydrate: 98.0%–102.0% on the dried basis
Hemi-pentahydrate: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 1**
[NOTE—Perform both *Procedure 1* and *Procedure 2*.]
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Diluted standard solution: ▲0.005 mg/mL of USP Risedronate Sodium RS and 0.5 µg/mL of USP Risedronate Related Compound A RS in *Mobile phase* from the *Standard solution*▲ (USP 1-May-2020)
System suitability
Samples: *Standard solution* and *Diluted standard solution*
Suitability requirements
Resolution: NLT 2.3 between risedronate related compound A and risedronate, *Standard solution*
Tailing factor: NMT 1.6 for the risedronate peak, *Standard solution*
Relative standard deviation: NMT 1.0% for the risedronate peak, ▲(USP 1-May-2020) *Standard solution*; NMT 15% for the risedronate related compound A peak, ▲(USP 1-May-2020) *Diluted standard solution*
Analysis
Samples: *Sample solution* and *Diluted standard solution*
Calculate the percentage of each impurity in the portion of Risedronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of risedronate from the *Diluted standard solution*
C_S = concentration of USP Risedronate Sodium RS in the *Diluted standard solution* (mg/mL)
C_U = concentration of Risedronate Sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Table 1

Name	Relative Retention Time	Relative Response Factor
3-Pyridyl acetic acid	0.22	1.65
▲Risedronate related compound A▲ (USP 1-May-2020) (2-Pyridinyl isomer)	0.84	1.0
Risedronate sodium	1.0	—

Acceptance criteria: ▲The reporting threshold is 0.05%.▲ (USP 1-May-2020)
Any individual impurity: NMT 0.10%
[NOTE—Disregard the peak due to the sodium ion, eluting at about 1.6 min, and any peak observed in the blank.▲▲ (USP 1-May-2020)]

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 2**
Mobile phase: Transfer 16.15 g of dibasic potassium phosphate and 0.46 g of edetate disodium to a 1-L beaker, and dissolve in about 400 mL of water. Add 1 vial of commercially available tetrabutylammonium dihydrogen phosphate buffered solution in methanol[1] and 1 mL of hydrochloric acid. Adjust with 1 N sodium hydroxide or 1 N hydrochloric acid, as necessary, to a pH of 7.5 ± 0.1, and dilute with water to 480 mL. Add 20 mL of methanol, mix well, pass the solution through a nylon filter of 0.45-µm pore size, and degas.
Diluent: Transfer 0.46 g of edetate disodium to a 1-L beaker, and dissolve in 500 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5 ± 0.1.
Standard solution: ▲0.005 mg/mL▲ (USP 1-May-2020) of USP Risedronate Related Compound B RS in *Diluent*
Diluted standard solution: 0.5 µg/mL of USP Risedronate Related Compound B RS in *Diluent* from the *Standard solution*
Sample solution: 2 mg/mL of Risedronate Sodium in *Diluent*, using sonication if necessary
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 263 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *Diluted standard solution*
Suitability requirements
▲▲ (USP 1-May-2020)
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: ▲▲ (USP 1-May-2020) NMT 10%, ▲▲ (USP 1-May-2020) *Diluted standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Disregard any peak eluting before risedronate related compound B. The risedronate peak elutes unretained at the void volume.]
Calculate the percentage of each impurity in the portion of Risedronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

[1] Available from Waters Corp. as Part #85101 (PIC A).

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r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of risedronate related compound B from the *Standard solution*
C_S = concentration of USP Risedronate Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Risedronate Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of risedronate related compound B as a free acid, 530.20
M_{r2} = molecular weight of risedronate related compound B as a tetrahydrate disodium salt, 646.22

Acceptance criteria: ▲The reporting threshold is 0.05%.▲ (USP 1-May-2020)
Risedronate related compound B: NMT 0.10%
Individual impurities: NMT 0.10%
Total impurities: NMT 0.50%, *Procedure 1* and *Procedure 2* being combined. [NOTE—Disregard any peak observed in the blank.▲▲ (USP 1-May-2020)]

SPECIFIC TESTS

Change to read:

- **WATER DETERMINATION** ⟨921⟩▲▲ (USP 1-May-2020)**:** Perform the test by direct introduction of solid sample into the titrator.▲▲ (USP 1-May-2020)
Acceptance criteria: 11.9%–13.9%; where it is labeled as a hemi-pentahydrate

Change to read:

- **LOSS ON DRYING**
(See *Thermal Analysis* ⟨891⟩.)
▲▲ (USP 1-May-2020)
Sample: 7–15 mg of Risedronate Sodium
Heating rate: 10°/min in a stream of nitrogen at a flow rate of about 40 mL/min
Temperature range: Ambient temperature to 250°
Acceptance criteria: 5.5%–7.5%; where it is labeled as a monohydrate

ADDITIONAL REQUIREMENTS

- **LABELING:** Label to indicate whether it is the monohydrate or the hemi-pentahydrate form.
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Risedronate Sodium RS
USP Risedronate Related Compound A RS
▲▲ (USP 1-May-2020)[1-hydroxy-2-(2-pyridinyl)ethylidene] bis(phosphonic acid) monohydrate.
$C_7H_{11}NO_7P_2 \cdot {}^{\blacktriangle}H_2O$ 301.13▲ (USP 1-May-2020)
USP Risedronate Related Compound B RS
Cyclic dimer;
Disodium tetrahydrate salt;
[3,6-Bis[(3-pyridinyl)methyl]-2,5-dihydroxy-2,5-dioxido-1,4,2,5-dioxadiphosphorinane-3,6-diyl]bis[phosphonic acid] disodium tetrahydrate salt.
$C_{14}H_{16}N_2O_{12}P_4Na_2 \cdot 4H_2O$ 646.22

Risedronate Sodium Tablets

DEFINITION
Risedronate Sodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of risedronate sodium ($C_7H_{10}NNaO_7P_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer a quantity of Tablets, equivalent to 50–75 mg of risedronate sodium, to a suitable flask. Add 10 mL of water, and shake. Pass first through a suitable paper filter, and then through a nylon filter of 0.45-µm pore size. Add 10 mL of 0.2 M cupric chloride solution, mix well, and allow the solution to stand for about 10 min. Add 2 mL of dehydrated alcohol, mix well, and allow the solution to stand for a minimum of 1 h, to form a blue precipitate of the copper complex. Collect the precipitate using a nylon filter of 0.45-µm pore size, wash it with 10 mL of dehydrated alcohol, and allow it to dry on the filter. [NOTE—Dry the precipitate under ambient conditions; do not heat the precipitate. A modest change of color (from blue to green) may be observed upon drying.]
Standard: Dissolve 50 mg of USP Risedronate Sodium RS in 10 mL of water, and pass the solution through a nylon filter of 0.45-µm pore size. Proceed as directed for the *Sample*, beginning with "Add 10 mL of 0.2 M cupric chloride solution..."
Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
For Tablets labeled to contain 5–35 mg of risedronate sodium
Mobile phase: 1.8 g/L of edetate disodium in water. Adjust with 1 N sodium hydroxide to a pH of 9.5 ± 0.1.
System suitability solution: 0.15 mg/mL of anhydrous USP Risedronate Sodium RS and 7.5 µg/mL of USP Risedronate Related Compound C RS in *Mobile phase*
Standard solution: 0.1–0.15 mg/mL of anhydrous USP Risedronate Sodium RS in *Mobile phase*
Sample stock solution: Transfer 10 Tablets to a suitable volumetric flask, add *Mobile phase* to about 60% of the volume of the flask, shake for about 10 min, then sonicate for a minimum of 5 min. Cool the solution to room temperature, and dilute with *Mobile phase* to volume to obtain a solution having a known concentration of about 0.5–1.5 mg/mL.
Sample solution: Nominally 0.1–0.15 mg/mL of risedronate sodium in *Mobile phase*, dilute from the *Sample stock solution*. Pass a portion of this solution through a nylon filter of 0.22-µm pore size, discarding the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 263 nm
Column: 4.0-mm × 25-cm; 10-µm packing L48
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

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Suitability requirements
Resolution: NLT 2.5 between risedronate related compound C and risedronate, *System suitability solution*
Relative standard deviation: NMT 2.0% for three replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of risedronate sodium ($C_7H_{10}NNaO_7P_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of risedronate from the *Sample solution*
r_S = peak response of risedronate from the *Standard solution*
C_S = concentration of USP Risedronate Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of risedronate sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

For Tablets labeled to contain at least 75 mg of risedronate sodium
Mobile phase: 1.8 g/L of edetate disodium in water. Adjust with 1 N sodium hydroxide to a pH of 9.5 ± 0.1.
Standard solution: 0.25 mg/mL of anhydrous USP Risedronate Sodium RS and 4 µg/mL of USP Risedronate Related Compound A RS in *Mobile phase*
Sample stock solution: Transfer 10 Tablets to a suitable container. Add 400 mL of *Mobile phase,* cap, and mechanically shake for 5–15 min, using an orbital or other suitable shaker. [NOTE—Additional sonication for 5–15 min may be performed if necessary.]
Sample solution: Nominally 0.2–0.3 mg/mL of risedronate sodium in *Mobile phase,* dilute from the *Sample stock solution.* Pass a portion of this solution through a nylon filter of 0.45-µm pore size, discarding the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 263 nm
Column: 4.0-mm × 25-cm; 10-µm packing L48
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between risedronate related compound A and risedronate peaks
Tailing factor: NMT 1.5 for risedronate peak
Relative standard deviation: NMT 1.5% for the risedronate peak from three replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of risedronate sodium ($C_7H_{10}NNaO_7P_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of risedronate from the *Sample solution*
r_S = peak response of risedronate from the *Standard solution*
C_S = concentration of USP Risedronate Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of risedronate sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
For Tablets labeled to contain 5, 30, or 35 mg
Medium: Water; 500 mL, deaerated
Apparatus 2: 50 rpm, paddles coated with Teflon
Time: 30 min
Mobile phase: Prepare as directed in the *Assay.*
Standard stock solution: 1 mg/mL of anhydrous USP Risedronate Sodium RS in *Medium*
Standard solution: (0.002 × *L*) mg/mL of USP Risedronate Sodium RS in *Medium* from the *Standard stock solution,* where *L* is the Tablet label claim in mg
Sample solution: Use a portion of the solution under test, filter if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 263 nm
Column: 4.0-mm × 5-cm; 10-µm packing L48
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of risedronate sodium ($C_7H_{10}NNaO_7P_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of risedronate from the *Sample solution*
r_S = peak response of risedronate from the *Standard solution*
C_S = concentration USP Risedronate Sodium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium,* 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of risedronate sodium is dissolved.
For Tablets labeled to contain at least 75 mg
Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm, paddles coated with Teflon
Time: 45 min
Standard solution: 0.12 mg/mL of anhydrous USP Risedronate Sodium RS in *Medium*
Sample solution: Use a portion of the solution under test. Dilute with *Medium,* if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Wavelength of maximum absorption at about 263 nm, with a background correction at 400 nm
Cell: 5 mm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of risedronate sodium ($C_7H_{10}NNaO_7P_2$) dissolved:

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$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of risedronate sodium is dissolved.

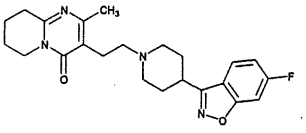
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Risedronate Related Compound A RS
2-Pyridinil isomer [1-hydroxy-2-(2-pyridinyl)ethyl idene] bis(phosphonic acid) monohydrate.
$C_7H_{11}NO_7P_2$ 283.12
USP Risedronate Related Compound C RS
[2-(3-Pyridinyl)ethylidene-1,1]bis(phosphonic acid).
$C_7H_{11}NO_6P_2$ 267.11
USP Risedronate Sodium RS

Risperidone



$C_{23}H_{27}FN_4O_2$ 410.48
4*H*-Pyrido[1,2-*a*]pyrimidin-4-one, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-;
3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidino]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [106266-06-2].

DEFINITION

Risperidone contains NLT 98.0% and NMT 102.0% of risperidone ($C_{23}H_{27}FN_4O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 5 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 6.0, and pass the solution through a suitable filter.
Solution A: Acetonitrile and tetrahydrofuran (80:20)
Mobile phase: *Buffer* and *Solution A* (80:20)
Standard stock solution: 1 mg/mL of USP Risperidone RS in solution prepared as follows. Dissolve a suitable amount of USP Risperidone RS in 20% of the final volume with methanol. Dilute with *Mobile phase* to final volume. Sonication may be used to aid in dissolution.
Standard solution: 0.2 mg/mL of USP Risperidone RS in *Mobile phase* from *Standard stock solution*
System suitability solution: 20 µg/mL of USP Risperidone Related Compound G RS in *Standard solution*
Sample stock solution: 1 mg/mL of Risperidone in solution prepared as follows. Dissolve a suitable amount of Risperidone in 20% of the final volume with methanol. Dilute with *Mobile phase* to final volume. Sonication may be used to aid in dissolution.
Sample solution: 0.2 mg/mL of Risperidone in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of risperidone
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for risperidone and risperidone related compound G are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between risperidone and risperidone related compound G, *System suitability solution*
Tailing factor: NMT 2.0 for risperidone, *Standard solution*
Relative standard deviation: NMT 1.0% for risperidone, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of risperidone ($C_{23}H_{27}FN_4O_2$) in the portion of Risperidone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Risperidone RS in the *Standard solution* (mg/mL)
C_U = concentration of Risperidone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 2.0 g of Risperidone
Acceptance criteria: NMT 0.1%

• **ORGANIC IMPURITIES**
Solution A: 5 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 6.0, and pass the solution through a suitable filter.
Solution B: Acetonitrile and methanol (40:60)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.01	70	30
12	65	35
18	65	35

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
25	35	65
35	30	70
40	30	70
42	70	30
50	70	30

System suitability solution: 20 µg/mL of USP Risperidone Related Compound G RS and 10 mg/mL of USP Risperidone RS in methanol. Sonication may be used to aid in dissolution.
Standard solution: 20 µg/mL of USP Risperidone RS in methanol. Sonication may be used to aid in dissolution.
Sample solution: 10 mg/mL of Risperidone in methanol. Sonication may be used to aid in dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Identify the peaks using *Table 2*.]
Suitability requirements
Resolution: NLT 1.5 between risperidone and risperidone related compound G, *System suitability solution*
Tailing factor: NMT 2.0 for risperidone, *Standard solution*
Relative standard deviation: NMT 5.0% for risperidone, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Risperidone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of risperidone from the *Standard solution*
C_S = concentration of USP Risperidone RS in the *Standard solution* (mg/mL)
C_U = concentration of Risperidone in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.
[NOTE—Disregard the impurity peaks less than 0.05%.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
E-Oxime[a]	0.52	1.0	0.20
Z-Oxime[b]	0.64	1.0	0.20
9-Hydroxyrisperidone[c]	0.71	1.0	0.20
Desfluoro risperidone[d, e]	0.79	1.0	0.10
Risperidone difluoroketone[f]	0.90	1.4	0.10
5-Fluororisperidone[g]	0.94	1.0	0.20
Risperidone	1.00	1.0	—
Risperidone related compound G[e]	1.08	2.5	0.10
6-Methylrisperidone[h]	1.44	1.0	0.20
Any unspecified individual impurity	—	1.0	0.10
Total impurities	—	—	0.30

[a] 3-(2-[4-[(*E*)-(2,4-Difluorophenyl)(hydroxyimino)methyl]piperidin-1-yl]ethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.
[b] 3-[2-[4-[(*Z*)-(2,4-Difluorophenyl)(hydroxyimino)methyl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.
[c] (9*RS*)-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.
[d] 3-[2-[4-(Benzisoxazol-3-yl)piperidin-1-yl]ethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.
[e] This impurity may not be relevant to all manufacturing processes.
[f] 3-[2-[4-(2,4-Difluorobenzoyl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.
[g] 3-[2-[4-(5-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (also known as 5-fluororisperidone).
[h] (6*RS*)-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2,6-dimethyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 80° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Risperidone RS
USP Risperidone Related Compound G RS
3-[2-[4-(4-Fluoro-2-hydroxybenzoyl)piperidin-1-yl] ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-α] pyrimidin-4-one hydrochloride.
$C_{23}H_{28}FN_3O_3 \cdot HCl$ 448.94

Risperidone Oral Solution

DEFINITION
Risperidone Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$). It may contain a suitable preservative.

IDENTIFICATION
The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 5.0 g/L of *ammonium* acetate in *water*
Mobile phase: *Acetonitrile* and *Buffer* (11:39)
System suitability solution: 0.25 mg/mL of USP Risperidone Related Compounds Mixture RS prepared as follows. Weigh USP Risperidone Related Compounds

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Mixture RS into a suitable volumetric flask. Dissolve first in 20% of the flask volume of *methanol*. Add 20% of the flask volume of *water* followed by 50% of the flask volume of *Buffer*, and allow to cool to room temperature. Dilute with *methanol* to volume.

Standard stock solution: 1 mg/mL of USP Risperidone RS in methanol

Standard solution: 0.2 mg/mL of USP Risperidone RS prepared from the *Standard stock solution* as follows. Transfer 5.0 mL of the *Standard stock solution* to a 25-mL volumetric flask. Add 5.0 mL of *water* followed by 12.5 mL of *Buffer*, and allow to cool to room temperature. Dilute with *methanol* to volume.

Sample solution: Nominal concentration of 0.2 mg/mL of risperidone prepared from Oral Solution as follows. Transfer an amount of Oral Solution, equivalent to 5 mg of risperidone to a 25-mL volumetric flask, add 12.5 mL of *Buffer*, fill with methanol almost to volume, and mix well. Allow to cool to room temperature, and dilute with *methanol* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 10-cm; 3-µm packing *L1*

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: 2 times the retention time of risperidone

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between bicyclorisperidone and *Z*-oxime, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of risperidone from the *Sample solution*

r_S = peak response of risperidone from the *Standard solution*

C_S = concentration of USP Risperidone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of risperidone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 5 µg/mL of USP Risperidone RS in methanol

Standard solution: 1 µg/mL of USP Risperidone RS from the *Standard stock solution* prepared as follows. Transfer 5.0 mL of the *Standard stock solution* to a 25-mL volumetric flask. Add 5.0 mL of *water* followed by 12.5 mL of *Buffer*, and allow to cool to room temperature. Dilute with *methanol* to volume.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between bicyclorisperidone and *Z*-oxime, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of risperidone from the *Standard solution*

C_S = concentration of USP Risperidone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of risperidone in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Risperidone *cis*-*N*-oxide[a]	0.33	0.97	0.50
Bicyclorisperidone[b]	0.43	0.67	0.50
Z-Oxime[c]	0.53	—	—
Risperidone	1.0	—	—
Any unspecified degradation product	—	1.0	0.20
Total impurities	—	—	1.0

[NOTE—Process impurity, not to be quantified in the drug product. It is used to establish system suitability only.]

[a] *cis*-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one,*N*-oxide monohydrate.

[b] 3-(4-Fluoro-2-hydroxyphenyl)-1-[2-(6,7,8,9-tetrahydro-2-methyl-4-oxo-4*H*-pyrido-[1,2-a]pyrimidin-3-yl)ethyl]-1-aza-2-azoniabicyclo[2.2.2]oct-2-ene salt.

[c] (*Z*)-3-[2-[4-(2,4-Difluorophenyl)(hydroxyimino)methyl]-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one.

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 2.0–4.0

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 10^1 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature. Do not freeze.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Risperidone RS

USP Risperidone Related Compounds Mixture RS

Contains a mixture of the following four compounds: Risperidone.

USP Monographs

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Risperidone *cis*-*N*-oxide: *cis*-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one, *N*-oxide.
Bicyclorisperidone: 3-(4-Fluoro-2-hydroxyphenyl)-1-[2-(6,7,8,9-tetrahydro-2-methyl-4-oxo-4*H*-pyrido-[1,2-*a*]pyrimidin-3-yl)ethyl]-2-aza-1-azoniabicyclo[2.2.2]oct-2-ene iodide.
Z-Oxime: (*Z*)-3-[2-[4-(2,4-Difluorophenyl)(hydroxyimino)methyl]-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.

Risperidone Tablets

» Risperidone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$).

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Risperidone RS
3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidino]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [CAS-106266-06-2]. 410.48

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197K⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)—
Test solution—Grind an appropriate number of Tablets to prepare a 550 ± 50 µg per mL solution of risperidone in ethyl acetate. Shake the solution for 30 minutes, and centrifuge for 20 minutes. Evaporate 5 mL of the supernatant with the aid of a stream of nitrogen to 2 mL on a warm water bath. Add 150 ± 50 mg of KBr powder, mix well, and evaporate to dryness. Grind the dried mixture, and press a small amount into a transparent pellet ⟨197K⟩.
Standard solution—Grind about 2 mg of USP Risperidone RS with about 200 mg of KBr powder, and press into a transparent pellet.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 500 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Determine the amount of $C_{23}H_{27}FN_4O_2$ dissolved by employing the following method.
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (65:35), and add 1 mL of trifluoroacetic acid to each 1 L of the mixture. Adjust with ammonium hydroxide to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP Risperidone RS in *Medium,* and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration of about 0.006 mg per mL.
Test solution—Use portions of the solution under test, and pass through a suitable filter having a porosity of 35 µm.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 237-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute.
Chromatograph the *Standard solution* as directed for *Procedure:* the retention time of risperidone is about 2.1 minutes, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of $C_{23}H_{27}FN_4O_2$ dissolved by the formula:

$$\frac{r_U \times C_S \times 500 \times 100}{r_S \times L}$$

in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of USP Risperidone RS in the *Standard solution;* 500 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and L is the Tablet label claim in mg.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{23}H_{27}FN_4O_2$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩—
Mobile phase and *Chromatographic system*—Proceed as directed for *Dissolution.*
Standard solution—Dissolve an accurately weighed quantity of USP Risperidone RS in a suitable volumetric flask, and dilute quantitatively with 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 0.03 mg of risperidone per mL.
Test solution—Transfer one Tablet into a 100-mL volumetric flask, add 50 mL of 0.1 N hydrochloric acid, and shake mechanically for about 30 minutes. Dilute with 0.1 N hydrochloric acid to volume, and mix. Pass a portion of this solution through a suitable filter having a 0.2-µm or finer porosity, and use the filtrate.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the risperidone peak. Calculate the quantitiy, in mg, of risperidone ($C_{23}H_{27}FN_4O_2$) in the portion of Tablets taken by the formula:

$$C(r_U/r_S)100$$

in which C is the concentration, in mg per mL, of USP Risperidone RS in the *Standard solution;* and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution,* respectively.

Related compounds—
Mobile phase and *Diluent*—Proceed as directed in the *Assay.*
Standard solution—Prepare as directed for the *Standard preparation* in the *Assay.*
Diluted sodium hydroxide—To 1 L of water in a beaker, add 0.1 N sodium hydroxide dropwise to obtain a pH of about 8.5.
Diluted hydrogen peroxide—Dilute 1 mL of hydrogen peroxide with water to 500 mL.
Peak identification solution—Suspend 10 mg of USP Risperidone RS in 10 mL of *Diluted sodium hydroxide* in a 100-mL volumetric flask. Store the flask at 90° for 24 hours. Cool the solution to room temperature. Add 10 mL of aqueous *Diluted hydrogen peroxide* to the flask, and store at 90° for an additional two hours. Cool the mixture to room temperature, and dilute with methanol to volume.

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Test solution—Use the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* Chromatograph about 20 µL of the *Peak identification solution,* record the peak responses as directed for *Procedure,* and identify the peaks using the relative retention times given in *Table 1:* the resolution, *R,* between the *trans-N*-oxide and *cis-N*-oxide is not less than 1.2.

[NOTE—The approximate relative retention times given in *Table 1* are for identification purposes only.]

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(1/R)(r_i/r_S)$$

in which R is the appropriate relative response factor as listed in *Table 1;* r_i is the peak response for each impurity in the *Test solution;* and r_S is the peak response of risperidone in the *Test solution:* not more than 0.3% of any individual unidentified impurity is found, not more than 0.5% of any individual specified impurity is found, and not more than 1.0% of total impurities is found.

Assay—

Diluent—Prepare a degassed mixture of methanol and water (80:20).

Solution A—Prepare a filtered and degassed mixture of water, acetonitrile, and trifluroacetic acid (80: 19.5: 0.1). Adjust with ammonium hydroxide to a pH of 3.0.

Solution B—Prepare a filtered and degassed mixture of water, methanol, and trifluoroacetic acid (61: 39: 0.1). Adjust with ammonium hydroxide to a pH of 3.0.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer an accurately weighed quantity of USP Risperidone RS to a suitable volumetric flask, and dissolve in and dilute quantitatively with *Diluent* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately weighed portion of not fewer than 10 Tablets to a volumetric flask that can accommodate a final concentration of 0.1 mg of risperidone per mL. Add an appropriate amount of water equivalent to 20% of the total volume of the volumetric flask, and mechanically shake for about 30 minutes. Add a volume of methanol equivalent to 60% of the total volume of the volumetric flask, and mechanically shake for about 30 minutes. Dilute with methanol to volume, and mix to obtain the final 0.1 mg per mL concentration. Pass a portion of this solution through a suitable filter having a 0.45-µm or finer porosity, and use the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 2.5 mL per minute. The column is maintained at room temperature. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–8	100	0	isocratic
8–16	100→0	0→100	linear gradient
16–20	0	100	isocratic
20–21	0→100	100→0	linear gradient
21–30	100	0	re-equilbration

Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor of risperidone is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the risperidone peak. Calculate the percentage of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$) in the portion of Tablets taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Risperidone RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of risperidone in the *Assay preparation;* and r_U and r_S are the peak responses of risperidone obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Risperidone Orally Disintegrating Tablets

DEFINITION

Risperidone Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$).

IDENTIFICATION

Change to read:

• **A.** ▲(USP 1-May-2019) The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained by the *Assay*

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*▲(USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Acetonitrile, trifluoroacetic acid, and water (200: 1.5: 800). Adjust with ammonium hydroxide to a pH of 3.0.

Diluent: Methanol and 0.1 N hydrochloric acid (40:60)

System suitability solution: 0.1 mg/mL of USP Risperidone Related Compounds Mixture RS prepared as follows. ▲Transfer a suitable quantity of USP Risperidone Related Compounds Mixture RS to a suitable volumetric flask and dissolve▲(USP 1-May-2019) in 20% of the flask volume of methanol. Dilute with *Diluent* to volume.

Standard solution: 0.1 mg/mL of USP Risperidone RS in *Diluent*

Sample solution: ▲Nominally▲(USP 1-May-2019) 0.1 mg/mL of risperidone in *Diluent* from NLT 13 Tablets. [NOTE—Sonicate for 30 min.]

USP Monographs

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Table 1 (Risperidone Tablets)

Peak Identfication	Approximate Relative Retention Time *(RRT)*	Relative Response Factor *(R)*	Limit of Impurity
Bicyclorisperidone[1]	0.68	0.81	Not more than 0.5%
Risperidone	1.0	1.0	—
Risperidone *trans-N*-oxide[2]	1.65	—	Not quantified. Used for identification and system suitability check only.
Risperidone *cis-N*-oxide[3]	1.81	0.95	Not more than 0.5%
Any other unspecified degradation product	—	1.0	Not more than 0.3%
Total impurities	—	—	Not more than 1.0%

[1] 3-(4-fluoro-2-hydroxyphenyl)-1-[2-(6,7,8,9-tetrahydro-2-methyl-4-oxo-4*H*-pyrido-[1,2-a]pyrimidin-3-yl)ethyl]-2-aza-1-azoniabicyclo[2.2.2]oct-2-ene iodide.
[2] *trans*-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one, *N*-oxide monohydrate.
[3] *cis*-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one, *N*-oxide monohydrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
Column: 3.0-mm × 15-cm; 3.5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 10 µL
Run time: ▲NLT 2.2▲ (USP 1-May-2019) times the retention time of risperidone
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—For relative retention times, see ▲*Table 1*.▲ (USP 1-May-2019)]
Suitability requirements
Resolution: NLT 1.8 between *Z*-oxime and bicyclorisperidone, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲the labeled amount of risperidone▲ (USP 1-May-2019) ($C_{23}H_{27}FN_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of risperidone from the *Sample solution*
r_S = peak response of risperidone from the *Standard solution*
C_S = concentration of USP Risperidone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of risperidone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩
Test 1: NMT 30 s
Test 2: NMT 60 s. [NOTE—If the product complies with this test, the labeling indicates that the product meets USP *Disintegration Test 2*.]

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 10 min
Buffer: 8.7 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 7.8.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Standard solution: (*L*/500) mg/mL of USP Risperidone RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass 10 mL of the solution under test through a suitable ▲▲ (USP 1-May-2019) filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 28 ± 3°
Flow rate: 2 mL/min
Injection volume: 20 µL. [NOTE—Use 40 µL for Tablets labeled to contain 0.5 mg of risperidone.]
Run time: ▲NLT▲ (USP 1-May-2019) 2 times the retention time of risperidone
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲the labeled amount of risperidone▲ (USP 1-May-2019) ($C_{23}H_{27}FN_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of risperidone is dissolved.

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
▲▲ (USP 1-May-2019)

USP Monographs

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IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_{U1}/r_{U2}) \times (1/F) \times 100$$

r_{U1} = peak response of any individual impurity from the *Sample solution*

r_{U2} = peak response of risperidone from the *Sample solution*

F = relative response factor (see ▲▲ (USP 1-May-2019) *Table 1*)

Acceptance criteria: ▲See *Table 1*.▲ (USP 1-May-2019)

▲▲ (USP 1-May-2019) **Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Z-Oxime[a, b]	0.59	1.0	—
Bicyclorisperidone[c]	0.66	0.86	0.3
Risperidone	1.0	1.0	—
Risperidone *cis*-*N*-oxide[d]	1.7	0.97	0.5
Any unspecified degradation product	—	1.0	0.2
▲Total impurities	—	—	1.0▲ (USP 1-May-2019)

[a] Process impurity; it is used to establish system suitability only.

[b] (*Z*)-3-[2-[4-(2,4-Difluorophenyl)(hydroxyimino)methyl]-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one.

[c] 3-(4-Fluoro-2-hydroxyphenyl)-1-[2-(6,7,8,9-tetrahydro-2-methyl-4-oxo-4*H*-pyrido-[1,2-a]pyrimidin-3-yl)ethyl]-2-aza-1-azoniabicyclo[2.2.2]oct-2-ene.

[d] *cis*-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one,*N*-oxide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Risperidone RS

4*H*-Pyrido[1,2-*a*]pyrimidin-4-one, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-.

3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidino] ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*] pyrimidin-4-one.

$C_{23}H_{27}FN_4O_2$ 410.48

USP Risperidone Related Compounds Mixture RS

Contains a mixture of the following four compounds: Risperidone.

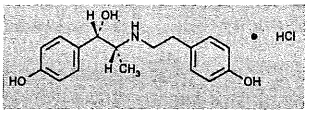
Risperidone *cis*-*N*-oxide: *cis*-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one, *N*-oxide.

Bicyclorisperidone: 3-(4-Fluoro-2-hydroxyphenyl)-1-[2-(6,7,8,9-tetrahydro-2-methyl-4-oxo-4*H*-pyrido-[1,2-*a*] pyrimidin-3-yl)ethyl]-2-aza-1-azoniabicyclo[2.2.2]oct-2-ene iodide.

Z-Oxime: (*Z*)-3-[2-[4-(2,4-Difluorophenyl) (hydroxyimino)methyl]-1-piperidinyl]ethyl-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.

Delete the following:

▲Ritodrine Hydrochloride



$C_{17}H_{21}NO_3 \cdot HCl$ 323.81

Benzenemethanol, 4-hydroxy-α-[1-[[2-(4-hydroxyphenyl) ethyl]amino]ethyl]-, hydrochloride, (*R**, *S**)-.

erythro-*p*-Hydroxy-α-[1-[(*p*-hydroxyphenethyl)amino]ethyl] benzyl alcohol hydrochloride [23239-51-2].

» Ritodrine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{17}H_{21}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨ 11 ⟩ —

USP Ritodrine Hydrochloride RS

Identification—

A: The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Ritodrine Hydrochloride RS.

B: The retention time of the ritodrine hydrochloride in the *Assay preparation* obtained in the *Assay* corresponds to that of the *Standard preparation* obtained in the *Assay*.

C: A solution (1 in 100) responds to the tests for *Identification Tests—General* ⟨191⟩, *Chloride*.

pH ⟨ 791 ⟩ : between 4.5 and 6.0, in a solution (1 in 50).

Loss on drying ⟨ 731 ⟩ : Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨ 281 ⟩ : not more than 0.2%.

Related compounds—

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay*.

Test preparation—Prepare a solution containing about 1 mg of Ritodrine Hydrochloride in each mL of *Mobile phase*.

Diluted test preparation—Quantitatively dilute a suitable volume of the *Test preparation* with *Mobile phase* to obtain a solution having a known concentration of 0.01 mg per mL of ritodrine hydrochloride.

Procedure—Chromatograph the *Test preparation* and the *Diluted test preparation*, as directed in the *Assay*. The relative retention times are about 0.3 for tyramine, 0.65 for *erythro*-1-(4-ketocyclohexyl)-2-[(1-hydroxyphenethyl)amino] propanol-1, 0.85 for

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erythro-p-hydroxy-[1-(4-ketocyclohexyleth yl)amino]ethyl benzyl alcohol, 1.0 for ritodrine, 1.15 for *threo* diastereomer of ritodrine, and 2.3 for *p*-hydroxy-β-(*p*-hydroxyphenethyl) amino]propiophenone. Determine the peak responses for ritodrine and for the related compounds from the chromatograms obtained from the *Diluted test preparation* and the *Test preparation*, respectively. Calculate the percentage of related compounds found: not more than 0.5% of any individual impurity and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Dissolve 6.6 g of dibasic ammonium phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and mix with 300 mL of methanol. Adjust by the addition of phosphoric acid to a pH of 3.0, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ritodrine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer about 200 mg of Ritodrine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

System suitability preparation—Dissolve about 20 mg of Ritodrine Hydrochloride in about 50 mL of *Mobile phase*. Add 5.6 mL of sulfuric acid, dilute with *Mobile phase* to 100 mL, and mix. Heat a portion of this solution for about 2 hours at about 85°, and then cool to room temperature. Cautiously mix 10.0 mL of the cooled solution with 8.0 mL of sodium hydroxide solution (1 in 10), and allow to cool. This solution contains ritodrine and its *threo* diastereomer.

Chromatographic system—The chromatograph is equipped with a 4.6-mm × 25-cm stainless steel column that contains packing L7 and an UV detector that monitors absorption at 214 nm. Chromatograph about 50 µL of the *System suitability preparation:* the resolution between ritodrine and its *threo* diastereomer is not less than 1.0. [NOTE—Chromatograms obtained as directed for this test, exhibit relative retention times of 1.0 for ritodrine and approximately 1.2 for the *threo* diastereomer.]

Procedure—Separately inject equal volumes (20 to 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph (see *Chromatography* ⟨621⟩) by means of a suitable sampling valve. Record the chromatograms and measure the peak responses. Calculate the quantity, in mg, of $C_{17}H_{21}NO_3 \cdot HCl$ in the portion of Ritodrine Hydrochloride taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ritodrine Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the peak responses for Ritodrine Hydrochloride obtained from the *Assay preparation* and the *Standard preparation*, respectively. ▲ (USP 1-May-2020)

Delete the following:

▲Ritodrine Hydrochloride Injection

» Ritodrine Hydrochloride Injection is a sterile solution of Ritodrine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{21}NO_3 \cdot HCl$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass. Store at room temperature, preferably below 30°.

USP Reference standards ⟨ 11 ⟩ —
USP Ritodrine Hydrochloride RS

Identification—The retention time of the ritodrine hydrochloride in the *Assay preparation* obtained in the *Assay* corresponds to that of the *Standard preparation* obtained in the *Assay*.

Bacterial Endotoxins Test ⟨ 85 ⟩ —It contains not more than 0.5 USP Endotoxin Unit per mg.

pH ⟨ 791 ⟩ : between 4.8 and 5.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, *Standard preparation*, and *System suitability preparation*—Proceed as directed in the *Assay* under *Ritodrine Hydrochloride*.

Assay preparation—Dilute an accurately measured volume of Injection, equivalent to about 20 mg of ritodrine hydrochloride, with *Mobile phase* to 100.0 mL, and mix.

Chromatographic system—Proceed as directed in the *Assay* under *Ritodrine Hydrochloride* except that the liquid chromatograph is equipped with a 275-nm detector.

Procedure—Proceed as directed in the *Assay* under *Ritodrine Hydrochloride*. Calculate the quantity, in mg, of $C_{17}H_{21}NO_3 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(100C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein. ▲ (USP 1-May-2020)

Delete the following:

▲Ritodrine Hydrochloride Tablets

» Ritodrine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{21}NO_3 \cdot HCl$.

Packaging and storage—Preserve in tight containers. Store at room temperature, preferably below 30°.

USP Reference standards ⟨ 11 ⟩ —
USP Ritodrine Hydrochloride RS

Identification—The retention time of the Tablets in the *Assay preparation* obtained in the *Assay* corresponds to that of the *Standard preparation* obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.

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Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{17}H_{21}NO_3 \cdot HCl$ dissolved by employing the procedure set forth in the *Assay*, using a filtered portion of the solution under test as the *Assay preparation* in comparison with a Standard solution having a known concentration of USP Ritodrine Hydrochloride RS in the same *Medium*.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{17}H_{21}NO_3 \cdot HCl$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Transfer 1 Tablet to a 200-mL volumetric flask, add about 5 mL of water, and stir until disintegration is complete. Add about 150 mL of methanolic sulfuric acid solution (0.005 M sulfuric acid in methanol), continue stirring for an additional 15 minutes, dilute with methanolic sulfuric acid solution to volume, and mix. Filter a portion of the mixture, discarding the first 20 mL of the filtrate. Dissolve an accurately weighed quantity of USP Ritodrine Hydrochloride RS in methanolic sulfuric acid solution, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 50 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 276 nm, with a suitable spectrophotometer, using methanolic sulfuric acid solution as the blank. Calculate the quantity, in mg, of $C_{17}H_{21}NO_3 \cdot HCl$ in the Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

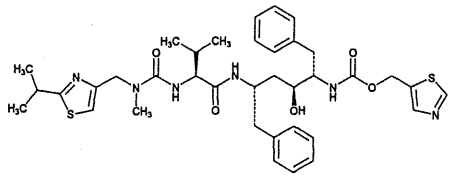
in which T is the labeled quantity, in mg, of ritodrine hydrochloride in the Tablet, C is the concentration, in µg per mL, of USP Ritodrine Hydrochloride RS in the Standard solution, D is the concentration, in µg per mL, of ritodrine hydrochloride in the solution from the Tablet based upon the labeled quantity per Tablet and the extent of dilution, and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—
Mobile phase, *Standard preparation*, and *System suitability preparation*—Proceed as directed in the *Assay* under *Ritodrine Hydrochloride*.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of ritodrine hydrochloride, to a 100-mL volumetric flask. Add about 70 mL of *Mobile phase*, stir for about 15 minutes, dilute with *Mobile phase* to volume, mix, and filter a portion to remove any particulate matter.
Chromatographic system—Proceed as directed in the *Assay* under *Ritodrine Hydrochloride Injection*.
Procedure—Proceed as directed in the *Assay* under *Ritodrine Hydrochloride*. Calculate the quantity, in mg, of $C_{17}H_{21}NO_3 \cdot HCl$ in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which the terms are as defined therein. ▲ (USP 1-May-2020)

Ritonavir



$C_{37}H_{48}N_6O_5S_2$ 720.94
2,4,7,12-Tetraazatridecan-13-oic acid, 10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-5-thiazolylmethyl ester [5*S*-(5*R**,8*R**,10*R**,11*R**)]-;
5-Thiazolylmethyl [(α*S*)-α-[(1*S*,3*S*)-1-hydroxy-3-[(2*S*)-2-[3-[(2-isopropyl-4-thiazolyl)methyl]-3-methylureido]3-methylbutyramido]-4-phenylbutyl]phenethyl]carbamate [155213-67-5].

DEFINITION
Ritonavir contains NLT 97.0% and NMT 102.0% of ritonavir ($C_{37}H_{48}N_6O_5S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* is within 2% of the retention time of the major peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 4.1 mg/mL of monobasic potassium phosphate in water
Solution B: Acetonitrile, tetrahydrofuran (inhibitor-free), *n*-butanol, and *Solution A* (18:8:5:69)
Mobile phase: *Solution B*
Diluent: Acetonitrile and *Solution A* (1:1)
Standard stock solution: 2.0 mg/mL of USP Ritonavir RS in *Diluent*. [NOTE—This solution may be kept for 5 days if refrigerated.]
Standard solution 1: 0.10 mg/mL of USP Ritonavir RS from the *Standard stock solution* diluted with *Diluent*
Standard solution 2: 0.025 mg/mL of USP Ritonavir RS from *Standard solution 1* diluted with *Diluent*
Sample solution: 0.025 mg/mL of Ritonavir in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 3-µm packing L26
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: 40 min
System suitability
Sample: *Standard solution 2*
Suitability requirements
Capacity factor, *k'*: NLT 13
Column efficiency: NLT 5000 theoretical plates
Tailing factor: 0.8–1.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentage of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Ritonavir taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ritonavir RS in *Standard solution 2* (mg/mL)
C_U = concentration of Ritonavir in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%, determined on 1.0 g
- **ORGANIC IMPURITIES**

Ritonavir is alkali sensitive. All glassware should be prerinsed with distilled water before use to remove residual detergent contamination.

Solution A, Solution B, Mobile phase, Diluent, Standard stock solution, and **Standard solution 1:** Prepare as directed in the *Assay*.

Solution C: Acetonitrile, tetrahydrofuran (inhibitor-free), *n*-butanol, and *Solution A* (47:8:5:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
60	100	0
120	0	100
120.1	100	0
155	100	0

Identity solution: 1 mg/mL of USP Ritonavir Related Compounds Mixture RS in *Diluent*

Standard solution 2: 5 µg/mL of USP Ritonavir RS from *Standard solution 1* in *Diluent*. [NOTE—This is stable for 48 h.]

Sample solution: 1 mg/mL of Ritonavir in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 3-µm packing L26

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time

Standard solution 2: 40 min

System suitability

Samples: *Identity solution* and *Standard solution 2* [NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.0 between hydroxyritonavir and hydantoin aminoalcohol peaks, *Identity solution*

Peak-to-valley ratio: NLT 1 for ritonavir and the 4-hydroxy isomer, *Identity solution*

Capacity factor, *k'*: NLT 13, *Standard solution 2*

Column efficiency: NLT 5000 theoretical plates, *Standard solution 2*

Tailing factor: 0.8–1.2, *Standard solution 2*

Relative standard deviation: NMT 3.0%, *Standard solution 2*

Analysis

Samples: *Diluent*, *Identity solution*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Ritonavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from *Standard solution 2*
C_S = concentration of *Standard solution 2* (mg/mL)
C_U = concentration of Ritonavir in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mixture of ureidovaline and *N*-deacylvaline ritonavir[a]	0.07	1.0	0.1
Acetamidoalcohol[b]	0.15	1.0	0.1
2,5-Thiazolylmethyl-dicarbamate[c]	0.24	1.37	0.1
Hydroxyritonavir[d]	0.36	1.0	0.3
Hydantoin aminoalcohol[e]	0.39	0.73	0.1
Ritonavir hydroperoxide[f]	0.45	1.0	0.1
Hydantoin-oxazolidinone derivative[g]	0.47	0.76	0.1
Ethyl analog[h]	0.64	1.0	0.1
Mixture of BOC-aminoalcohol and isobutoxycarbonyl aminoalcohol[i]	0.81	0.74	0.1
Oxazolidinone derivative[j]	0.87	0.53	0.1
Ureidovaline isobutyl ester[k]	0.94	1.0	0.1
4-Hydroxy isomer[l]	1.05	1.0	0.1
3*R*-Epimer[m]	1.11	1.0	0.3
Aminoalcohol urea derivative[n]	1.14	1.0	0.1
3*R*,5*R*-Diastereomer[o]	1.23	1.0	0.1
5*R*-Epimer[p]	1.32	1.0	0.1
Diacyl valine urea[q]	1.62	1.0	0.1
Divalinyl analog[r]	2.87	0.73	0.2
O-Acyl ritonavir[s]	3.20	1.0	0.1
Any other individual impurity	—	1.0	0.1

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	1.0

[a] Ureidovaline is [*N*-methyl[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl-L-valine and *N*-deacylvaline ritonavir is thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[b] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[c] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate.
[d] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-2-(3-{[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[e] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.
[f] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-(3-{[2-(2-hydroperoxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[g] (4*S*,5*S*)-Thiazol-5-ylmethyl 4-benzyl-5-{(*S*)-2-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl}-2-oxooxazolidine-3-carboxylate.
[h] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[i] BOC-aminoalcohol is thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate and isobutoxycarbonyl aminoalcohol is thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[j] (*S*)-*N*-[(*S*)-1-[(4*S*,5*S*)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide.
[k] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[l] Thiazol-5-ylmethyl (2*S*,4*S*,5*S*)-4-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[m] Thiazol-5-ylmethyl (2*S*,3*R*,5*S*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[n] Bis(thiazol-5-ylmethyl) (2*S*,2′*S*,3*S*,3′*S*,5*S*,5′*S*)-5,5′-carbonylbis(azanediyl) bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.
[o] Thiazol-5-ylmethyl (2*S*,3*R*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[p] Thiazol-5-ylmethyl (2*S*,3*S*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[q] (3*S*,4*S*,6*S*,10*S*,13*S*,15*S*,16*S*)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.
[r] (2*S*,2′*S*)-*N*,*N*′-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis(2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide).
[s] (*S*)-[(5*S*,8*S*,10*S*,11*S*)-8,11-Dibenzyl-5-isopropyl-1-(2-isopropylthiazol-4-yl)-2-methyl-3,6,13-trioxo-15-(thiazol-5-yl)-14-oxa-2,4,7,12-tetraazapentadecan-10-yl] 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%, determined on 0.500 g

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 5° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ritonavir RS
  USP Ritonavir Related Compounds Mixture RS

Ritonavir Capsules

DEFINITION
Ritonavir Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 4.1 g/L of monobasic potassium phosphate
Diluent: Acetonitrile and *Buffer* (50:50)
Mobile phase: Acetonitrile, methanol, tetrahydrofuran (stabilizer-free), and *Buffer* (7:4:4:25). Separately filter the *Buffer* and the pre-mixed solvents before combining to make the *Mobile phase*.
Standard solution: 25 µg/mL of USP Ritonavir RS in *Diluent*
Sample stock solution: Nominally 1 mg/mL of ritonavir prepared as follows. Transfer Capsules (NLT 5) equivalent to 500 mg of ritonavir into a 500-mL volumetric flask, add about 250 mL of *Diluent*, and shake for at least 30 min or until the Capsules have visually disintegrated. Add 150 mL of acetonitrile, allow to cool to room temperature, and dilute to volume with *Diluent*.
Sample solution: Nominally 25 µg/mL of ritonavir in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: NLT 15
Tailing factor: 0.8–1.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

▲Test 1▲ (RB 1-Apr-2018)
Medium: 0.1 N hydrochloric acid with 25 mM polyoxyethylene 10 lauryl ether, 900 mL
Apparatus 2: 50 rpm, with sinkers
Time: 30 min
Buffer: 4.1 g/L of monobasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (55:45). Adjust with phosphoric acid to a pH of 4.0 ± 0.1.
Standard stock solution: 5.2 mg/mL of USP Ritonavir RS in methanol
Standard working solution: 104 µg/mL of USP Ritonavir RS in *Medium*

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Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)
L = ritonavir label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) is dissolved.

▲**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid with 25 mM polyoxyethylene 10 lauryl ether; 900 mL, degassed
Apparatus 2: 50 rpm, with sinker
Time: 20 and 120 min
Solution A: Water and phosphoric acid (98:2)
Buffer: Water adjusted with *Solution A* to a pH of 3.5
Mobile phase: Acetonitrile, methanol, and *Buffer* (500:100:400).
Standard stock solution: 0.56 mg/mL of USP Ritonavir RS in methanol
Standard solution: 0.11 mg/mL of USP Ritonavir RS in *Medium* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.8 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of ritonavir from the *Sample solution*
r_S = peak response of ritonavir from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

C_i = concentration of ritonavir in the portion of sample withdrawn at the specified time point i (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of ritonavir (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point i (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (min)	Tolerances ▲▲ (ERR 1-Nov-2018)
1	20	20%–40%
2	120	NLT 80% ▲(Q)▲ (ERR 1-Nov-2018)▲ (RB 1-Apr-2018)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
[Note—Ritonavir is alkali sensitive. All glassware should be pre-rinsed with distilled water before use to remove residual detergent contamination.]
Buffer A: 4.1 g/L of monobasic potassium phosphate
Buffer B: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate
Solution A: Acetonitrile and *Buffer A* (50:50)
Solution B: Acetonitrile and *Buffer A* (65:35)
Solution C: Butyl alcohol and *Buffer A* (8:92)
Mobile phase: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer B* (18:5:8:69). Adjust apparent pH to 6.3 ± 0.1 with 1 M phosphoric acid or 1 M potassium hydroxide if necessary.
Cleaning solution: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer A* (30:8:13:49)
Standard stock solution: 0.1 mg/mL of USP Ritonavir RS in *Solution A*
Standard solution: 10 µg/mL of USP Ritonavir RS in *Solution C* from *Standard stock solution*
Peak identification solution: Transfer 5–10 g from contents of Capsules into a suitable sealed container. Add an amount of citric acid equivalent to 1% of the Capsule weight taken, and mix until dissolved. Seal the container, and heat at 60° for about 24 h. Transfer about 2 g to a 100-mL volumetric flask, and dilute with *Solution B* to volume. Transfer 5.0 mL of the solution to a 50-mL centrifuge tube that has been previously rinsed with methanol and dried. Add 20.0 mL of heptane, and seal the tube with a stopper. Shake vigorously until a uniform emulsion is obtained, making sure to vent periodically. The emulsion formed yields distinct layers when centrifuged.

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The top layer (clear heptane) and the bottom layer (clear sample solution) are separated by a viscous white cloudy layer. The middle layer is part of the heptane layer. Carefully remove the clear heptane layer and the middle layer. Pass the bottom layer through a solid phase extraction cartridge containing strong anion-exchange packing in acetate form as described below.

Sample stock solution: Nominally 2 mg/mL of ritonavir prepared as follows. Empty the contents of Capsules (NLT 6) into a suitable container, and accurately weigh and transfer an equivalent to 200 mg of ritonavir to a 100-mL volumetric flask. Dissolve and dilute with *Solution B* to volume.

Sample solution: Nominally 1 mg/mL of ritonavir prepared as follows. Transfer 25.0 mL of *Sample stock solution* into a 50-mL volumetric flask, and dilute with *Solution C* to volume. Add 15.0 mL of this solution into a 50-mL centrifuge tube that has been previously rinsed with methanol and dried. Add 20.0 mL of heptane, and seal the tube with a stopper. Shake vigorously until a uniform emulsion is obtained, making sure to vent periodically. The emulsion formed yields distinct layers when centrifuged. The top layer (clear heptane) and the bottom layer (clear sample solution) are separated by a viscous white cloudy layer. The middle layer is part of the heptane layer. Carefully remove the clear heptane layer and the middle layer. Pass the bottom layer through a solid phase extraction cartridge containing strong anion-exchange packing in acetate form as described below.

Condition a solid phase extraction cartridge with methanol and *Solution B* two separate times, and dry for 10 min under low vacuum. Add 5.0 mL of the clear sample solution into the reservoir. Collect the sample solution at a slow rate into a 5-mL volumetric flask using low vacuum. Dilute with *Solution B* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 3-µm packing L26. Wash the column after each injection of the *Peak identification solution* and each injection of the *Sample solution* with *Cleaning solution* for about 26 min, and equilibrate with *Mobile phase* for about 30 min. Store in *Cleaning solution* after the analysis is completed.

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: 1.8 times the retention time of ritonavir

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: NLT 13

Tailing factor: 0.8–1.2

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Peak identification solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of ritonavir from the *Standard solution*

C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)

F = relative response factor (see ▲*Table 2*▲ (RB 1-Apr-2018))

Acceptance criteria: See ▲*Table 2*. Reporting threshold is▲ (RB 1-Apr-2018) 0.05%.

▲Table 2▲ (RB 1-Apr-2018)

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Ureidovaline[a, b]	0.03	—	—
N-Deacylvaline ritonavir[c, d]	0.11	1.0	0.4
Acetamidoalcohol[b, e]	0.15	1.0	0.1
2,5-Thiazolylmethyldicarbamate[b, f]	0.24	1.37	0.1
Hydroxyritonavir[d, g]	0.36	1.0	0.2
Hydantoin aminoalcohol[d, h]	0.39	0.73	0.9
Ritonavir hydroperoxide[d, i]	0.44	—	—
Ethanol adduct[d, i]	0.45	0.66	0.3
Hydantoin-oxazolidinone derivative[b, k]	0.50	0.76	0.2
Ethyl analog[b, l]	0.64	1.0	0.1
Geo-isomer[d, m]	0.74	1.0	0.3
BOC-aminoalcohol[b, n]	0.81	—	—
Isobutoxycarbonyl aminoalcohol[b, o]		0.74	0.1
Oxazolidinone derivative[d, p]	0.87	0.53	1.0
Ureidovaline isobutyl ester[b, q]	0.94	1.0	0.1
Ritonavir	1.00	—	—
4-Hydroxy isomer[b, r]	1.05	1.0	0.1
3*R*-Epimer[b, s]	1.11	1.0	0.3
Aminoalcohol urea derivative[b, t]	1.14	1.0	0.1
3*R*,5*R*-Diastereomer[b, u]	1.23	1.0	0.1
5*R*-Epimer[b, v]	1.32	1.0	0.1
Diacyl valine urea[b, w]	1.70	1.0	0.1
Any other individual impurity	—	—	0.2
Total process impurity	—	—	0.8

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Table 2▲ (RB 1-Apr-2018) *(continued)*

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	3.0

[a] [*N*-Methyl[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl-L-valine (not quantified by this method due to solvent front and placebo interferences).
[b] Process impurity.
[c] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[d] Degradation impurity.
[e] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[f] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate.
[g] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-2-(3-{[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[h] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.
[i] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-(3-{[2-(2-hydroperoxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (report as ethanol adduct due to possible co-elution).
[j] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-ethoxycarbonylamino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[k] (4*S*,5*S*)-Thiazol-5-ylmethyl 4-benzyl-5-{(*S*)-2-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl}-2-oxooxazolidine-3-carboxylate.
[l] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-(3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[m] (*S*)-{(2*S*,3*S*,5*S*)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl} 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[n] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (may co-elute with isobutoxycarbonyl aminoalcohol; report as isobutoxycarbonyl aminoalcohol).
[o] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[p] (*S*)-*N*-[(*S*)-1-[(4*S*,5*S*)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide.
[q] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[r] Thiazol-5-ylmethyl (2*S*,4*S*,5*S*)-4-hydroxy-5-[(*S*)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[s] Thiazol-5-ylmethyl (2*S*,3*R*,5*S*)-3-hydroxy-5-[(*S*)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[t] Bis(thiazol-5-ylmethyl) (2*S*,2′*S*,3*S*,3′*S*,5*S*,5′*S*)-5,5′-carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.
[u] Thiazol-5-ylmethyl (2*S*,3*R*,5*R*)-3-hydroxy-5-[(*S*)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[v] Thiazol-5-ylmethyl (2*S*,3*S*,5*R*)-3-hydroxy-5-[(*S*)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[w] (3*S*,4*S*,6*S*,10*S*,13*S*,15*S*,16*S*)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g. It meets the requirements of the test for absence of *Escherichia coli* and *Salmonella*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 2° and 8°.

Add the following:

▲• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. ▲ (RB 1-Apr-2018)

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ritonavir RS

Ritonavir Oral Solution

DEFINITION

Ritonavir Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 4.1 g/L of monobasic potassium phosphate
Mobile phase: Acetonitrile, methanol, tetrahydrofuran (stabilizer-free), and *Buffer* (17.5: 10: 10: 62.5). Filter the required solutions individually prior to use.
Diluent: Acetonitrile and *Buffer* (50:50)
Standard solution: 25 µg/mL of USP Ritonavir RS in *Diluent*
Sample solution: Nominally 25 µg/mL of ritonavir in *Diluent* from a measured volume of Oral Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ritonavir in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

[NOTE—Ritonavir is alkali sensitive. All glassware should be prerinsed with distilled water before use to remove residual detergent contamination.]
Buffer A: 4.1 g/L of monobasic potassium phosphate
Buffer B: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate
Solution A: Acetonitrile and *Buffer A* (50:50)
Solution B: Acetonitrile and *Buffer A* (65:35)

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Solution C: Butyl alcohol and *Buffer A* (8:92)
Mobile phase: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer B* (18:5:8:69). Adjust with 1 M phosphoric acid or 1 M potassium hydroxide to a pH of 6.3 ± 0.1, if necessary.
Cleaning solution: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer A* (30:8:13:49)
Peak identification solution: Transfer 5–10 g of Oral Solution to a suitable sealed container. Add an amount of citric acid equivalent to 1% by weight of Oral Solution taken, and mix until dissolved. Seal the container, and heat at 70° for 24 h. Transfer 5.0 mL of the thermally degraded sample to a 200-mL volumetric flask. Dissolve and dilute with *Solution B* to volume.
Standard stock solution: 0.1 mg/mL of USP Ritonavir RS in *Solution A*
Standard solution: 10 µg/mL of USP Ritonavir RS in *Solution C* from the *Standard stock solution*
Sample stock solution: Nominally 2 mg/mL of ritonavir in *Solution B* prepared as follows. Transfer a measured volume of Oral Solution equivalent to 400 mg of ritonavir to a 200-mL volumetric flask, and dilute with *Solution B* to volume.
Sample solution: Nominally 1 mg/mL of ritonavir prepared as follows. Transfer 25.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Solution C* to volume. Transfer 15.0 mL of the solution to a 50-mL centrifuge tube that has been previously rinsed with methanol and dried. Add 20.0 mL of heptane, then stopper the tube. Shake the tube vigorously until a uniform emulsion is obtained, making sure to vent periodically. Centrifuge the resulting emulsion for about 5 min. Carefully aspirate off the top layer (heptane), leaving the clear bottom layer (*Sample solution*) in the tube. The centrifuged emulsion will have three distinct layers. The top layer (clear heptane) and the bottom layer (*Sample solution*) are separated by a viscous white cloudy layer. The middle layer should be considered part of the top layer for removal by aspiration. Repeat the extraction steps, and analyze the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 3-µm packing L26. Wash the column with the *Cleaning solution* after each injection of the *Peak identification solution* and each injection of the *Sample solution* for about 26 min, and equilibrate with *Mobile phase* for about 30 min. Store in the *Cleaning solution* after the analysis is completed.
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: 1.8 times the retention time of ritonavir
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.2
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Peak identification solution*, *Standard solution*, and *Sample solution*
[NOTE—Determine the relative retention value (r) for the components listed in *Table 1* as directed in *Chromatography* ⟨621⟩, using the time measured at the baseline deflection of the *Standard solution* chromatogram as the void volume (t_M).]
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of ritonavir from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05%.

Table 1

Name	Relative Retention (r)	Relative Response Factor	Acceptance Criteria, NMT (%)
Ureidovaline[a, b]	0.03	—	—
N-Deacylvaline ritonavir[c, d]	0.11	1.0	0.7
Acetamidoalcohol[b, e]	0.15	1.0	0.1
Hydroxypropyl carbamate analog[d]		0.59	0.4
2,5-Thiazolylmethyldicarbamate[b, f]	0.24	1.37	0.1
Hydroxyritonavir[d, g]	0.36	1.0	0.5
Hydantoin aminoalcohol[d, h]	0.39	0.73	0.5
Ritonavir hydroperoxide[d, i]	0.44	1.0	0.2
Ethyl carbamate analog[d, l]	0.45	0.66	1.0
Hydantoin-oxazolidinone derivative[b, k]	0.50	0.76	0.2
Ethyl analog[b, l]	0.64	1.0	0.1
O-Acyl isomer[d, m]	0.74	1.0	0.2
BOC-aminoalcohol[b, n]		—	—
Isobutoxycarbonyl aminoalcohol[b, o]	0.81	0.74	0.1
Oxazolidinone derivative[d, p]	0.87	0.53	0.2
Ureidovaline isobutyl ester[b, q]	0.94	1.0	0.1
Ritonavir	1.00	—	—
4-Hydroxy isomer[b, r]	1.05	1.0	0.1
3*R*-Epimer[b, s]	1.11	1.0	0.3
Aminoalcohol urea derivative[b, t]	1.14	1.0	0.1
3*R*,5*R*-Diastereomer[b, u]	1.23	1.0	0.1
5*R*-Epimer[b, v]	1.32	1.0	0.1
Diacyl valine urea[b, w]	1.70	1.0	0.1
Any individual unspecified degradation product	—	1.0	0.2
Total process impurity	—	—	1.2

USP Monographs

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Table 1 *(continued)*

Name	Relative Retention (*r*)	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	3.0

[a] {*N*-Methyl[(2-isopropyl-4-thiazolyl)methyl]amino}carbonyl-L-valine (not quantified by this method due to solvent front and placebo interferences; controlled as a synthetic impurity in the drug substance).
[b] Process impurity.
[c] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[d] Degradation product.
[e] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[f] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate. (If two peaks appear with a relative retention of 0.24, the second peak is identified as the 2,5-thiazolylmethyl dicarbamate impurity. If a single peak with a relative retention of 0.24 appears, report as *Hydroxypropyl carbamate analog*.)
[g] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-2-(3-{[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[h] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.
[i] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-(3-{[2-(2-hydroperoxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (unspecified degradant; report as *Ethyl carbamate analog* due to possible coelution).
[j] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-ethoxycarbonylamino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (possible coelution with ritonavir hydroperoxide).
[k] (4*S*,5*S*)-Thiazol-5-ylmethyl 4-benzyl-5-{(*S*)-2-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl}-2-oxooxazolidine-3-carboxylate.
[l] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[m] (*S*)-{(2*S*,3*S*,5*S*)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl} 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate (unspecified degradant).
[n] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (may coelute with isobutoxycarbonyl aminoalcohol; report as isobutoxycarbonyl aminoalcohol using a relative response factor of 0.74).
[o] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[p] (*S*)-*N*-[(*S*)-1-[(4*S*,5*S*)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide (unspecified degradant).
[q] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[r] Thiazol-5-ylmethyl (2*S*,4*S*,5*S*)-4-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[s] Thiazol-5-ylmethyl (2*S*,3*R*,5*S*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[t] Bis(thiazol-5-ylmethyl) (2*S*,2′*S*,3*S*,3′*S*,5*S*,5′*S*)-5,5′-carbonylbis(azanediyl) bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.
[u] Thiazol-5-ylmethyl (2*S*,3*R*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[v] Thiazol-5-ylmethyl (2*S*,3*S*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[w] (3*S*,4*S*,6*S*,10*S*,13*S*,15*S*,16*S*)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.

SPECIFIC TESTS

- **ALCOHOL CONTENT**

Internal standard solution: Transfer 10.0 mL of butyl alcohol to a 200-mL volumetric flask and dilute with methanol to volume.
Internal standard identity solution: Dilute 5.0 mL of the *Internal standard solution* with methanol to 100 mL.
Standard stock solution: 4.0% (v/v) of dehydrated alcohol in methanol
Standard solution: 0.4% (v/v) of dehydrated alcohol prepared as follows. Transfer 10.0 mL of the *Standard stock solution* and 5 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with methanol to volume.
Sample stock solution: Transfer 5.0 mL of Oral Solution to a 50-mL volumetric flask with the aid of several portions of methanol, and dilute with methanol to volume.
Sample solution: Transfer 10.0 mL of the *Sample stock solution* and 5.0 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused silica capillary; 1-µm layer of phase G16
Temperatures
Injection port: 185°
Detector: 220°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	10	145	6
145	20	200	9.75

Carrier gas: Helium
Flow rate: 4.5 mL/min
Makeup gas flow: 30 mL/min
Injection volume: 1 µL
Injection type: Split injection with a split ratio, 4:1
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.2 for the alcohol peak
Relative standard deviation: NMT 3.0% for the peak area ratio of alcohol to butyl alcohol
Analysis
Samples: *Internal standard identity solution*, *Standard solution*, and *Sample solution*
Calculate the percentage of alcohol in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times C \times D$$

R_U = peak area ratio of alcohol to butyl alcohol from the *Sample solution*
R_S = average peak area ratio of alcohol to butyl alcohol from the *Standard solution*
C = concentration of dehydrated alcohol in the *Standard solution* (% v/v)
D = dilution factor used to prepare the *Sample solution*, 100

Acceptance criteria: 40%–47% (v/v) of alcohol (C_2H_6O)

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

USP Monographs

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ritonavir RS

Ritonavir Tablets

DEFINITION
Ritonavir Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 4.1 g/L of monobasic potassium phosphate
Solution A: Acetonitrile and *Buffer* (50:50)
Solution B: Acetonitrile, butyl alcohol, water, and *Buffer* (65:15:10:10)
Mobile phase: Acetonitrile, methanol, tetrahydrofuran (stabilizer-free), and *Buffer* (17.5: 10: 10: 62.5). Filter the required solutions individually before use.
Standard solution: 0.1 mg/mL of USP Ritonavir RS in *Solution A*
Sample stock solution: Nominally 1 mg/mL of ritonavir prepared as follows. Transfer Tablets (NLT 5) equivalent to 500 mg of ritonavir into a 500-mL volumetric flask. Fill the flask half full with *Solution B*, and mechanically shake for at least 60 min or until the Tablets have visually disintegrated. Dilute with *Solution B* to volume, and stir for 30 min. Transfer a sufficient quantity of this solution to a centrifuge tube, and centrifuge for about 15 min. Use the supernatant to prepare the *Sample solution*.
Sample solution: Nominally 0.1 mg/mL of ritonavir in *Solution A* from the supernatant of *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: 15–24
Tailing factor: 0.8–1.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.06 M polyoxyethylene 10 lauryl ether; 900 mL
Apparatus 2: 75 rpm
Time: 120 min
Buffer: 4.1 g/L of monobasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (55:45). Adjust with phosphoric acid to a pH of 4.0 ± 0.1.
Standard stock solution: 1.11 mg/mL of USP Ritonavir RS in methanol
Standard working solution: 111 µg/mL of USP Ritonavir RS in *Medium* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.9–1.5
Capacity factor: Greater than 3.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)
L = label claim for ritonavir (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Ritonavir is alkali sensitive. All glassware should be pre-rinsed with distilled water before use to remove residual detergent contamination.
Buffer A: 4.1 g/L of monobasic potassium phosphate
Buffer B: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate
Solution A: Acetonitrile and *Buffer A* (50:50)
Solution B: Acetonitrile, butyl alcohol, water, and *Buffer A* (65:15:10:10)
Solution C: Acetonitrile, butyl alcohol, and *Buffer A* (15:5:80)
Mobile phase: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer B* (18:5:8:69) adjusted with 1 M phosphoric acid or 1 M potassium hydroxide, if necessary, to an apparent pH of 6.3 ± 0.1
Cleaning solution: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer A* (30:8:13:49)
Standard stock solution: 0.05 mg/mL of USP Ritonavir RS in *Solution A*
Standard solution: 2.5 µg/mL of USP Ritonavir RS in *Solution C* from *Standard stock solution*

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System suitability stock solution: 1 mg/mL of USP Ritonavir Related Compounds Mixture RS in *Solution B*
System suitability solution: 0.5 mg/mL of USP Ritonavir Related Compounds Mixture RS in *Solution C* from *System suitability stock solution*
Sample stock solution: Nominally 1 mg/mL prepared as follows. Transfer Tablets (NLT 5) equivalent to 500 mg of ritonavir into a 500-mL volumetric flask. Fill the flask half full with *Solution B*, and mechanically shake for at least 60 min or until the Tablets have visually disintegrated. Dilute with *Solution B* to volume, and stir for 30 min. Transfer a sufficient quantity of this solution to a centrifuge tube, and centrifuge for 15 min. Use the supernatant to prepare the *Sample solution*.
Sample solution: Nominally 0.5 mg/mL of ritonavir in *Solution C* from the supernatant of *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 3-µm packing L26. Wash the column after each injection of the *Sample solution* with *Cleaning solution* for about 26 min, and equilibrate with *Mobile phase* for about 30 min. Store in *Cleaning solution* after the analysis is completed.
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: 2.4 times the retention time of ritonavir
System suitability
Samples: *Standard solution* and *System suitability solution*
See *Table 1* for relative retention values. Disregard all peaks occurring before the *N*-deacylvaline ritonavir peak.
Suitability requirements
Resolution: Greater than 0.7 between the hydroxyritonavir and hydantoin aminoalcohol peaks, *System suitability solution*
Capacity factor: Greater than 10.8, *Standard solution*
Tailing factor: 0.8–1.2, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of ritonavir from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)
F = relative response factor *(See Table 1)*

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05%.

Table 1

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Deacylvaline ritonavir[a, b]	0.11	1.0	0.2
Acetamidoalcohol[c, d]	0.15	—	—

Table 1 *(continued)*

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
2,5-Thiazolylmethyldicarbamate[d, e]	0.24	—	—
Hydroxyritonavir[b, f]	0.36	1.0	0.3
Hydantoin aminoalcohol[b, g]	0.39	0.73	2.6
Ritonavir hydroperoxide[b, h]	0.44	1.0	0.2
Hydantoin-oxazolidinone derivative[d, i]	0.50	—	—
Ethyl analog[d, j]	0.64	—	—
Geo-isomer[b, k]	0.74	1.0	0.2
BOC-aminoalcohol[d, l]			
Isobutoxycarbonyl aminoalcohol[d, m]	0.81	—	—
Oxazolidinone derivative[b, n]	0.87	0.53	0.3
Ureidovaline isobutyl ester[d, o]	0.94	—	—
Ritonavir	1.00	—	—
4-Hydroxy isomer[d, p]	1.05	—	—
3*R*-Epimer[d, q]	1.11	—	—
Aminoalcohol urea derivative[d, r]	1.14	—	—
3*R*,5*R*-Diastereomer[d, s]	1.23	—	—
5*R*-Epimer[d, t]	1.32	—	—
Diacyl valine urea[d, u]	1.70	—	—
Any individual unspecified degradation product	—	1.0	0.2

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Table 1 *(continued)*

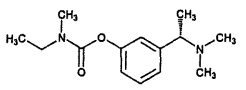
Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	3.5

[a] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[b] Degradation product.
[c] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[d] Process impurity included in this table for peak identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product nor included in the total impurities.
[e] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate. (Two peaks may be detected with a relative retention value of 0.24. The first peak is considered as an unknown impurity and the second as 2,5-thiazolylmethyldicarbamate.)
[f] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-2-(3-{[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[g] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.
[h] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-(3-{[2-(2-hydroperoxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[i] (4*S*,5*S*)-Thiazol-5-ylmethyl 4-benzyl-5-((*S*)-2-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl)-2-oxooxazolidine-3-carboxylate.
[j] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[k] (*S*)-((2*S*,3*S*,5*S*)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy) carbonylamino]hexan-3-yl} 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[l] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (may co-elute with isobutoxycarbonyl aminoalcohol).
[m] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (may co-elute with BOC-aminoalcohol).
[n] (*S*)-*N*-[(*S*)-1-[(4*S*,5*S*)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide.
[o] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[p] Thiazol-5-ylmethyl (2*S*,4*S*,5*S*)-4-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[q] Thiazol-5-ylmethyl (2*S*,3*R*,5*S*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[r] Bis(thiazol-5-ylmethyl) (2*S*,2′*S*,3*S*,3′*S*,5*S*,5′*S*)-5,5′-carbonylbis(azanediyl) bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.
[s] Thiazol-5-ylmethyl (2*S*,3*R*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl) methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[t] Thiazol-5-ylmethyl (2*S*,3*S*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl) methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.
[u] (3*S*,4*S*,6*S*,10*S*,13*S*,15*S*,16*S*)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at or below 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ritonavir RS
  USP Ritonavir Related Compounds Mixture RS

Rivastigmine



$C_{14}H_{22}N_2O_2$ 250.34

Ethylmethylcarbamic acid, 3-[(*S*)-1-(dimethylamino)ethyl] phenyl ester;
(*S*)-3-[1-(Dimethylamino)ethyl]phenyl ethyl(methyl) carbamate [123441-03-2].

DEFINITION

Rivastigmine contains NLT 98.0% and NMT 102.0% of rivastigmine ($C_{14}H_{22}N_2O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Enantiomeric Purity*.

ASSAY

- **PROCEDURE**

[NOTE—Protect solutions containing rivastigmine from light.]

Buffer: 8.9 g/L of dibasic sodium phosphate dihydrate in water (0.05 M)

Mobile phase: Methanol and *Buffer* (58:42), adjusted with phosphoric acid to a pH of 8.45. [NOTE—Let the solution cool to room temperature before pH adjustment.]

System suitability solution: 1 mg/mL of USP Rivastigmine Tartrate RS, and 1 µg/mL each of USP Rivastigmine Related Compound B RS, USP Rivastigmine Related Compound C RS, and USP Rivastigmine Related Compound D RS in *Mobile phase*

Standard solution: 1.0 mg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*

Sample solution: 0.625 mg/mL of Rivastigmine in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.0-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for rivastigmine related compound C, rivastigmine related compound D, rivastigmine related compound B, and rivastigmine are 0.37, 0.56, 0.68, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between rivastigmine related compound D and rivastigmine related compound B, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of rivastigmine ($C_{14}H_{22}N_2O_2$) in the portion of Rivastigmine taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rivastigmine Tartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Rivastigmine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of rivastigmine, 250.34
M_{r2} = molecular weight of rivastigmine tartrate, 400.42

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

[NOTE—Protect solutions containing rivastigmine from light.]

Buffer, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.0025 mg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*

Sensitivity solution: 0.5 µg/mL of USP Rivastigmine Tartrate RS in *Mobile phase* from the *Standard solution*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—The relative retention times for rivastigmine related compound C, rivastigmine related compound D, rivastigmine related compound B, and rivastigmine are 0.37, 0.56, 0.68, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between rivastigmine related compound D and rivastigmine related compound B, *System suitability solution*

Signal-to-noise ratio: NLT 10 for rivastigmine, *Sensitivity solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Rivastigmine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of rivastigmine from the *Standard solution*
C_S = concentration of USP Rivastigmine Tartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Rivastigmine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of rivastigmine, 250.34
M_{r2} = molecular weight of rivastigmine tartrate, 400.42
F = relative response factor, see *Table 1*

Acceptance criteria: See *Table 1*. Disregard the system peak eluting at the relative retention time of about 0.45.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenol impurity (rivastigmine related compound C)	0.37	1.7	0.3
Nor impurity[a]	0.68	1.0	0.1
Rivastigmine	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] (*S*)-3-[1-(Dimethylamino)ethyl]phenyl dimethylcarbamate (racemic mixture is rivastigmine related compound B).

- **ENANTIOMERIC PURITY**

[NOTE—Protect solutions containing rivastigmine from light.]

Buffer: Dissolve 1.78 g of dibasic sodium phosphate dihydrate and 1.38 g of monobasic sodium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 6.0.

Mobile phase: Transfer 20.0 mL of acetonitrile and 205 µL of *N,N*-dimethyloctylamine into a 1000-mL volumetric flask, and dilute with *Buffer* to volume.

System suitability solution: 0.1 mg/mL of USP Rivastigmine Tartrate RS and 0.1 µg/mL of USP Rivastigmine Tartrate R-Isomer RS in *Mobile phase*

Standard solution: 0.1 µg/mL of USP Rivastigmine Tartrate R-Isomer RS in *Mobile phase*

Sample solution: 0.0625 mg/mL of Rivastigmine in *Mobile phase*

Sensitivity solution: 0.05 µg/mL of USP Rivastigmine Tartrate R-Isomer RS in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.0-mm × 10-cm; 5-µm packing L41

Flow rate: 0.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—The relative retention times for rivastigmine *R*-isomer and rivastigmine are 0.85 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 10%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Resolution: NLT 0.8 between rivastigmine *R*-isomer and rivastigmine, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of rivastigmine *R*-isomer in the portion of Rivastigmine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of rivastigmine *R*-isomer from the *Sample solution*
r_S = peak response of rivastigmine *R*-isomer from the *Standard solution*
C_S = concentration of USP Rivastigmine Tartrate R-Isomer RS in the *Standard solution* (mg/mL)

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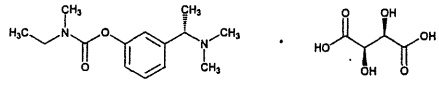
C_U = concentration of Rivastigmine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of rivastigmine, 250.34
M_{r2} = molecular weight of rivastigmine tartrate, 400.42

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers under inert gas protected from light. Store at 2°–8°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Rivastigmine RS
USP Rivastigmine Related Compound B RS
Nor impurity (racemic mixture);
(*RS*)-3-[1-(Dimethylamino)ethyl]phenyl dimethylcarbamate.
$C_{13}H_{20}N_2O_2$ 236.31
USP Rivastigmine Related Compound C RS
Phenol impurity;
(*S*)-3-[1-(Dimethylamino)ethyl]phenol.
$C_{10}H_{15}NO$ 165.23
USP Rivastigmine Related Compound D RS
Acetylphenol impurity;
3-Acetylphenyl ethyl(methyl)carbamate.
$C_{12}H_{15}NO_3$ 221.25
USP Rivastigmine Tartrate RS
USP Rivastigmine Tartrate R-Isomer RS
(*R*)-3-[1-(Dimethylamino)ethyl]phenyl ethyl (methyl) carbamate tartrate.
$C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$ 400.42

Rivastigmine Tartrate



$C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$ 400.42
Ethylmethylcarbamic acid, 3-[(*S*)-1-(dimethylamino)ethyl] phenyl ester, (2*R*,3*R*)-2,3-dihydroxybutanedioate;
(*S*)-3-[1-(Dimethylamino)ethyl]phenyl ethylmethylcarbamate, hydrogen tartrate [129101-54-8].
Rivastigmine 250.34
[123441-03-2].

DEFINITION
Rivastigmine Tartrate contains NLT 98.0% and NMT 102.0% of the labeled amount of $C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Organic Impurities, Procedure 2: Enantiomeric Purity*.

ASSAY
• **PROCEDURE**
Buffer: 8.6 mg/mL of monobasic ammonium phosphate. Adjust with ammonia solution to a pH of 7.0.
Mobile phase: Methanol, acetonitrile, and *Buffer* (15:15:70)
System suitability solution: 0.05 mg/mL each of USP Rivastigmine Related Compound A RS and USP Rivastigmine Related Compound B RS in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Rivastigmine Tartrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection size: 20 µL
[NOTE—The flow rate may be adjusted to 1.5 mL/min, if needed, to achieve a recommended retention time of rivastigmine at about 10 min.]
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between rivastigmine related compound A and rivastigmine related compound B, *System suitability solution*
Column efficiency: NLT 5000 theoretical plates, *Standard solution*
Tailing factor: NMT 3.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$ in the portion of Rivastigmine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
ORGANIC IMPURITIES
• **Procedure 1**
Mobile phase and **System suitability solution:** Proceed as directed in the *Assay*.
Standard solution: 1.0 µg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Rivastigmine Tartrate in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*.
(See *Chromatography* ⟨621⟩, *System Suitability*.)
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between rivastigmine related compound A and rivastigmine related compound B, *System suitability solution*
Relative standard deviation: NMT 10%, *Standard solution*
Analysis [NOTE—The run time is 8 times the retention time of the rivastigmine peak.]
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of any individual impurity in the portion of Rivastigmine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rivastigmine Tartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Rivastigmine Tartrate in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tartrate	0.18	—	Disregard
Phenol impurity[a]	0.28	1.6	0.3
DPTTA[b]	0.46	0.83	0.15
Nor impurity[c]	0.57	1.2	0.15
Rivastigmine	1.0	1.0	—
Carbamate impurity[d]	4.1	1.3	0.15
Ether impurity[e]	6.5	1.4	0.15
Any other impurity	—	1.0	0.1

[a] (*S*)-3-[1-(Dimethylamino)ethyl]phenol.
[b] (+)-Di-(*p*-toluoyl)-D-tartaric acid (rivastigmine related compound A).
[c] (*S*)-3-[1-(Dimethylamino)ethyl]phenyl dimethylcarbamate (racemic mixture is rivastigmine related compound B).
[d] 3-Nitrophenyl ethyl(methyl)carbamate.
[e] (*S*)-*N*,*N*-Dimethyl-1-[3-(4-nitrophenoxy)phenyl]ethanamine.

• Procedure 2: Enantiomeric Purity
Buffer: Transfer 1.78 g of dibasic sodium phosphate dihydrate and 1.38 g of monobasic sodium phosphate into a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Adjust with phosphoric acid to a pH of 6.0.
Mobile phase: Transfer 20 mL of acetonitrile and 205 µL of *N*,*N*-dimethyloctylamine to a 1000-mL volumetric flask, and dilute with *Buffer* to volume.
Standard solution: 0.1 µg/mL of USP Rivastigmine Tartrate R-Isomer RS in *Mobile phase*
Sensitivity solution: 0.05 µg/mL of USP Rivastigmine Tartrate R-Isomer RS in *Mobile phase*, *Standard solution*
System suitability solution: 100 µg/mL of USP Rivastigmine Tartrate RS and 0.1 µg/mL of USP Rivastigmine Tartrate R-Isomer RS in *Mobile phase*
Sample solution: 100 µg/mL of Rivastigmine Tartrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.0-mm × 10-cm; packing L41
Flow rate: 0.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution*, *Sensitivity solution*, and *System suitability solution*
Suitability requirements
Resolution: NLT 0.8 between the enantiomer peaks, *System suitability solution*
[NOTE—The elution order is the *R*-enantiomer, followed by the rivastigmine peak, which is the *S*-enantiomer.]
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 10%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the *R*-enantiomer in the portion of Rivastigmine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *R*-enantiomer from the *Sample solution*
r_S = peak response of *R*-enantiomer from the *Standard solution*
C_S = concentration of *R*-enantiomer in the *Standard solution* (µg/mL)
C_U = concentration of Rivastigmine Tartrate in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.3% of the *R*-enantiomer

SPECIFIC TESTS
• WATER DETERMINATION, *Method Ia* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• PACKAGING AND STORAGE: Preserve in tight containers, and store at room temperature.
• USP REFERENCE STANDARDS ⟨11⟩
USP Rivastigmine Tartrate RS
USP Rivastigmine Related Compound A RS
(+)-Di-(*p*-toluoyl)-D-tartaric acid.
$C_{20}H_{18}O_8$ 386.35
USP Rivastigmine Related Compound B RS
(*RS*)-3-[1-(Dimethylamino)ethyl]phenyl dimethylcarbamate.
$C_{13}H_{20}N_2O_2$ 236.32
USP Rivastigmine Tartrate R-Isomer RS

Rivastigmine Tartrate Capsules

DEFINITION
Rivastigmine Tartrate Capsules contain an amount of Rivastigmine Tartrate equivalent to NLT 94.0% and NMT 105.0% of the labeled amount of rivastigmine ($C_{14}H_{22}N_2O_2$).

IDENTIFICATION
• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• PROCEDURE
Buffer: 8.6 mg/mL of monobasic ammonium phosphate in water. Adjust with ammonia solution to a pH of 7.0.
Mobile phase: Methanol, acetonitrile, and *Buffer* (15:15:70)
Standard solution: 0.064 mg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*. [NOTE—Use a small amount of methanol (about 2% of the final volume) to facilitate dissolution before diluting with *Mobile phase* to volume, and use sonication if necessary.]

USP Monographs

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System suitability solution: 0.01 mg/mL each of USP Rivastigmine Related Compound A RS and USP Rivastigmine Related Compound B RS in *Mobile phase*

Sample solution: Remove as completely as possible the contents of NLT 20 Capsules, and mix. Transfer a weighed portion of the combined contents, equivalent to about 48 mg of rivastigmine, to a 250-mL volumetric flask. Add 25 mL of methanol and 60 mL of *Mobile phase*, and sonicate for 15 min to disperse the contents. Dilute with *Mobile phase* to volume, mix well, and centrifuge. Dilute a portion of the supernatant with *Mobile phase* to obtain a solution having a concentration of 0.038 mg/mL of rivastigmine, based on the label claim.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for rivastigmine related compound A, rivastigmine related compound B, and rivastigmine are 0.46, 0.57, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between rivastigmine related compound A and rivastigmine related compound B, *System suitability solution*
Column efficiency: NLT 5000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.3, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{22}N_2O_2$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of rivastigmine from the *Sample solution*
r_S = peak response of rivastigmine from the *Standard solution*
C_S = concentration of USP Rivastigmine Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of rivastigmine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of rivastigmine, 250.34
M_{r2} = molecular weight of rivastigmine tartrate, 400.42

Acceptance criteria: 94.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1
Medium: Water; 500 mL, deaerated
Apparatus 2: 50 rpm, with sinkers, if necessary
Time: 30 min
Buffer, Mobile phase, and **System suitability solution:** Prepare as directed in the *Assay*.
Standard solution: 0.192 mg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*. Further dilute with *Medium* to obtain a solution having a concentration similar to that expected in the *Sample solution*.
Sample solutions: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few mL.
Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.
[NOTE—Use an injection size of 100 µL.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{22}N_2O_2$ dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
M_{r1} = molecular weight of rivastigmine, 250.34
M_{r2} = molecular weight of rivastigmine tartrate, 400.42
V = volume of *Medium* (mL), 500
L = Capsule label claim (mg)

Tolerances: NLT 75% (Q) of the labeled amount of $C_{14}H_{22}N_2O_2$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Deaerated water; 500 mL
Apparatus 2: 75 rpm
Time: 15 min
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: 0.192 mg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*. Further dilute with *Medium* to obtain a solution having a concentration similar to that expected in the *Sample solution*.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few milliliters.
Chromatographic system: Proceed as directed in the *Assay*. [NOTE—Use an injection volume of 100 µL.]
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Tailing factor: NMT 2.3
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rivastigmine ($C_{14}H_{22}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
M_{r1} = molecular weight of rivastigmine, 250.34
M_{r2} = molecular weight of rivastigmine tartrate, 400.42
V = volume of *Medium* (mL), 500
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of rivastigmine ($C_{14}H_{22}N_2O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Buffer, Mobile phase, and **System suitability solution:** Prepare as directed in the *Assay*.
Standard solution: 1.6 µg/mL of USP Rivastigmine Tartrate RS in *Mobile phase*
Sample solution: Remove as completely as possible the contents of NLT 20 Capsules, and mix. Transfer a weighed

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portion of the combined contents, equivalent to 25 mg of rivastigmine, to a 25-mL volumetric flask. Disperse in 10 mL of *Mobile phase*, and sonicate for 15 min. Dilute with *Mobile phase* to volume, mix well, and centrifuge. Use the supernatant.

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between rivastigmine related compound A and rivastigmine related compound B, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Identify the peaks using the relative retention times provided in *Impurity Table 1*.]

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of rivastigmine from the *Standard solution*

C_S = concentration of USP Rivastigmine Tartrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of rivastigmine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of rivastigmine, 250.34

M_{r2} = molecular weight of rivastigmine tartrate, 400.42

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenol impurity[a]	0.28	1.6	0.6
Rivastigmine	1.0	1.0	—
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.0

[a] (*S*)-3-[1-(Dimethylamino)ethyl]phenol.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Rivastigmine Tartrate RS

USP Rivastigmine Related Compound A RS

(+)-Di-(*p*-toluoyl)-D-tartaric acid.

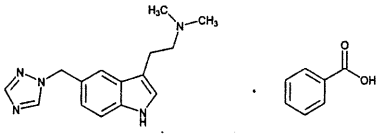
$C_{20}H_{18}O_8$ 386.35

USP Rivastigmine Related Compound B RS

(*RS*)-3-[1-(Dimethylamino)ethyl]phenyl dimethylcarbamate.

$C_{13}H_{20}N_2O_2$ 236.32

Rizatriptan Benzoate



$C_{22}H_{25}N_5O_2$ 391.47

1*H*-Indole-3-ethanamine, *N*,*N*-dimethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-, monobenzoate;

3-[2-(Dimethylamino)ethyl]-5-(1*H*-1,2,4-triazol-1-ylmethyl) indole monobenzoate [145202-66-0].

DEFINITION

Rizatriptan Benzoate contains NLT 98.0% and NMT 102.0% of $C_{22}H_{25}N_5O_2$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)

[NOTE—If the spectra obtained show differences, dissolve the substance to be examined and the Reference Standard separately in methanol, evaporate to dryness, and record the new spectra using the residues.]

- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Use silanized autosampler vials and freshly prepared solutions.]

Solution A: Add 1.0 mL of trifluroacetic acid to 1 L of a solution of acetonitrile and water (4:21), and mix.

Solution B: Acetonitrile and trifluroacetic acid (1000:1)

Standard solution: 1 mg/mL of USP Rizatriptan Benzoate RS in *Solution A*

Sample solution: 1 mg/mL of Rizatriptan Benzoate in *Solution A*

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8.0	100	0
17.0	70	30
20.0	70	30
20.1	100	0
23.0	100	0

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L11

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for rizatriptan and benzoic acid are 1.0 and about 2.1, respectively.]

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Suitability requirements
Tailing factor: NMT 3.5 for rizatriptan
Relative standard deviation: NMT 0.73% for the rizatriptan peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{25}N_5O_2$ in the portion of Rizatriptan Benzoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of rizatriptan from the *Sample solution*
r_S = peak response of rizatriptan from the *Standard solution*
C_S = concentration of USP Rizatriptan Benzoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Rizatriptan Benzoate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

• **Procedure**
Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay*.
Sample solution: 1 mg/mL of Rizatriptan Benzoate in *Solution A*
System suitability solution: 1 mg/mL of USP Rizatriptan Benzoate System Suitability Mixture RS in *Solution A*
Sensitivity solution: 0.5 µg/mL of Rizatriptan Benzoate obtained by suitable dilution of the *Sample solution* with *Solution A*
Chromatographic system: Prepare as directed in the *Assay*.
System suitability
Sample: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for rizatriptan, rizatriptan impurity C, and benzoic acid are 1.0, about 1.3, and about 2.1, respectively.]
Suitability requirements
Resolution: NLT 2.0 between rizatriptan and rizatriptan impurity C, *System suitability solution*
Signal-to-noise ratio: NLT 10 for the rizatriptain peak, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Rizatriptan Benzoate taken:

$$\text{Result} = [r_U/(r_T - r_{BA})] \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the areas of all the peaks from the *Sample solution*
r_{BA} = area of the benzoic acid peak from the *Sample solution*

Acceptance criteria
Any individual impurity: NMT 0.10%
Total: NMT 0.3%. [NOTE—Disregard any impurity that is less than 0.05%.]

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in well-closed containers at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Rizatriptan Benzoate RS
USP Rizatriptan Benzoate System Suitability Mixture RS
Mixture of rizatriptan benzoate and at least 0.1% of rizatriptan impurity C. Rizatriptan impurity C is 2-{5-[(1*H*-1,2,4-triazol-1-yl)methyl]-1*H*-indol-2-yl}-*N*,*N*-dimethylethanamine.
($C_{15}H_{19}N_5$ 269.35)

Rizatriptan Benzoate Tablets

DEFINITION

Rizatriptan Benzoate Tablets contain an amount of rizatriptan benzoate ($C_{15}H_{19}N_5 \cdot C_7H_6O_2$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020)
Sample: Grind a suitable number of Tablets containing NLT 125 mg of rizatriptan. Transfer a suitable amount of powder equivalent to NLT 100 mg of rizatriptan to a suitable flask to obtain a 5 mg/mL solution. Add 75% of the flask volume of methanol. Shake vigorously to disperse the powder. Dilute with methanol to volume. Pass a portion through a suitable filter. Evaporate the filtrate under a stream of nitrogen. Prepare a mull of the residue with mineral oil.
Acceptance criteria: The spectrum of the *Sample* corresponds to that of a similarly prepared Standard.
• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: Dissolve 1.7 g of monobasic potassium phosphate and 0.9 g of sodium 1-hexanesulfonate to a 1-L volumetric flask. Add 900 mL of water. Adjust with 50% (w/w) sodium hydroxide solution to a pH of 6.8. Dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (15:85)
Diluent: Acetonitrile and 0.05 M ammonium acetate (20:80)
Standard solution: 0.06 mg/mL of USP Rizatriptan Benzoate RS in *Diluent*
Sample stock solution: Nominally 0.2 mg/mL of rizatriptan in *Diluent* prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask. Add 10%–20% of the flask volume of *Diluent*. Swirl until the Tablets have disintegrated completely. Dilute with *Diluent* to volume. Stir the solution for 90–150 min. Centrifuge a portion of the resulting solution. Use the supernatant to prepare the *Sample solution*.
Sample solution: Nominally 0.04 mg/mL of rizatriptan from the supernatant of the *Sample stock solution* and *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm

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Column: 4.6-mm × 25-cm; 5-µm packing L1. [NOTE—A suitable guard column may be used if necessary.]
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2.5 times the retention time of rizatriptan
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Tailing factor: NMT 3 for the rizatriptan peak
Relative standard deviation: NMT 2.0% for the rizatriptan peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of rizatriptan from the *Sample solution*
r_S = peak response of rizatriptan from the *Standard solution*
C_S = concentration of USP Rizatriptan Benzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of rizatriptan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of rizatriptan, 269.35
M_{r2} = molecular weight of rizatriptan benzoate, 391.47

Acceptance criteria: 90.0%–110.0% of the labeled amount of rizatriptan

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water, deaerated if necessary; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Analyze the sample under test using either the *Instrumental procedure* or the *Chromatographic procedure*.
Instrumental procedure
Standard solution: (*L*/625) mg/mL of USP Rizatriptan Benzoate RS in *Medium*, where *L* is the label claim of rizatriptan in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 10-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: 226 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Rizatriptan Benzoate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of rizatriptan, 269.35
M_{r2} = molecular weight of rizatriptan benzoate, 391.47
L = label claim of rizatriptan (mg/Tablet)

Chromatographic procedure
Buffer: 1.36 g/L of monobasic potassium phosphate. Adjust the pH of the solution with phosphoric acid to 2.5.
Mobile phase: Acetonitrile and *Buffer* (10:90)
Standard stock solution: 0.4 mg/mL of USP Rizatriptan Benzoate RS in *Medium*
Standard solution: (*L*/900) mg/mL of rizatriptan from the *Standard stock solution* and *Medium*, where *L* is the label claim of rizatriptan in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 10-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L10
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of rizatriptan
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the rizatriptan peak
Relative standard deviation: NMT 2.0% for the rizatriptan peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of rizatriptan from the *Sample solution*
r_S = peak response of rizatriptan from the *Standard solution*
C_S = concentration of USP Rizatriptan Benzoate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of rizatriptan, 269.35
M_{r2} = molecular weight of rizatriptan benzoate, 391.47
L = label claim of rizatriptan (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: A solution containing rizatriptan, benzoic acid, and rizatriptan *N*-oxide prepared as follows. Rinse a suitable flask with hydrogen peroxide. Heat the flask in an oven at 60° for about 10 min. Allow the flask to cool and rinse with water. Transfer 5 mL of the *Standard solution*. Add 0.2 mL of hydrogen peroxide. Mix well and heat in an oven at 60° for about 30 min. Allow to stand for 24 h. [NOTE—This solution is stable for at least 7 days at room temperature.]
Sensitivity solution: 0.06 µg/mL of USP Rizatriptan Benzoate RS from *Standard solution* and water
System suitability
Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between benzoic acid and rizatriptan *N*-oxide, *System suitability solution*

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Tailing factor: NMT 3 for the rizatriptan peak, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual degradation product
r_T = sum of peak responses of rizatriptan and all degradation products excluding benzoic acid and process impurities

Acceptance criteria: See *Table 1*. Disregard any impurity less than 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, (%)
Benzoic acid[a]	0.3	—
Rizatriptan *N*-oxide[b]	0.44	0.5
Rizatriptan	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products[c]	—	0.75

[a] Not an impurity; counter ion of the drug substance, included for information only.
[b] 2-(5-[(1*H*-1,2,4-Triazol-1-yl)methyl]-1*H*-indol-3-yl)-*N*,*N*-dimethylethanamine oxide.
[c] Do not include benzoic acid or any known process impurities.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Rizatriptan Benzoate RS

Rizatriptan Benzoate Orally Disintegrating Tablets

DEFINITION
Rizatriptan Benzoate Orally Disintegrating Tablets contain an amount of rizatriptan benzoate ($C_{15}H_{19}N_5 \cdot C_7H_6O_2$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$).

IDENTIFICATION
- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Transfer 3.4 g of monobasic potassium phosphate and 2.0 g of 1-heptanesulfonic acid, sodium salt to a 1-L volumetric flask. Add 900 mL of *water*. Adjust with 50% (w/w) sodium hydroxide to a pH of 7.5, and dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (16:84)
Diluent: Acetonitrile and 0.025 M monobasic potassium phosphate (10:90)
Standard solution: 0.07 mg/mL of USP Rizatriptan Benzoate RS in *Diluent*
Sample stock solution: Nominally 0.5 mg/mL of rizatriptan in *Diluent* prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask. Add 25%–50% of the flask volume of *Diluent*, and swirl until the Tablets have disintegrated completely. Dilute with *Diluent* to volume.
Sample solution: Nominally 0.05 mg/mL of rizatriptan free base from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of rizatriptan
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Tailing factor: NMT 3.5
Relative standard deviation: NMT 2.0% for the rizatriptan peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rizatriptan Benzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of rizatriptan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of rizatriptan free base, 269.35
M_{r2} = molecular weight of rizatriptan benzoate, 391.47

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Test 1
Medium: *water*; 900 mL
Apparatus 2: 50 rpm
Time: 5 min
Standard solution: (*L*/625) mg/mL of USP Rizatriptan Benzoate RS in *Medium*, where *L* is the label claim of rizatriptan in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 10-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelengths: 278 and 320 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

USP Monographs

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A_U = absorbance of rizatriptan from the *Sample solution*, corrected for the absorbance at 320 nm
A_S = absorbance of rizatriptan from the *Standard solution*, corrected for the absorbance at 320 nm
C_S = concentration of USP Rizatriptan Benzoate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of rizatriptan free base, 269.35
M_{r2} = molecular weight of rizatriptan benzoate, 391.47
L = label claim of rizatriptan (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) is dissolved.

Test 2: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water, deaerated; 900 mL
Apparatus 2: 50 rpm
Time: 10 min
Buffer: Dissolve 2.7 g of monobasic potassium phosphate in 1 L of water. Add 2 mL of triethylamine and adjust with diluted phosphoric acid (1 in 10) to a pH of 5.0.
Mobile phase: Methanol and *Buffer* (10:90)
Standard solution: (L/625) mg/mL of USP Rizatriptan Benzoate RS in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of rizatriptan
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rizatriptan Benzoate RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of rizatriptan, 269.35
M_{r2} = molecular weight of rizatriptan benzoate, 391.47
V = volume of *Medium* (900 mL)
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of rizatriptan ($C_{15}H_{19}N_5$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability stock solution A: Solution containing rizatriptan *N*-oxide prepared as follows. Rinse a suitable flask with hydrogen peroxide. Heat the flask in an oven for about 10 min. Allow the flask to cool and rinse with water. Transfer 5 mL of *Standard solution* to the flask, add 0.2 mL of hydrogen peroxide, and tightly stopper. Mix well and heat in an oven at 60° for about 30 min. Allow to stand for 24 h. [Note—This solution is stable for at least 7 days at room temperature.]
System suitability stock solution B: Solution containing an *N*-methyl adduct of rizatriptan prepared as follows. Dissolve 10 mg of USP Rizatriptan Benzoate RS in 1 mL of methanol in a suitable round-bottom flask. Add 1 mL of dimethylcarbonate and mix by swirling. Reflux the resulting solution over a heating mantle at 125° for NLT 2 h. Allow the solution to cool, and dilute 1 mL of the resulting solution with *Diluent* to 100 mL. [Note—This solution is stable for 3 months under refrigerated conditions.] Transfer 10 mL of the resulting solution to a suitable container with a stopper. Add 0.2 mL of 6 M sodium hydroxide solution, and allow the solution to remain at room temperature for NLT 2 h. Neutralize the solution with 0.3 mL of 6 M hydrochloric acid.
System suitability solution: *System suitability stock solution A, System suitability stock solution B,* and *Standard solution* (4:4:2)
Sensitivity solution: 0.07 µg/mL of USP Rizatriptan Benzoate RS from the *Standard solution* in *Diluent*
System suitability
Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2 between *N*-methyl adduct and rizatriptan, *System suitability solution*
Tailing factor: NMT 3.5, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each individual degradation product from the *Sample solution*
r_T = sum of all the areas of rizatriptan and all the degradation products excluding the benzoic acid peak and process impurities from the *Sample solution*

Acceptance criteria: See *Table 1*. Reporting threshold for the impurities is 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzoic acid[a]	0.2	—
Rizatriptan *N*-oxide[b]	0.3	0.5
Aspartame[c]	0.4	—
Rizatriptan desmethyl[d]	0.8	0.4
Rizatriptan *N*-methyl adduct[e]	0.9	1.5
Rizatriptan	1.0	—
Any individual unspecified degradation product	—	0.2

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Table 1 *(continued)*

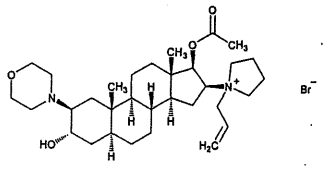
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total degradation products[f]	—	2.0

[a] This is the counterion. It is not to be reported or included in the total degradation products for the drug product.
[b] 2-{5-[(1*H*-1,2,4-Triazol-1-yl)methyl]-1*H*-indol-3-yl}-*N*,*N*-dimethylethanamine oxide.
[c] Excipient; may not be present in all sample formulations.
[d] 2-{5-[(1*H*-1,2,4-Triazol-1-yl)methyl]-1*H*-indol-3-yl}-*N*-methylethanamine. [NOTE—May not be present in all formulations.]
[e] 2-{5-[(1*H*-1,2,4-Triazol-1-yl)methyl]-1*H*-indol-3-yl}-*N*,*N*,*N*-trimethylethan-1-aminium.
[f] Process impurities are controlled in the drug substance and are not to be reported or included in the total degradation products for the drug product.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Rizatriptan Benzoate RS

Rocuronium Bromide



$C_{32}H_{53}BrN_2O_4$ 609.68

Pyrrolidinium, 1-[(2β,3α,5α,16β,17β)-17-(acetyloxy)-3-hydroxy-2-(4-morpholinyl)androstan-16-yl]-1- (2-propenyl)-, bromide;
1-Allyl-1-(3α,17β-dihydroxy-2β-morpholino-5α-androstan-16β-yl)pyrrolidinium bromide, 17-acetate [119302-91-9].

DEFINITION

Rocuronium Bromide contains NLT 98.0% and NMT 102.0% of rocuronium bromide ($C_{32}H_{53}BrN_2O_4$), calculated on the anhydrous and 2-propanol-free or acetic acid-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the rocuronium bromide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Bromide*
  Sample solution: 10 mg/mL
  Acceptance criteria: Meets the requirements of test *B*

ASSAY

- **PROCEDURE**
  Diluent: Acetonitrile and water (90:10)
  Buffer: 4.53 g/L of tetramethylammonium hydroxide pentahydrate. Adjust the solution with phosphoric acid to a pH of 7.4.
  Mobile phase: Acetonitrile and *Buffer* (90:10)
  Standard solution: 1 mg/mL of USP Rocuronium Bromide RS in *Diluent*
  Sample solution: 1 mg/mL of Rocuronium Bromide in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L3
  Column temperature: 30°
  Flow rate: 2 mL/min
  Injection volume: 5 µL
  System suitability
  [NOTE—The system may need equilibration for 4 h.]
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of rocuronium bromide ($C_{32}H_{53}BrN_2O_4$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rocuronium Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Rocuronium Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and 2-propanol-free or acetic acid-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Diluent, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Peak identification solution: 1 mg/mL of USP Rocuronium Peak Identification Mixture RS in *Diluent*
  Standard solution: 0.01 mg/mL of USP Rocuronium Bromide RS in *Diluent*
  Sample solution: 10 mg/mL of Rocuronium Bromide in *Diluent*
  Run time: 2.5 times the retention time for rocuronium
  System suitability
  [NOTE—The system may need equilibration for 4 h.]
  Sample: *Peak identification solution*
  Suitability requirements
  Peak-to-valley ratio: The ratio of the height of the rocuronium related compound H peak to the height of the valley between the rocuronium related compound H peak and the rocuronium peak is NLT 1.5.
  Resolution: NLT 3.5 between rocuronium and rocuronium related compound C
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of each impurity in the portion of Rocuronium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of rocuronium bromide from the *Standard solution*

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C_S = concentration of USP Rocuronium Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Rocuronium Bromide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.
[NOTE—Disregard any peak eluting before rocuronium bromide related compound A, and any peak with an area less than 0.5 times that of the principal peak from the *Standard solution*.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Rocuronium related compound A[a]	0.20	2.1	0.2
Rocuronium related compound G[b]	0.44	2.3	0.1
Rocuronium related compound F[c]	0.75	0.79	0.1
Rocuronium related compound B[d]	0.80	1.0	0.3
Rocuronium related compound D[e]	0.90	1.0	0.1
Rocuronium related compound H[f]	0.95	2.9	0.1
Rocuronium bromide	1.0	—	—
Rocuronium related compound C[g]	1.20	1.0	0.3
Rocuronium related compound E[h]	1.53	1.0	0.1
Any individual unspecified impurity	—	—	0.10
Total impurities	—	—	1.5

[a] 3α-Hydroxy-2β-(morpholin-4-yl)-16β-(pyrrolidin-1-yl)-5α-androstan-17β-yl acetate.
[b] 2β-(Morpholin-4-yl)-16β-(pyrrolidin-1-yl)-5α-androstan-3α,17β-diol.
[c] 1-[3α,17β-Bis(acetyloxy)-2β-(pyrrolidin-1-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium.
[d] 1-[3α,17β-Bis(acetyloxy)-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium.
[e] 1-[3α-(Acetyloxy)-17β-hydroxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium.
[f] 1-[17β-(Acetyloxy)-2-(morpholin-4-yl)-3-oxo-5α-androst-1-en-16β-yl]-1-(prop-2-enyl)pyrrolidinium.
[g] 1-[3α,17β-Dihydroxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium.
[h] 1-[17β-(Acetyloxy)-3α-hydroxy-2β-(pyrrolidin-1-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium.

• **LIMIT OF 2-PROPANOL**
[NOTE—Perform this test only if 2-propanol is a known organic manufacturing process impurity.]
Standard stock solution: Transfer 35.0 µL of ethyl ether, 32.0 µL of 2-propanol, and 19.0 µL of methylene chloride to a 100-mL volumetric flask containing 90 mL of dimethylformamide (DMF), and dilute with DMF to volume.
Standard solution: Transfer 2.5 mL of the *Standard stock solution* to a 25-mL volumetric flask containing 20 mL of DMF, and dilute with DMF to volume.
Dilute standard solution: Transfer 1.0 mL of the *Standard solution* and 4.0 mL of water to a 20-mL headspace vial. Immediately close the vial with a cap, and mix.
Sample solution: Transfer 50 mg of Rocuronium Bromide to a 20-mL headspace vial. Dissolve in 1.0 mL of DMF. Add 4 mL of water, immediately close the vial with a cap, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 60-m fused silica; coated with a 1.8-µm layer of liquid phase G43
Temperatures
Injector: 140°
Detector: 280°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	8
50	20	250	8

Carrier gas: Helium with a linear velocity of 55 cm/s or nitrogen with a linear velocity of 25 cm/s
Injection type: Split ratio, 1:6
Head space autosampler
Sample equilibration temperature: 90°
Sample equilibration time: 15 min
Transfer line temperature: 140°
System suitability
Sample: *Dilute standard solution*
[NOTE—The relative retention times for ethyl ether, 2-propanol, and methylene chloride are 0.87, 1.0, and 1.08, respectively.]
Suitability requirements
Resolution: NLT 1.0 between ethyl ether and 2-propanol; NLT 1.0 between 2-propanol and methylene chloride
Relative standard deviation: NMT 10.0% for the 2-propanol peak
Analysis
Samples: *Dilute standard solution* and *Sample solution*
Calculate the percentage of 2-propanol in the portion of Rocuronium Bromide taken:

$$\text{Result} = [(r_U/r_S) \times (V \times D/W) \times 100]/F$$

r_U = peak response of 2-propanol from the *Sample solution*
r_S = peak response of 2-propanol from the *Dilute standard solution*
V = volume of 2-propanol taken to prepare the *Standard stock solution* (µL)
D = relative density of 2-propanol, 0.786 mg/µL
W = weight of Rocuronium Bromide taken to prepare the *Sample solution* (mg)
F = dilution factor for the *Standard solution*, 1000

Acceptance criteria: NMT 1.0%
• **LIMIT OF ACETIC ACID**
[NOTE—Perform this test only if acetic acid is a known organic manufacturing process impurity.]
Mobile phase: 6.1 g of sodium perchlorate in 800 mL of water. Adjust with 1 N sulfuric acid to a pH of 2.0. Dilute to 1 L.
Standard solution: 0.2 mg/mL of glacial acetic acid in *Mobile phase*

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Sample solution: 6.0 mg/mL of Rocuronium Bromide in *Mobile phase*. [NOTE—Sonication may be necessary to completely dissolve the rocuronium bromide.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time of acetic acid is about 3.8 min.]
Suitability requirements
Column efficiency: NLT 5000 theoretical plates
Tailing factor: NMT 1.8
Relative standard deviation: NMT 5.0% for three injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetic acid in the portion of Rocuronium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for acetic acid from the *Sample solution*
r_S = peak response for acetic acid from the *Standard solution*
C_S = concentration of acetic acid in the *Standard solution* (mg/mL)
C_U = concentration of Rocuronium Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5.0%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 4.0%
• **PH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 7.0–9.5
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in 0.05 M hydrochloric acid
Acceptance criteria: 28.5°–32.0°, measured on the anhydrous and solvent-free basis at 20°
• **COLOR AND ACHROMICITY** ⟨631⟩
Reference solution: Mix 33 mL of *Matching Fluid G* and 67 mL of water.
Sample solution: 10 mg/mL of Rocuronium Bromide in water
Analysis: Proceed as directed for *Color and Achromicity* ⟨631⟩.
Acceptance criteria: The *Sample solution* is not more intensely colored than the *Reference solution*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture. Store in the freezer. If the article contains acetic acid, store it between 2° and 8°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Rocuronium Bromide RS
USP Rocuronium Peak Identification Mixture RS
Mixture of approximately 0.2% to 0.4% each of rocuronium related compound A, rocuronium related compound B, rocuronium related compound C, rocuronium related compound D, rocuronium related compound E, rocuronium related compound F, rocuronium related compound G, and rocuronium related compound H in a matrix of rocuronium bromide.

Ropinirole Tablets

DEFINITION
Ropinirole Tablets contain ropinirole hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ropinirole free base ($C_{16}H_{24}N_2O$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

• **PROCEDURE**
Buffer: 3.85 g/L of ammonium acetate. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile, methanol, and *Buffer* (7:3:40)
System suitability solution: 0.1 mg/mL of USP Ropinirole Hydrochloride RS and 0.5 µg/mL of USP Ropinirole Related Compound B RS in *Buffer*
Standard solution: 0.1 mg/mL of USP Ropinirole Hydrochloride RS in *Buffer*
Sample solution: Nominally 0.1 mg/mL of ropinirole in *Buffer* ▲▲ (USP 1-Dec-2019) prepared as follows. ▲Transfer NLT 5 Tablets into a suitable volumetric flask and▲ (USP 1-Dec-2019) add 50% of the flask volume with *Buffer*. Shake mechanically for 30 min. Dilute with *Buffer* to volume. Pass a portion of the supernatant through a suitable membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Dec-2019)
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: ▲NLT▲ (USP 1-Dec-2019) 2 times the retention time of ropinirole
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between ropinirole and ropinirole related compound B, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ropinirole in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ropinirole, 260.37
M_{r2} = molecular weight of ropinirole hydrochloride, 296.84

Acceptance criteria: 90.0%–110.0% of ropinirole free base

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: 2.9 g/L of sodium citrate dihydrate and 3.3 g/L of anhydrous citric acid in water, pH 4.0; 500 mL
Apparatus 1: 50 rpm
Time: 15 min
Mobile phase: Acetonitrile and *Medium* (1:4)
Standard solution: ▲0.0045 mg/mL▲ (USP 1-Dec-2019) of USP Ropinirole Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discarding the first few milliliters. Dilute with *Medium* to a concentration similar to the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 3.0-mm × 7-cm; 5-µm packing L1
Flow rate: 0.6 mL/min
Injection volume: 50 µL
Run time: ▲NLT▲ (USP 1-Dec-2019) 3 times the retention time of ropinirole
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times (M_{r1}/M_{r2}) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor for the *Sample solution*
M_{r1} = molecular weight of ropinirole, 260.37
M_{r2} = molecular weight of ropinirole hydrochloride, 296.84
V = volume of *Medium*, 500 mL

Tolerances: NLT 85% (*Q*) of the labeled amount of ropinirole is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 2.1 g/L of citric acid in water. Adjust with a solution containing 121.2 g/L of tris (hydroxymethyl) methylamine in water to a pH of 4.0▲ (USP 1-Dec-2019); 500 mL, deaerated.
Apparatus 1: 50 rpm
Time: 15 min
Standard solution: (*L*/500) ▲mg/mL▲ (USP 1-Dec-2019) of USP Ropinirole Hydrochloride RS in *Medium*, in which *L* is the label claim in mg/Tablet
Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
Sample solution: Pass a portion of the solution through a ▲suitable▲ (USP 1-Dec-2019) filter of 15- to 20-µm pore size, discarding the first few milliliters. ▲[NOTE—A polyethylene filter may be suitable.]▲ (USP 1-Dec-2019)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: ▲1 mL/min▲ (USP 1-Dec-2019)
Injection volume: 200 µL for Tablets with a label claim of 0.25, 0.5, 1.0, and 2.0 mg/Tablet; 100 µL for all other strengths
▲Run time: NLT 1.3 times the retention time of ropinirole▲ (USP 1-Dec-2019)
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of ropinirole, 260.37
M_{r2} = molecular weight of ropinirole hydrochloride, 296.84
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of ropinirole is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Buffer: 1.8 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 7.4.
Solution A: Methanol and *Buffer* (20:80)
Solution B: Methanol and *Buffer* (80:20)
Diluent: Dissolve 5 g of sodium dodecyl sulfate in 800 mL of water. Adjust with phosphoric acid or sodium hydroxide to a pH of 6.8. Add 200 mL of methanol, and mix.
Mobile phase: See *Table 1*.

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
8	85	15
30	30	70
40	10	90
60	10	90
60.1	85	15
70	85	15

System suitability solution: 0.3 µg/mL of USP Ropinirole Hydrochloride RS in *Diluent* and 0.5 µg/mL of USP Ropinirole Related Compound B RS in *Diluent*

Standard solution: 0.2 µg/mL of ropinirole prepared from USP Ropinirole Hydrochloride RS in *Diluent*

Sample solution: Nominally 100 µg/mL of ropinirole from ▲NLT 20 Tablets▲ (USP 1-Dec-2019) prepared as follows. ▲Finely powder the Tablets and▲ (USP 1-Dec-2019) transfer a portion to a suitable volumetric flask. Add 70% of the flask volume with *Diluent*. Shake mechanically for 30 min. Dilute with *Diluent* to volume. Pass a portion of the supernatant through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 100 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between ropinirole and ropinirole related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of ropinirole in the *Standard solution* (µg/mL)
C_U = nominal concentration of ropinirole in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Monopropyl ropinirole[a]	0.24	1.1	0.6
Ropinirole *N*-oxide[b]	0.27	1.0	0.5
Cyclopentanylindolinone[c]	0.55	1.0	0.5
Hydroxy ropinirole[d]	0.64	0.33	0.5
Ropinirole related compound B	0.95	1.4	0.6
Ropinirole	1.00	—	—
Ethyl ropinirole[e]	1.20	—	—
Propylidene ropinirole[f]	1.35	1.6	0.4
Any unspecified degradation product	—	1.0	0.3
Total impurities	—	—	2.0

[a] 4-[2-(Propylamino)ethyl]indolin-2-one.
[b] *N*-[2-(2-Oxoindolin-4-yl)ethyl]-*N*-propylpropan-1-amine oxide.
[c] 1,2a,3,4-Tetrahydro-2*H*-cyclopenta(cd)indol-2-one.
[d] 4-[2-(Dipropylamino)ethyl]-1-hydroxy-1,3-dihydro-2*H*-indol-2-one.
[e] 4-[2-(Dipropylamino)ethyl]-1-ethyl-1,3-dihydro-2*H*-indol-2-one; process impurity included for identification only.
[f] (*Z*)-4-[2-(Dipropylamino)ethyl]-3-propylideneindolin-2-one.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ropinirole Hydrochloride RS
  USP Ropinirole Related Compound B RS
  4-[2-(Dipropylamino)ethyl]indoline-2,3-dione hydrochloride.
  $C_{16}H_{22}N_2O_2 \cdot HCl$ 310.82

Ropinirole Extended-Release Tablets

DEFINITION
Ropinirole Extended-Release Tablets contain ropinirole hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ropinirole free base ($C_{16}H_{24}N_2O$).

IDENTIFICATION
- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: Dissolve 4.5 g of dibasic sodium phosphate dihydrate in 900 mL of water. Adjust with phosphoric acid to a pH of 7.0. Dilute with water to 1 L.
  Mobile phase: Methanol and *Buffer* (75:25)
  Dilute phosphoric acid: Dissolve 0.7 mL of phosphoric acid in 1 L of water.
  Diluent: Acetonitrile and *Dilute phosphoric acid* (80:20)

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System suitability solution: 0.1 mg/mL of USP Ropinirole Hydrochloride RS and 0.003 mg/mL of USP Ropinirole Related Compound B RS in *Diluent*. Sonication may be used to aid dissolution.
Standard solution: 0.11 mg/mL of USP Ropinirole Hydrochloride RS in *Diluent*. Sonication may be used to aid dissolution.
Sample solution: 0.05–0.2 mg/mL of ropinirole prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask containing 75% of the flask volume of *Diluent*. Sonicate for NLT 30 min. Allow to cool to room temperature. Dilute with *Diluent* to volume. Pass a portion of the solution through a nylon filter of 0.45-µm pore size and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 12.5-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.5 times the retention time of ropinirole
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between ropinirole related compound B and ropinirole, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ropinirole from the *Sample solution*
r_S = peak response of ropinirole from the *Standard solution*
C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ropinirole in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ropinirole free base, 260.37
M_{r2} = molecular weight of ropinirole hydrochloride, 296.84

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1
Solution A: 121.2 g/L of trishydroxymethylaminomethane in water
Buffer 1: Dissolve 2.1 g of citric acid monohydrate in 900 mL of water. Adjust with *Solution A* to a pH of 4.0. Dilute with water to 1 L.
Buffer 2: Dissolve 3.9 g of ammonium acetate in 900 mL of water. Adjust with phosphoric acid to a pH of 2.5. Dilute with water to 1 L.
Medium: *Buffer 1*; 500 mL. Deaerate as appropriate.
Apparatus 2: 100 rpm with tablet holder. See *Figure 1*.

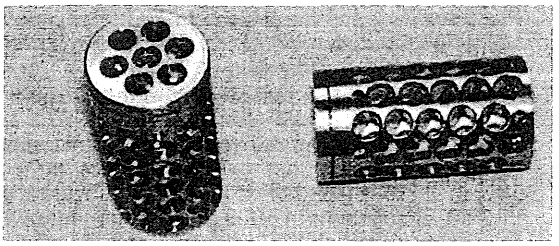


Figure 1. 37-mm(l) × 19-mm(d) stainless steel sinker; screw cap drilled with seven 4-mm holes, bottom drilled with seven 5-mm holes, 12 longitudinal series of 5-mm holes alternately starting and ending with one 3-mm hole, polished electrochemically or with a suitably validated alternative

Times: 2, 12, and 24 h
Mobile phase: Acetonitrile, methanol, and *Buffer 2* (14:6:80)
Standard solution: (*L*/400) mg/mL of USP Ropinirole Hydrochloride RS in *Medium* where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL for 12-mg Tablets; 100 µL for all other strengths
Run time: NLT 2 times the retention time of ropinirole
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim, mg/Tablet
M_{r1} = molecular weight of ropinirole free base, 260.37
M_{r2} = molecular weight of ropinirole hydrochloride, 296.84

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 20
2	12	45–65
3	24	NLT 80

USP Monographs

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The percentages of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Solution A: 121.2 g/L of trishydroxymethylaminomethane in water

Buffer 1: Dissolve 2.1 g of citric acid monohydrate and 11.7 mL of *Solution A* in 1000 mL of water. Adjust with *Solution A* to a pH of 4.0.

Buffer 2: Dissolve 4.2 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 6.5.

Medium: *Buffer 1*; 500 mL

Apparatus 2: 100 rpm

Times: 2, 6, 12, and 24 h

Mobile phase: Acetonitrile and *Buffer 2* (20:80)

Standard solution: (*L*/500) mg/mL of USP Ropinirole Hydrochloride RS in *Medium* where *L* is the label claim in mg/Tablet

Sample solution: Centrifuge a portion of the solution under test.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of ropinirole

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of ropinirole ($C_{16}H_{24}N_2O$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of ropinirole from the *Sample solution*
r_S = peak response of ropinirole from the *Standard solution*
C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

C_i = concentration of ropinirole in the *Sample solution* at the specified time point (mg/mL)
L = label claim, mg/Tablet
M_{r1} = molecular weight of ropinirole free base, 260.37
M_{r2} = molecular weight of ropinirole hydrochloride, 296.84
V = volume of *Medium*, 500 mL
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	14–34
2	6	42–62
3	12	68–88
4	24	NLT 85

The percentages of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Buffer: 1.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Medium: 0.1 N hydrochloric acid VS; 500 mL

Apparatus 2: 100 rpm, with sinkers

Times: 1, 6, 12, and 24 h

Mobile phase: Acetonitrile and *Buffer* (10:90)

Standard solution: (*L*/500) mg/mL of USP Ropinirole Hydrochloride RS in *Medium* where *L* is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of ropinirole

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of ropinirole ($C_{16}H_{24}N_2O$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of ropinirole and 3-oxo ropinirole from the *Sample solution*. [NOTE—The relative retention times for ropinirole and 3-oxo ropinirole are 1.0 and 1.21, respectively.]
r_S = peak response of ropinirole from the *Standard solution*
C_S = concentration of USP Ropinirole Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved at each time point (*i*):

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$$Result_1 = C_1 \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

$$Result_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

C_i = concentration of ropinirole in the *Sample solution* at the specified time point (mg/mL)
L = label claim, mg/Tablet
M_{r1} = molecular weight of ropinirole free base, 260.37
M_{r2} = molecular weight of ropinirole hydrochloride, 296.84
V = volume of *Medium*, 500 mL
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 25
2	6	40–60
3	12	65–85
4	24	NLT 80

The percentages of the labeled amount of ropinirole ($C_{16}H_{24}N_2O$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 0.05% (v/v) trifluoroacetic acid in water
Solution B: Acetonitrile and methanol (80:20)
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	84	16
23	84	16
36	40	60
36.1	84	16
50	84	16

Diluent 1: Acetonitrile and *Solution A* (80:20)
Diluent 2: *Diluent 1* and *Solution A* (20:80)
System suitability solution: 0.03 mg/mL of USP Ropinirole Hydrochloride RS and 0.001 mg/mL of USP Ropinirole Related Compound B RS in *Diluent 2*. Sonication may be used to aid dissolution.
Sensitivity solution: 0.015 µg/mL of USP Ropinirole Hydrochloride RS in *Diluent 2*
Standard solution: 0.15 µg/mL of USP Ropinirole Related Compound B RS in *Diluent 2*
Sample stock solution: Nominally 0.13–0.14 mg/mL of ropinirole from a suitable number of Tablets containing 20–50 mg of ropinirole prepared as follows. Homogenize an appropriate number of Tablets in a suitable volume of *Diluent 1*. Pass a portion of the solution through a nylon filter of 0.45-µm pore size and use the filtrate.
Sample solution: Nominally 26–28 µg/mL of ropinirole from the *Sample stock solution* and *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between ropinirole related compound B and ropinirole, *System suitability solution*
Relative standard deviation: NMT 10% for ropinirole related compound B, *Standard solution*
Signal-to-noise ratio: NLT 10 for ropinirole, *Sensitivity solution*
Analysis
Samples: *Standard solution and Sample solution*
Calculate the percentage of ropinirole related compound B in the portion of Tablets taken:

$$Result = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ropinirole related compound B from the *Sample solution*
r_S = peak response of ropinirole related compound B from the *Standard solution*
C_S = concentration of USP Ropinirole Related Compound B RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ropinirole in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of ropinirole related compound B free base, 274.36
M_{r2} = molecular weight of ropinirole related compound B hydrochloride, 310.82

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$Result = (r_U/F)/[\Sigma(r_U/F) + r_R] \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
F = relative response factor for the corresponding degradation product from *Table 5*
r_R = peak response of ropinirole from the *Sample solution*

Acceptance criteria: See *Table 5*. Disregard peaks less than 0.05%.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ropinirole monopropyl[a]	0.42	1.0	0.5
Ropinirole related compound B	0.89	—	0.5

USP Monographs

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Table 5 *(continued)*

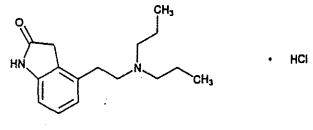
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ropinirole *N*-hydroxymethyl[b]	0.94	0.71	0.5
Ropinirole	1.00	—	—
Ropinirole *N*-oxide[c]	1.31	1.0	0.5
Ropinirole methylene dimer[d]	1.82	1.0	0.5
Propylidene ropinirole[e,f]	1.96	2.0	—
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.5

[a] 4-[2-(Propylamino)ethyl]indolin-2-one.
[b] 4-[2-(Dipropylamino)ethyl]-1-(hydroxymethyl)indolin-2-one.
[c] *N*-[2-(2-Oxoindolin-4-yl)ethyl]-*N*-propylpropan-1-amine oxide.
[d] 4-[2-(Dipropylamino)ethyl]-3-({4-[2-(dipropylamino)ethyl]-2-oxo-2,3-dihydro-1*H*-indol-3-yl}methyl)-2,3-dihydro-1*H*-indol-2-one.
[e] (*Z*)-4-[2-(Dipropylamino)ethyl]-3-propylideneindolin-2-one.
[f] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ropinirole Hydrochloride RS
  USP Ropinirole Related Compound B RS
  4-[2-(Dipropylamino)ethyl]indoline-2,3-dione hydrochloride.
  $C_{16}H_{22}N_2O_2 \cdot HCl$ 310.82

Ropinirole Hydrochloride



$C_{16}H_{24}N_2O \cdot HCl$ 296.84
2*H*-Indol-2-one, 4-[2-(dipropylamino)ethyl]-1,3-dihydro-, monohydrochloride;
4-[2-(Dipropylamino)ethyl]-2-indolinone monohydrochloride [91374-20-8].

DEFINITION

Ropinirole Hydrochloride contains NLT 98.0% and NMT 102.0% of ropinirole hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
  Sample: 20 mg/mL
  Acceptance criteria: Meets the requirements of test *A*

ASSAY

- **PROCEDURE**
  Buffer: 1.88 g of sodium 1-hexanesulfonate and 1 g of phosphoric acid in 1 L of water. Adjust with dilute triethylamine solution (1 mL/10 mL) to a pH of 6.5.
  Diluent: Acetonitrile and water (20:80)
  Mobile phase: Acetonitrile and *Buffer* (20:80)
  Standard solution: 0.1 mg/mL of USP Ropinirole Hydrochloride RS in *Diluent*. Sonication may be used to aid dissolution.
  Sample solution: 0.1 mg/mL of Ropinirole Hydrochloride in *Diluent*. Sonication may be used to aid dissolution.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 215 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Column temperature: 30°
  Flow rate: 1.0 mL/min
  Injection volume: 10 µL
  Run time: 2.5 times the retention time of ropinirole
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.6
  Relative standard deviation: NMT 1.5%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of ropinirole hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$) in the portion of Ropinirole Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF Pd**
  [NOTE—Perform this test if Pd is a known inorganic impurity.]
  Solution A: Dissolve 2.4 g of sodium hydroxide in 20 mL of water, and dilute with glycerol to 100 mL.
  Solution B: 40 mg/mL of thioacetamide in water. [NOTE—The solution may be filtered if necessary.]
  Buffer: Carefully add 27 mL of hydrochloric acid to 25 g of ammonium acetate, and dilute with water to 100 mL.
  Pd standard solution: 10 ppm of Pd from commercially available ICP grade 1000 ppm Pd stock solution
  Standard solution: Pipet 1.0 mL of *Pd standard solution* into a platinum crucible, and gently evaporate to dryness.

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Moisten the residue with 0.5 mL of sulfuric acid, and heat with a Bunsen burner until fumes are no longer emitted. Place the crucible in a muffle furnace at 700°, and heat for 5 additional min. Cool, add 5 mL of hydrochloric acid to the residue, and evaporate to dryness. Add 1 mL of 2 M hydrochloric acid and, if necessary, dissolve the residue with gentle heating. When cool, transfer the contents to a 50-mL beaker. Wash the crucible twice with 1-mL aliquots of water, and add the washings to the beaker. Pass the solution through a membrane filter of 2-µm pore size into a centrifuge tube.

Sample solution: Transfer 1 g of Ropinirole Hydrochloride to a platinum crucible, and heat over a Bunsen burner until the material is completely carbonized. Moisten the contents with 0.5 mL of sulfuric acid, and heat over a Bunsen burner until fumes are no longer emitted. Place the crucible in a muffle furnace at 700°, and heat until all the organic material has been destroyed. When cool, add 5 mL of hydrochloric acid to the residue, and evaporate to dryness. Add 1 mL of 2 M hydrochloric acid and, if necessary, dissolve the residue with gentle heating. When cool, transfer the contents to a 50-mL beaker. Wash the crucible twice with 1-mL aliquots of water, and add the washings to the beaker. Pass the solution through a membrane filter of 2-µm pore size into a centrifuge tube.

Analysis: To the filtered *Standard solution* and *Sample solution* add 1 mL of *Solution A*, 0.2 mL of *Solution B*, and 2 mL of *Buffer*. Cap the centrifuge tubes securely, and incubate at 37° for 2 h. Pass the *Sample solution* and *Standard solution* through a membrane filter of 2-µm pore size, wash the membrane with 2-mL aliquots of water, and allow the membrane to dry.

Acceptance criteria: The intensity of the spot due to the sample is not more intense than the spot due to the standard (10 ppm).

• **ORGANIC IMPURITIES, PROCEDURE 1**

If methylene ropinirole and propylidene ropinirole are known process impurities, *Organic Impurities, Procedure 2* is recommended. If ropinirole indole derivative and ropinirole oxime are known process impurities, *Organic Impurities, Procedure 3* is recommended.

Buffer and **Diluent:** Proceed as directed in the *Assay*.

Solution A: Acetonitrile and *Buffer* (15:85)

Solution B: Acetonitrile and water (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
30	60	40
50	60	40
55	100	0
65	100	0

System suitability solution: 1 mg/mL of USP Ropinirole Hydrochloride RS and 1.5 µg/mL of USP Ropinirole Related Compound B RS in *Diluent*

Sample solution: 1 mg/mL of Ropinirole Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between ropinirole and ropinirole related compound B

Tailing factor: NMT 2.0 for the ropinirole peak

Relative standard deviation: NMT 2.0% for the ropinirole peak

Analysis

Sample: *Sample solution*

Calculate the percentage of any individual impurity in the portion of Ropinirole Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of responses of all the peaks from the *Sample solution*

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Open ring nitro derivative[a]	0.39	0.72	0.15
Monopropyl ropinirole[b]	0.58	1.1	0.15
N-hydroxyropinirole[c]	0.84	0.65	0.15
Ropinirole related compound B	0.97	0.66	0.15
Ropinirole	1.0	—	—
Any individual unknown impurity	—	1.0	0.10
Total impurities[d]	—	—	1.0

[a] 2-{2-[2-(Dipropylamino)ethyl]-6-nitrophenyl}acetic acid.
[b] 4-[2-(Propylamino)ethyl]indolin-2-one.
[c] 4-[2-(Dipropylamino)ethyl]-1-hydroxyindolin-2-one.
[d] Includes impurities greater than or equal to 0.05%.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: 3.85 g/L of ammonium acetate. Adjust with phosphoric acid to a pH of 2.5.

Solution B: Acetonitrile and methanol (70:30)

Diluent: *Solution A* and *Solution B* (84:16)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	84	16
16	84	16
36	20	80
37	84	16
51	84	16

System suitability solution: 0.05 mg/mL each of USP Ropinirole Hydrochloride RS, USP Ropinirole Related

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Compound A RS, and USP Ropinirole Related Compound B RS in *Diluent*
Standard solution: 3 µg/mL of USP Ropinirole Related Compound B RS in *Diluent*
Sample solution: 1 mg/mL of Ropinirole Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between ropinirole related compound A and ropinirole related compound B; NLT 3.0 between ropinirole related compound B and ropinirole, *System suitability solution*
Tailing factor: NMT 1.5 for ropinirole, *System suitability solution*
Relative standard deviation: NMT 5.0% for the ropinirole related compound B peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ropinirole related compound B in the portion of Ropinirole Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ropinirole related compound B from the *Sample solution*
r_S = peak response of USP Ropinirole Related Compound B RS from the *Standard solution*
C_S = concentration of USP Ropinirole Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Ropinirole Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Monopropyl ropinirole[a]	0.37	0.2
Ropinirole related compound A[b]	0.70	—
Ropinirole related compound B	0.85	0.3
Ropinirole	1.0	—
Methylene ropinirole[c]	1.39	0.2
Ropinirole isohexyl analog[d]	1.64	0.3
Propylidene ropinirole[e]	1.70	0.2
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] 4-[2-(Propylamino)ethyl]indolin-2-one.
[b] Only used for system suitability and peak identification. This impurity is not to be reported and not to be included in total impurities.
[c] 4-[2-(Dipropylamino)ethyl]-3-methyleneindolin-2-one.
[d] 4-[2-(2-Methylpentylamino)ethyl]indolin-2-one.
[e] (*Z*)-4-[2-(Dipropylamino)ethyl]-3-propylideneindolin-2-one.

• **ORGANIC IMPURITIES, PROCEDURE 3**
Solution A: Dissolve 3.95 g of ammonium bicarbonate in 990 mL of water. Add 4 mL of 25% ammonia, and dilute with water to 1000 mL. The pH is 9.2–9.3.
Solution B: Acetonitrile
Diluent: Acetonitrile and water (32:68)
Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	68	32
12	68	32
28	20	80
34	20	80
34.1	68	32
40	68	32

System suitability solution: 1 mg/mL of USP Ropinirole Hydrochloride RS and 0.01 mg/mL of USP Ropinirole Related Compound B RS in *Diluent*
Standard solution: 1.2 µg/mL of USP Ropinirole Hydrochloride RS in *Diluent*
Sample solution: 1.2 mg/mL of Ropinirole Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between ropinirole and ropinirole related compound B, *System suitability solution*
Tailing factor: 0.8–1.5 for ropinirole, *Standard solution*
Relative standard deviation: NMT 5.0% for ropinirole, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Ropinirole Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
F = relative response factor (see *Table 6*)

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Acceptance criteria: See *Table 6*.

Table 6

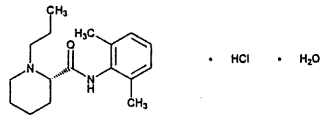
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ropinirole oxime[a]	0.69	1.0	0.15
Ropinirole	1.0	—	—
Ropinirole related compound B	1.2	1.0	0.15
Ropinirole indole derivative[b]	2.0	4.0	0.10
Any individual unspecified impurity	—	—	0.10
Total impurities	—	—	0.50

[a] 4-[2-(Dipropylamino)ethyl]-3-(hydroxyimino)indolin-2-one.
[b] *N,N*-Dipropyl-*N*-(2-indol-4-ylethyl)amine.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
• **LABELING:** The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ropinirole Hydrochloride RS
USP Ropinirole Related Compound A RS
4-(2-Hydroxyethyl)indolin-2-one.
$C_{10}H_{11}NO_2$ 177.20
USP Ropinirole Related Compound B RS
4-[2-(Dipropylamino)ethyl]indoline-2,3-dione hydrochloride.
$C_{16}H_{22}N_2O_2 \cdot HCl$ 310.82

Ropivacaine Hydrochloride



$C_{17}H_{26}N_2O \cdot HCl \cdot H_2O$ 328.88
(*S*)-(−)-1-Propylpiperidine-2-carboxylic acid (2,6-dimethylphenyl)amide hydrochloride monohydrate;
(*S*)-(−)-1-Propyl-2′,6′-pipecoloxylidine hydrochloride monohydrate [132112-35-7].

DEFINITION
Ropivacaine Hydrochloride contains NLT 98.5% and NMT 101.0% of $C_{17}H_{26}N_2O \cdot HCl$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: 10 mg/mL

ASSAY
• **PROCEDURE**
Sample solution: Dissolve 1000 mg of Ropivacaine Hydrochloride in 10 mL of water and 40 mL of alcohol. Add 1.0 mL of 1 N hydrochloric acid.
Analysis: Titrate with 1 N sodium hydroxide VS. Two equivalence points are obtained; the difference in titrant volume corresponds to the amount of ropivacaine hydrochloride (see *Titrimetry* ⟨541⟩). Each mL of 1 N sodium hydroxide is equivalent to 310.9 mg of anhydrous ropivacaine hydrochloride ($C_{17}H_{26}N_2O \cdot HCl$).
Acceptance criteria: 98.5%–101.0% on the anhydrous basis

IMPURITIES
ORGANIC IMPURITIES
• **Procedure 1**
Buffer solution: Combine 1.3 mL of monobasic sodium phosphate solution (138 g/L) and 32.5 mL of disodium hydrogen phosphate dihydrate solution (89 g/L), and dilute with water to 1 L. The pH of this solution is 8.0. Make adjustments if necessary.
Mobile phase: Acetonitrile and *Buffer solution* (1:1)
System suitability solution: 10 µg/mL of each of USP Ropivacaine Hydrochloride RS and USP Bupivacaine Hydrochloride RS in *Mobile phase*
Sample solution 1: 2.75 mg/mL of Ropivacaine Hydrochloride in *Mobile phase*
Sample solution 2: 2.75 µg/mL of Ropivacaine Hydrochloride from *Sample solution 1* diluted with *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
[NOTE—Check the stability of the baseline by injecting *Mobile phase*. Run the chromatogram for at least 15 min.]
Samples: *System suitability solution* and *Sample solution 2*
[NOTE—The relative retention times for ropivacaine and bupivacaine are about 1.0 and 1.6, respectively.]
Suitability requirements
Resolution: NLT 6 between ropivacaine and bupivacaine, *System suitability solution*
Signal-to-noise ratio: NLT 10 for ropivacaine, *Sample solution 2*
Analysis
Samples: *System suitability solution* and *Sample solution 1*
Calculate the percentage of each impurity in the portion of Ropivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria
Bupivacaine: NMT 0.2%
Any other individual impurity: Less than 0.1%
Total impurities: NMT 0.5%
• **Procedure 2: Limit of Ropivacaine Related Compound A**
Buffer solution, Mobile phase, and **Chromatographic system:** Prepare as directed in *Procedure 1*.

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Standard solution: 0.13 µg/mL of USP Ropivacaine Related Compound A RS in *Mobile phase*
Sample solution: 10 mg/mL of Ropivacaine Hydrochloride in *Mobile phase*
System suitability
Sample: *Standard solution*
Suitability requirements
Signal-to-noise ratio: NLT 10 for ropivacaine related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The response for any peak corresponding to ropivacaine related compound A (2,6-dimethylaniline) in the *Sample solution* is not greater than the response of the major peak in the *Standard solution* (NMT 10 ppm).

• **Procedure 3: Enantiomeric Purity**

Background electrolyte solution: 9.31–10.29 mg/mL of phosphoric acid in water. The pH is between 2.9 and 3.1. If necessary, adjust the pH with triethanolamine to 2.9–3.1.
Run buffer: 13.3 mg/mL of heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin in *Background electrolyte solution*. [NOTE—This solution is freshly prepared and passed through a 0.45-µm filter.]
System suitability solution: 15 µg/mL of each USP Ropivacaine Hydrochloride RS and USP Ropivacaine Related Compound B RS in water
Sample solution 1: 2 mg/mL of Ropivacaine Hydrochloride in water
Sample solution 2: 0.01 mg/mL of ropivacaine hydrochloride from *Sample solution 1* diluted with water
Capillary rinsing procedure: Use separate *Run buffer* vials for capillary rinse and sample analysis. Rinse the capillary with water for 1 min, with 0.1 N sodium hydroxide for 10 min, and with water for 3 min. If a new or dry capillary is being used, increase the sodium hydroxide rinse time to 30 min. Rinse the capillary between injections as follows: water for 1 min, 0.1 N sodium hydroxide for 4 min, and water for 1 min, then *Run buffer* for 4 min. Rinse times are based on a rinse pressure of 1 bar.
Capillary electrophoresis system
Detector: UV 206 nm
Column: 50-µm × 72-cm fused silica
Column temperature: 30°
Applied voltage: 375 V/cm
Initial ramping: 500 V/s, positive polarity, and a resulting current of 40–45 µA
Injection size: Equal volumes
System suitability
Samples: *System suitability solution* and *Sample solution 2*
[NOTE—The relative migration times for ropivacaine related compound B (R enantiomer) and ropivacaine (S enantiomer) are about 0.96 and 1.0, respectively.]
Suitability requirements
Signal-to-noise ratio: NLT 10, *Sample solution 2*
Resolution: NLT 3.7 between ropivacaine related compound B and ropivacaine, *System suitability solution*
[NOTE—The analysis run time is about 30 min. If needed, increase the resolution by increasing the concentration of heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin or by lowering the system temperature.]
Analysis
Samples: *Run buffer*, water, and *Sample solution 1*
Inject *Run buffer* and water to ensure there are no interfering peaks (50 mbar for 5.0 s followed by injection of *Run buffer* at 50 mbar for 1.0 s). Inject *Sample solution 1* into the electrophoresis system, record the electropherograms, and measure the peak responses for ropivacaine and ropivacaine related compound B.
Calculate the percentage of ropivacaine related compound B in the portion of Ropivacaine Hydrochloride taken:

$$\text{Result} = (r_R/M_R)/(r_S/M_S) \times 100$$

r_R = peak response of ropivacaine related compound B from *Sample solution 1*
M_R = migration time of ropivacaine related compound B (min)
r_S = peak response of ropivacaine from *Sample solution 1*
M_S = migration time of ropivacaine (min)

[NOTE—After the analysis, rinse the capillary for 10 min with 0.1 N sodium hydroxide, then for 10 min with water. Dry the capillary before storage.]
Acceptance criteria: NMT 0.5% of ropivacaine related compound B

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** The level of bacterial endotoxins is such that the requirements under the relevant dosage form monograph(s) in which Ropivacaine Hydrochloride is used can be met. Where the label states that Ropivacaine Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirements under the relevant dosage form monograph(s) in which Ropivacaine Hydrochloride is used can be met.

• **COLOR**
Sample solution: Transfer an aliquot of Ropivacaine Hydrochloride, 480–500 mg, into a 25-mL volumetric flask, and dissolve in and dilute with water to volume. Pass the solution through a 5-µm polyvinylidene filter (PVDF).
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelengths: 405 and 436 nm
Cell: 5 cm
Analysis
Sample: *Sample solution*
Immediately measure the absorbance of the *Sample solution*, using water as the reference.
Acceptance criteria: NMT 0.030 at 405 nm, and NMT 0.025 at 436 nm

• **CLARITY**
Hydrazine sulfate solution: 10 mg/mL of hydrazine sulfate in water. Allow to stand 4–6 h.
Hexamethylenetetramine solution: Transfer 2.5 g of hexamethylenetetramine to a 100-mL glass-stoppered flask, and dissolve in 25 mL of water.
Opalescence standard stock suspension: To the flask containing the *Hexamethylenetetramine solution*, add 25.0 mL of *Hydrazine sulfate solution*, mix, and allow to stand for 24 h. This suspension is stable for up to 2 months when stored in a glass container free from surface defects. The suspension must not adhere to the flask and must be well mixed before use.
Opalescence standard suspension: Dilute 15.0 mL of the *Opalescence standard stock suspension* with water to 1000 mL. This suspension should be freshly prepared and may be stored for NMT 24 h.
Standard suspension 1: *Opalescence standard suspension* and water (5:95). Shake before use.
Standard suspension 2: *Opalescence standard suspension* and water (10:90). Shake before use.

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Sample solution: 480–500 mg of Ropivacaine Hydrochloride in a 25-mL volumetric flask. Dilute with water to volume.

Analysis:

Samples: *Standard suspension 1*, *Standard suspension 2*, and *Sample solution*

Use identical tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm. The depth of the layer is 40 mm. Compare the solutions in diffused daylight 5 min after the preparation of *Standard suspension 1* and *Standard suspension 2*, viewing vertically against a black background. The diffusion of light must be such that *Standard suspension 1* can readily be distinguished from water, and *Standard suspension 2* can readily be distinguished from *Standard suspension 1*.

Acceptance criteria: The *Sample solution* is considered clear if its clarity is the same as that of water or if its opalescence is not more pronounced than that of *Standard suspension 1*.

- **pH** ⟨791⟩: 4.5–6.0, in a solution (10 mg/mL)
- **Water Determination,** *Method Ia* ⟨921⟩: 5.0%–6.0%. Perform the determination on 0.0900–0.1100 g of sample.
- **Sterility Tests** ⟨71⟩: Where the label states that Ropivacaine Hydrochloride is sterile, it meets the requirements.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP Reference Standards** ⟨11⟩

USP Bupivacaine Hydrochloride RS
USP Ropivacaine Hydrochloride RS
USP Ropivacaine Related Compound A RS
2,6-Dimethylaniline hydrochloride.
$C_8H_{12}ClN$ 157.64
[CAS-21436-98-6].
USP Ropivacaine Related Compound B RS
(*R*)-Ropivacaine hydrochloride monohydrate; (*R*)-(+)-1-propylpiperidine-2-carboxylic acid (2,6-dimethylphenyl)-amide hydrochloride monohydrate.
$C_{17}H_{26}N_2O$ 328.89

Ropivacaine Hydrochloride Injection

» Ropivacaine Hydrochloride Injection is a sterile solution of Ropivacaine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ropivacaine hydrochloride ($C_{17}H_{26}N_2O \cdot HCl$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type 1 glass or of suitable plastic.

USP Reference standards ⟨11⟩—

USP Ropivacaine Hydrochloride RS
USP Ropivacaine Related Compound A RS
2,6-Dimethylaniline hydrochloride.
$C_8H_{12}ClN$ 157.64
[CAS-21436-98-6].
USP Ropivacaine Related Compound B RS
(*R*)-Ropivacaine hydrochloride monohydrate; (*R*)-(+)-1-propylpiperidine-2-carboxylic acid (2,6-dimethylphenyl)-amide hydrochloride monohydrate.
$C_{17}H_{26}N_2O$ 328.89

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *System suitability solution*, as obtained in the test for *Enantiomeric purity*.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 60 USP Endotoxin Units per g of ropivacaine hydrochloride.

Particulate Matter in Injections ⟨788⟩: meets the requirements for injections.

Sterility Tests ⟨71⟩: It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 4.0 and 6.0.

Limit of 2,6-dimethylaniline (ropivacaine related compound A, base)—

pH 8.0 Buffer solution and *Mobile phase*—Prepare as directed in the *Assay*.

Standard solution—Prepare as directed for *Standard preparation* in the *Assay*.

Test solution—Dilute accurately the Injection with *Mobile phase* to obtain a concentration of 2.0 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between ropivacaine related compound A and ropivacaine is not less than 5; and the signal-to-noise ratio for ropivacaine related compound A is not less than 10.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The peak response of ropivacaine related compound A obtained from the *Test solution* is not greater than the corresponding response obtained from the *Standard solution* (not more than 0.01% of ropivacaine related compound A base is found).

Enantiomeric purity—

pH 7.2 Buffer solution—Transfer 7.5 mL of 1 M monobasic sodium phosphate solution and 28.5 mL of 0.5 M dibasic sodium phosphate dihydrate solution into a 1-L volumetric flask, and dilute with water to volume. Adjust the resulting solution to a pH of 7.2, if necessary.

Mobile phase—Transfer 35 mL of isopropyl alcohol into a 500-mL volumetric flask, dilute with *pH 7.2 Buffer solution* to volume, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve suitable quantities of USP Ropivacaine Hydrochloride RS and USP Ropivacaine Related Compound B RS in water, and dilute quantitatively, and stepwise, with water to obtain a solution containing about 75 µg per mL and 0.75 µg per mL, respectively.

Test solution—Dilute the Injection with *Mobile phase* to a concentration of about 75 µg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 10-cm column that contains packing L41. The

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flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between ropivacaine related compound B (*R* enantiomer) and ropivacaine (*S* enantiomer) is not less than 1.5. [NOTE—For the purpose of identification, the relative retention times are about 0.75 for ropivacaine related compound B and 1.0 for ropivacaine.]

Procedure—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of ropivacaine related compound B (*R* enantiomer) in the portion of Injection taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response of ropivacaine related compound B (*R* enantiomer); and r_s is the sum of the peak responses of ropivacaine (*S* enantiomer) and ropivacaine related compound B (*R* enantiomer) obtained from the *Test solution:* not more than 2.0% of ropivacaine related compound B (*R* enantiomer) is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

pH 8.0 Buffer solution—Transfer 1.3 mL of 1 M monobasic sodium phosphate solution and 32.5 mL of 0.5 M dibasic sodium phosphate dihydrate solution to a 1-L volumetric flask. Dilute with water to volume, and mix. Adjust the resulting solution to a pH of 8.0, if necessary.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *pH 8.0 Buffer solution* (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Ropivacaine Hydrochloride RS and USP Ropivacaine Related Compound A RS in *Mobile phase,* and dilute quantitatively, and stepwise, with *Mobile phase* to obtain a solution having known concentrations of about 0.25 mg per mL of USP Ropivacaine Hydrochloride RS and about 0.26 µg per mL of USP Ropivacaine Related Compound A RS.

Assay preparation—Dilute accurately the Injection with *Mobile phase* to obtain a concentration of about 0.25 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 3.9-mm × 15-cm column that contains 5- or 10-µm packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections, calculated for the ropivacaine peak, is not more than 1.0%; and the resolution, *R,* between ropivacaine related compound A and ropivacaine is not less than 5.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ropivacaine hydrochloride ($C_{17}H_{26}N_2O \cdot HCl$) in each mL of Injection taken by the formula:

$$C\,D(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ropivacaine Hydrochloride RS in the *Standard preparation; D* is the dilution factor, in mL, for the *Assay preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Rose Water Ointment

DEFINITION

Prepare Rose Water Ointment as follows.

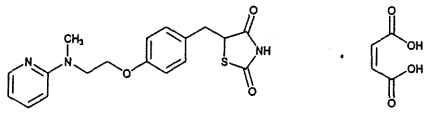
Cetyl Esters Wax	125 g
White Wax	120 g
Almond Oil	560 g
Sodium Borate	5 g
Stronger Rose Water	25 mL
Purified Water	165 mL
Rose Oil	200 µL
To make about	1000 g

Reduce the *Cetyl Esters Wax* and the *White Wax* to small pieces, melt them on a steam bath, add the *Almond Oil,* and continue heating until the temperature of the mixture reaches 70°. Dissolve the *Sodium Borate* in the *Purified Water* and the *Stronger Rose Water,* warmed to 70°, and gradually add the warm aqueous phase to the melted oil phase, stirring rapidly and continuously until it has cooled to about 45°. Then incorporate the *Rose Oil.* [NOTE—Rose Water Ointment is free from rancidity. If the Ointment has been chilled, warm it slightly before attempting to incorporate other ingredients (see *5.20.20. Added Substances, Excipients, and Ingredients in Official Products* in the *General Notices and Requirements.*]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers.

Rosiglitazone Maleate



$C_{18}H_{19}N_3O_3S \cdot C_4H_4O_4$ 473.50

(±)-5-[*p*-[2-(Methyl-2-pyridylamino)ethoxy]benzyl]-2,4-thiazolidinedione maleate (1:1);

(*RS*)-5-{[4-({2-[Methyl(2-pyridinyl)amino]ethyl}oxy) phenyl] methyl}-1,3-thiazolidine-2,4-dione (*Z*)-2-butenedioate [155141-29-0].

DEFINITION

Rosiglitazone Maleate contains NLT 98.0% and NMT 102.0% of $C_{18}H_{19}N_3O_3S \cdot C_4H_4O_4$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

USP Monographs

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ASSAY

• **PROCEDURE**

Buffer: Dissolve 5.75 g of phosphoric acid in 800 mL water, adjust with 4 N sodium hydroxide to a pH of 3.0, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (25:75)

System suitability solution: Transfer 2.5 mg of USP Rosiglitazone Maleate RS and 1 mg of USP Rosiglitazone Related Compound A RS to a 50-mL volumetric flask, dissolve in 1 mL of stabilizer-free tetrahydofuran, and dilute with *Mobile phase* to volume.

Standard solution: 0.05 mg/mL of USP Rosiglitazone Maleate RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Rosiglitazone Maleate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: Greater than 2.0 between rosiglitazone and rosiglitazone related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of rosiglitazone maleate ($C_{18}H_{19}N_3O_3S \cdot C_4H_4O_4$) in the portion of Rosiglitazone Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Rosiglitazone Maleate RS in the *Standard solution* (mg/mL)

C_U = concentration of Rosiglitazone Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

OTHER COMPONENTS

• **CONTENT OF MALEIC ACID**

Buffer: Prepare 0.1 M sodium phosphate buffer as follows. Add 11.5 g of phosphoric acid to 800 mL of water, adjust with 2 N sodium hydroxide to a pH of 3.0, and dilute with water to 1 L.

Mobile phase: Methanol and *Buffer* (50:50)

Diluent: Methanol and water (50:50)

System suitability solution: 0.1 µg/mL of USP Fumaric Acid RS and 0.04 mg/mL of USP Rosiglitazone Maleate RS in *Diluent*

Standard solution: 0.01 mg/mL of USP Maleic Acid RS in *Diluent*

Sample solution: 0.04 mg/mL of Rosiglitazone Maleate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 212 nm

Column: 4.6-mm × 15-cm; 5-µm packing L14

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for rosiglitazone, maleic acid, and fumaric acid are 0.5, 1.0, and 1.8, respectively.]

Suitability requirements

Resolution: NLT 2.0 between maleic acid and fumaric acid, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of maleic acid in the portion of Rosiglitazone Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of maleic acid from the *Sample solution*

r_S = peak response of maleic acid from the *Standard solution*

C_S = concentration of USP Maleic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Rosiglitazone Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 23.5%–26.0%

IMPURITIES

• **RESIDUE ON IGNITION,** ⟨281⟩: NMT 0.2%, using an ignition temperature of 800 ± 25°

• **ORGANIC IMPURITIES**

[NOTE—Protect the *System suitability solution* and *Sample solution* from light.]

Buffer 1: Prepare 0.05 M dibasic potassium phosphate buffer as follows. Dissolve 11.4 g of dibasic potassium phosphate trihydrate in 800 mL of water, adjust with a mixture of phosphoric acid and water (1:1) to a pH of 7.0, and dilute with water to 1 L.

Solution A: Acetonitrile and *Buffer 1* (30:70)

Solution B: Acetonitrile and *Buffer 1* (70:30)

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25.0	100	0
50.0	0	100

Buffer 2: Prepare 0.05 M monobasic potassium phosphate buffer by dissolving 6.8 g of monobasic potassium phosphate in 1 L of water.

Diluent: Acetonitrile and *Buffer 2* (30:70)

System suitability solution: 0.5 mg/mL of USP Rosiglitazone Maleate RS in *Diluent*, using sonication, if necessary, to dissolve. [NOTE—USP Rosiglitazone Maleate RS contains rosiglitazone related compound A as a minor component.]

Sample solution: 0.5 mg/mL of Rosiglitazone Maleate in *Diluent*, using sonication, if necessary, to dissolve

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

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Detector: UV 246 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—Identify the peak due to rosiglitazone related compound A based on its relative retention time shown in *Table 2*.]
Suitability requirements
Resolution: NLT 2.0 between rosiglitazone and rosiglitazone related compound A
Analysis
Sample: *Sample solution*
Calculate the percentage of any individual impurity in the portion of Rosiglitazone Maleate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the peak responses from the *Sample solution*, except for the peak response of maleic acid

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Maleic acid	0.09	Disregard
Impurity 1[a]	0.15	0.1
Impurity 2[b]	0.81	0.5
Rosiglitazone	1.0	—
Rosiglitazone related compound A[c]	1.15	0.5
Any other individual impurity	—	0.1
Total impurities	—	1.0

[a] 2-(5-{[4-({2-[Methyl(2-pyridinyl)amino]ethyl}oxy)phenyl]methyl}-2,4-dioxo-1,3-thiazolidin-3-yl)butanedioic acid.
[b] 3-[4-({2-[Methyl(2-pyridinyl)amino]ethyl}oxy)phenyl]propanamide.
[c] (5*Z*)-5-{[4-({2-[Methyl(2-pyridinyl)amino]ethyl}oxy)phenyl]methylidene}-1,3-thiazolidine-2,4-dione.

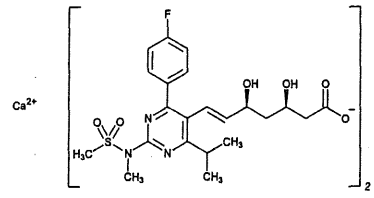
SPECIFIC TESTS

- **WATER DETERMINATION,** *Method 1a* ⟨921⟩: NMT 0.5%
[NOTE—Because maleic acid will react with methanol thereby producing water, both titrant and solvent must be methanol free.]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Fumaric Acid RS
USP Maleic Acid RS
USP Rosiglitazone Maleate RS
USP Rosiglitazone Related Compound A RS
(5*Z*)-5-{[4-({2-[Methyl(2-pyridinyl)amino]ethyl}oxy)phenyl]methylidene}-1,3-thiazolidine-2,4-dione.
$C_{18}H_{17}N_3O_3S$ 355.41

Rosuvastatin Calcium



$Ca(C_{22}H_{27}FN_3O_6S)_2$ 1001.14
6-Heptenoic acid, 7-[4-(4-fluorophenyl)-6-(1-methylethyl)-2-[methyl(methylsulfonyl)amino]-5-pyrimidinyl]-3,5-dihydroxy-, calcium salt (2:1), (3*R*,5*S*,6*E*);
[*S*-[*R**,*S**-(*E*)]]-7-[4-(4-Fluorophenyl)-6-(1-methylethyl)-2-[methyl(methylsulfonyl)amino]-5-pyrimidinyl]-3,5-dihydroxy-6-heptenoic acid, calcium salt (2:1);
Calcium (3*R*,5*S*,*E*)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido)pyrimidin-5-yl)-3,5-dihydroxyhept-6-enoate salt (1:2) [147098-20-2].

DEFINITION

Rosuvastatin Calcium contains NLT 97.0% and NMT 103.0% of rosuvastatin calcium [$Ca(C_{22}H_{27}FN_3O_6S)_2$], calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A OR 197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Enantiomeric Purity*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Calcium*
Sample solution: 8 mg/mL of Rosuvastatin Calcium in a mixture of methanol and water (1:1)
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
Protect all solutions containing rosuvastatin calcium and its related compounds from light.
Solution A: Acetonitrile, 1% (v/v) aqueous trifluoroacetic acid, and water (290:10:700)
Solution B: Acetonitrile, 1% (v/v) aqueous trifluoroacetic acid, and water (750:10:240)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	100	0
50	60	40
60	0	100
70	0	100
71	100	0
80	100	0

Diluent: Acetonitrile and water (25:75)
System suitability solution A: Dissolve 10 mg of Rosuvastatin Calcium in 10 mL of 1% trifluoroacetic acid in ethyl acetate. Heat at 40° for 1 h. Cool, and transfer to a

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separatory funnel. Add 2 mL of 1 M sodium hydroxide, and shake for 30 s. Allow the layers to separate, and discard the lower aqueous layer. Add 2 mL of water, and shake for 10 s. Allow the layers to separate, and discard the aqueous layer. Transfer 2 mL of the retained organic layer to a 50-mL standard flask, add 12 mL of acetonitrile, and dilute with water to volume. This solution contains predominantly rosuvastatin lactone.

System suitability solution B: Dissolve 10 mg of Rosuvastatin Calcium in a 50-mL volumetric flask in 10 mL of 1% trifluoroacetic acid in acetonitrile. Stopper, and heat at 40° for 1 h. Cool, add 20 mL of water, and neutralize with 1 M sodium hydroxide to a pH of 6–8. Dilute with water to volume. This solution contains predominantly rosuvastatin diastereomers.

System suitability solution C: 0.25 mg/mL each of USP Rosuvastatin Related Compound A RS and USP Rosuvastatin Related Compound B RS in a mixture of acetonitrile and water (1:1)

System suitability solution D: 0.04 mg/mL of USP Rosuvastatin Related Compound C RS in a mixture of acetonitrile and water (1:1)

System suitability solution E: Heat 250 mg of Rosuvastatin Calcium at 50° for 7 days in suitable glassware with a porous cover. Dissolve 50 mg of the heated rosuvastatin calcium in 11 mL of acetonitrile in a 50-mL standard flask. Add 5 mL of *System suitability solution A*, 3 mL of *System suitability solution B*, 1 mL of *System suitability solution C*, and 1 mL of *System suitability solution D*. Dilute with water to volume.

Standard solution: 0.7 mg/mL of USP Rosuvastatin Calcium RS in *Diluent*

Sample solution: 0.7 mg/mL of Rosuvastatin Calcium in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 3.0-mm × 15-cm; 3-µm packing L1

Column temperature: 40°

Flow rate: 0.75 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution E* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between the rosuvastatin and rosuvastatin diastereomer peaks, *System suitability solution E*

Tailing factor: NMT 1.5 for the rosuvastatin peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of rosuvastatin calcium $[Ca(C_{22}H_{27}FN_3O_6S)_2]$ in the portion of Rosuvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of rosuvastatin from the *Sample solution*

r_S = peak response of rosuvastatin from the *Standard solution*

C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (mg/mL)

C_U = concentration of Rosuvastatin Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous and solvent-free basis

IMPURITIES

• ORGANIC IMPURITIES

Protect all solutions containing rosuvastatin calcium and its related compounds from light.

Mobile phase, Diluent, System suitability solution E, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Standard solution: 0.0014 mg/mL of USP Rosuvastatin Calcium RS in *Diluent*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Rosuvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of rosuvastatin from the *Standard solution*

C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (mg/mL)

C_U = concentration of Rosuvastatin Calcium in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Rosuvastatin related compound A	0.9	1.00	0.2
Rosuvastatin	1.0	1.00	—
Rosuvastatin diastereomers[a]	1.1	1.00	0.5
Rosuvastatin ketone[b]	1.5	0.71	0.8
Rosuvastatin lactone[c]	1.7	1.00	0.15
Rosuvastatin related compound B[d]	2.2	1.00	—
Rosuvastatin dehydro analog[e]	1.8	1.00	0.15
Rosuvastatin related compound C[d]	2.6	1.00	—
Any unspecified impurity	—	1.00	0.10
Total impurities	—	—	1.5

[a] (3*RS*,5*RS*,*E*)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid.

[b] (*R*,*E*)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido) pyrimidin-5-yl]-3-hydroxy-5-oxohept-6-enoic acid.

[c] *N*-[4-(4-Fluorophenyl)-5-((*E*)-2-[(2*S*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]vinyl)-6-isopropylpyrimidin-2-yl]-*N*-methylmethanesulfonamide.

[d] Classified as any unspecified impurity.

[e] (*S*,2*ZE*,6*E*)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido)pyrimidin-5-yl]-5-hydroxyhepta-2,6-dienoic acid.

• ENANTIOMERIC PURITY

Prepare all solutions containing rosuvastatin calcium and its related compounds in amber glassware.

Mobile phase: Acetonitrile and 0.1% (v/v) trifluoroacetic acid in water (25:75)

Diluent: Prepare as directed in the *Assay*.

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System suitability solution: 1 mg/mL of USP Rosuvastatin Calcium RS and 0.004 mg/mL of USP Rosuvastatin Enantiomer RS in *Diluent*
Standard solution: 0.005 mg/mL of USP Rosuvastatin Calcium RS in *Diluent*
Sample solution: 1 mg/mL of Rosuvastatin Calcium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 15-cm; 5-µm packing L107
Column temperature: 35°
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for rosuvastatin enantiomer and rosuvastatin are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the rosuvastatin enantiomer and rosuvastatin peaks
Tailing factor: NMT 1.8 for the rosuvastatin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of rosuvastatin enantiomer in the portion of Rosuvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of rosuvastatin enantiomer from the *Sample solution*
r_S = peak response of rosuvastatin from the *Standard solution*
C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (mg/mL)
C_U = concentration of Rosuvastatin Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.15%

• **LIMIT OF CHLORIDE**
Sample: 150 mg of Rosuvastatin Calcium
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.01 M silver nitrate
Endpoint detection: Potentiometric
Blank: 60 mL of water and 5 mL of 10% (v/v) aqueous nitric acid
Analysis: Dissolve the *Sample* in 60 mL of water, and add 5 mL of 10% (v/v) aqueous nitric acid. Titrate with *Titrant*.
Calculate the percentage of chloride in the portion of Rosuvastatin Calcium taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mmol/mL)
F = equivalency factor, 35.45 mg/mmol
W = *Sample* weight (mg)

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*, *Method Ia* or *Method Ic*: NMT 6%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Rosuvastatin Calcium RS
USP Rosuvastatin Enantiomer RS
Calcium (3*S*,5*R*,*E*)-7-[4-(4-fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate salt (1:2).
$Ca(C_{22}H_{27}FN_3O_6S)_2$ 1001.14
USP Rosuvastatin Related Compound A RS
Calcium (3*R*,5*S*,*E*)-7-{4-(4-fluorophenyl)-2-[(2-hydroxy-*N*,2-dimethylpropyl)sulfonamide]-6-isopropylpyrimidin-5-yl}-3,5-dihydroxyhept-6-enoate salt (1:2).
$Ca(C_{25}H_{33}FN_3O_7S)_2$ 1117.30
USP Rosuvastatin Related Compound B RS
Calcium (3*R*,5*S*,*E*)-7-(4-(4-fluorophenyl)-2-{2-[4-(4-fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido)pyrimidin-5-yl]-2-hydroxy-*N*-methylethylsulfonamido}-6-isopropylpyrimidin-5-yl)-3,5-dihydroxyhept-6-enoate salt (1:2).
$Ca(C_{38}H_{45}F_2N_6O_9S_2)_2$ 1703.93
USP Rosuvastatin Related Compound C RS
tert-Butyl 2-[(4*R*,6*S*)-6-{(*E*)-2-[4-(4-fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido)pyrimidin-5-yl]vinyl}-2,2-dimethyl-1,3-dioxan-4-yl]acetate.
$C_{29}H_{40}FN_3O_6S$ 577.71

Rosuvastatin Tablets

DEFINITION
Rosuvastatin Tablets contain NLT 90% and NMT 110% of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$).

IDENTIFICATION
• **A.** The UV absorption spectra of the rosuvastatin peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Protect all solutions containing rosuvastatin from light.
Solution A: 1% trifluoroacetic acid in water
Mobile phase: Acetonitrile, *Solution A*, and water (37:1:62)
Diluent: Acetonitrile and water (25:75)
Standard stock solution: 1 mg/mL of USP Rosuvastatin Calcium RS prepared as follows. To a suitable amount of USP Rosuvastatin Calcium RS in a suitable volumetric flask, add water equal to about 50% of the flask volume. Vigorously mix or sonicate the flask to dissolve the material. Add acetonitrile equal to about 25% of the total volume and then dilute with water to volume.
Standard solution: 25 µg/mL of USP Rosuvastatin Calcium RS in *Diluent* from the *Standard stock solution*
Sample solution: Nominally 25 µg/mL of rosuvastatin prepared as follows. Transfer a suitable number of Tablets, NLT 5 Tablets for 80-mg Tablet strength and NLT 10 Tablets for all other Tablet strengths, into a suitable extraction flask. Add water and vigorously mix to disintegrate the Tablets. Add acetonitrile and mix

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vigorously. Add more water to obtain a 25:75 composition of acetonitrile and water. Pass the solution through a suitable filter. Dilute the filtrate with *Diluent*, if necessary, to the desired concentration.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm. For *Identification A*, use a diode array detector in the range of 200–440 nm.
Column: 3.2-mm × 25-cm; 5-µm packing L1. [NOTE—A suitable guard column may be used.]
Column temperature: 40°
Flow rate: 0.75 mL/min
Injection volume: 10 µL
Run time: NLT 1.3 times the retention time of rosuvastatin

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak response of rosuvastatin from the *Sample solution*
r_S = peak response of rosuvastatin from the *Standard solution*
C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of rosuvastatin in the *Sample solution* (µg/mL)
M = number of moles of rosuvastatin per mole of rosuvastatin calcium, 2
M_{r1} = molecular weight of rosuvastatin, 481.54
M_{r2} = molecular weight of rosuvastatin calcium, 1001.14

Acceptance criteria: 90%–110%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Protect all solutions containing rosuvastatin from light.
Test 1
Medium: Citrate buffer, pH 6.6 (prepare a solution of 14.7 g/L of sodium citrate dihydrate and 0.33 g/L of anhydrous citric acid; adjust if necessary with sodium citrate or citric acid to a pH of 6.6); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Diluent: Acetonitrile and water (25:75)
Mobile phase: Acetonitrile, water, and phosphoric acid (400:600:1)
Standard stock solution: 1 mg/mL of USP Rosuvastatin Calcium RS in *Diluent*
Standard solution: A solution of concentration similar to the *Sample solution* in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention time of rosuvastatin

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak response of rosuvastatin from the *Sample solution*
r_S = peak response of rosuvastatin from the *Standard solution*
C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M = number of moles of rosuvastatin per mole of rosuvastatin calcium, 2
M_{r1} = molecular weight of rosuvastatin, 481.54
M_{r2} = molecular weight of rosuvastatin calcium, 1001.14

Tolerances: NLT 75% (Q) of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets *Dissolution Test 2*.
Medium: 0.05 M citrate buffer pH 6.6 (to a solution of 10.5 g/L of citric acid monohydrate, add 5.9 g/L of sodium hydroxide and mix; adjust with 0.2 M sodium hydroxide or 0.2 M hydrochloric acid to a pH of 6.6); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: Dissolve 2.72 g of potassium dihydrogen phosphate in 1 L of water and add 2 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Buffer* (30:70)
Standard stock solution: 0.5 mg/mL of USP Rosuvastatin Calcium RS in *Medium*. Sonication may be necessary for complete dissolution.
Standard solution: (L/900) mg/mL of USP Rosuvastatin Calcium RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of rosuvastatin

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak response of rosuvastatin from the *Sample solution*
r_S = peak response of rosuvastatin from the *Standard solution*
C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M = number of moles of rosuvastatin per mole of rosuvastatin calcium, 2
M_{r1} = molecular weight of rosuvastatin, 481.54
M_{r2} = molecular weight of rosuvastatin calcium, 1001.14

Tolerances: NLT 80% (Q) of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets *Dissolution Test 3*.
Medium: 0.05 M citrate buffer pH 6.6 (dissolve 63.0 ▲g▲ (ERR 1-Jun-2019) of citric acid monohydrate and 35.2 g of sodium hydroxide into 6 L of water and mix; adjust if necessary with sodium hydroxide or citric acid to a pH of 6.6); 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard stock solution: 0.044 mg/mL of USP Rosuvastatin Calcium RS in *Medium*. Sonication may be necessary for complete dissolution.
Standard solution: (L/900) mg/mL of USP Rosuvastatin Calcium RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet. [NOTE—The *Standard stock solution* is the *Standard solution* for 40-mg Tablets.]
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: 241 nm
Cell: 1.0 cm (for 5-mg and 10-mg Tablets) and 0.2 cm (for 20-mg and 40-mg Tablets)
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times [M \times (M_{r1}/M_{r2})] \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M = number of moles of rosuvastatin per mole of rosuvastatin calcium, 2
M_{r1} = molecular weight of rosuvastatin, 481.54
M_{r2} = molecular weight of rosuvastatin calcium, 1001.14

Tolerances: NLT 80% (Q) of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that it meets *Dissolution Test 4*.
Medium: 0.05 M sodium citrate buffer pH 6.6 (prepare a solution of 14.7 g/L of sodium citrate dihydrate and 0.33 g/L of anhydrous citric acid; adjust if necessary with 10% w/v sodium citrate dihydrate solution or 10% w/v anhydrous citric acid solution to a pH of 6.6); 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Solution A: Acetonitrile and water (25:75)
Mobile phase: Acetonitrile, phosphoric acid, and water (40: 0.1: 60)
Standard stock solution: 1.04 mg/mL of USP Rosuvastatin Calcium RS in *Solution A*. Sonication may be necessary for complete dissolution.
Standard solution: (L/900) mg/mL of USP Rosuvastatin Calcium RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 5-cm; 3.5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: NLT 3.5 times the retention time of rosuvastatin
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak response of rosuvastatin from the *Sample solution*
r_S = peak response of rosuvastatin from the *Standard solution*
C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M = number of moles of rosuvastatin per mole of rosuvastatin calcium, 2
M_{r1} = molecular weight of rosuvastatin, 481.54
M_{r2} = molecular weight of rosuvastatin calcium, 1001.14

Tolerances: NLT 80% (Q) of the labeled amount of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Protect all solutions containing rosuvastatin calcium from light.
Mobile phase and **Diluent:** Prepare as directed in the *Assay*.
System suitability stock solution: 50 µg/mL each of USP Rosuvastatin Calcium RS and rosuvastatin diastereomers in acidic water prepared as follows. To a suitable amount of USP Rosuvastatin Calcium RS in a suitable volumetric flask, add water equal to about 50% of the flask volume. Add 1 M hydrochloric acid equal to about 10% of the total volume. Heat in a water bath at 60° for 2 h and neutralize by adding

USP Monographs

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1 M sodium hydroxide. Cool to room temperature and add acetonitrile equal to about 25% of the total volume. Dilute with water to volume.

System suitability solution: 25 µg/mL each of USP Rosuvastatin Calcium RS and rosuvastatin diastereomers prepared by mixing *System suitability stock solution* and *Diluent* (1:1)

Standard solution: 10 µg/mL of USP Rosuvastatin Calcium RS in *Diluent*

Sample solution: Nominally 1 mg/mL of rosuvastatin prepared as follows. Transfer a number of Tablets per *Table 1* into a suitable extraction flask. Add water, and mix vigorously to disintegrate the Tablets. Add acetonitrile and mix vigorously followed by an additional amount of water to obtain a final composition of acetonitrile and water (1:3). Pass the solution through a suitable filter.

Table 1

Tablet Strength (mg)	Number of Tablets	Volumetric Flask Size (mL)	Water (mL)	Acetonitrile (mL)
2.5	40	100	50	25
5	20	100	50	25
10	10	100	50	25
20	10	200	100	50
40	12	500	250	125
80	6	500	250	125

Chromatographic system: Proceed as directed in the *Assay*, except for *Run time*.

Run time: NLT 2.5 times the retention time of rosuvastatin

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between rosuvastatin and rosuvastatin diastereomers, *System suitability solution*

Tailing factor: NMT 1.8, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of rosuvastatin from the *Standard solution*

C_S = concentration of USP Rosuvastatin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of rosuvastatin in the *Sample solution* (mg/mL)

M = number of moles of rosuvastatin per mole of rosuvastatin calcium, 2

M_{r1} = molecular weight of rosuvastatin, 481.54

M_{r2} = molecular weight of rosuvastatin calcium, 1001.14

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Rosuvastatin related compound A	0.9	—	—
Rosuvastatin	1.0	—	—
Rosuvastatin diastereomers[a, b]	1.1	—	—
Rosuvastatin ketone[c]	1.6	0.71	2.1
Rosuvastatin lactone[d]	2.3	1.0	1.5
Rosuvastatin ethyl ester (if present)[e]	3.8	1.0	0.5
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	3.6

[a] (3*RS*,5*RS*,*E*)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid.

[b] Process impurity controlled in the drug substance monograph. Provided for information only; the content is not calculated, not reported, and not included in the total impurities.

[c] (*R*,*E*)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido) pyrimidin-5-yl]-3-hydroxy-5-oxohept-6-enoic acid.

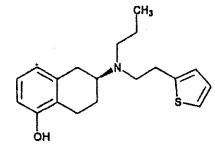
[d] *N*-[4-(4-Fluorophenyl)-5-{(*E*)-2-[(2*S*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]vinyl}-6-isopropylpyrimidin-2-yl]-*N*-methylmethanesulfonamide.

[e] Ethyl (3*R*,5*S*,*E*)-7-[4-(4-fluorophenyl)-6-isopropyl-2-(*N*-methylmethylsulfonamido)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Rosuvastatin Calcium RS

Rotigotine



$C_{19}H_{25}NOS$ 315.47

1-Naphthalenol, 5,6,7,8-tetrahydro-6-[propyl[2-(2-thienyl) ethyl]amino-(6*S*)-;
(-)-(*S*)-5,6,7,8-Tetrahydro-6-[propyl[2-(2-thienyl)ethyl] amino]-1-naphthol;
(6*S*)-6-[Propyl(2-(2-thienyl)ethyl)amino]-5,6,7,8-tetrahydro-1-naphthalenol [99755-59-6].

DEFINITION

Rotigotine contains NLT 98.0% and NMT 102.0% of rotigotine ($C_{19}H_{25}NOS$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
  Standard: USP Rotigotine RS
  Acceptance criteria: Meets the requirements

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• **B.** The retention time of the major peak of the *Identification solution* corresponds to the rotigotine–*S* isomer peak of the *System suitability solution*, as obtained in the *Limit of Rotigotine R-Enantiomer* test.

ASSAY

• **PROCEDURE**

Solution A: 0.3 mL of trifluoroacetic acid in 1 L of water
Solution B: 0.2 mL of trifluoroacetic acid in 1 L of acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	82	18
2	82	18
24	50	50
24.1	82	18
27	82	18

Diluent A: 1 mL of trifluoroacetic acid in 1 L of water
Diluent B: 1 mL of trifluoroacetic acid in 1 L of acetonitrile
Standard stock solution: 1.3 mg/mL of prepared as follows. Transfer a suitable amount of to a suitable volumetric flask. Add 5% of the flask volume of *Diluent B* and 40% of the flask volume of *Diluent A*. Sonicate to promote dissolution. [NOTE—A sonication time of NLT 20 min with shaking at 5-min intervals may be suitable.] Dilute with *Diluent A* to volume.
Standard solution: 650 µg/mL of from *Standard stock solution* in *Diluent A*
System suitability solution: 0.005 mg/mL each of USP Rotigotine Related Compound G RS and USP Rotigotine Related Compound H RS in *Standard solution*
Sample solution: 600 µg/mL of Rotigotine in *Diluent A* prepared as follows. Transfer a suitable amount of Rotigotine to a suitable volumetric flask. Add 5% of the flask volume of *Diluent B* followed by 40% of the flask volume of *Diluent A*. Sonicate to promote dissolution. [NOTE—A sonication time of NLT 20 min with shaking at 5-min intervals may be suitable.] Dilute with *Diluent A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.2 between rotigotine related compound G and rotigotine related compound H
Tailing factor: NMT 3.0 for rotigotine
Relative standard deviation: NMT 0.73% for rotigotine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of rotigotine ($C_{19}H_{25}NOS$) in the portion of Rotigotine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of in the *Standard solution* (µg/mL)
C_U = concentration of Rotigotine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of rotigotine, 315.47
M_{r2} = molecular weight of rotigotine hydrochloride, 351.93

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **LIMIT OF ROTIGOTINE *R*-ENANTIOMER**

Diluent A: Absolute alcohol, methanol, and 2-propanol (90:5:5)
Diluent B: 1 mL of diethylamine in 1 L of *Diluent A*
Mobile phase: Heptane, *Diluent A*, and diethylamine (98: 2: 0.1)
System suitability solution: 0.1 mg/mL of USP Rotigotine Racemate RS in *Diluent B*
Sample solution: 2 mg/mL of Rotigotine in *Diluent B*
Identification solution: 0.05 mg/mL of Rotigotine from the *Sample solution* in *Diluent B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 10-µm packing L40. [NOTE—A 4.6-mm × 5-cm guard column with L40 packing may be used.]
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: About 1.5 times the retention time of rotigotine
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for rotigotine *R*-isomer and rotigotine are 0.9 and 1.0, respectively. Use the chromatogram from the *Identification solution* for *Identification B*.]
Suitability requirements
Resolution: NLT 1.5 between rotigotine *R*-isomer and rotigotine
Tailing factor: NMT 2.0 for rotigotine
Analysis
Samples: *Sample solution* and *Identification solution*
Calculate the percentage of rotigotine *R*-isomer in the portion of Rotigotine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of rotigotine *R*-isomer from the *Sample solution*
r_T = sum of the peak responses of rotigotine *R*-isomer and rotigotine from the *Sample solution*

Acceptance criteria: NMT 0.15% of rotigotine *R*-isomer

• **ORGANIC IMPURITIES**

Mobile phase, Diluent A, Diluent B, Standard stock solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.65 µg/mL of from *Standard stock solution* and *Diluent A*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.2 between rotigotine related compound G and rotigotine related compound H, *System suitability solution*

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Tailing factor: NMT 2.0 for rotigotine, *Standard solution*
Relative standard deviation: NMT 5.0% for rotigotine, *Standard solution*

Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Rotigotine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of in the *Standard solution* (µg/mL)
C_U = concentration of Rotigotine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of rotigotine, 315.47
M_{r2} = molecular weight of rotigotine hydrochloride, 351.93

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desthienylethyl rotigotine[a]	0.2	0.30
Rotigotine related compound C[b]	0.6	0.30
Ethylrotigotine[c]	0.79	0.15
Rotigotine	1.0	—
Acetyl rotigotine[d]	1.46	0.15
Rotigotine related compound G	1.62	0.30
Rotigotine related compound H[e]	1.67	—
Rotigotine-*O*-tosylate[f]	2.42	0.15
Rotigotine-*O*-thienylethyl[g]	2.60	0.15
Any individual, unspecified impurity	—	0.10
Total impurities	—	1.0

[a] (*S*)-6-(Propylamino)-5,6,7,8-tetrahydronaphthalen-1-ol.
[b] (*S*)-6-{[2-(Thiophen-2-yl)ethyl]amino}-5,6,7,8-tetrahydronaphthalen-1-ol.
[c] (*S*)-6-(Ethyl[2-(thiophen-2-yl)ethyl]amino)-5,6,7,8-tetrahydronaphthalen-1-ol.
[d] (6*S*)-6-[Propyl[2-(thiophen-2-yl)ethyl]amino]-5,6,7,8-tetrahydronaphthalen-1-yl acetate.
[e] Included for *System suitability* evaluation only.
[f] (*S*)-6-(Propyl[2-(thiophen-2-yl)ethyl]amino)-5,6,7,8-tetrahydronaphthalen-1-yl 4-methylbenzenesulfonate.
[g] (*S*)-*N*-Propyl-5-[2-(thiophen-2-yl)ethoxy]-*N*-[2-(thiophen-2-yl)ethyl]-1,2,3,4-tetrahydronaphthalen-2-amine.

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: NMT 0.2%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Rotigotine RS

(6*S*)-6-[Propyl(2-(2-thienyl)ethyl)amino]-5,6,7,8-tetrahydronaphthalen-1-ol hydrochloride.
$C_{19}H_{25}NOS \cdot HCl$ 351.93

USP Rotigotine Related Compound G RS
(*S*)-6-{Bis[2-(thiophen-2-yl)ethyl]amino}-5,6,7,8-tetrahydronaphthalen-1-ol hydrochloride.
$C_{22}H_{25}NOS_2 \cdot HCl$ 420.03

USP Rotigotine Related Compound H RS
(*S*)-5-Methoxy-*N*-propyl-*N*-[2-(thiophen-2-yl)ethyl]-1,2,3,4-tetrahydronaphthalen-2-amine.
$C_{20}H_{27}NOS$ 329.50

USP Rotigotine Racemate RS
(*RS*)-6-{Propyl[2-(thiophen-2-yl)ethyl]amino}-5,6,7,8-tetrahydronaphthalen-1-ol.
$C_{19}H_{25}NOS$ 315.47

Add the following:

▲Rotigotine Transdermal System

DEFINITION
Rotigotine Transdermal System contains NLT 90.0% and NMT 110.0% of the labeled amount of rotigotine ($C_{19}H_{25}NOS$).

IDENTIFICATION
- **A.** The UV spectrum of the rotigotine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Extraction solvent: Prepare a mixture of 2-propanol and *tert*-butylmethylether (80:20). Add 1 mL of methanesulfonic acid to 1 L of the solvent mixture.
Solution A: 0.5 mL of methanesulfonic acid in 1 L of water
Solution B: 0.5 mL of methanesulfonic acid in 1 L of acetonitrile
Mobile phase: *Solution A* and *Solution B* (65:35)
Diluent: 1 mL of methanesulfonic acid in 1 L of water
Standard stock solution: 0.5 mg/mL of USP Rotigotine Hydrochloride RS in *Extraction solvent*. [NOTE—Sonication in a cooled ultrasonic bath may be used to aid in dissolution.]
Standard solution: Mix 3 mL of the *Standard stock solution* with 7 mL of *Diluent*.
Sample stock solution: Nominally 0.45 mg/mL of rotigotine from NLT 10 Transdermal Systems prepared as follows. Transfer the required number of Transdermal Systems without the release liner to a suitable flask containing *N* mL of *Extraction solvent*, where *N* is the total area of the Transdermal Systems taken. Close the flask and sonicate the solution for NLT 10 min in a sonicator maintained at 20°, shaking the flask intermittently to ensure the silicone matrix is completely dissolved. [NOTE—Sonication time may be extended if needed to enable complete dissolution of the silicone matrix.]
Sample solution: Mix 3 mL of *Sample stock solution* with 7 mL of *Diluent*. Centrifuge the solution and use the clear supernatant. [NOTE—A centrifuge speed of NLT 4000 rpm for NLT 15 min may be used.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

USP Monographs

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Mode: LC
Detector: UV 272 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.0-mm × 7.5-cm; 4-µm packing L7
Column temperature: 30°
Flow rate: 2 mL/min
Injection volume: 10 µL
Run time: NLT 4 times the retention time of rotigotine

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.2
Relative standard deviation: NMT 1.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of rotigotine ($C_{19}H_{25}NOS$) in each Transdermal System taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/N) \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rotigotine Hydrochloride RS in the *Standard stock solution* (mg/mL)
V = volume of the *Sample stock solution* (mL)
N = number of Transdermal System units used to prepare the *Sample stock solution*
M_{r1} = molecular weight of rotigotine, 315.47
M_{r2} = molecular weight of rotigotine hydrochloride, 351.93
L = label claim (nominal content) of rotigotine in each Transdermal System (mg/unit)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DRUG RELEASE** ⟨724⟩
Medium: Dissolve 6.9 g of monobasic sodium phosphate in 1 L of water. Adjust with a suitable concentration of sodium hydroxide or phosphoric acid to a pH of 4.5; 900 mL, deaerated.
Apparatus 5: 50 rpm; Transdermal System on metal disk
Times: 15, 30, 60, and 180 min
Solution A, Solution B, and **Mobile phase:** Prepare as directed in the *Assay*.
Standard solution: (L/800) mg/mL of USP Rotigotine Hydrochloride RS in *Medium*, where L is the label claim in mg/unit
Sample solution: Withdraw 2 mL from the vessel at the specified times.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 272 nm
Column: 4.0-mm × 7.5-cm; 4-µm packing L7
Column temperature: 30°
Flow rate: 2 mL/min
Injection volume: 50 µL
Run time: NLT 2.5 times the retention time of rotigotine

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.2 for rotigotine
Relative standard deviation: NMT 2.0% for rotigotine

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount (nominal content) of rotigotine ($C_{19}H_{25}NOS$) released at each time point *i*:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rotigotine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (nominal content) of rotigotine in the Transdermal System (mg/unit)
M_{r1} = molecular weight of rotigotine, 315.47
M_{r2} = molecular weight of rotigotine hydrochloride, 351.93

Acceptance criteria: See *Table 1*.

Table 1

Time Point (*i*)	Time (min)	Rotigotine Released (%)
1	15	14–34
2	30	27–47
3	60	45–65
4	180	NLT 85

The percentages of the labeled amount of rotigotine ($C_{19}H_{25}NOS$) released at the times specified conform to *Drug Release* ⟨724⟩, *Acceptance Table 1*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Extraction solvent, Solution A, Solution B, Diluent, and **Standard stock solution:** Prepare as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2.0	95	5
35	40	60
38	40	60
39	95	5

Impurities stock solution: 0.1 mg/mL each of USP Rotigotine Related Compound C RS and USP Rotigotine Related Compound K RS in 2-propanol
System suitability stock solution: 0.15 mg/mL of USP Rotigotine Hydrochloride RS and 0.0025 mg/mL each of USP Rotigotine Related Compound C RS and USP Rotigotine Related Compound K RS from suitable volumes of *Standard stock solution* and *Impurities stock solution* in *Extraction solvent*
System suitability solution: Mix 3 mL of *System suitability stock solution* with 7 mL of *Diluent*.

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Sensitivity stock solution: 0.25 µg/mL of USP Rotigotine Hydrochloride RS from *Standard stock solution* in *Extraction solvent*

Sensitivity solution: Mix 3 mL of *Sensitivity stock solution* with 7 mL of *Diluent*.

Sample stock solution: [NOTE—Lacquer removal is recommended to minimize the interferences from the lacquer with the degradation product peaks as follows. Remove the lacquer from the backing foil of each Transdermal System with 4% (v/v) glacial acetic acid in methanol. Dry the lacquer-removed Transdermal Systems for NLT 1 h at room temperature.] Nominally 0.45 mg/mL of rotigotine from NLT 3 Transdermal Systems prepared as follows. Transfer the required number of (lacquer removed, if necessary) Transdermal Systems without the release liner to a suitable flask containing *n* mL of *Extraction solvent*, where *n* is the total area (cm^2) of the Transdermal Systems taken. Close the flask and sonicate the solution for NLT 10 min in a sonicator maintained at 20°, shaking the flask intermittently to ensure the silicone matrix is completely dissolved. [NOTE—Sonication time may be extended if needed to enable complete dissolution of the silicone matrix.]

Sample solution: Mix 3 mL of *Sample stock solution* with 7 mL of *Diluent*. Centrifuge the solution and use the clear supernatant. [NOTE—A centrifuge speed of NLT 4000 rpm for NLT 15 min may be used.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L10. [NOTE—A guard column with dimensions 4-mm × 1-cm with 5-µm L3 packing is recommended to minimize interference from the adhesive.]
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 80 µL

System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—See *Table 3* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between rotigotine related compound K and rotigotine related compound C, *System suitability solution*
Signal-to-noise ratio: NLT 10 for rotigotine, *Sensitivity solution*

Analysis
Sample: *Sample solution*
Calculate the percentage of each specified or unspecified degradation product in the portion of the Transdermal System taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each specified or unspecified degradation product from the *Sample solution*
r_T = sum of all the peak responses (including the rotigotine peak) from the *Sample solution*
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

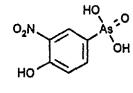
Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desthienylethyl rotigotine[a]	0.36	1.0	0.40
Rotigotine related compound K	0.6	3.5	0.20
Rotigotine related compound C	0.7	1.0	0.60
Rotigotine	1.0	—	—
Any individual unspecified degradation product	—	1.0	0.20
Total degradation products	—	—	1.0

[a] (*S*)-6-(Propylamino)-5,6,7,8-tetrahydronaphthalen-1-ol.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** The label states the total amount of rotigotine in the Transdermal System and the release rate, in mg/day, for the duration of the application of one system.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Rotigotine Hydrochloride RS
  (6*S*)-6-[Propyl(2-(2-thienyl)ethyl)amino]-5,6,7,8-tetrahydro-1-naphthalenol hydrochloride.
  $C_{19}H_{25}NOS \cdot HCl$ 351.93
  USP Rotigotine Related Compound C RS
  (*S*)-6-{[2-(Thiophen-2-yl)ethyl]amino}-5,6,7,8-tetrahydronaphthalen-1-ol.
  $C_{16}H_{19}NOS$ 273.39
  USP Rotigotine Related Compound K RS
  7,8-Dihydronaphthalen-1-ol.
  $C_{10}H_{10}O$ 146.19 ▲(USP 1-May-2020)

Roxarsone



$C_6H_6AsNO_6$ 263.04
Arsonic acid, (4-hydroxy-3-nitrophenyl)-.
4-Hydroxy-3-nitrobenzenearsonic acid [121-19-7].

» Roxarsone contains not less than 98.0 percent and not more than 101.0 percent of $C_6H_6AsNO_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Roxarsone RS

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests ⟨197⟩, Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 8 µg per mL.
Medium: 0.1 N hydrochloric acid in methanol.

Loss on drying ⟨731⟩—Dry it at 100° for 6 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%.

Limit of trivalent arsenic—Transfer 2.50 g of Roxarsone to a 250-mL conical flask, add 5.0 mL of a solution containing 0.1237 mg of anhydrous arsenic trioxide per mL, 10 mL of water, and 4.0 mL of 5 N sodium hydroxide, and swirl to dissolve. Add 5.0 mL of glacial acetic acid, and a stirring bar, and titrate with 0.0025 N iodine VS, determining the endpoint potentiometrically. Perform the same procedure on a second 2.50-g portion of Roxarsone, except to replace the 5.0 mL of arsenic trioxide solution with 5.0 mL of water. Calculate the percentage of trivalent arsenic (As^{+++}) in the Roxarsone taken by the formula:

$$(0.4623/25)[V_B/(V_A - V_B)]$$

in which 0.4623 is the arsenic equivalent of the 5.0 mL of arsenic trioxide solution, and V_A and V_B are the volumes, in mL, of 0.0025 N iodine consumed in the first and second titrations, respectively. Not more than 0.05% is found.

Content of total arsenic—Transfer about 200 mg of Roxarsone, accurately weighed, to a clean, dry 300-mL Kjeldahl flask, and clamp the flask at an angle of 45°. Add 5 mL of sulfuric acid, 10 mL of nitric acid, 5 mL of a saturated solution of sodium sulfate, and several glass beads. Heat to boiling, heat strongly until dense white fumes are produced, and continue heating until the volume is reduced to a few mL. Allow to cool, and cautiously rinse the neck of the flask with about 5 mL of water. Boil again, and continue heating for 30 minutes after the water has boiled away. Allow to cool, add 100 mL of water and 4 mL of 50% potassium iodide solution, and boil to expel the iodine vapors. Continue boiling until the solution becomes colorless, occasionally adding water to maintain the volume at about 60 mL. Add 60 mL of water, and immediately cool the solution. Add 2 drops of phenolphthalein TS, neutralize with 5 N sodium hydroxide, and then make slightly acidic (pH 6.5 to 7.0) by adding a small quantity of 18 N sulfuric acid. Transfer this solution to a beaker with the aid of a water rinse, add 4 g of sodium bicarbonate, and stir with a stirring bar. Titrate with 0.05 N iodine VS, determining the endpoint potentiometrically. Each mL of 0.05 N iodine is equivalent to 1.873 mg of arsenic (As): between 28.0% and 28.8% is found, calculated on the dried basis.

Assay—

Mobile phase—Prepare a degassed mixture of water, methanol, and 0.17% (v/v) phosphoric acid (700:300:60). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 50 mg of USP Roxarsone RS, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with 1.2 N sodium hydroxide to volume, and mix. Transfer 10.0 mL of this stock solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.1 mg of USP Roxarsone RS per mL.

Assay preparation—Transfer about 50 mg of Roxarsone, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with 1.2 N sodium hydroxide to volume, and mix. Transfer 10.0 mL of this stock solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_6H_6AsNO_6$ in the portion of Roxarsone taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Roxarsone RS in the *Standard preparation*, and r_U and r_S are the roxarsone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Rubidium Chloride Rb 82 Injection

» Rubidium Chloride Rb 82 Injection is a sterile solution, suitable for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{82}Rb expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. It is obtained by elution from a strontium 82-rubidium 82 generator system. ^{82}Rb, with a half-life of 76 seconds, is a short-lived positron-emitting radionuclide formed by the radioactive decay of the parent nuclide ^{82}Sr. Strontium Sr 82 with a half-life of 25.5 days is produced by the proton irradiation of rubidium or spallation of molybdenum. The chemical form of the Injection is ^{82}RbCl. [NOTE—Elute with additive-free Sodium Chloride Injection only. Discard the first 50 mL of the eluate each day the generator is eluted.]

Packaging, storage, and labeling—Requirements for packaging, storage, and labeling do not apply; Rubidium Chloride Rb 82 Injection is obtained by elution from the generator and is administered by direct infusion.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than $175/V$ USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

Radionuclide identification (see *Radioactivity* ⟨821⟩)—[NOTE—Perform this test quickly, because of the rapid decay of the ^{82}Rb.] The gamma-ray spectrum of eluted ^{82}Rb exhibits photopeaks at 511 and 777 keV.

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pH ⟨791⟩: between 4.0 and 8.0.

Radionuclidic purity—Using a suitable counting assembly, determine the radioactivity of each radionuclidic impurity, in kBq per MBq (or µCi per mCi), of Rb 82 in the generator eluate by use of a calibrated system as directed under *Radioactivity* ⟨821⟩. [NOTE—For the following tests, use the generator eluate containing ^{82} Rb that has been allowed to decay for 1 hour after the end of elution.]

Sr 82 and *Rb 83*—Obtain a gamma-ray spectrum of the hour-old eluate, and measure the activities of the radionuclidic impurities directly from the spectrum. Sr 82 exhibits photopeaks at 511 and 777 keV and decays with a radioactive half-life of 25.5 days. Rb 83 exhibits a photopeak at 530 keV and decays with a radioactive half-life of 86.2 days. The activity levels of Sr 82 and Rb 83 are not more than 0.02 kBq per MBq (0.02 µCi per mCi) and not more than 0.05 kBq per MBq (0.05 µCi per mCi) of Rb 82 at the end of elution, respectively.

Sr 85—Obtain a gamma spectrum of the hour-old eluate, and, using the same system and geometry, obtain a gamma spectrum of a pure Rb 82 specimen (generator eluate containing ^{82} Rb taken within 10 minutes of elution). Sr 85 exhibits a major photopeak at 514 keV and decays with a radioactive half-life of 64.8 days. Sr 85 may be determined by subtraction of the 511 and 777 keV peaks in the pure Rb 82, from the 511–514 keV and 777 keV peaks in the hour-old eluate. The activity level of Sr 85 is not more than 0.2 kBq per MBq (0.2 µCi per mCi) of Rb 82 at the end of elution.

Other gamma-ray emitters—The total of other gamma-ray emitting radionuclidic impurities does not exceed 0.005 kBq per MBq (0.005 µCi per mCi) of Rb 82 at the end of elution.

Chemical purity—

Electrolyte solution—Transfer 107 g of ammonium chloride, 25 g of gelatin, and 42 mL of hydrochloric acid to a 500-mL volumetric flask. Add about 450 mL of water, and sonicate until a clear solution is obtained. Dilute with water to volume, and mix.

Tin stock standard solution—Dissolve 100 mg of metallic tin (Sn), accurately weighed, in 10 mL of dilute hydrochloric acid (1 in 2), and dilute with water to 100 mL.

Tin standard solution A—Transfer 0.5 mL of *Tin stock standard solution* to a 50-mL volumetric flask and dilute with 0.1 N hydrochloric acid to volume.

Tin standard solution B—Transfer 1.0 mL of *Tin standard solution A* to a 50-mL volumetric flask. Add 10.0 mL of 0.9% sodium chloride solution, dilute with *Electrolyte solution* to volume, and mix.

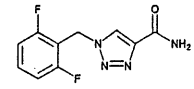
Test solution—Obtain a 50-mL eluate from the generator, and allow to stand for at least 1 hour to allow for the complete decay of ^{82} Rb. Transfer 10.0 mL of the eluate to a 50-mL volumetric flask, dilute with the *Electrolyte solution* to volume, and mix.

Procedure—Transfer a portion of the *Test solution* to a polarographic cell, and deaerate by bubbling nitrogen through the solution for 5 minutes. Insert the dropping mercury electrode of a suitable polarograph, and obtain the differential pulse polarogram from –0.15 to –0.75 volts, at a current range of 0.5 µA, using a saturated calomel electrode as the reference electrode and a platinum wire as the auxiliary electrode (see *Polarography* ⟨801⟩). Similarly, transfer a portion of the *Tin standard solution B* to a polarographic cell and obtain the polarogram. A peak at –0.52 volts indicates the presence of tin. The peak height of the *Test solution* is not greater than that of the *Tin standard solution B* (1 µg per mL).

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation for *Container Content* ⟨697⟩.

Assay for radioactivity—Using a suitable counting assembly , determine the radioactivity, in MBq (or in µCi) per mL, of the Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Rufinamide



$C_{10}H_8F_2N_4O$ 238.19

1*H*-1,2,3-Triazole-4-carboxamide, 1-[(2,6-difluorophenyl) methyl]-;

1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxamide [106308-44-5].

DEFINITION

Rufinamide contains NLT 98.0% and NMT 102.0% of $C_{10}H_8F_2N_4O$, calculated on the as-is basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Use amber glassware for all volumetric solutions.

Diluent: Methanol and tetrahydrofuran (50:50)

Mobile phase: Methanol, tetrahydrofuran, and water (12:5:83)

System suitability stock solution: 0.8 mg/mL of USP Rufinamide RS and 4 µg/mL each of USP Rufinamide Related Compound A RS and USP Rufinamide Related Compound B RS in *Diluent*. [NOTE—USP Rufinamide Related Compound B RS is used for peak identification purposes only.]

System suitability solution: 0.2 mg/mL of USP Rufinamide RS and 0.8 µg/mL each of USP Rufinamide Related Compound A RS and USP Rufinamide Related Compound B RS in water from the *System suitability stock solution*

Standard stock solution: 0.8 mg/mL of USP Rufinamide RS in *Diluent*

Standard solution: 0.2 mg/mL of USP Rufinamide RS in water from the *Standard stock solution*

Sample stock solution: 0.8 mg/mL of Rufinamide in *Diluent*

Sample solution: 0.2 mg/mL of Rufinamide in water from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 12.5-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 2.6 times the retention time of the rufinamide peak

System suitability

[NOTE—For relative retention times refer to *Table 1* in *Organic Impurities*.]

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Sample: *System suitability solution*
Suitability requirements
Tailing factor: NMT 1.2 for rufinamide
Resolution: NLT 2.5 between rufinamide and rufinamide related compound A
Relative standard deviation: NMT 1.0% for rufinamide and NMT 5.0% for rufinamide related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of rufinamide ($C_{10}H_8F_2N_4O$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rufinamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Rufinamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the as-is basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Organic Impurities**
Use amber glassware for all volumetric solutions.
Diluent, Mobile phase, System suitability stock solution, System suitability solution, Standard stock solution, Sample stock solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.8 µg/mL of USP Rufinamide RS in water from the *Standard stock solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of any individual impurity in the portion of Rufinamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of rufinamide from the *Standard solution*
C_S = concentration of USP Rufinamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Rufinamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Rufinamide	1.0	—
Rufinamide related compound A[a]	1.2	0.30
Rufinamide related compound B[b]	1.9	0.10
Any other individual impurity	—	0.05
Total impurities	—	0.50

[a] 1-(2-Fluorobenzyl)-1*H*-1,2,3-triazole-4-carboxamide.
[b] Methyl 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Rufinamide RS
USP Rufinamide Related Compound A RS
1-(2-Fluorobenzyl)-1*H*-1,2,3-triazole-4-carboxamide.
$C_{10}H_9FN_4O$ 220.20
USP Rufinamide Related Compound B RS
Methyl 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate.
$C_{11}H_9F_2N_3O_2$ 253.20

Rufinamide Tablets

DEFINITION
Rufinamide Tablets contain an amount of Rufinamide equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of rufinamide ($C_{10}H_8F_2N_4O$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: 2.7 g/L of potassium dihydrogen phosphate in water
Diluent: Acetonitrile, methanol, and water (40:50:10)
Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (15:5:80)
System suitability stock solution: 0.8 mg/mL of USP Rufinamide RS, and 0.02 mg/mL each of USP Rufinamide Related Compound A RS and USP Rufinamide Related Compound B RS in *Diluent*. [Note—USP Rufinamide Related Compound B RS is used for identification purposes only.]
System suitability solution: 0.08 mg/mL of USP Rufinamide RS, and 2 µg/mL each of USP Rufinamide Related Compound A RS and USP Rufinamide Related Compound B RS, in *Buffer* from the *System suitability stock solution*
Standard stock solution: 0.8 mg/mL of USP Rufinamide RS in *Diluent*
Standard solution: 0.08 mg/mL of USP Rufinamide RS in *Buffer* from the *Standard stock solution*
Sample stock solution: Nominally 0.8 mg/mL of rufinamide in *Diluent* from a portion of NLT 20 finely powdered Tablets. Sonicate for 10 min, and shake for 15 min. Centrifuge a portion of the suspension.
Sample solution: 0.08 mg/mL of rufinamide in *Buffer*, from a portion of suspension obtained from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
Run time: 2.3 times the retention time of the rufinamide peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—For relative retention times refer to *Table 9* in *Organic Impurities*.]

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Suitability requirements
Resolution: NLT 1.5 between rufinamide and rufinamide related compound A, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of rufinamide ($C_{10}H_8F_2N_4O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Rufinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of rufinamide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium 1: 0.1 N hydrochloric acid
Medium 2: pH 6.8 phosphate buffer
Apparatus 4: With 22.6-mm cell, glass beads in the cone, with Tablet laying on the beads. Insert 320–350 mg of glass wool in the filter insert and then a glass microfiber filter of 2.7-µm pore size and a glass microfiber filter of 0.7-µm pore size.
Times: 5 and 12 h for the 200-mg Tablets; 6 and 16 h for the 400-mg Tablets
Flow rate: 16 mL/min, pulsating
Test intervals, media, and sample solutions for the 200-mg Tablets: See *Table 1*.

Table 1

Samples	Interval (min)	Volume (mL)	Medium
1	60	50	1
2	120	50	2
1	60	50	2
3	120	50	2

Test intervals (I_i): See *Table 2*.

Table 2

Interval	Time (min)
I_1	0–60
I_2	60–180
I_3	180–300
I_4	300–360
I_5	360–480
I_6	480–600
I_7	600–720

Sample solutions (V_i): See *Table 3*.

Table 3

V_1	eluate of test interval I_1; volume = 960 mL
V_2 to V_3	eluate of test interval I_2 to I_3; volume = 1920 mL, each
V_4	eluate of test interval I_4; volume 960 mL
V_5 to V_7	eluate of test interval I_5 to I_7; volume = 1920 mL, each

Test intervals, media, and sample solutions for the 400-mg Tablets: See *Table 4*.

Table 4

Samples	Interval (min)	Volume (mL)	Medium
1	60	50	1
1	60	50	2
3	120	50	2
1	120	50	2
2	180	50	2

Test intervals (I_i): See *Table 5*.

Table 5

Interval	Time (min)
I_1	0–60
I_2	60–120
I_3	120–240
I_4	240–360
I_5	360–480
I_6	480–600
I_7	600–780
I_8	780–960

Sample solutions (V_i): See *Table 6*.

Table 6

V_1	eluate of test interval I_1; volume = 960 mL
V_2	eluate of test interval I_2; volume = 960 mL
V_3 to V_6	eluate of test interval I_3 to I_6; volume = 1920 mL, each
V_7 to V_8	eluate of test interval I_7 to I_8; volume = 2880 mL, each

Mobile phase: Water, methanol, tetrahydrofuran, and acetic acid (100: 50: 13: 0.12), with the addition of 206 mg of sodium pentanesulfonate, monohydrate
Standard stock solution: 600 µg/mL of USP Rufinamide RS in methanol
Standard solution 1: 60 µg/mL of rufinamide in *Medium 1* from the *Standard stock solution*
Standard solution 2: 60 µg/mL of rufinamide in *Medium 2* from the *Standard stock solution*
Standard solution 3: 12 µg/mL of rufinamide prepared as follows. Transfer 10 mL of the *Standard stock solution* to a 500-mL volumetric flask, add 40 mL of methanol, and dilute with *Medium 2* to volume.
Standard solution 4: 6 µg/mL of rufinamide in *Medium 2* from *Standard solution 3*

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 10-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: 1.4 times the retention time of the rufinamide peak
System suitability
Sample: *Standard solution 1*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Calculate the percentage of the labeled amount of rufinamide ($C_{10}H_8F_2N_4O$) [$f(S_i)$] dissolved in the *Sample solution* (S_i) by the following steps:
Calculate the regression line for the *Standard solutions*:

$$y = ax + b$$

y = peak area of rufinamide from the *Standard solution*
x = concentration of rufinamide in the *Standard solution* (µg/mL)

$$f(S_i) = [(y - b)/a] \times [(V_i)/(1000 \times L)] \times 100$$

y = peak area of rufinamide from the *Sample solution*
b = y-intercept
a = slope
L = label claim (mg/Tablet)
V_i = volume of *Sample solution* (mL)

Cumulative percentage of the Tablet label claim dissolved:

$$F(I_j) = \sum_{i=1}^{j} f(S_i)$$

i,j = indices of test interval

Tolerances
For Tablets labeled to contain 200 mg: See *Table 7*.

Table 7

Time (h)	Amount Released
5	NLT 60%
12	NLT 80%

For Tablets labeled to contain 400 mg: See *Table 8*.

Table 8

Time (h)	Amount Released
6	NLT 60%
16	NLT 80%

The percentages of the labeled amount of rufinamide dissolved in the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES
• **Organic Impurities**
Buffer, Diluent, Mobile phase, Sample stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability stock solution: 0.8 mg/mL of USP Rufinamide RS, and 0.02 mg/mL each of USP Rufinamide Related Compound A RS and USP Rufinamide Related Compound B RS in *Diluent*. [Note—USP Rufinamide Related Compound B RS is used for identification purposes.]
System suitability solution: 0.08 mg/mL of USP Rufinamide RS, and 2 µg/mL each of USP Rufinamide Related Compound A RS and USP Rufinamide Related Compound B RS, in *Buffer* from the *System suitability stock solution*
Standard stock solution: 0.8 mg/mL of USP Rufinamide RS in *Diluent*
Standard solution: 0.4 µg/mL of USP Rufinamide RS from the *Standard stock solution* prepared as follows. Pipet a suitable volume of *Standard stock solution* to a volumetric flask. Add *Diluent* to fill 10% of final volume, and dilute with *Buffer* to volume.
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—For relative retention times refer to *Table 9*.]
Suitability requirements
Resolution: NLT 1.5 between rufinamide and rufinamide related compound A, *System suitability solution*
Tailing factor: NMT 1.5 for rufinamide, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of rufinamide from the *Standard solution*
C_S = concentration of USP Rufinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of rufinamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 9*.

Table 9

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Rufinamide	1.0	—
Rufinamide related compound A[a]	1.2	—
Rufinamide related compound B[b]	1.8	—
Any individual unspecified degradation product	—	0.1
Total impurities	—	0.5

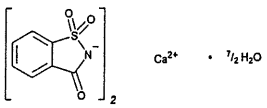
[a] 1-(2-Fluorobenzyl)-1*H*-1,2,3-triazole-4-carboxamide.
[b] Methyl 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate.

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Rufinamide RS
  USP Rufinamide Related Compound A RS
  1-(2-Fluorobenzyl)-1*H*-1,2,3-triazole-4-carboxamide.
  $C_{10}H_9FN_4O$ 220.20
  USP Rufinamide Related Compound B RS
  Methyl 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate.
  $C_{11}H_9F_2N_3O_2$ 253.20

Saccharin Calcium



$C_{14}H_8CaN_2O_6S_2 \cdot 3½H_2O$ 467.48

$C_{14}H_8CaN_2O_6S_2$ 404.44

1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide, calcium salt, hydrate (2:7);
1,2-Benzisothiazolin-3-one 1,1-dioxide calcium salt hydrate (2:7) [6381-91-5].
Anhydrous [6485-34-3].

DEFINITION

Change to read:

Saccharin Calcium contains ▲NLT 98.0% and NMT 102.0%▲ (USP 1-May-2020) of saccharin calcium ($C_{14}H_8CaN_2O_6S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)
  Sample: Dry at 105° to constant weight.
  Acceptance criteria: Meets the requirements
- **B.**
  Sample solution: 100 mg/mL
  Analysis: To the *Sample solution* add 2 drops of methyl red TS, and neutralize with 6 N ammonium hydroxide. Add 3 N hydrochloric acid, dropwise, until the solution is acid to the indicator. Add ammonium oxalate TS.
  Acceptance criteria: A white precipitate is formed when the ammonium oxalate is added. This precipitate is insoluble in 6 N acetic acid but dissolves in hydrochloric acid.
- **C.** Calcium salts moistened with hydrochloric acid impart a transient yellowish-red color to a nonluminous flame.

ASSAY

Change to read:

- **PROCEDURE**
  ▲Solution A: 50 mM dibasic potassium phosphate (K_2HPO_4) buffer in 0.1% (v/v) phosphoric acid solution
  Solution B: Methanol
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
7.0	90	10
8.0	5	95
10.0	5	95
10.1	90	10
15.0	90	10

Diluent: Methanol and water (50:50 v/v)
System suitability solution: 0.1 mg/mL of phthalic anhydride and 0.1 mg/mL of USP Saccharin Calcium RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Saccharin Calcium RS in *Diluent*
Sample solution: 0.1 mg/mL of Saccharin Calcium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 20 ± 5°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Run time: 15 min
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The retention times for phthalic anhydride and saccharin calcium are about 6.3 and 7.3 min, respectively. Phthalic anhydride is a potential impurity.]
Suitability requirements
Resolution: NLT 1.5 between the phthalic anhydride and saccharin calcium peaks, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73% for five replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of saccharin calcium in the portion of Saccharin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of saccharin calcium from the *Sample solution*
r_S = peak area of saccharin calcium from the *Standard solution*
C_S = concentration of USP Saccharin Calcium RS in the *Standard solution* (mg/mL)
C_U = concentration of Saccharin Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis▲ (USP 1-May-2020)

IMPURITIES

- **LIMIT OF TOLUENESULFONAMIDES**
  Internal standard solution: 0.25 mg/mL of caffeine in methylene chloride
  Standard stock solution: 20.0 µg/mL of USP *o*-Toluenesulfonamide RS and 20.0 µg/mL of USP *p*-Toluenesulfonamide RS in methylene chloride

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Standard solution: Evaporate 5.0 mL of *Standard stock solution* to dryness in a stream of nitrogen. Dissolve the residue in 1.0 mL of the *Internal standard solution*.
Sample stock solution: 200 mg/mL in water. If necessary, adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 7–8 before final dilution.
Sample solution: Shake 50 mL of the *Sample stock solution* with four quantities each of 50 mL of methylene chloride. Combine the lower layers, dry over anhydrous sodium sulfate, and filter. Wash the filter and the sodium sulfate with 10 mL of methylene chloride. Combine the solution and the washings, and evaporate almost to dryness in a water bath at a temperature not exceeding 40°. Using a small quantity of methylene chloride, quantitatively transfer the residue into a suitable 10-mL tube, evaporate to dryness in a stream of nitrogen, and dissolve the residue in 1.0 mL of the *Internal standard solution*.
Blank solution: Evaporate 200 mL of methylene chloride to dryness in a water bath at a temperature not exceeding 40°. Dissolve the residue in 1 mL of methylene chloride.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 10-m fused silica; coated with a 2-µm film of phase G3
Temperatures
Injector: 250°
Column: 180°
Detector: 250°
Carrier gas: Nitrogen
Flow rate: 10 mL/min
Injection volume: 1 µL
Injection type: Split ratio, 2:1
System suitability
Samples: *Standard solution* and *Blank solution*
[NOTE—The substances are eluted in the following order: *o*-toluenesulfonamide, *p*-toluenesulfonamide, and caffeine.]
Suitability requirements: There are no peaks present in the *Blank solution* at the retention times of the *Internal standard solution*, *o*-toluenesulfonamide, and *p*-toluenesulfonamide; *Blank solution*
Resolution: NLT 1.5 between *o*-toluenesulfonamide and *p*-toluenesulfonamide, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: See *Table 2*. If any peaks due to *o*-toluenesulfonamide and *p*-toluenesulfonamide appear in the chromatogram obtained with the *Sample solution*, the ratio of their areas to that of the *Internal standard solution* is NMT the corresponding ratio in the chromatogram obtained with the *Standard solution*.

Table 2

Name	Acceptance Criteria, NMT (ppm)
o-Toluenesulfonamide	10
p-Toluenesulfonamide	10

• **LIMIT OF BENZOATE AND SALICYLATE**
Sample solution: 50 mg/mL
Analysis: To 10 mL of the *Sample solution* add 5 drops of 6 N acetic acid and 3 drops of ferric chloride TS.
Acceptance criteria: No precipitate or violet color appears.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 15.0%

Change to read:

• **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩
▲Matching fluid A: Cobaltous chloride CS, ferric chloride CS, cupric sulfate CS, and water (0.1: 0.4: 0.1: 4.4)▲ (USP 1-May-2020)
Sample solution: 40 mg/mL in sulfuric acid
▲maintained at**▲** (USP 1-May-2020) 48°–50° for 10 min
Acceptance criteria: The *Sample solution* has no more color than *Matching fluid A*, when viewed against a white background.
• **CLARITY OF SOLUTION**
[NOTE—The *Sample solution* is to be compared to *Reference suspension A* and to water in diffused daylight 5 min after preparation of *Reference suspension A*.]
Diluent: 200-g/L solution of sodium acetate
Hydrazine solution: 10.0 mg/mL of hydrazine sulfate. [NOTE—Allow to stand for 4–6 h.]
Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.
Primary opalescent suspension: Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]
Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension* and dilute to 1000 mL. [NOTE—This suspension should not be used beyond 24 h after preparation.]
Reference suspension A: *Opalescence standard* and water (1 in 20)
Reference suspension B: *Opalescence standard* and water (1 in 10)
Sample solution: 200 mg/mL in *Diluent*
Analysis
Samples: *Diluent*, *Reference suspension A*, *Reference suspension B*, *Sample solution*, and water
Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Reference suspension A*, *Reference suspension B*, water, and *Diluent* to separate matching test tubes. Compare solutions in diffused daylight, viewing vertically against a black background (see *Visual Comparison* ⟨630⟩). [NOTE—The diffusion of light must be such that *Reference suspension A* can readily be distinguished from water, and that *Reference suspension B* can readily be distinguished from *Reference suspension A*.]
Acceptance criteria: The *Sample solution* shows the same clarity as that of water, or *Diluent*, or its opalescence is NMT that of *Reference suspension A*.

Change to read:

• **COLOR OF SOLUTION**
Diluent A: 200-g/L solution of sodium acetate
Diluent B: 10-g/L solution of hydrochloric acid
Standard stock solution: Ferric chloride CS, Cobaltous chloride CS, cupric sulfate CS, and *Diluent B* (3.0: 3.0: 2.4: 1.6)
Standard solution: *Standard stock solution* and *Diluent B* (1:99). [NOTE—Prepare the **▲***Standard stock solution* and**▲** (USP 1-May-2020) *Standard solution* immediately before use.]

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Sample solution: Use the *Sample solution* from the test for *Clarity of Solution*.

Analysis

Samples: *Diluent A*, *Standard solution*, *Sample solution*, and water

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of the *Standard solution*, *Diluent A*, and water to separate matching test tubes. Compare solutions in diffused daylight, viewing vertically against a white background (see *Visual Comparison* ⟨630⟩).

Acceptance criteria: The *Sample solution* has the appearance of water or *Diluent A*, or is not more intensely colored than the *Standard solution*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **LABELING:** Where the quantity of saccharin calcium is indicated in the labeling of any preparation containing Saccharin Calcium, this shall be expressed in terms of saccharin ($C_7H_5NO_3S$).

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Saccharin Calcium RS

USP *o*-Toluenesulfonamide RS

▲▲ (USP 1-May-2020)

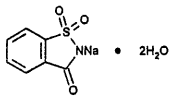
USP *p*-Toluenesulfonamide RS

▲▲ (USP 1-May-2020)

Saccharin Sodium

Change to read:

▲▲ (USP 1-Dec-2019)



$C_7H_4NNaO_3S \cdot 2H_2O$ 241.20

$C_7H_4NNaO_3S$ 205.17

1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide, sodium salt, dihydrate;

1,2-Benzisothiazolin-3-one 1,1-dioxide sodium salt dihydrate [6155-57-3].

Anhydrous [128-44-9].

DEFINITION

Change to read:

Saccharin Sodium contains ▲NLT 98.0% and NMT 102.0%▲ (USP 1-Dec-2019) of saccharin sodium ($C_7H_4NNaO_3S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Dry at 105° to constant weight.

Acceptance criteria: Meets the requirements

- **B.**

Sample solution: 100 mg/mL

Potassium pyroantimonate solution: Dissolve 2 g of potassium pyroantimonate in 95 mL of hot water. Cool quickly, and add 50 mL of a potassium hydroxide solution (50 mg/mL) and 1 mL of sodium hydroxide solution (8.5 in 100). Allow to stand for 24 h, filter, and dilute with water to 150 mL.

Analysis: To 10 mL of the *Sample solution* add 2 mL of 15% potassium carbonate, and heat to boiling. No precipitate is formed. Add 4 mL of *Potassium pyroantimonate solution*, and heat to boiling. Allow to cool in ice water and, if necessary, rub the inside of the test tube with a glass rod.

Acceptance criteria: A dense precipitate is formed.

Change to read:

- ▲▲ (USP 1-Dec-2019)**C.** Sodium salts impart an intense yellow color to a nonluminous flame.▲▲ (USP 1-Dec-2019)

ASSAY

Change to read:

- **PROCEDURE**

▲Solution A: 50 mM dibasic potassium phosphate (K_2HPO_4) buffer in 0.1% (v/v) phosphoric acid solution

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
7.0	90	10
8.0	5	95
10.0	5	95
10.1	90	10
15.0	90	10

Diluent: Methanol and water (50:50 v/v)

System suitability solution: 0.1 mg/mL of phthalic anhydride and 0.1 mg/mL of USP Saccharin Sodium RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Saccharin Sodium RS in *Diluent*

Sample solution: 0.1 mg/mL of Saccharin Sodium in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Column temperature: 20 ± 5°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Run time: 15 min

System suitability

Samples: *System suitability solution* and *Standard solution*

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[NOTE—The retention times for phthalic anhydride and saccharin sodium are about 6.3 and 7.3 min, respectively. Phthalic anhydride is a potential impurity.]

Suitability requirements

Resolution: NLT 1.5 between the phthalic anhydride and saccharin sodium peaks, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 0.73% for five replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of saccharin sodium in the portion of Saccharin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of saccharin sodium from the *Sample solution*

r_S = peak area of saccharin sodium from the *Standard solution*

C_S = concentration of USP Saccharin Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Saccharin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis ▲(USP 1-Dec-2019)

IMPURITIES

Change to read:

• ▲▲(USP 1-Dec-2019)**LIMIT OF TOLUENESULFONAMIDES**

Internal standard solution: 0.25 mg/mL of caffeine in methylene chloride

Standard stock solution: 20.0 µg/mL of USP *o*-Toluenesulfonamide RS and 20.0 µg/mL of USP *p*-Toluenesulfonamide RS in methylene chloride

Standard solution: Evaporate 5.0 mL of *Standard stock solution* to dryness in a stream of nitrogen. Dissolve the residue in 1.0 mL of the *Internal standard solution*.

Sample stock solution: 200 mg/mL in water. If necessary, adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 7–8 before final dilution.

Sample solution: Shake 50 mL of the *Sample stock solution* with four quantities each of 50 mL of methylene chloride. Combine the lower layers, dry over anhydrous sodium sulfate, and filter. Wash the filter and the sodium sulfate with 10 mL of methylene chloride. Combine the solution and the washings, and evaporate almost to dryness in a water bath at a temperature not exceeding 40°. Using a small quantity of methylene chloride, quantitatively transfer the residue into a suitable 10-mL tube, evaporate to dryness in a stream of nitrogen, and dissolve the residue in 1.0 mL of the *Internal standard solution*.

Blank solution: Evaporate 200 mL of methylene chloride to dryness in a water bath at a temperature not exceeding 40°. Dissolve the residue in 1 mL of methylene chloride.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 10-m fused silica; coated with a 2-µm film of phase G3

Temperatures

Injection port: 250°

Column: 180°

Detector: 250°

Carrier gas: Nitrogen

Flow rate: 10 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 2:1

System suitability

Samples: *Standard solution* and *Blank solution*

[NOTE—The substances are eluted in the following order: *o*-toluenesulfonamide, *p*-toluenesulfonamide, and caffeine.]

Suitability requirements: No peaks at the retention times for the internal standard, *o*-toluenesulfonamide, or *p*-toluenesulfonamide, *Blank solution*

Resolution: NLT 1.5 between *o*-toluenesulfonamide and *p*-toluenesulfonamide, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: See *Table 2*. If any peaks due to *o*-toluenesulfonamide and *p*-toluenesulfonamide appear in the chromatogram of the *Sample solution*, the ratio of their areas to that of caffeine (internal standard) is NMT the corresponding ratio in the chromatogram of the *Standard solution*.

Table 2

Name	Acceptance Criteria, NMT (ppm)
o-Toluenesulfonamide	10
p-Toluenesulfonamide	10

▲▲(USP 1-Dec-2019)

• **LIMIT OF BENZOATE AND SALICYLATE**

Sample solution: 50 mg/mL

Analysis: To 10 mL of the *Sample solution* add 5 drops of 6 N acetic acid, and then add 3 drops of ferric chloride TS.

Acceptance criteria: No precipitate or violet color appears.

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 15.0%

Change to read:

• **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩

Matching fluid A: Cobaltous chloride CS, ferric chloride TS, cupric sulfate CS, and water (0.1: 0.4: 0.1: 4.4)

Sample solution: 40 mg/mL in sulfuric acid ▲▲(USP 1-Dec-2019) maintained at 48°–50° for 10 min

Acceptance criteria: The *Sample solution* has no more color than *Matching fluid A*, when viewed against a white background.

• **ACIDITY OR ALKALINITY**

Sample solution: 100 mg/mL in carbon dioxide-free water

Analysis: To 10 mL of the *Sample solution* add 1 drop of phenolphthalein TS.

Acceptance criteria: No red or pink color is produced. Then add 1 drop of 0.1 N sodium hydroxide: a red or pink color is produced.

• **CLARITY OF SOLUTION**

[NOTE—The *Sample solution* is to be compared to *Reference suspension A* in diffused daylight 5 min after preparation of *Reference suspension A*.]

Hydrazine solution: 10.0 mg/mL of hydrazine sulfate in water. [NOTE—Allow to stand for 4–6 h.]

Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

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Primary opalescent suspension: Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]
Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension*, dilute with water to 1000 mL, and mix. [NOTE—This suspension should not be used beyond 24 h after preparation.]
Reference suspension A: *Opalescence standard* and water (1 in 20)
Reference suspension B: *Opalescence standard* and water (1 in 10)
Sample solution: 200 mg/mL in water
Analysis
Samples: *Reference suspension A, Reference suspension B, Sample solution,* and water
Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Reference suspension A, Reference suspension B,* and water to separate matching test tubes. Compare solutions in diffused daylight, viewing vertically against a black background (see *Visual Comparison* ⟨630⟩).
[NOTE—The diffusion of light must be such that *Reference suspension A* can readily be distinguished from water, and that *Reference suspension B* can readily be distinguished from *Reference suspension A.*]
Acceptance criteria: The *Sample solution* shows the same clarity as that of water, or its opalescence is NMT that of *Reference suspension A.*

Change to read:

• **COLOR OF SOLUTION**
Diluent: 10-g/L solution of hydrochloric acid
Standard stock solution: Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and *Diluent* (3.0: 3.0: 2.4: 1.6)
Standard solution: *Standard stock solution* and *Diluent* (1 in 100). [NOTE—Prepare the ▲*Standard stock solution* and▲ (USP 1-Dec-2019) *Standard solution* immediately before use.]
Sample solution: Use the *Sample solution* from the test for *Clarity of Solution.*
Analysis
Samples: *Standard solution, Sample solution,* and water
Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of the *Standard solution* and water to separate, matching test tubes. Compare the solutions in diffused daylight, viewing vertically against a white background (see *Visual Comparison* ⟨630⟩).
Acceptance criteria: The *Sample solution* has the appearance of water or is not more intensely colored than the *Standard solution.*

ADDITIONAL REQUIREMENTS

Change to read:

• ▲▲ (USP 1-Dec-2019)**PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.▲▲ (USP 1-Dec-2019)

Change to read:

• ▲▲ (USP 1-Dec-2019)**LABELING:** Where the quantity of saccharin sodium is indicated in the labeling of any preparation containing Saccharin Sodium, this shall be expressed in terms of saccharin ($C_7H_5NO_3S$).▲▲ (USP 1-Dec-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Saccharin Sodium RS
USP *o*-Toluenesulfonamide RS
▲▲ (USP 1-Dec-2019)
USP *p*-Toluenesulfonamide RS
▲▲ (USP 1-Dec-2019)

Saccharin Sodium Oral Solution

» Saccharin Sodium Oral Solution contains the equivalent of not less than 95.0 percent and not more than 105.0 percent of the labeled amount of saccharin ($C_7H_5NO_3S$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Saccharin RS

Identification—
A: Transfer a volume of Oral Solution, equivalent to about 100 mg of saccharin, to a small dish, evaporate to dryness, and gently fuse the residue over a small flame until it no longer evolves ammonia. Allow the residue to cool, dissolve in 20 mL of water, neutralize the solution with 3 N hydrochloric acid, and filter: the addition of 1 drop of ferric chloride TS to the filtrate produces a violet color.
B: It responds to the tests for *Sodium* ⟨191⟩.
pH ⟨791⟩: between 3.0 and 5.0.

Assay—
Mobile phase—Mix 5 mL of 10 percent tetramethylammonium hydroxide solution and 400 mL of water in a 500-mL volumetric flask, adjust with phosphoric acid to a pH of 4.0, and mix. Add 50 mL of methanol to the solution, dilute with water to volume, mix, and degas the solution.
Standard preparation—Add to an accurately weighed quantity of USP Saccharin RS an amount of 0.1 N sodium hydroxide sufficient to dissolve the solid, and dilute quantitatively with water to obtain a solution having a known concentration of about 1.2 mg of saccharin per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 120 mg of saccharin, to a 100-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 257-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph three replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph using a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses

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for the major peaks. Calculate the quantity, in mg, of saccharin ($C_7H_5NO_3S$) in each mL of the Oral Solution taken by the formula:

$$(100C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Saccharin RS in the *Standard preparation*, V is the volume, in mL, of Oral Solution taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Saccharin Sodium Tablets

» Saccharin Sodium Tablets contain the equivalent of not less than 95.0 percent and not more than 110.0 percent of the labeled amount of saccharin ($C_7H_5NO_3S$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Saccharin RS

Completeness of solution—Place 5 Tablets in a 250-mL beaker containing 150 mL of water at 25°. Stir for 5 minutes: all of the Tablets dissolve to give a clear or practically clear solution.

Identification—

A: Dissolve a quantity of Tablets, equivalent to about 1 g of saccharin, in 10 mL of water, filter if necessary, and to the solution add 5 mL of 3 N hydrochloric acid: a white precipitate of saccharin is formed. Collect the precipitate on a filter, wash with small portions of cold water until the last washing is practically free from chloride, and dry it at 105° for 2 hours: the saccharin so obtained melts between 226° and 230°, the procedure for *Class I* being used (see *Melting Range or Temperature* ⟨741⟩).

[NOTE—Use the saccharin obtained in *Identification* test *A* for *Identification* tests *B* and *C*.]

B: Dissolve about 100 mg in 5 mL of sodium hydroxide solution (1 in 20), evaporate to dryness, and gently fuse the residue over a small flame until it no longer evolves ammonia. Allow the residue to cool, dissolve in 20 mL of water, neutralize with 3 N hydrochloric acid, and filter: the addition of a drop of ferric chloride TS to the filtrate produces a violet color.

C: Mix 20 mg with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a suitable liquid bath of 200° for 3 minutes. Allow it to cool, and add 10 mL of water and an excess of 1 N sodium hydroxide: a fluorescent green liquid results.

Limit of ammonium salts—Dissolve a quantity of powdered Tablets, equivalent to about 300 mg of saccharin, in 5 mL of water, and warm with 3 mL of 1 N sodium hydroxide: the odor of ammonia is not perceptible.

Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of saccharin, to a 100-mL volumetric flask, add about 50 mL of water, agitate until the Tablet material is dissolved or evenly dispersed, dilute with water to volume, and mix. Transfer 10.0 mL to a separator, add 2 mL of 3 N hydrochloric acid, and extract with five 20-mL portions of a solvent composed of chloroform and alcohol (9:1). Collect the combined extracts in a beaker, and evaporate on a steam bath to dryness with the aid of a current of air. Dissolve the residue in 10 mL of sodium hydroxide solution (1 in 250), transfer the solution with the aid of water to a 200-mL volumetric flask, dilute with water to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Saccharin RS in the same medium having a known concentration of about 50 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 269 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of saccharin ($C_7H_5NO_3S$) in the portion of Tablets taken by the formula:

$$(2C)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Saccharin RS in the Standard solution; and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

Safflower Oil

[8001-23-8].

DEFINITION

Safflower Oil is the refined fixed oil yielded by the seed of *Carthamus tinctorius* L. (Fam. Compositae). A suitable antioxidant may be added.

IDENTIFICATION

- **A. IDENTITY BY FATTY ACID COMPOSITION**

Analysis: Proceed as directed in the test for *Fats and Fixed Oils* ⟨401⟩, *Fatty Acid Composition*.

Acceptance criteria: Meets the composition profile of fatty acids in *Table 1*

- **B. IDENTITY BY TRIGLYCERIDE PROFILE**

Analysis: Proceed as directed in *Identification of Fixed Oils by Thin-Layer Chromatography* ⟨202⟩.

Acceptance criteria: Meets the requirements in the chapter

IMPURITIES

- **ALKALINE IMPURITIES**

Sample: 10 mL of Safflower Oil

Analysis: Mix 10 mL of freshly opened acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. Add the *Sample*, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid VS to change the color of the upper layer to yellow.

Acceptance criteria: NMT 0.1 mL of 0.01 N hydrochloric acid is required.

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Acid Value (Free Fatty Acids)* ⟨401⟩**:** The free fatty acids in 10 g require for neutralization NMT 2.5 mL of 0.020 N sodium hydroxide.
- **FATS AND FIXED OILS,** *Fatty Acid Composition* ⟨401⟩

Standard solution, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Fatty Acid Composition*.

1 N methanolic potassium hydroxide solution: Dissolve 34 g of potassium hydroxide in sufficient methanol to produce 500 mL. Allow to settle for 24 h, and decant the clear solution.

Sample solution: Place 1 g of Safflower Oil in a small conical flask fitted with a reflux attachment. Add 10 mL of methanol and 0.5 mL of *1 N methanolic potassium hydroxide solution*. Reflux the mixture for 10 min, cool, and transfer to a separator with the aid of 15 mL of *n*-heptane. Shake

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with 10 mL of saturated sodium chloride solution, and allow to separate. Transfer the lower layer to another separator, and shake it with 10 mL of *n*-heptane. Wash the combined organic layers with 10 mL of water, dry over anhydrous sodium sulfate, and filter. Use a suitable portion of the filtrate.

Analysis: Inject a suitable volume into the chromatograph, and measure the nine main peak areas of the methyl esters of the fatty acids. The order of elution is myristate, palmitate, stearate, oleate, linoleate, linolenate, arachidate, eicosenoate, and behenate.

Calculate the percentage of myristate, palmitate, stearate, oleate, linoleate, linolenate, arachidate, eicosenoate, or behenate in the portion of Safflower Oil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of myristate, palmitate, stearate, oleate, linoleate, linolenate, arachidate, eicosenoate, or behenate

r_T = total area of the nine peaks

Acceptance criteria: It exhibits the composition profiles of fatty acids shown in *Table 1*.

Table 1

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
14	0	≤1.0
16	0	2–10
18	0	1–10
18	1	8–21
18	2	68–83
18	3	≤1.5
20	0	≤0.5
20	1	≤0.5
22	0	≤1.0

- **FATS AND FIXED OILS,** *Unsaponifiable Matter* ⟨401⟩: NMT 1.5%
- **LIMIT OF PEROXIDE**

Solvent A: Chloroform and glacial acetic acid (40:60)

Potassium iodide solution: Prepare a saturated solution of potassium iodide in freshly boiled and cooled water, and store it protected from light. Discard the solution if it gives a color on the addition of *Solvent A* and starch TS.

Sample: 10 g of Safflower Oil

Analysis: Transfer the *Sample* to a conical flask, add 30 mL of *Solvent A*, and swirl to dissolve. Add 0.5 mL of *Potassium iodide solution*, and swirl the flask for 1 min, accurately timed. Add 30 mL of water, and titrate with 0.01 N sodium thiosulfate VS, with vigorous agitation, to a light yellow color. Add 0.5 mL of starch TS, and continue the titration until the blue color has disappeared. Perform a blank test, and make any necessary correction.

Calculate the peroxide content, in mEq/kg, in the portion of Safflower Oil taken:

$$\text{Result} = [(V \times N)/W] \times F$$

V = volume of sodium thiosulfate required in the titration (mL)

N = normality of the sodium thiosulfate VS

W = weight of Safflower Oil taken (g)

F = conversion factor, 1000 g/kg

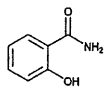
Acceptance criteria: NMT 10.0 mEq/kg

- **WATER DETERMINATION,** *Method Ic* ⟨921⟩: NMT 0.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and prevent exposure to excessive heat.
- **LABELING:** Label it to indicate the name and concentration of any additive.

Salicylamide



$C_7H_7NO_2$ 137.14

Benzamide, 2-hydroxy-;
2-Hydroxybenzamide [65-45-2].

DEFINITION

Salicylamide contains NLT 98.0% and NMT 102.0% of salicylamide ($C_7H_7NO_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% Formic acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	92	8
6.0	55	45
6.5	5	95
8.0	5	95
8.01	92	8
12.0	92	8

Diluent: Acetonitrile and water (50:50)

System suitability solution: 1.0 mg/mL of USP Salicylamide RS and 0.001 mg/mL of USP Salicylic Acid Related Compound B RS in *Diluent*

Standard solution: 0.2 mg/mL of USP Salicylamide RS in *Diluent*

Sample solution: 0.2 mg/mL of Salicylamide in *Diluent*. Pass through a suitable filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 300 nm

Column: 2.1-mm × 10-cm; 1.8-µm packing L1

Column temperature: 35°

Flow rate: 0.3 mL/min

Injection volume: 1 µL

USP Monographs

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System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between salicylic acid related compound B and salicylamide, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylamide ($C_7H_7NO_2$) in the portion of Salicylamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Salicylamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Salicylamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL each of USP Salicylamide RS and USP Salicylic Acid RS in *Diluent*
Sample solution: 1.0 mg/mL of Salicylamide in *Diluent*. Pass through a suitable filter of 0.2-µm pore size.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between salicylic acid related compound B and salicylamide, *System suitability solution*
Relative standard deviation: NMT 2.8%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid in the portion of Salicylamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the salicylic acid peak from the *Sample solution*
r_S = peak response of the salicylic acid peak from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Salicylamide in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Salicylamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of salicylamide from the *Standard solution*
C_S = concentration of USP Salicylamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Salicylamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

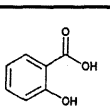
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound B[a]	0.93	—
Salicylamide	1.00	—
Salicylic acid	1.33	0.1
Individual unspecified impurity	—	0.10
Total impurities	—	1

[a] For identification only.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Salicylamide RS
USP Salicylic Acid RS
USP Salicylic Acid Related Compound B RS
4-Hydroxyisophthalic acid.
$C_8H_6O_5$ 182.13

Salicylic Acid



$C_7H_6O_3$ 138.12
Benzoic acid, 2-hydroxy-;
Salicylic acid [69-72-7].

DEFINITION
Salicylic Acid contains NLT 98.0% and NMT 102.0% of salicylic acid ($C_7H_6O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, glacial acetic acid, and water (40:1:60), prepared by adding 10 mL of glacial acetic acid to a solution containing 400 mL of methanol and 600 mL of water
Standard solution: 0.5 mg/mL of USP Salicylic Acid RS in *Mobile phase*. Sonicate to dissolve as needed.
Sample solution: 0.5 mg/mL of Salicylic Acid in *Mobile phase*. Sonicate to dissolve as needed.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Salicylic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Salicylic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.05%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample: 1.5 g
Analysis: Heat the *Sample* with 75 mL of water until it is dissolved. Cool, add water to restore the original volume, and filter.
Acceptance criteria: A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid. (0.014%)

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample solution: 1.0 g in alcohol and water (1:1)
Acceptance criteria: No more sulfate than corresponds to 0.2 mL of 0.020 N sulfuric acid (0.02%)

- **ORGANIC IMPURITIES**

Mobile phase: Prepare as directed in the *Assay*.
Diluent: Methanol, glacial acetic acid, and water (70:4:30)
Standard solution: 0.5 mg/mL of USP Salicylic Acid RS, 0.05 mg/mL of USP Salicylic Acid Related Compound A RS, 0.025 mg/mL of USP Salicylic Acid Related Compound B RS, and 0.01 mg/mL of USP Phenol RS in *Diluent*
Peak identification solution: 0.01 mg/mL of USP Phenol RS in *Diluent*
Sample solution: 50 mg/mL of Salicylic Acid in *Diluent*. Sonicate until completely dissolved.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 2 µL
System suitability
Samples: *Standard solution* and *Peak identification solution*
Suitability requirements
[NOTE—Identify the elution order of phenol by comparing with the *Peak identification solution*.]
Resolution: NLT 2.0 between any two peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of salicylic acid related compound A, salicylic acid related compound B, and phenol in the portion of Salicylic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid related compound A, salicylic acid related compound B, or phenol from the *Sample solution*
r_S = peak response of salicylic acid related compound A, salicylic acid related compound B, or phenol from the *Standard solution*
C_S = concentration of USP Salicylic Acid Related Compound A RS, USP Salicylic Acid Related Compound B RS, or USP Phenol RS in the *Standard solution* (mg/mL)
C_U = concentration of Salicylic Acid in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Salicylic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of salicylic acid related compound B from the *Standard solution*
C_S = concentration of USP Salicylic Acid Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Salicylic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. [NOTE—The elution order for salicylic acid related compound B and phenol may switch.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A	0.35	0.1
Salicylic acid related compound B	0.45	0.05
Phenol	0.50	0.02
Salicylic acid	1.0	—
Any other individual impurity	—	0.05
Total impurities	—	0.2

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over silica gel for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Phenol RS
USP Salicylic Acid RS
USP Salicylic Acid Related Compound A RS
4-Hydroxybenzoic acid.
$C_7H_6O_3$ 138.12

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USP Salicylic Acid Related Compound B RS
4-Hydroxyisophthalic acid.
$C_8H_6O_5$ 182.13

Salicylic Acid Collodion

DEFINITION
Salicylic Acid Collodion contains NLT 9.5% and NMT 11.5% of salicylic acid ($C_7H_6O_3$).
Salicylic Acid Collodion is prepared as follows.

Salicylic Acid	100 g
Flexible Collodion, a sufficient quantity to make	1000 mL

Dissolve the *Salicylic Acid* in about 750 mL of *Flexible Collodion*. Add a sufficient quantity of the *Flexible Collodion* to bring the preparation to final volume, and mix.

ASSAY
• **PROCEDURE**
Sample: 5 mL
Solution A: Ether and alcohol (3:1) neutralized to bromothymol blue TS
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 400-mL beaker, rinse the measuring device with three 10-mL portions of *Solution A*, and add the rinsings to the beaker. Add, with stirring, 5 mL of 100 mg/mL sodium lauryl sulfate solution that was previously neutralized with 0.1 N hydrochloric acid to the distinct yellow color of bromothymol blue TS, and add 20 mL of water. Mix, add 5 drops of bromothymol blue TS, and titrate with the *Titrant*. Each mL of *Titrant* is equivalent to 13.81 mg of salicylic acid ($C_7H_6O_3$).
Acceptance criteria: 9.5%–11.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight containers. Store at controlled room temperature, remote from fire.

Salicylic Acid Topical Foam

» Salicylic Acid Topical Foam contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_7H_6O_3$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Salicylic Acid RS
Identification—The retention time of the salicylic acid peak of the *Assay preparation* observed in the chromatogram obtained in the *Assay* corresponds to the retention time of the salicylic acid peak of the *Standard preparation*.
pH ⟨791⟩: between 5.0 and 6.0.

Assay—
Mobile phase—To 225 mg of tetramethylammonium hydroxide pentahydrate add 700 mL of water, 150 mL of methanol, 150 mL of acetonitrile, and 1.0 mL of glacial acetic acid, mix, filter, and degas.

Internal standard solution—Dissolve benzoic acid in methanol to obtain a solution having a concentration of about 8 mg per mL.

Standard preparation—Transfer about 20 mg of USP Salicylic Acid RS, accurately weighed, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer an accurately weighed portion of Topical Foam, equivalent to about 20 mg of salicylic acid, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. Cool in an ice bath to below room temperature and filter, discarding the first few mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph four replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 3.0%, the resolution factor between salicylic acid and benzoic acid is not less than 3.0, and the tailing factors for the salicylic acid and benzoic acid peaks are not more than 2.0.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention times are about 2.5 minutes for salicylic acid and 5.5 minutes for benzoic acid. Calculate the quantity, in mg, of $C_7H_6O_3$ in the portion of Topical Foam taken by the formula:

$$(100C)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*, and R_U and R_S are the ratios of the peak responses for salicylic acid to the peak responses for benzoic acid obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Salicylic Acid Gel

» Salicylic Acid Gel is Salicylic Acid in a suitable viscous hydrophilic vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_7H_6O_3$. It may contain alcohol.

Packaging and storage—Preserve in collapsible tubes or in tight containers, preferably at controlled room temperature.
Identification—Filter 5 mL of the solution obtained by titration in the *Assay*. Add 1 mL of ferric chloride TS to the filtrate: a violet color is produced. Add 1 mL of 6 N acetic acid: the violet color does not change. Add 1 mL of 6 N hydrochloric acid: the violet color is discharged. A small amount of white precipitate may appear.
Alcohol Determination ⟨611⟩ ***(if present)*:** from 90.0% to 110.0% of the labeled amount of C_2H_5OH.
Assay—To 25 mL of diluted alcohol add 1 drop of phenolphthalein TS and sufficient 0.1 N sodium hydroxide to produce a faint pink color. Add 5.0 g of Gel, accurately weighed, and stir. Titrate the dispersion with 0.1 N sodium hydroxide VS until a pink color is produced. [NOTE—Reserve this solution for the *Identification* test.] Each mL of 0.1 N sodium hydroxide is equivalent to 13.81 mg of $C_7H_6O_3$.

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Salicylic Acid Plaster

DEFINITION

Salicylic Acid Plaster is a uniform mixture of Salicylic Acid in a suitable base, spread on paper, cotton cloth, or other suitable backing material. The plaster mass contains NLT 90.0% and NMT 110.0% of the labeled amount of salicylic acid ($C_7H_6O_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Methanol and 2% (v/v) phosphoric acid in water (1:1)
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	100	0	1.0
5.5	100	0	1.0
5.6	0	100	2.0
7.1	0	100	2.0
7.2	100	0	2.0
9.4	100	0	2.0
9.5	100	0	1.0
10.0	100	0	1.0

Diluent: Tetrahydrofuran and hydrochloric acid (99:1)
Standard solution: 0.2 mg/mL of USP Salicylic Acid RS prepared as follows. Transfer USP Salicylic Acid RS to a suitable volumetric flask, and add *Diluent* equivalent to 12.5% of the final volume to dissolve. Dilute with *Solution A* to volume. Protect from light.
Sample solution: Transfer Plaster (scrape it from the fabric if needed), equivalent to 40 mg of salicylic acid, to a 200-mL volumetric flask, and add 25 mL of *Diluent* to dissolve. Sonicate if necessary to facilitate dissolution. Dilute with *Solution A* to volume. Mix, filter, and discard the first few mL. Protect from light.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 306 nm
Column: 4.6-mm × 15-cm; 5-µm packing L11
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Plaster taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of salicylic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store between 20° and 25°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Salicylic Acid RS

Salmeterol Inhalation Powder

DEFINITION

Salmeterol Inhalation Powder for use in dry powder inhalers contains an amount of salmeterol xinafoate ($C_{25}H_{37}NO_4 \cdot C_{11}H_8O_3$) equivalent to NLT 90% and NMT 110% of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Delivered-Dose Uniformity*.

ASSAY

- **PROCEDURE**

Buffer: 0.15 M ammonium acetate
Mobile phase: Methanol, *Buffer*, and formic acid (65: 35: 0.5)
Diluent: Methanol and water (70:30)
System suitability solution: 11 µg/mL of USP Salmeterol Resolution Check RS in *Diluent*
Standard solution: 9 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*
Sample solution: Nominally 6 µg/mL of salmeterol free base from NLT 12 unit doses in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: 4.6-mm × 20-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 200 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for salmeterol and salmeterol-deoxy are 1.0 and 1.4, respectively.]
Suitability requirements
Tailing factor: NMT 1.5 for the salmeterol peak
Resolution: NLT 2.0 between salmeterol and salmeterol-deoxy
Relative standard deviation: NMT 2% for the salmeterol peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

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C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of salmeterol free base in the *Sample solution* (µg/mL), based on the target emitted dose of 47 µg
M_{r1} = molecular weight of salmeterol free base, 415.57
M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: 90%–110%

PERFORMANCE TESTS

• **PARTICLE SIZE DISTRIBUTION BY CASCADE IMPACTION**

Sampling apparatus: Modified *Apparatus 3* (*Figure 1*) in *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩ with a modified *Induction Port* (*Figure 2*), and *Preseparator Top* (*Figure 3*) are to be used.

Buffer: 0.0025 M sodium dodecylsulfate in 1% (v/v) of glacial acetic acid

Mobile phase: Methanol and *Buffer* (100:5). [NOTE—The ratio of methanol to *Buffer* can be varied up to 100:15.]

System suitability solution: 0.1 µg/mL of USP Salmeterol Related Compound A RS and 1.4 µg/mL of USP Salmeterol Xinafoate RS in methanol

Standard solution: 0.7 µg/mL of USP Salmeterol Xinafoate RS in methanol

Sample solutions: Discharge the 10 unit doses into the cascade impaction sampling apparatus described above. Operate the pump for 3 s at an airflow rate of 60 L/min for each dose discharged. Detach the inhaler, and rinse each piece of the apparatus with methanol into a separate suitable volumetric flask. The final expected concentration of salmeterol should be in the range of 0.1–1 µg/mL. Dilute with methanol to volume. Repeat these steps on one additional sample preparation for a total of two *Sample solutions*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence
Excitation: 225 nm
Emission: 305 nm
Column: 3.0-mm × 5-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.5–1.5 mL/min
Injection volume: 100 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for salmeterol related compound A and salmeterol are 0.67 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between salmeterol and salmeterol related compound A, *System suitability solution*
Relative standard deviation: NMT 2%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solutions*
Calculate the quantity, in µg/unit dose, of salmeterol free base ($C_{25}H_{37}NO_4$) in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/N) \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solutions*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)
V = total volume of the *Sample solutions* (mL)
N = number of unit doses discharged into the apparatus, 10
M_{r1} = molecular weight of salmeterol free base, 415.57
M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: The mean of the two samples for the mass of drug recovered from the cascade impactor component groupings are given in *Table 1*.

Table 1. Amount of Salmeterol in Cascade Impactor Stage Groupings

Apparatus Component	Salmeterol Deposited (µg/dose)
Mouthpiece adaptor, induction port, preseparator, and Stage 0	32–40
Stages 1–5	9.0–14
Stages 3 and 4	4.0–8.0
Stages 6, 7, and filter	NMT 1

• **DELIVERED-DOSE UNIFORMITY** ⟨601⟩

Sampling apparatus: Use the apparatus (*Figure 4A*) with the modified glass sampling device (*Figure 4B*).

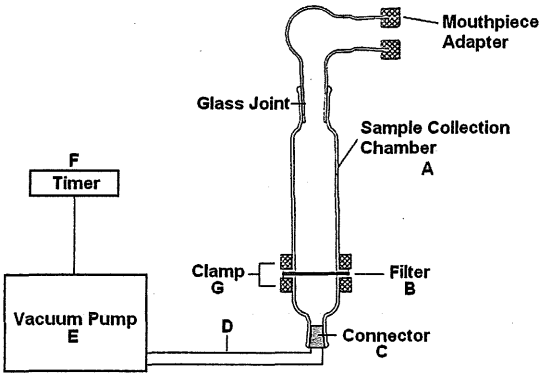


Figure 4A. Sampling apparatus.

Buffer, Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in *Particle Size Distribution by Cascade Impaction*.

System suitability solution: 0.1 µg/mL of USP Salmeterol Related Compound A RS and 0.7 µg/mL of USP Salmeterol Xinafoate RS in methanol

Standard solution: 0.36 µg/mL of USP Salmeterol Xinafoate RS in methanol

Sample solutions: Nominally 0.25 µg/mL of salmeterol free base in methanol prepared as follows. Discharge a single unit dose into the apparatus shown in *Figure 4A*. Operate the pump for 2 s at an airflow of 60 L/min to collect the dose. Detach the inhaler, and rinse the mouthpiece adaptor and each piece of the sample collection chamber with methanol. Place the filter and washings into a container, and sonicate for NMT 5 min. Transfer the contents to a 200 mL volumetric flask, and dilute with methanol to volume. Prepare 9 additional samples from 9 additional unit doses. For multi-dose inhalers, 1 dose must be collected from 10 inhalers with the 10 doses collected across the minimum number of recommended doses on the label of the inhaler.

Analysis
Samples: *Standard solution* and *Sample solutions*

USP Monographs

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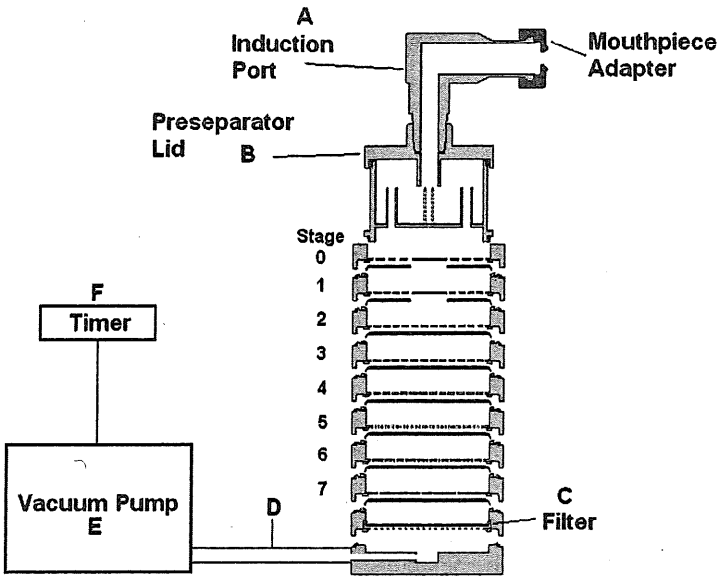


Figure 1. Cascade impaction sampling apparatus (modified *Apparatus 3* in ⟨601⟩) including *Induction Port* and *Preseparator Top*. (Salmeterol Inhalation Powder)

Calculate the percentage of the target emitted dose of salmeterol ($C_{25}H_{37}NO_4$) delivered by the Inhalation Powder in each of the *Sample solutions*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solutions*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Salmeterol xinafoate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of salmeterol free base in the *Sample solutions* (µg/mL) based on target emitted dose of 47 µg
M_{r1} = molecular weight of salmeterol free base, 415.57
M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria

The mean content of salmeterol of 10 doses is 85%–115% of the target emitted dose.
NMT 1 dose is outside 80%–120% of the target emitted dose.
No dose is outside 75%–125% of the target emitted dose.
If the first and second requirements described are not met, test an additional 20 unit doses.
The mean dose of salmeterol of 30 doses is NLT 85% and NMT 115% of the target emitted dose.
NMT 3 doses are outside 80%–120% of the target emitted dose.
No dose is outside 75%–125% of the target emitted dose.

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Diluent: Methanol and water (70:30)
Solution A: Trifluoroacetic acid in water (0.05%, v/v)
Solution B: Trifluoroacetic acid in acetonitrile (0.05%, v/v)
Mobile phase: See *Table 2*.

Table 2

Time	Solution A (%)	Solution B (%)
0	60	40
5	60	40
40	15	85
40.1	60	40
45[a]	60	40

[a] This time may be increased to ensure equilibration.

Protect the *Sample solution* and *Standard solution* from light.
System suitability solution: 0.1 mg/mL of USP Salmeterol System Suitability RS in *Diluent*
Standard solution: 2.5 µg/mL of USP Salmeterol Related Compound H RS in *Diluent*
Sample solution: Nominally 50 µg/mL of salmeterol free base from NLT 10 unit doses prepared as follows. Weigh, and transfer the contents of the appropriate number of unit doses to a suitable volumetric flask. Add 80% of the flask volume of *Diluent*, and sonicate for 5 min. Cool to room temperature, and dilute with *Diluent* to volume. Allow the undissolved material to settle, and inject the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*.
[NOTE—The relative retention times are given in *Table 3*.]
Suitability requirements
Resolution: NLT 2 between salmeterol-*N*-alkyl and salmeterol related compound H, *System suitability solution*

USP Monographs

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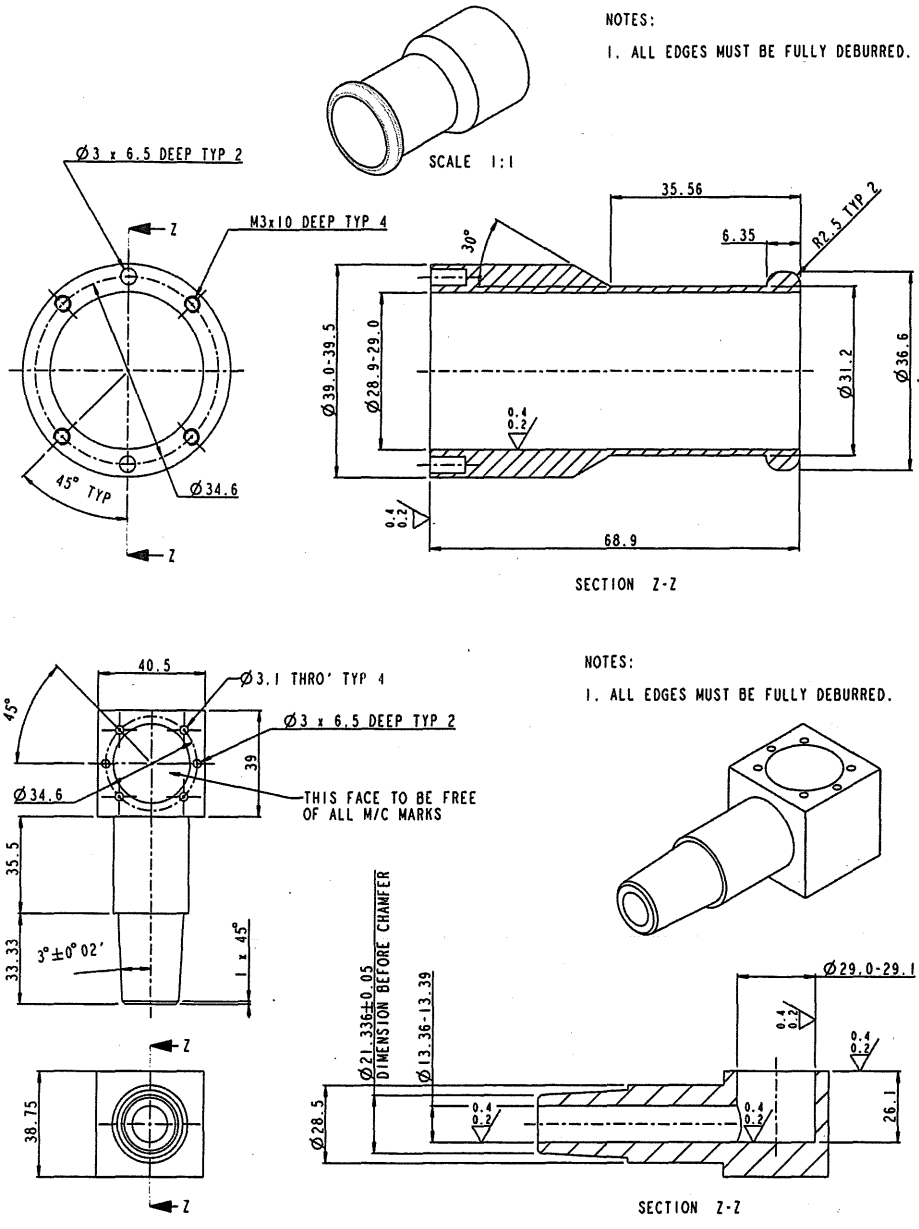


Figure 2. Expanded view of the modified *Induction Port.* (Salmeterol Inhalation Powder)

Relative standard deviation: NMT 2 for salmeterol related compound H, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of salmeterol related compound H in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (W_N/W_U) \times (1/L) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of salmeterol related compound H in the *Standard solution*

C_S = concentration of USP Salmeterol Related Compound H RS in the *Standard solution* (µg/mL)

V = volume of the *Sample solution* (mL)

W_N = theoretical unit dose weight (mg/unit dose)

W_U = weight of Inhalation Powder in the *Sample solution* (mg)

L = label claim of salmeterol free base (µg/unit dose)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salmeterol related compound B[a]	0.59	—
Salmeterol phenylpropoxy[a,b]	0.77	—

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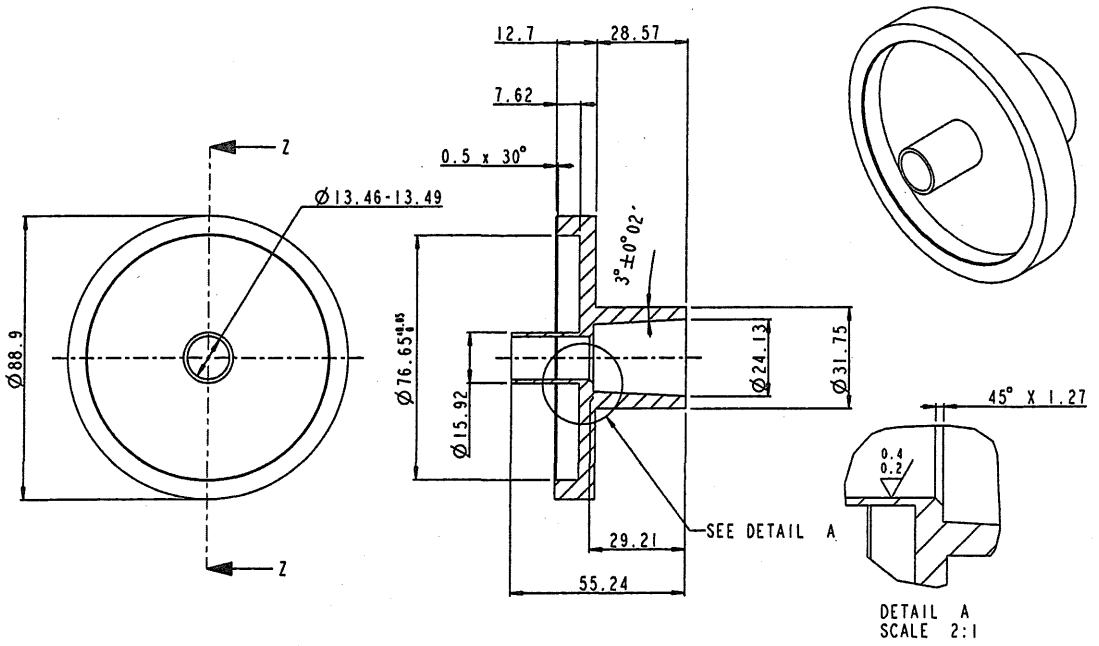


Figure 3. Expanded view of the *Preseparator Top* (Salmeterol Inhalation Powder)

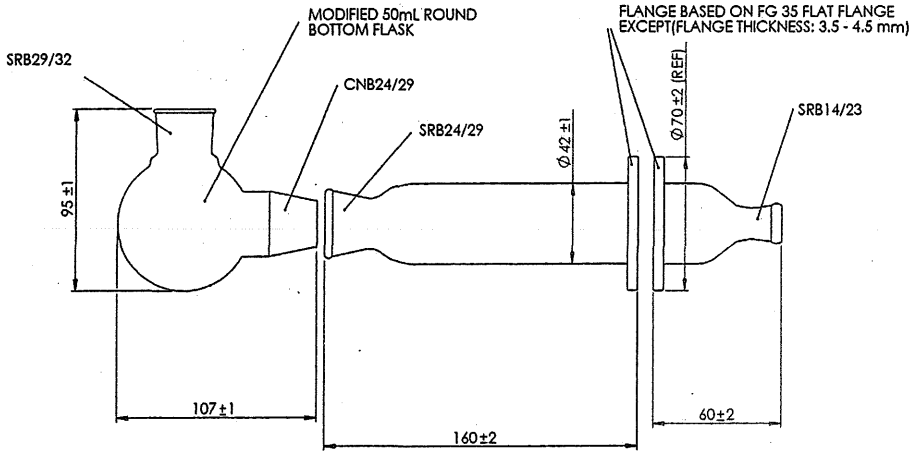


Figure 4B. Expanded view of the modified glass sample collection apparatus. (Salmeterol Inhalation Powder)

Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salmeterol-phenyl-2-butoxy[a, c]	0.93	—
Salmeterol-*O*-alkyl[a, d]	0.97	—
Salmeterol	1.0	—
Salmeterol-deoxy[a, e]	1.52	—
▲Hydroxynaphthoic acid▲ (ERR 1-Jun-2019)	1.63	—
Salmeterol *N*-alkyl[a, f]	2.25	—
Salmeterol related compound H	2.33	1.8
Any unspecified degradation product	—	0.2
Total impurities[g]	—	2.4

[a] Included for identification purposes only. Process impurities controlled in the drug substance and not to be included in the total impurities.
[b] 4-{1-Hydroxy-2-[6-(3-phenylpropoxy)hexylamino]ethyl}-2-(hydroxymethyl) phenol.
[c] 4-{1-Hydroxy-2-[6-(4-phenylbutan-2-yloxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.
[d] 4-{1-Hydroxy-2-[4-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-(hydroxymethyl)phenoxy]ethyl}-2-(hydroxymethyl)phenol.
[e] 4-{1-Hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-methylphenol.
[f] 4-{1-Hydroxy-2-[(2-hydroxy-5-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino] ethyl}benzyl)[6-(4-phenylbutoxy)hexyl]amino]ethyl}-2-(hydroxymethyl) phenol.
[g] Includes all degradation product peaks ≥0.05%.

USP Monographs

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SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella* species, and bile-tolerant Gram-negative bacteria in 1 g of bulk powder. The total aerobic microbial count does not exceed 10^1 cfu/g of bulk powder. The total aerobic yeast and mold count does not exceed 10^1 cfu/g of bulk powder.
- **FOREIGN PARTICULATE MATTER**

Particulate Matter in Injections ⟨788⟩, *Method 2* describes details of the test apparatus to be used for the determination of particulate matter using a microscopic particle count test methodology. Samples should be carefully prepared to avoid environmental contamination, and testing should be performed with suitable controls, including the appropriate use of blank determinations.

Diluent: Methanol and water (65:35), passed through a filter of 0.45-µm pore size

Filter: Mixed cellulose and ester filter; 25-mm diameter and 0.45-µm pore size

Sample solution: Transfer the contents of 4 unit doses from each of 2 inhalers into a suitable receptacle, and dissolve the drug and excipient particles in about 75 mL of *Diluent*.

Analysis

Sample: *Sample solution*

Pass the *Sample solution* through the *Filter*, and allow the *Filter* to dry under conditions that will limit particulate contamination. Using a microscopic particle count test method, enumerate the number of particles present in the sample solution.

Calculate the number of particles per dose:

$$\text{Result} = (N_{<10} + N_{10\text{–}100} + N_{>100})/8$$

$N_{<10}$ = total number of particles <10 µm
$N_{10\text{–}100}$ = total number of particles between 10 and 100 µm
$N_{>100}$ = total number of particles >100 µm

Acceptance criteria: See *Table 4*.

Table 4

Particle Size Range (µm)	Number of Particles Per Dose (NMT)
<10	200
10–100	100
>100	10
Total	300

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature, in a dry place away from direct heat or sunlight.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Salmeterol Related Compound A RS
4-[1-Hydroxy-2-(4-phenylbutylamino)ethyl]-2-(hydroxymethyl)phenol.
$C_{19}H_{25}NO_3$ 315.41

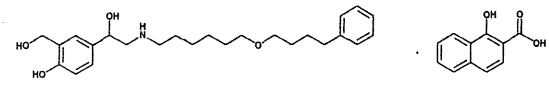
USP Salmeterol Related Compound H RS
1-Hydroxy-4-[2-hydroxy-5-(1-hydroxy-2-{[6-(4-phenylbutoxy)hexyl]amino}ethyl)benzyl]-2-naphthoic acid, monohydrate.
$C_{36}H_{43}NO_6 \cdot H_2O$ 603.76

USP Salmeterol Resolution Check RS
This is a mixture of salmeterol xinafoate, salmeterol related compound H, and salmeterol-deoxy, also known as
4-{1-Hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-methylphenol.

USP Salmeterol System Suitability RS
This is a mixture of salmeterol xinafoate, salmeterol related compound H, and salmeterol-*N*-alkyl, also known as
4-{1-Hydroxy-2-[(2-hydroxy-5-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}benzyl)[6-(4-phenylbutoxy)hexyl]amino]ethyl}-2-(hydroxymethyl)phenol.

USP Salmeterol Xinafoate RS

Salmeterol Xinafoate



$C_{25}H_{37}NO_4 \cdot C_{11}H_8O_3$ 603.75

1,3-Benzenedimethanol, 4-hydroxy-α¹-[[[6-(4-phenylbut oxy) hexyl]amino]methyl]-, (±)-, 1-hydroxy-2-naphthalenecarboxylate (salt);
(±)-4-Hydroxy-α¹-[[[6-(4-phenylbutoxy)hexyl]amino]methyl]-*m*-xylene-α,α′-diol 1-hydroxy-2-naphthoate (salt) [94749-08-3].

DEFINITION

Salmeterol Xinafoate contains NLT 98.0% and NMT 102.0% of salmeterol xinafoate ($C_{25}H_{37}NO_4 \cdot C_{11}H_8O_3$), calculated on the water- and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—It is recommended that solutions containing salmeterol be protected from light.]

Buffer A: 0.1 M sodium dodecyl sulfate

Buffer B: 0.1 M ammonium acetate

Mobile phase: Acetonitrile, *Buffer A*, and *Buffer B* (52:24:24). Adjust with glacial acetic acid to a pH of 3.8. [NOTE—This may need as much as 75 mL of glacial acetic acid for each liter.]

System suitability solution: 0.25 mg/mL of USP Salmeterol Xinafoate RS and 0.02 mg/mL of USP Salmeterol Related Compound B RS in *Mobile phase*

Standard solution: 0.25 mg/mL of USP Salmeterol Xinafoate RS in *Mobile phase*

Sample solution: 0.25 mg/mL of Salmeterol Xinafoate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

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[NOTE—The relative retention times for salmeterol related compound B and salmeterol are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between salmeterol and salmeterol related compound B

Relative standard deviation: NMT 1.0% for salmeterol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of salmeterol xinafoate ($C_{25}H_{37}NO_4 \cdot C_{11}H_8O_3$) in the portion of Salmeterol Xinafoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (mg/mL)

C_U = concentration of Salmeterol Xinafoate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the water- and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

[NOTE—It is recommended that solutions containing salmeterol be protected from light.]

Buffer A, Buffer B, and **Chromatographic system:** Proceed as directed in the *Assay*.

Diluent: Acetonitrile and water (50:50)

Solution A: Acetonitrile, *Buffer A*, and *Buffer B* (52:24:24). Adjust with glacial acetic acid to a pH of 3.8.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
16.0	100	0
36.0	30	70
45.0	30	70
45.1	100	0
50[a]	100[a]	0[a]

[a] The required equilibration time may be modified to achieve a stable baseline.

System suitability solution: 5.0 mg/mL of USP Salmeterol Xinafoate RS and 0.3 mg/mL each of USP Salmeterol Related Compound A RS and USP Salmeterol Related Compound B RS in *Diluent*

Sample solution: 5.0 mg/mL of Salmeterol Xinafoate in *Diluent*

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.0 between salmeterol and salmeterol related compound B

Tailing factor: NMT 2.5 for salmeterol

Analysis

[NOTE—Disregard the peak due to hydroxynaphthoic acid and any peaks from blank injections.]

Sample: *Sample solution*

Calculate the percentage of any individual impurity in the portion of Salmeterol Xinafoate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. [NOTE—Calculate the total impurities from the sum of all impurity peaks greater than or equal to 0.05%.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydroxynaphthoic acid[a]	0.2	—
Salmeterol related compound A	0.3	0.2
Salmeterol-phenylethoxy[b]	0.5	0.1
Salmeterol-phenylpropoxy[c]	0.7	0.1
Salmeterol-*O*-alkyl[d]	0.8	0.3
Salmeterol related compound B	0.9	0.1
Salmeterol	1.0	—
Salmeterol-deoxy[e]	1.6	0.2
Salmeterol-*N*-alkyl[f]	2.7	0.2
Any unspecified impurity	—	0.10
Total impurities	—	0.9

[a] 1-Hydroxy-naphthalene-2-carboxylic acid. This is the counter ion of salmeterol and is included for identification only.

[b] 4-[1-Hydroxy-2-(6-phenethoxyhexylamino)ethyl]-2-(hydroxymethyl)phenol.

[c] 4-{1-Hydroxy-2-[6-(3-phenylpropoxy)hexylamino]ethyl}-2-(hydroxymethyl) phenol.

[d] 4-{1-Hydroxy-2-[4-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-(hydroxymethyl)phenoxy]ethyl}-2-(hydroxymethyl)phenol.

[e] 4-{1-Hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-methylphenol.

[f] 4-{1-Hydroxy-2-[(2-hydroxy-5-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino] ethyl}benzyl)[6-(4-phenylbutoxy)hexyl]amino]ethyl}-2-(hydroxymethyl) phenol.

SPECIFIC TESTS

- **WATER DETERMINATION ⟨921⟩, *Method I***

Sample: 0.5 g

Acceptance criteria: NMT 0.25%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at a temperature not exceeding 30°.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Salmeterol Xinafoate RS

USP Salmeterol Related Compound A RS

4-[1-Hydroxy-2-(4-phenylbutylamino)ethyl]-2-(hydroxymethyl)phenol.

$C_{19}H_{25}NO_3$ 315.41

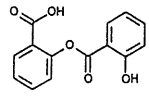
USP Salmeterol Related Compound B RS

4-{1-Hydroxy-2-[6-(4-phenylbutan-2-yloxy)hexylamino] ethyl}-2-(hydroxymethyl)phenol.

$C_{25}H_{37}NO_4$ 415.57

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Salsalate



$C_{14}H_{10}O_5$ 258.23
Benzoic acid, 2-hydroxy-, 2-carboxyphenyl ester.
Disalicylic acid.
Salicylsalicylic acid.
Salicylic acid, bimolecular ester [552-94-3].

» Salsalate contains not less than 98.0 percent and not more than 102.0 percent of total salicylates, expressed as the sum of the percentages of salsalate, salicylic acid, and trisalicylic acid, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Salsalate RS
USP Salicylic Acid RS
USP Trisalicylic Acid RS
$C_{21}H_{14}O_7$ 378.34

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: ***197M***▲ (CN 1-May-2020)
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.10%.
Chloride ⟨221⟩—Dissolve 1.4 g in 6 mL of methanol, warming if necessary to effect solution. Dilute with water to 50 mL to precipitate the salsalate, allow to stand for 5 minutes, and filter. A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.20 mL of 0.010 N hydrochloric acid (0.01%).
Sulfate ⟨221⟩—A 17-mL portion of the filtrate prepared for the test for *Chloride* shows no more sulfate than corresponds to 0.50 mL of 0.010 N sulfuric acid (0.05%).

Limit of dimethylaniline—
Internal standard solution—Prepare a solution in methylene chloride containing 0.4 mg of indene per mL.
Standard preparation—Transfer about 50 mg of *N,N*-dimethylaniline, accurately weighed, to a 100-mL volumetric flask, dilute with *Internal standard solution* to volume, insert the stopper securely, and mix.
Test preparation—Transfer about 5 g of Salsalate, accurately weighed, to a 125-mL separator fitted with a cotton pledget in its stem. Add 50 mL of water and 6 mL of 6 N ammonium hydroxide, and swirl until dissolved. Add 5.0 mL of *Internal standard solution*, insert the stopper into the separator, and shake for 1 minute. Keep the separator stoppered, and allow the layers to separate. Loosen the stopper, and drain most of the lower phase into a screw-capped test tube. Use this solution as the *Test preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a split injector with a 10:1 split ratio, and a 30-m × 0.53-mm capillary column, the internal wall of which is coated with a 1.0-µm film of phase G42. Maintain the column at 105°, the injector at 250°, and the detector block at 250°, and use helium as the carrier gas, at a flow rate of about 13 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.75 for indene and 1.0 for *N,N*-dimethylaniline, the resolution, *R*, between the indene peak and the *N,N*-dimethylaniline peak is not less than 2.0, and the relative standard deviation for replicate injections is not more than 3%.
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Inject equal volumes (about 1 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Indene elutes before *N,N*-dimethylaniline. Calculate the percentage of *N,N*-dimethylaniline in the portion of Salsalate taken by the formula:

$$0.5(C/W)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of *N,N*-dimethylaniline in the *Standard preparation*, *W* is the weight, in g, of Salsalate taken to prepare the *Test preparation*, and R_U and R_S are the ratios of the response of the *N,N*-dimethylaniline peak to that of the indene peak obtained from the *Test preparation* and the *Standard preparation*, respectively. The limit is 0.05%.

Isopropyl, ethyl, and methyl salicylates—
Standard stock solution—Prepare a solution in chromatographic *n*-heptane containing 0.20 mg of isopropyl salicylate, 0.50 mg of ethyl salicylate, and 0.50 mg of methyl salicylate per mL.
Standard preparation—Transfer to a suitable screw-capped test tube 2.0 g of Salsalate, add 10 mL of 1 N sodium hydroxide and 2 mL of chromatographic *n*-heptane, shake until dissolved, and allow the layers to separate. Draw off and discard all of the upper layer. To the lower layer add 2.0 mL of *Standard stock solution*, shake for 1 minute, and allow the layers to separate. Use the upper layer as the *Standard preparation*.
Test preparation—Transfer 2.0 g of Salsalate to a suitable screw-capped test tube, add 10 mL of 1 N sodium hydroxide and 2.0 mL of chromatographic *n*-heptane, shake until dissolved, and allow the layers to separate. Use the upper layer as the *Test preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a split injector with a 10:1 split ratio, and a 30-m × 0.53-mm capillary column, the internal wall of which is coated with a 1.0-µm film of phase G42. Maintain the column at 120° and the injector and detector block at about 250°. Helium is used as the carrier gas, flowing at the rate of about 13 mL per minute.
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Inject equal volumes (about 1 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.65 for methyl salicylate, 0.9 for ethyl salicylate, and 1.0 for isopropyl salicylate. The response of any isopropyl salicylate peak obtained from the *Test preparation* is not greater than that obtained from the *Standard preparation* (0.02%), the response of any ethyl salicylate peak obtained from the *Test preparation* is not greater than that obtained from the *Standard preparation* (0.05%), and the response of any methyl salicylate peak obtained from the *Test preparation* is not greater than that obtained from the *Standard preparation* (0.05%).
Chromatographic purity—Using the chromatograms obtained in the *Assay*, calculate the percentage of each impurity, other than salicylic acid and trisalicylic acid, in the portion of Salsalate taken by the formula:

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$$10{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salsalate RS in the *Salsalate standard preparation*, W is the weight, in mg, of Salsalate taken to prepare the *Assay stock solution*, r_U is the response of the particular impurity peak obtained from the *Assay stock solution*, and r_S is the salsalate peak response obtained from the *Salsalate standard preparation:* not more than 0.2% of each other impurity is found.

Related compounds—The percentages of salicylic acid and trisalicylic acid, determined as directed in the *Assay*, do not exceed 0.5% and 2.5%, respectively.

Assay—

Mobile phase—Prepare a suitable filtered and degassed mixture of methanol, water, and phosphoric acid (650:350:1), and adjust with phosphoric acid or 1 N sodium hydroxide, if necessary, to a pH of 3.1. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of water, acetonitrile, and phosphoric acid (540:460:1).

Salsalate standard preparation—Dissolve an accurately weighed quantity of USP Salsalate RS in *Diluent* to obtain a stock solution having a known concentration of about 1 mg per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.02 mg per mL.

Salicylic acid standard preparation—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Diluent* to obtain a stock solution having a known concentration of about 0.5 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.005 mg of USP Salicylic Acid RS per mL.

Trisalicylic acid standard preparation—Dissolve an accurately weighed quantity of USP Trisalicylic Acid RS in *Diluent* to obtain a stock solution having a known concentration of about 0.5 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.025 mg of USP Trisalicylic Acid RS per mL.

Resolution solution—Prepare a solution in *Diluent* containing about 0.02 mg of USP Salsalate RS per mL, 0.02 mg of USP Salicylic Acid RS per mL, and 0.04 mg of USP Trisalicylic Acid RS per mL.

Assay stock solution—Transfer about 100 mg of Salsalate, accurately weighed, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Sonicate if necessary to effect the solution.

Assay preparation—Transfer 2.0 mL of the *Assay stock solution* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 236-nm detector and a 4-mm × 15-cm column that contains 5-µm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.55 for salicylic acid, 1.0 for salsalate, and 1.5 for trisalicylic acid, and the resolution, R, between the salicylic acid and salsalate peaks and between the salsalate and trisalicylic acid peaks is not less than 2.0. Chromatograph the *Salicylic acid standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation of the salicylic acid peak responses for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Salsalate standard preparation*, the *Salicylic acid standard preparation*, the *Trisalicylic acid standard preparation*, the *Assay stock solution*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—Continue chromatography after each injection for a period of time not less than the retention time of trisalicylic acid.] Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Salsalate taken by the formula:

$$10{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Salicylic acid standard preparation*, W is the weight, in mg, of the portion of Salsalate taken, and r_U and r_S are the responses of the salicylic acid peak obtained from the *Assay stock solution* and the *Salicylic acid standard preparation*, respectively. Calculate the percentage of trisalicylic acid ($C_{21}H_{14}O_7$) in the portion of Salsalate taken by the formula:

$$10{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Trisalicylic Acid RS in the *Trisalicylic acid standard preparation*, and r_U and r_S are the responses of the trisalicylic acid peaks obtained from the *Assay stock solution* and the *Trisalicylic acid standard preparation*, respectively. Calculate the percentage of salsalate ($C_{14}H_{10}O_5$) in the portion of Salsalate taken by the formula:

$$500{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salsalate RS in the *Salsalate standard preparation*, and r_U and r_S are the salsalate peak responses obtained from the *Assay preparation* and the *Salsalate standard preparation*, respectively.

Salsalate Capsules

» Salsalate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{14}H_{10}O_5$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Salsalate RS
USP Salicylic Acid RS
USP Trisalicylic Acid RS
$C_{21}H_{14}O_7$ 378.34

Identification—Transfer a quantity of Capsule contents, equivalent to about 500 mg of salsalate, to a stoppered glass test tube. Add 20 mL of ether to the tube, close the tube tightly, shake by mechanical means for 10 minutes, and filter. Evaporate the filtrate to dryness using a stream of nitrogen: the IR absorption spectrum of a mineral oil dispersion of the residue thus obtained, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Salsalate RS.

Disintegration ⟨701⟩: 30 minutes, simulated gastric fluid TS (without pepsin) being used.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Limit of salicylic acid—

Mobile phase, Diluent, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard preparation—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Diluent* to obtain a stock

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solution having a known concentration of about 0.5 mg per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.015 mg per mL.

Test preparation—Use the *Assay stock solution* prepared as directed in the *Assay*.

Procedure—Proceed as directed for *Procedure* in the *Assay*, except to inject equal volumes (about 10 µL) of the *Standard preparation* and the *Test preparation*. Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Capsules taken by the formula:

$$10{,}000(C/O_T)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*, O_T is the quantity, in mg, of salsalate in the portion of Capsules taken based on the labeled amount, and r_U and r_S are the salicylic acid peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 1.5% is found.

Assay—

Mobile phase , *Diluent*, *Salsalate standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Salsalate*.

Assay preparation—Transfer as completely as possible the contents of not less than 20 Capsules to a suitable tared container, and weigh. Mix, and transfer an accurately weighed portion of the powder, equivalent to about 100 mg of salsalate, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Sonicate for about 10 minutes, and mix. Filter a portion of this solution through a suitable filter of 0.5 µm or finer porosity. Use the clear filtrate as the *Assay stock solution*. Transfer 2.0 mL of the *Assay stock solution* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix (*Assay preparation*).

Procedure—Separately inject equal volumes (about 10 µL) of the *Salsalate standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the salsalate peaks. Calculate the quantity, in mg, of salsalate ($C_{14}H_{10}O_5$) in the portion of Capsules taken by the formula:

$$5000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salsalate RS in the *Salsalate standard preparation*, and r_U and r_S are the responses of the salsalate peaks obtained from the *Assay preparation* and the *Salsalate standard preparation*, respectively.

Salsalate Tablets

» Salsalate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{14}H_{10}O_5$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Salsalate RS

USP Salicylic Acid RS

Identification—Transfer a quantity of finely powdered Tablets, equivalent to about 500 mg of salsalate, to a stoppered glass test tube. Add 20 mL of ether to the tube, close the tube tightly, shake by mechanical means for 10 minutes, and filter. Evaporate the filtrate to dryness using a stream of nitrogen: the IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Salsalate RS.

Dissolution ⟨711⟩—

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

Medium: 0.25 M pH 7.4 phosphate buffer, prepared by mixing 5.175 g of monobasic sodium phosphate and 30.17 g of anhydrous dibasic sodium phosphate with water to obtain 1000 mL of solution, and adjusting by the dropwise addition of 50% sodium hydroxide solution to a pH of 7.40 ± 0.05; 900 mL.

Apparatus 2: 50 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{14}H_{10}O_5$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 308 nm of filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Salsalate RS in the same *Medium*.

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{14}H_{10}O_5$ is dissolved in 60 minutes.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.05 M, pH 7.5 phosphate buffer; prepared by mixing 40.83 g of monobasic potassium phosphate and 120 mL of 2 N sodium hydroxide with water to obtain 6 L of solution, and adjusting by the dropwise addition of 2 N sodium hydroxide or phosphoric acid to a pH of 7.50 ± 0.05; 900 mL.

Apparatus 2: 100 rpm.

Time and *Procedure*—Proceed as directed for *Test 1*.

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{14}H_{10}O_5$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Limit of salicylic acid—

Mobile phase, *Diluent*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Diluent* to obtain a stock solution having a known concentration of about 0.5 mg per mL. Transfer 3.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.03 mg per mL.

Test solution—Use the *Assay stock preparation* prepared as directed in the *Assay*.

Procedure—Proceed as directed for *Procedure* in the *Assay*, except to inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution*. Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Tablets taken by the formula:

$$10{,}000(C/O_T)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*; O_T is the quantity, in mg, of salsalate in the portion of Tablets taken based on the labeled amount; and r_U and r_S are the salicylic acid peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.

Assay—

Mobile phase, *Diluent*, *Salsalate standard preparation*, *Salicylic acid standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Salsalate*.

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Assay stock preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of salsalate, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Sonicate for about 10 minutes, and mix. Pass a portion of this solution through a suitable filter of 0.5-µm or finer porosity. Use the clear filtrate.

Assay preparation— Transfer 2.0 mL of the *Assay stock preparation* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Salsalate*. Calculate the quantity, in mg, of $C_{14}H_{10}O_5$ in the portion of Tablets taken by the formula:

$$5000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salsalate RS in the *Salsalate standard preparation;* and r_U and r_S are the responses of the salsalate peaks obtained from the *Assay preparation* and the *Salsalate standard preparation,* respectively.

Samarium Sm 153 Lexidronam Injection

» Samarium Sm 153 Lexidronam Injection is a sterile aqueous solution suitable for intravenous injection that contains ^{153}Sm in the form of a complex with ethylenediaminetetramethyl enephosphonic acid (EDTMP). It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{153}Sm expressed in megabecquerels per mL (or in millicuries per mL) at the date and time indicated in the labeling. Not less than 99 percent of the Sm-153 is complexed by EDTMP. It contains no antimicrobial agents.

Packaging and storage—Preserve in adequately shielded single-dose containers in a freezer.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{153}Sm complexed with EDTMP expressed as total megabecquerels (or millicuries) and the concentration as megabecquerels per mL (or millicuries per mL) at the time of calibration; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{153}Sm is 46.3 hours. The labeling indicates that it should not be diluted or mixed with other solutions, that it is to be thawed at room temperature before administration, and that it is to be used within 8 hours of thawing.

Radionuclide identification ⟨821⟩—Samarium-153 decays by beta emission to stable Europium 153 as follows: 640 keV (30%), 710 keV (50%), and 810 keV (20%). The average emission energy is 233 keV. Its gamma-ray spectrum is identical to that of a specimen of known purity of ^{153}Sm that exhibits major photopeaks having energies of 70 keV and 103 keV.

Bacterial Endotoxins Test ⟨85⟩—The limit of endotoxin content is not more than 175/V USP Endotoxin Unit per mL of Injection, in which V is the maximum recommended total dose, in mL, at the expiration time.

pH ⟨791⟩: between 7.0 and 8.5.

Radionuclidic purity ⟨821⟩—Using a gamma ray spectrophotometer, determine the radionuclidic purity of the Injection: not less than 99.8% of the total radioactivity is present as Sm 153 at the time of expiry. The Europium 154 radioactivity is not more than 3.44 kBq per 37 MBq of Samarium 153 (or 0.0093% of total Samarium 153 at expiry). The sum of all other radionuclidic impurities is less than or equal to 0.1907% of the total Sm 153 at expiry.

Radiochemical purity ⟨821⟩—

Mobile phase—Transfer 8.0 g of sodium chloride, 0.2 g of monobasic potassium phosphate, 1.15 g of dibasic sodium phosphate, and 0.2 g of potassium chloride to a 1-liter volumetric flask, dilute with distilled water to volume, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatographic system (see *Chromatography* ⟨621⟩)—A 10-mm × 40-mm glass chromatographic column is packed using gravity flow with a strong cation-exchange resin* (prepared by mixing 5 g of resin with 25 mL of water in a suitable beaker) to a final resin volume of 0.5 mL.

Procedure—Transfer the packed column to an ion-chamber dose counter to determine the background count for Sm 153. Apply about 10 µL of the Injection onto the column and place it in the ion-chamber counter, and record the total radioactivity. Elute the complexed radioactivity using about 20 mL of *Mobile phase,* and record the radioactivity retained on the column. Subtract the background radioactivity from all measured radioactivity values. Calculate the percentage of complexed radioactivity in the portion of Injection taken by the formula:

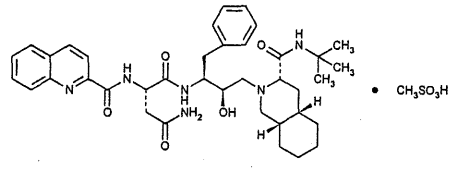
$$100(T - S/0.95)/T$$

in which T is the total amount of radioactivity; S is the quantity of free Sm-153 retained on the column; and 0.95 is the correction factor (5% of the uncomplexed Sm 153 passes through the column and into the Injection): not less than 99% of the Sm-153 is complexed by EDTMP.

Other requirements—Meets the requirements of *Injections and Implanted Drug Products* ⟨1⟩; not subject to *Container content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity in MBq (or mCi) per mL of Injection by use of a calibrated system: the activity is within ±10% of the labeled amount at the time of calibration.

Saquinavir Mesylate



$C_{38}H_{50}N_6O_5 \cdot CH_4O_3S$ 766.95

* Sephadex-CM C-25 brand of ion exchange resin is available from Aldrich Chemical (No. 27, 124-1).

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Butanediamide, N^1-[3-[3-[[(1,1-dimethylethyl)amino]carbonyl]octahydro-2(1*H*)-isoquinolinyl]-2-hydroxy-1-(phenylmethyl)propyl]-2-[(2-quinolinylcarbonyl)amino]-, [3*S*-[2[1*R**(*R**),2*S**],3α,4aβ,8aβ]]-, monomethanesulfonate (salt).
(*S*)-*N*-[(α*S*)-α-[(1*R*)-2-[(3*S*,4a*S*,8a*S*)-3-(*tert*-Butylcarbamoyl)octahydro-2(1*H*)-isoquinolyl]-1-hydroxyethyl]phenethyl]-2-quinaldamidosuccinamide monomethanesulfonate (salt) [149845-06-7].

» Saquinavir Mesylate contains not less than 98.5 percent and not more than 101.0 percent of $C_{38}H_{50}N_6O_5 \cdot CH_4O_3S$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Saquinavir Mesylate RS
USP Saquinavir Related Compound A RS
N-*tert*-Butyl-decahydro-2-[2(*R*)-hydroxy-4-phenyl-3(*S*)-[[*N*-(2-quinolylcarbonyl)-D-asparaginyl]amino]butyl]-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide.
$C_{38}H_{50}N_6O_5$ 670.84

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 12 µg per mL.
Medium: methanol.
The absorptivity of the sample preparation at 238 ± 2 nm, calculated on the anhydrous basis, is between 61.0 and 63.4.
C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Specific rotation ⟨781S⟩**:** between −66.8° and −69.6° (λ = 436 nm at 20°).
Test solution: 5 mg per mL, in methanol.
Water Determination, *Method I* ⟨921⟩**:** not more than 1.0%.
Residue on ignition ⟨281⟩**:** not more than 0.1%.

Chromatographic purity—
Triethylamine phosphate solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Use the *Standard preparation,* prepared as directed in the *Assay.*
Test solution—Use the *Assay preparation.*
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Saquinavir Mesylate taken by the formula:

$$100F(C_S/C_U)(r_i/r_S)$$

in which F is a response factor and is equal to 2 for peaks, if present, at a retention time of 0.32 relative to saquinavir, to 0.5 for peaks, if present, at retention times of about 0.38 and 0.53 relative to saquinavir, and to 1 for all other peaks; C_S is the concentration, in mg per mL, of USP Saquinavir Mesylate RS in the Standard solution; C_U is the concentration, in mg per mL, of Saquinavir Mesylate in the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for saquinavir obtained from the *Standard solution:* not more than 0.1% of any individual impurity is found; and not more than 0.5% of total impurities is found.

Assay—
Triethylamine phosphate solution—Transfer 10 mL of triethylamine to a 1-liter volumetric flask, dilute with water to volume, and mix. Adjust with phosphoric acid to a pH of 2.5, and filter.
Mobile phase—Prepare a filtered and degassed mixture of *Triethylamine phosphate solution,* tetrahydrofuran, and acetonitrile (14:5:1). [NOTE—Protect from light.] Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve suitable quantities of USP Saquinavir Related Compound A RS and USP Saquinavir Mesylate RS in *Mobile phase* to obtain a solution containing about 2 µg per mL and 0.25 mg per mL, respectively.
Standard preparation—Dissolve an accurately weighed quantity of USP Saquinavir Mesylate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.25 mg per mL.
Assay preparation—Transfer about 12.5 mg of Saquinavir Mesylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix for about 20 minutes.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 20°, and the flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.89 for saquinavir related compound A and 1.0 for saquinavir; and the resolution, *R,* between saquinavir related compound A and saquinavir is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 500 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{38}H_{50}N_6O_5 \cdot CH_4O_3S$ in the portion of Saquinavir Mesylate taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Saquinavir Mesylate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Saquinavir Capsules

» Saquinavir Capsules contain not less than 95.0 percent and not more than 105.0 percent of saquinavir ($C_{38}H_{50}N_6O_5$).

USP Monographs

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Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Saquinavir Mesylate RS
USP Saquinavir Related Compound A RS
N-tert-Butyl-decahydro-2-[2(*R*)-hydroxy-4-phenyl-3(*S*)-[[*N*-(2-quinolylcarbonyl)-D-asparaginyl]amino]butyl]-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide.
$C_{38}H_{50}N_6O_5$ 670.84

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Citrate buffer—Transfer 5.82 g of anhydrous dibasic sodium phosphate and 16.7 g of citric acid monohydrate to a 1-L volumetric flask. Dissolve in and dilute with water to volume.
Medium: Citrate buffer; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{38}H_{50}N_6O_5$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 240 nm on filtered portions of the solution under test, suitably diluted with *Medium,* in comparison with a Standard solution having a known concentration of USP Saquinavir Mesylate RS in the same *Medium.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{38}H_{50}N_6O_5$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Water Determination, *Method I* ⟨921⟩: not more than 3.0%.

Chromatographic purity—
Triethylamine phosphate solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Saquinavir Mesylate.*
Standard solution—Use the Standard preparation, prepared as directed in the *Assay* under *Saquinavir Mesylate.*
Test solution—Use the *Assay preparation.*
Procedure—Separately inject equal volumes (about 20 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Capsules taken by the formula:

$$10{,}000F(670.86/766.96)(C/W)(r_i/r_S)$$

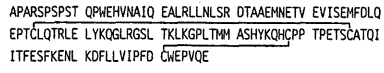
in which *F* is a response factor, and is equal to 2 for a peak, if present, at a retention time of 0.32 relative to saquinavir, to 0.5 for peaks, if present, at relative retention times of about 0.38 and 0.53, and to 1 for all other peaks; 670.86 and 766.96 are the molecular weights of saquinavir and saquinavir mesylate, respectively; *C* is the concentration, in mg per mL, of USP Saquinavir Mesylate RS in the *Standard solution; W* is the weight, in mg, of Capsule contents taken for the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for saquinavir obtained from the *Standard solution:* not more than 0.2% of any individual impurity is found, and not more than 1.0% of total impurities is found.

Assay—
Triethylamine phosphate solution,Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Saquinavir Mesylate.*
Assay preparation—Empty and combine the contents of not fewer than 10 Capsules. Transfer an amount of Capsule contents, equivalent to about 22 mg of Saquinavir, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of saquinavir ($C_{38}H_{50}N_6O_5$) in the portion of Capsules taken by the formula:

$$(670.86/766.96)(100C)(r_U/r_S)$$

in which 670.86 and 766.96 are the molecular weights of saquinavir and saquinavir mesylate, respectively; *C* is the concentration, in mg per mL, of USP Saquinavir Mesylate RS in the *Standard preparation;* and r_U and r_S are the peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Sargramostim



$C_{639}H_{1002}N_{168}O_{196}S_8$ (protein moiety) 14,414
[123774-72-1].

DEFINITION
Sargramostim is a single-chain, glycosylated polypeptide of 127 amino acid residues expressed from *Saccharomyces cerevisiae.* The glycoprotein primarily consists of three molecular species having relative molecular weights of approximately 19,500; 16,800; and 15,500 due to different levels of glycosylation. Sargramostim has the property of generating granulocyte, macrophage, and mixed granulocyte macrophage colonies from hematopoietic progenitor cells found in bone marrow. It possesses the primary sequence of the natural form of granulocyte-macrophage colony-stimulating factor with a substitution in the amino acid residue at position 23 (Leu_{23} in place of Arg_{23}). It has a biological potency of NLT 73.0% and NMT 146.0% of the potency stated on the label, the potency being 5.6 million USP Sargramostim Units/mg of protein. The presence of host cell DNA and host cell protein impurities in Sargramostim is process-specific; the limits of these impurities are determined by validated methods.

IDENTIFICATION
- **A.** The retention times of the peaks of the *Sample solution* do not differ by more than 0.5 min from those of the *Standard solution,* as obtained in the test for *Chromatographic Purity.*
- **B. PEPTIDE MAPPING**

Solution A: Trifluoroacetic acid and water (1:1000), filtered and degassed
Solution B: Trifluoroacetic acid and acetonitrile (1:1000), filtered and degassed
Mobile phase: See *Table 1.*

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	65	35
50	35	65

Digestion solution: Dissolve 29.4 mg of calcium chloride and 1.8 mg of β-alanine in 2 mL of water. Adjust with hydrochloric acid to a pH of 4.0. Add 0.4 mg of trypsin, and mix.

Standard solution: 500 µg/mL of USP Sargramostim RS in water. Transfer 100 µL of this solution to a clean test tube, and add 11 µL of pH 7.6 buffer solution (see *Reagents, Indicators, and Solutions—Buffer Solutions*) containing 0.1 M tris(hydroxymethyl)aminomethane. Add 25 µL of *Digestion solution*, and incubate at 37° for 2 h. Quench the reaction by adding 3 µL of 20% trifluoroacetic acid.

Sample solution: 500 µg/mL of Sargramostim in water. Transfer 100 µL of this solution to a clean test tube, and add 11 µL of pH 7.6 buffer solution (see *Reagents, Indicators, and Solutions—Buffer Solutions*) containing 0.1 M tris(hydroxymethyl)aminomethane. Add 25 µL of *Digestion solution*, and incubate at 37° for 2 h. Quench the reaction by adding 3 µL of 20% trifluoroacetic acid.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Column temperature: Ambient

Flow rate: 1 mL/min

Injection volume: 100 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Equilibrate the system with a *Mobile phase* consisting of 100% *Solution A*. Measure the responses for the eight major peaks as defined in the USP Sargramostim RS Data Sheet.

Acceptance criteria: The retention times of the peak responses of the *Sample solution* correspond to those of the *Standard solution* if the retention times of the corresponding peaks do not differ by more than 0.3 min; the peak area ratios for peaks 4, 8, and 10 are between 0.7 and 1.3; and no additional significant peaks or shoulders are found.

ASSAY

• **PROCEDURE**

Iscove's Modified Dulbecco's medium: Prepare a mixture of the ingredients in the quantities shown in *Table 2* in sufficient water to obtain 1 L of medium, and sterilize by filtration.

Table 2

Calcium chloride	165.00 mg
Potassium chloride	330.00 mg
Potassium nitrate	0.076 mg
Magnesium sulfate	97.67 mg
Sodium chloride	4505.00 mg
Sodium bicarbonate	3024.00 mg
Monobasic sodium phosphate	125.00 mg
Sodium selenite	0.0173 mg
Glucose	4500.00 mg

Table 2 *(continued)*

HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid)	5958.00 mg
Phenol red	15.00 mg
Sodium pyruvate	110.00 mg
L-Alanine	25.00 mg
L-Arginine hydrochloride	84.00 mg
L-Asparagine	28.40 mg
L-Aspartic acid	30.00 mg
L-Cystine dihydrochloride	91.24 mg
L-Glutamic acid	75.00 mg
L-Glutamine	584.00 mg
Glycine	30.00 mg
L-Histidine hydrochloride	42.00 mg
L-Isoleucine	105.00 mg
L-Leucine	105.00 mg
L-Lysine hydrochloride	146.00 mg
L-Methionine	30.00 mg
L-Phenylalanine	66.00 mg
L-Proline	40.00 mg
L-Serine	42.00 mg
L-Threonine	95.00 mg
L-Tryptophan	16.00 mg
L-Tyrosine disodium	103.79 mg
L-Valine	94.00 mg
Biotin	0.013 mg
Calcium pantothenate	4.00 mg
Choline chloride	4.00 mg
Cyanocobalamin	0.013 mg
Folic acid	4.00 mg
Inositol	7.20 mg
Niacinamide	4.00 mg
Pyridoxal hydrochloride	4.00 mg
Riboflavin	0.40 mg
Thiamine	4.00 mg

Medium A: Prepare a mixture of *Iscove's Modified Dulbecco's medium* with 10% heat-inactivated fetal bovine serum as described in *Table 3*.

Table 3

Iscove's Modified Dulbecco's medium	500 mL
Fetal bovine serum (inactivated at 56° for 30 min)	50 mL
Penicillin, streptomycin, and L-glutamine mixture containing, in each mL, 5000 units of penicillin G potassium, 5000 µg of streptomycin sulfate, and 29.2 mg of L-glutamine	5 mL
Gentamicin (50 mg/mL)	0.5 mL
2-Mercaptoethanol	0.5 mL

Prepare aseptically, sterilize by filtration, and store at 2°–8°. Use within 1 month.

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Medium B: 1 µg/mL of USP Sargramostim RS in *Medium A*. Prepare aseptically, sterilize by filtration, and store at 2°–8°. Use within 1 month.

Standard solution: 100 ng/mL of USP Sargramostim RS in *Medium A*. Dispense aseptically in equal portions, and store at –60° or below. Use within 24 months. Store thawed portions at a temperature between 2° and 8°, and use within 1 month. At the time of use, dilute with *Medium A* to obtain a solution having a known concentration of 2 ng/mL.

Sample solution: 2 ng/mL of Sargramostim in *Medium A*

Cell culture preparation: Prepare cell cultures of TF-1 cells (ATCC CRL-2003). Passage the cultures every 3–4 days, using a 1:10 subculture of the cells for up to 3 months. After 3 months, initiate a new culture. Use *Medium A* containing 0.5% *Medium B* for passage propagation and storage in the frozen state.

Cell suspension: Wash the cells three times in *Medium A*, and adjust the cell concentration to 5×10^4 cells/mL in *Medium A*.

Tritiated thymidine solution: 1.0 mL of a solution of tritiated thymidine with a specific activity of 20 Ci/mmol in 49 mL of *Medium A*. Store at 2°–8°. Use within 2 weeks.

Analysis: Use a 96-well, flat-bottom microtitration plate with wells arranged in 8 rows (labeled A–H), with 12 wells (numbered 1–12) in each row. Place 50 µL of *Medium A* in wells 2–12. Place 100 µL of the *Standard solution* or each *Sample solution* or *Medium A* (negative control) in well 1. Make serial dilutions by transferring 50 µL from well 1 to well 2, and so on through well 12 (serial two-fold dilutions). Place 50 µL of the *Cell suspension* in each well, and incubate the microtitration plate for 72 h at 37° in a 10% carbon dioxide incubator. Following incubation, add 25 µL of *Tritiated thymidine solution* to each well, and return the plate to the same incubator for an additional 4–5 h. Before harvesting the cells on a filter mat, prewet the mat filter, using distilled water. [NOTE—The prewetting minimizes background radiation noise.] Using a multiple, automated sample harvesting system, place the incubated plate under the harvesting system. Fill the wells to the top with deionized water. Aspirate the water, and pass it through the collecting filter mat. Repeat the procedure at least five times or until all the cells have been fully harvested. When all wells have been fully harvested, pour 5–10 mL of alcohol on the plate tray, and aspirate the methanol. Repeat the procedure if further drying of the filter mat is desired. [NOTE—The alcohol helps to dry out the filter mat by carrying away the wash fluid.] Remove the filter mat, and repeat the procedure until all plates under test have been harvested. Dry the filter mat in a drying oven for about 30 min. Place the completely dry filter mats in a beta counter, and determine the amount of radioactivity in each cell well. Convert the amount of incorporated radioactivity in each well to a percentage of the maximum incorporated radioactivity. If fewer than five values are between 3% and 97% of the maximum revision, repeat the *Assay*. Using the least-squares method of regression analysis, plot the slope of each test specimen versus the slope of the standard, excluding any values exceeding the maximum of each dilution set. Calculate the USP Sargramostim Units in each mL of the *Sample solution* in terms of the dilution that gives half-maximal activity. To convert this value to units of protein/mg, divide the USP Sargramostim Units/mL by the weight, in mg/mL, of protein in the initial undiluted solution.

Acceptance criteria: The potency is 73.0%–146.0% of the potency stated on the label in terms of USP Sargramostim Units/mg of protein.

SPECIFIC TESTS

• **CHROMATOGRAPHIC PURITY**

Solution A: Trifluoroacetic acid and water (1:1000), filtered and degassed

Solution B: Trifluoroacetic acid and acetonitrile (1:1000), filtered and degassed

Solution C: Dissolve 116.9 g of sodium chloride in 2 L of water, adding 2 mL of trifluoroacetic acid.

Mobile phase: See *Table 4*. Make adjustments if necessary (see *Chromatography* ⟨621⟩, *System Suitability*).

Table 4

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	55	25	20
40	15	65	20

Standard solution: 1 mg/mL of USP Sargramostim RS in water

Sample solution: 1 mg/mL of Sargramostim in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: Ambient

Flow rate: 1 mL/min

Injection volume: 50 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Equilibrate the system with a *Mobile phase* consisting of 55% *Solution A*, 25% *Solution B*, and 20% *Solution C*. After injection of the solution under test, the composition is changed linearly. The major peaks are from hyperglycosylated sargramostim and from the three glycosylated forms of sargramostim, as indicated in the USP Sargramostim RS Data Sheet.

Calculate the percentage of hyperglycosylated sargramostim in the *Sample solution*:

$$\text{Result} = (r_U / r_T) \times 100$$

r_U = peak response of hyperglycosylated sargramostim

r_T = sum of all the peak responses of sargramostim

Calculate the percentage of each of the three glycosylated forms of sargramostim in the *Sample solution*:

$$\text{Result} = (r_U / r_T) \times 100$$

r_U = peak response of each individual glycosylated form of sargramostim

r_T = sum of the peak responses of all three glycosylated forms of sargramostim

Acceptance criteria: The peak responses of the *Sample solution* correspond to those of the *Standard solution*, and no peaks or shoulders are present in the chromatogram of the *Sample solution* that are not present in the chromatogram of the *Standard solution*. NMT 5.6% of hyperglycosylated sargramostim. The percentages of each of the three glycosylated forms of sargramostim, in order of elution, are 25%–42%, 14%–32%, and 35%–53%.

• **PROTEIN CONTENT**

Standard solutions: 100, 200, 400, 600, 800, and 1000 µg/mL of USP Sargramostim RS in water

USP Monographs

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Sample solution: Between 250 and 500 µg/mL of Sargramostim in water
BCA reagent: Dissolve 10 g of bicinchoninic acid, 20 g of sodium carbonate monohydrate, 1.6 g of sodium tartrate, 4 g of sodium hydroxide, and 9.5 g of sodium bicarbonate in water. Adjust, if necessary, with sodium hydroxide or sodium bicarbonate to a pH of 11.25. Dilute with water to 1 L, and mix.
Copper sulfate reagent: 40 mg/mL of cupric sulfate
Copper–BCA reagent: *Copper sulfate reagent* and *BCA reagent* (1:50). [NOTE—If a commercially available kit is used, follow the manufacturer's instructions for preparation of the *Copper–BCA reagent.*]
Blank: Water
Analytical wavelength: 562 nm
Analysis: Mix 0.1 mL each of *Standard solution, Sample solution,* and *Blank* with 2 mL of the *Copper–BCA reagent.* Incubate the solutions at 37° for 30 min, and allow to stand for 5 min at room temperature. Within 60 min after incubation, determine the absorbances of the *Standard solutions* and the *Sample solution* in 1-cm cells with a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩), using the *Blank* to set the instrument to zero. Plot the absorbances of the *Standard solutions* versus the concentrations, in µg/mL, of USP Sargramostim RS, and draw a straight line best fitting the plotted points. From the graph, determine the concentration, in µg/mL, of protein in the *Sample solution.*

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 1 cfu/mL.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 5 USP Endotoxin Units/mg

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in sealed containers, and store at a temperature of –20° or below.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sargramostim RS

Sargramostim for Injection

DEFINITION

Sargramostim for Injection is a sterile, lyophilized preparation of Sargramostim. Its biological activity is NLT 73.0% and NMT 146.0% of that stated on the label in USP Sargramostim Units. It contains NLT 90.0% and NMT 110.0% of the total protein content stated on the label.

IDENTIFICATION

- **A.** The retention times of the peaks of the *Sample solution* do not differ by more than 0.5 min from those of the *Standard solution,* as obtained in the test for *Chromatographic Purity.*
- **B. PEPTIDE MAPPING**

Solution A: Trifluoroacetic acid and water (1:1000), filtered and degassed
Solution B: Trifluoroacetic acid and acetonitrile (1:1000), filtered and degassed
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	65	35
50	35	65

Digestion solution: Dissolve 29.4 mg of calcium chloride and 1.8 mg of β-alanine in 2 mL of water. Adjust with hydrochloric acid to a pH of 4.0. Add 0.4 mg of trypsin, and mix.
Standard solution: 500 µg/mL of USP Sargramostim RS in water. Transfer 100 µL of this solution to a clean test tube, and add 11 µL of pH 7.6 buffer solution (see *Reagents, Indicators, and Solutions—Buffer Solutions*) containing 0.1 M tris(hydroxymethyl)aminomethane. Add 25 µL of *Digestion solution,* and incubate at 37° for 2 h. Quench the reaction by adding 3 µL of 20% trifluoroacetic acid.
Sample solution: 500 µg/mL of sargramostim in water. Transfer 100 µL of this solution to a clean test tube, and add 11 µL of pH 7.6 buffer solution (see *Reagents, Indicators, and Solutions—Buffer Solutions*) containing 0.1 M tris(hydroxymethyl)aminomethane. Add 25 µL of *Digestion solution,* and incubate at 37° for 2 h. Quench the reaction by adding 3 µL of 20% trifluoroacetic acid.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 10-µm packing L1
Column temperature: Ambient
Flow rate: 1 mL/min
Injection volume: 100 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Equilibrate the system with a *Mobile phase* consisting of 100% *Solution A.* Measure the responses for the eight major peaks as defined in the USP Sargramostim RS Data Sheet.
Acceptance criteria: The retention times of the peak responses of the *Sample solution* correspond to those of the *Standard solution* if the retention times of the corresponding peaks do not differ by more than 0.3 min; the peak area ratios for peaks 4, 8, and 10 are between 0.7 and 1.3; and no additional significant peaks or shoulders are found.

ASSAY

- **PROCEDURE**

Iscove's Modified Dulbecco's medium: Prepare a mixture of the ingredients in the quantities shown in *Table 2* in sufficient water to obtain 1 L of medium, and sterilize by filtration.

Table 2

Calcium chloride	165.00 mg
Potassium chloride	330.00 mg
Potassium nitrate	0.076 mg
Magnesium sulfate	97.67 mg
Sodium chloride	4505.00 mg
Sodium bicarbonate	3024.00 mg
Monobasic sodium phosphate	125.00 mg
Sodium selenite	0.0173 mg
Glucose	4500.00 mg
HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid)	5958.00 mg

USP Monographs

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Table 2 *(continued)*

Phenol red	15.00 mg
Sodium pyruvate	110.00 mg
L-Alanine	25.00 mg
L-Arginine hydrochloride	84.00 mg
L-Asparagine	28.40 mg
L-Aspartic acid	30.00 mg
L-Cystine dihydrochloride	91.24 mg
L-Glutamic acid	75.00 mg
L-Glutamine	584.00 mg
Glycine	30.00 mg
L-Histidine hydrochloride	42.00 mg
L-Isoleucine	105.00 mg
L-Leucine	105.00 mg
L-Lysine hydrochloride	146.00 mg
L-Methionine	30.00 mg
L-Phenylalanine	66.00 mg
L-Proline	40.00 mg
L-Serine	42.00 mg
L-Threonine	95.00 mg
L-Tryptophan	16.00 mg
L-Tyrosine disodium	103.79 mg
L-Valine	94.00 mg
Biotin	0.013 mg
Calcium pantothenate	4.00 mg
Choline chloride	4.00 mg
Cyanocobalamin	0.013 mg
Folic acid	4.00 mg
Inositol	7.20 mg
Niacinamide	4.00 mg
Pyridoxal hydrochloride	4.00 mg
Riboflavin	0.40 mg
Thiamine	4.00 mg

Medium A: Prepare a mixture of *Iscove's Modified Dulbecco's medium* with 10% heat-inactivated fetal bovine serum as described in *Table 3*.

Table 3

Iscove's Modified Dulbecco's medium	500 mL
Fetal bovine serum (inactivated at 56° for 30 min)	50 mL
Penicillin, streptomycin, and L-glutamine mixture containing, in each mL, 5000 units of penicillin G potassium, 5000 µg of streptomycin sulfate, and 29.2 mg of L-glutamine	5 mL
Gentamicin (50 mg/mL)	0.5 mL
2-Mercaptoethanol	0.5 mL

Prepare aseptically, sterilize by filtration, and store at 2°–8°. Use within 1 month.

Medium B: 1 µg/mL of USP Sargramostim RS in *Medium A*. Prepare aseptically, sterilize by filtration, and store at 2°–8°. Use within 1 month.

Standard solution: 100 ng/mL of USP Sargramostim RS in *Medium A*. Dispense aseptically in equal portions, and store at –60° or below. Use within 24 months. Store thawed portions at a temperature between 2° and 8°, and use within 1 month. At the time of use, dilute with *Medium A* to obtain a solution having a known concentration of 2 ng/mL.

Sample solution: Reconstitute Sargramostim for Injection in water, then dilute to a concentration of 2 ng/mL of sargramostim in *Medium A*.

Cell culture preparation: Prepare cell cultures of TF-1 cells (ATCC CRL-2003). Passage the cultures every 3–4 days, using a 1:10 subculture of the cells for up to 3 months. After 3 months, initiate a new culture. Use *Medium A* containing 0.5% *Medium B* for passage propagation and storage in the frozen state.

Cell suspension: Wash the cells three times in *Medium A*, and adjust the cell concentration to 5×10^4 cells/mL in *Medium A*.

Tritiated thymidine solution: 1 mL of a solution of tritiated thymidine with a specific activity of 20 Ci/mmol in 49 mL of *Medium A*. Store at 2°–8°. Use within 2 weeks.

Analysis: Use a 96-well, flat-bottom microtitration plate with wells arranged in 8 rows (labeled A–H) with 12 wells (numbered 1–12) in each row. Place 50 µL of *Medium A* in wells 2–12. Place 100 µL of the *Standard solution* or each *Sample solution* or *Medium A* (negative control) in well 1. Make serial dilutions by transferring 50 µL from well 1 to well 2, and so on through well 12 (serial two-fold dilutions). Place 50 µL of the *Cell suspension* in each well, and incubate the microtitration plate for 72 h at 37° in a 10% carbon dioxide incubator. Following incubation, add 25 µL of *Tritiated thymidine solution* to each well, and return the plate to the same incubator for an additional 4–5 h. Before harvesting the cells on a filter mat, prewet the mat filter, using distilled water. [NOTE—The prewetting minimizes background radiation noise.] Using a multiple, automated sample harvesting system, place the incubated plate under the harvesting system. Fill the wells to the top with deionized water. Aspirate the water, and pass it through the collecting filter mat. Repeat the procedure at least five times or until all the cells have been fully harvested. When all wells have been fully harvested, pour 5–10 mL of alcohol on the plate tray, and aspirate the methanol. Repeat the procedure if further drying of the filter mat is desired. [NOTE—The alcohol helps to dry out the filter mat by carrying away the wash fluid.] Remove the filter mat, and repeat the procedure until all plates under test have been harvested. Dry the filter mat in a drying oven for about 30 min. Place the completely dry filter mats in a beta counter, and determine the amount of radioactivity in each cell well. Convert the amount of incorporated radioactivity in each well to a percentage of the maximum incorporated radioactivity. If fewer than five values are between 3% and 97% of the maximum revision, repeat the *Assay*. Using the least-squares method of regression analysis, plot the slope of each test specimen versus the slope of the standard, excluding any values exceeding the maximum of each dilution set. Calculate the USP Sargramostim Units in each mL of the *Sample solution* in terms of the dilution that gives half-maximal activity. To convert this value to units of protein/mg, divide the USP Sargramostim Units/mL by the weight, in mg/mL, of protein in the initial undiluted solution.

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Acceptance criteria: The potency is 73.0%–146.0% of the potency stated on the label in terms of USP Sargramostim Units/mg of protein.

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements for *Content Uniformity*

SPECIFIC TESTS

- **CHROMATOGRAPHIC PURITY**

Solution A: Trifluoroacetic acid and water (1:1000), filtered and degassed

Solution B: Trifluoroacetic acid and acetonitrile (1:1000), filtered and degassed

Solution C: Dissolve 116.9 g of sodium chloride in 2 L of water, adding 2 mL of trifluoroacetic acid.

Mobile phase: See *Table 4*. Make adjustments if necessary (see *Chromatography* ⟨621⟩, *System Suitability*).

Table 4

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	55	25	20
40	15	65	20

Standard solution: 1 mg/mL of USP Sargramostim RS in water

Sample solution: Reconstitute Sargramostim for Injection in water to obtain a 1 mg/mL solution of sargramostim.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: Ambient

Flow rate: 1 mL/min

Injection volume: 50 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Equilibrate the system with a *Mobile phase* consisting of 55% *Solution A*, 25% *Solution B*, and 20% *Solution C*. After injection of the solution under test, the composition is changed linearly. The major peaks are from hyperglycosylated sargramostim and from the three glycosylated forms of sargramostim, as indicated in the USP Sargramostim RS Data Sheet.

Calculate the percentage of hyperglycosylated sargramostim in the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of hyperglycosylated sargramostim

r_T = sum of all the peak responses of sargramostim

Calculate the percentage of each of the three glycosylated forms of sargramostim in the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual glycosylated form of sargramostim

r_T = sum of all the peak responses of all three glycosylated forms of sargramostim

Acceptance criteria: The peak responses of the *Sample solution* correspond to those of the *Standard solution*, and no peaks or shoulders are present in the chromatogram of the *Sample solution* that are not present in the chromatogram of the *Standard solution*. NMT 5.6% of hyperglycosylated sargramostim. The percentages of each of the three glycosylated forms of sargramostim, in order of elution, are 25%–42%, 14%–32%, and 35%–53%.

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 50 USP Endotoxin Units/mg
- **SAFETY:** Meets the requirements in *Biological Reactivity Tests, In Vivo* ⟨88⟩, *Safety Tests—Biologicals*
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH** ⟨791⟩: 7.1–7.7, in the solution constituted as directed in the labeling
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in hermetic containers at a temperature between 2° and 8°.
- **LABELING:** Label it to state the biological activity in USP Sargramostim Units/vial and the amount of protein/vial.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sargramostim RS

Scaffold Bovine Dermis

DEFINITION

Scaffold Bovine Dermis is a remodelable collagen scaffold derived from fetal or neonatal bovine skin. It is presented to the physician as a flat white sheet that is cut to size and hydrated in room-temperature sterile saline solution before implantation. The sterile material is surgically secured, onlayed, and/or packed into deficient soft tissues such as skin, tendon, muscle, and dura mater. In order to provide antimicrobial protection of the Scaffold Bovine Dermis, silver nitrate solution may be added to the Scaffold Bovine Dermis, resulting in ionic silver as a component. The source fetal or neonatal bovine skin is mechanically and chemically processed to isolate the dermis and remove cells and cellular components. To prevent the transmission of infectious disease, the manufacturing process has been validated to inactivate viruses potentially present in the source material. To prevent the spread of transmissible spongiform encephalopathies, the source material is acquired from appropriate geographic locations in accordance with relevant guidelines subject to governmental oversight. The product is inspected and tested to ensure that the product meets specifications.

SPECIFIC TESTS

- **HISTOLOGICAL EVALUATION**

Solutions

1% Acidified alcohol solution: To 99 mL of 70% ethyl alcohol add 1 mL of hydrochloric acid (37.5%).

Potassium alum solution: Dissolve 100 g of potassium alum in 1000 mL of distilled water with the aid of heat and a magnetic stirrer.

Hematoxylin–alcohol solution: Dissolve 5 g of hematoxylin (see *Reagents, Indicators, and Solutions, Reagent Specifications*) in 50 mL of 100% ethyl alcohol at room temperature.

Hematoxylin solution: Slowly combine the 1000 mL of the *Potassium alum solution* with the 50 mL of the *Hematoxylin–alcohol solution*. Bring to a boil as rapidly as possible. Remove from heat, and slowly add 2.5 g of mercuric oxide. Return the solution to heat until it

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becomes dark purple, remove from heat, and cool in a sink of cold water.

Eosin solution: Dissolve 1.0 g of eosin Y, water soluble, in 100 mL of distilled water. Dissolve 1.0 g of phloxine B in 100.0 mL of distilled water. Combine 100 mL of eosin Y solution with 10 mL of phloxine B solution, 780 mL of 95% ethyl alcohol, and 4.0 mL of glacial acetic acid. [NOTE—Filter daily before use.]

Bluing agent: Dissolve 1.54 g of lithium carbonate in 100 mL of distilled water.

10% Neutral buffered formalin: To 6.5 g of dibasic sodium phosphate (anhydrous) and 4.0 g of monobasic sodium phosphate, add 900 mL of distilled water and 100 mL of formaldehyde (37%–40%).

Sample preparation and staining: Remove a sample of the finished product with an 8.0-mm biopsy punch. Place the sample in a labeled tissue cassette, and fix for 24 h in *10% Neutral buffered formalin*. Dehydrate the sample in sequential soaks of the following: 70% ethyl alcohol (45 min), 80% ethyl alcohol (45 min), 95% ethyl alcohol (90 min), 100% ethyl alcohol (180 min), and xylene (90 min). Embed the sample in melted paraffin, cool, and cut 5-µm-thick sections with a microtome. Collect sections on microscope slides. Deparaffinize the slides with xylene, and hydrate with distilled water. Stain in *Hematoxylin solution* for 6–15 min. Wash in running tap water for 2–5 min. Dip two times in *1% Acidified alcohol solution*. Wash briefly in tap water. Place in *Bluing agent* until the sections are bright blue. Wash in running tap water for 10 min. Place in 80% ethyl alcohol for 1–2 min. Dehydrate, and clear through two changes each of 95% ethyl alcohol, 100% ethyl alcohol, and xylene, 2 min each. Affix a coverslip over the tissue, using an appropriate resinous mounting medium. The nuclei stain blue, the cytoplasm stains from pink to red, and the collagen fibers stain from pink to red.

Microscopic and morphological characteristics: The collagen fibers of the Scaffold Bovine Dermis stain pink-red, and no evidence of cell nuclei or cytoplasm is apparent in prepared histological sections, as shown in the USP Bovine Acellular Dermal Matrix Reference Photomicrographs,[1] of products with acceptable histological appearance.

- **PROTEIN DETERMINATION**

Use the Kjeldahl nitrogen (protein) determination method to calculate the percent protein of the final product, as directed in *Nitrogen Determination* ⟨461⟩, with the following specifics. Suitable equipment and procedures are readily available.[2]

Digestion: Prepare a rack of 15–20 Kjeldahl digestion tubes. In each, place 2.0–2.2 g of the final product, 0.2 ± 0.05 g of ammonium sulfate, a metallic catalyst tablet,[3] and boiling chips.[4] Prepare a blank tube with catalyst tablets and boiling chips (reagent blank). To each tube add 15 mL of concentrated sulfuric acid, and then, very slowly, 3 mL of hydrogen peroxide (30%–35%). Place the digestion tubes on a digestion block, and heat to 410°. Digest for 60 ± 5 min. The mixture in the tubes should be a clear green.

Distillation: Add excess base (50% sodium hydroxide). Generally, for each 5 mL of concentrated sulfuric acid used in the digestion, 20 mL of 40% (w/w) sodium hydroxide is required to make the digest strongly alkaline (pH >11). Mix each tube, and let cool to room temperature. Distill each tube to collect approximately 125 mL of total distillate in a flask containing 25 mL of 4% boric acid. A reagent blank is run with each set.

Titration: Titrate the collected distillate with 0.2 N sulfuric acid to a neutral gray color endpoint. Record the volume of sulfuric acid used.

Calculation: Calculate the percentage of protein:

$$\text{Result} = [(\text{mL of sulfuric acid} - \text{mL of blank}) \times \text{N of sulfuric acid} \times A \times B]/\text{weight of sample (g)}$$

A = milliequivalent weight N × 100 (%), 1.4007
B = protein factor for meat, 6.25

Acceptance criteria: The percentage of protein in 2.0–2.2 g of the Scaffold Bovine Dermis sample is between 90.0% and 95.0%.

- **LIPID ANALYSIS**

Analysis: A standard Soxhlet extraction apparatus is required. Dry flasks in an oven/desiccator and weigh, recording the weight to the nearest 0.0001 g. Grind or cut into small pieces 3.0–4.0 g of test material, and place into a thimble. Record the weight of the test material to the nearest 0.0001 g. Place the thimble of material and 80–90 mL of petroleum ether into an extraction flask, and place into the Soxhlet extraction tube. Reflux for 4 h. Collect all the ether into the flask, and evaporate. Weigh the flask, recording the weight to the nearest 0.0001 g. For the weight of lipid, subtract the weight of the clean flask from the final weight of the flask. Calculate the percentage of lipid on the basis of the weight of the starting material.

Acceptance criteria: The percentage of lipid in 3.0–4.0 g of the Scaffold Bovine Dermis sample is between 0% and 1.5%.

- **LOSS ON DRYING** ⟨731⟩**:** Calculate the moisture content, with the following specifics. Mince approximately 5.0 g of Scaffold Bovine Dermis, and place it into an aluminum dish. Dry the sample in an air oven for 16–18 h at 100°–102°. Calculate the percentage of moisture in the sample taken:

$$\text{Dry matter \%} = [(\text{weight of dried sample + pan (g)} - \text{weight of pan (g)})/\text{g of sample}] \times 100$$

$$\text{Moisture \%} = 100 - \text{dry matter \%}$$

Acceptance criteria: The moisture loss is NLT 10.0% and NMT 12.0% of the original sample weight.

- **ASH DETERMINATION**

Analysis: Place a sample of the final product, about 5.0 g, in a kiln-dried porcelain crucible. Record the weight to the nearest 0.0001 g. Place the crucible containing the sample into an oven at 125° for 2–4 h. Then place the crucible containing the sample into a cool muffle furnace. Heat the furnace to 350°, and maintain the temperature until smoking ceases (generally about 20 min). Heat the furnace to 550°. Maintain the temperature for 2 h. Cool the crucible in a dessicator. Weigh the crucible, and record the weight to the nearest 0.0001 g. Calculate the percentage of ash:

$$\text{Result} = [(\text{weight of crucible + residue (g)} - \text{weight of crucible (g)})/\text{g of sample}] \times 100$$

Acceptance criteria: The percentage of ash is between 0% and 0.3%.

- **CARBOHYDRATE CONTENT**

Calculate the percentage of carbohydrates:

[1] These photomicrographs are available as a CD from the USP Reference Standards collection, available to the reader through USP Customer Services. To order these and other Reference Standards, call 1-800-227-8772 (U.S. and Canada), +1-301-881-0666 or 00-800-4875-5555 (select Europe); or go online to www.usp.org. Order item number 1535824.

[2] A suitable device and associated procedures can be obtained from Labconoco, 8811 Prospect Ave., Kansas City, MO 64132-2696.

[3] A suitable catalyst is Pro-Pac CT-37, Alfie Packers, 8901 J St., Omaha, NE 68127.

[4] Commonly referred to as Henger granules.

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Carbohydrate % = 100% – (protein % + lipid % + moisture % + ash %)

Acceptance criteria: The percentage of carbohydrates is equal to or less than 0.0%. Because this is a calculated value, influenced by the error inherent in the test methods above (*Protein Determination, Lipid Analysis, Loss on Drying,* and *Ash Determination*), a calculated value less than 0.0% is acceptable.

• **GEL ELECTROPHORESIS**

Solutions

Collagen extraction solution: Prepare a 0.5 M acetic acid solution containing 2 mM of ethylenediaminetetraacetic acid (EDTA).

2X Tris-glycine sample buffer: Prepare a 2X solution containing 63 mM Tris-HCl pH 6.8, 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.05% 2-mercaptoethanol, and 0.25% bromophenol blue.[5]

1X Tris-glycine sample buffer: Prepare a solution containing a mixture of *2X Tris-glycine sample buffer* and water (1:1).

SDS-PAGE running buffer: Prepare a solution containing 25 mM of Tris pH 8.3, 192 mM of glycine, and 0.1% of SDS.[6]

Polyacrylamide gel: Prepare a Tris-HCl polyacrylamide gel with a 4%–20% gradient.[7]

Molecular weight marker: Use a suitable molecular weight marker containing protein bands between 10 and 250 kilodaltons (kDa).

Staining solution: Prepare a solution containing 0.25% (w/v) Coomassie brilliant blue R-250 (see *Reagents, Indicators, and Solutions, Reagent Specifications*) in 10% acetic acid and 10% *n*-propanol.

Destain solution: Prepare a mixture of water, acetic acid, and *n*-propanol (8:1:1).

Collagen preparations: Mince 0.5 g of Scaffold Bovine Dermis final product. Weigh a sample of minced Scaffold Bovine Dermis, and add to a volume of *Collagen extraction solution* to obtain a concentration of 5 mg/mL (dry weight of Scaffold Bovine Dermis). Extract on a rocking platform at room temperature for 72 h.

Analysis: Dilute acid-extracted collagen samples in *2X Tris-glycine sample buffer* to a concentration of 0.5 mg/mL, and incubate for 5 min at 100°. Load the *Polyacrylamide gel* in the electrophoresis apparatus, and add *SDS-PAGE running buffer* to the top and bottom reservoirs. Load 10 µL of *Molecular weight marker* in the first well of the *Polyacrylamide gel* and 10 µL of *1X Tris-glycine sample buffer* in the second well. Load 10 µL (5 µg) of each *Collagen preparation* into subsequent gel wells. Attach the cathode and anode to the appropriate terminals, and apply 110 V to the gel. Run the gel until the bromophenol blue reaches the bottom of the gel. Remove the gel from the electrophoresis apparatus, and place it in a tray containing enough *Staining solution* to cover the gel. Incubate the gel for 3 h on a rocker at room temperature. Completely remove the *Staining solution* from the tray, cover the gel with *Destain solution*, and slowly rock the gel for 20 min. Remove the *Destain solution*, and repeat the destaining procedure three times. Inspect the gel for bands that have migrated from the origin.

System suitability: All *Molecular weight marker* bands between 20 and 200 kDa are present. The lane containing *1X Tris-glycine sample buffer* does not contain any bands.

Data analysis: Where a protein band appears in the gel, the molecular weight of this protein is determined by comparing the position of the band to that of the known *Molecular weight marker*.

Specificity and acceptance criteria: The lanes of the *Polyacrylamide gel* that correspond to Scaffold Bovine Dermis show four major protein bands. Two bands, when compared to the *Molecular weight marker*, appear at 96 and 94 kDa. These two bands correspond to the monomeric alpha 1 and alpha 2 chains of collagen Type I, respectively. Another two bands appear close together at 200 kDa, which correspond to alpha 1 and alpha 1/alpha 2 collagen dimers.

• **TENSILE STRENGTH**

Procedure: Cut test specimens 5-mm wide × 50-mm long from representative pieces from final product lots. Measure the thickness of the specimens. Test the specimens with a commercially available material test system.[8] Mount and align each specimen, gripping 1 cm of the test specimen on both ends to ensure a test specimen gauge length of 3 cm. Pull the grips apart at 30 mm/min while concurrently measuring the force exerted on the specimen. Record the maximum force (*N*) measured during the test. Calculate the tensile strength:

Tensile strength (N/mm^2) = maximum force (*N*)/5 (mm) × thickness (mm)

Acceptance criteria: The measured tensile strength for each lot is NLT 5 N/mm^2.

• **SUTURE RETENTION FORCE**

Analysis: Cut representative 1-cm × 1-cm test specimens from final product lots. Using an appropriate suture material (e.g., 4-0 polypropylene suture), thread the suture 3 mm from the edge of the sample in the center, and pull through. Clamp approximately 5 mm of the opposite, unsutured end of the test specimen in the upper pneumatic grip of a commercially available material test system.[9] The suture tails are hanging freely. Clamp the suture tails to the lower grip. Pull the grips apart at 20 mm/min while concurrently measuring the force exerted. Record the maximum force (*N*) measured.

Acceptance criteria: The suture retention force measured for each lot is NLT 5*N* for a 1-cm × 1-cm test sample of Scaffold Bovine Dermis.

• **THERMAL ANALYSIS**

Analysis: Heat a final product sample of approximately 10–20 mg at 2°/min from 30°–90°, hydrate with water, and measure the thermal characteristics of each processed sample with a differential scanning calorimeter as directed in *Thermal Analysis* ⟨891⟩.

Acceptance criteria: Scaffold Bovine Dermis displays a single thermal transition peak between 58° and 67°.

• **VISUAL INSPECTION**

Analysis: Visually inspect each piece of final product under a white light at a distance of 30–45 cm for color, the presence of particulates, and holes.

Acceptance criteria: Scaffold Bovine Dermis is white, and neither particulates nor holes are visible.

[5] A suitable sample buffer can be obtained from Invitrogen Corporation, 1600 Faraday Ave., P.O. Box 6482, Carlsbad, CA 92008.

[6] A suitable gel running buffer can be obtained from Bio-Rad Laboratories, 1000 Alfred Nobel Dr., Hercules, CA 94547.

[7] A suitable precast acrylamide gel can be obtained from Bio-Rad Laboratories, 1000 Alfred Nobel Dr., Hercules, CA 94547.

[8] A suitable material test system is available from Instron Corporation, 825 University Ave., Norwood, MA 02062-2643.

[9] A suitable material test system is available from Instron Corporation, 825 University Ave., Norwood, MA 02062-2643.

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• **HYDRATION RATE**
Analysis: Cut a sample of finished product lot approximately 1 cm × 1 cm. Fully hydrate the sample, as indicated by a change in color from white to gray.
Acceptance criteria: The sample should be fully hydrated in NMT 3 min when placed in room-temperature saline solution.

• **CONTENT OF SILVER**
Sample: Roll or fold a 4-cm × 4-cm sample of Scaffold Bovine Dermis into a labeled 50-mL Pyrex glass tube containing 30 mL of 6.0% nitric acid, and cap tightly. Heat the sample tube in a purified glycerin bath at 100°–105° for 16 h. Remove the sample tube from glycerin bath, and allow to cool to <30°. Transfer 0.5 mL of the dissolved *Sample* into a second Pyrex glass tube containing 30 mL of 2.0% nitric acid prepared freshly on the day of use, and cap tightly. The *Sample* should be stored at room temperature (15°–30°) until analysis is initiated.
Analysis: The *Sample* is analyzed for ionic silver via inductively coupled plasma–atomic emission spectrometry (ICP–AES). The instrument should be a computer-controlled emission spectrometer with background correction capability. Accurately weigh the *Sample*. Nebulize the *Sample*, and transport the resulting aerosol to the plasma torch. Element-specific spectra are produced by radio frequency inductively conducted plasma. Spectra are dispersed by a grating spectrometer, and the intensities of the line spectra are monitored at specific wavelengths by a photosensitive device. Photo currents from the photosensitive device are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the determination of silver. Background must be measured adjacent to the analyte wavelength during analysis. Four types of blanks are required for the analysis: The calibration blank is used in establishing the analytical curve. The laboratory reagent blank is used to assess possible contamination from the *Sample* preparation procedure. The laboratory fortified blank is used to assess routine laboratory performance. A rinse blank is used to flush the instrument uptake system and nebulizer between standards and to check solutions and samples to reduce memory interferences.
Calibration and standardization: Before using this method, the user must optimize the plasma operating conditions. The purpose of plasma optimization is to provide a maximum signal-to-background ratio. The use of a mass flow controller to regulate the nebulizer gas flow rate greatly facilitates the procedure.
Data analysis and calculations: Report results up to three significant figures as mg/kg on the dry weight basis. Calculate the concentration of silver in the *Sample*:

$$\text{Result} = [C \times V \times D]/W$$

C = concentration in extract (mg/L)
V = volume of extract (L)
D = dilution factor (undiluted = 1)
W = weight of sample aliquot extracted (g × 0.001 = kg)

Acceptance criteria: The content of ionic silver in the Scaffold Bovine Dermis must be more than 100.0 µg/cm² and less than 165.0 µg/cm² as determined by ICP–AES.

• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements.

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It meets the requirements as directed in ▲*Medical Devices—Bacterial Endotoxin and Pyrogen Tests* ⟨161⟩.▲ (CN 1-May-2019)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** The package is a sealed foil pouch that provides an effective moisture, light, gas, and sterility barrier. Store in clean, dry conditions between 15° and 30°.

• **LABELING:** Label it to indicate that it is derived from bovine origin. The product is labeled to indicate its intended clinical use. It is labeled with the dimensions of the product, the expiration date, the required storage conditions, the lot number, the part number, and the manufacturer's name and address. The label indicates that the product is sterile and nonpyrogenic and is designed for single-patient, one-time use. The labeling cautions the user to inspect the packaging for damage and to discard the product if the packaging has been compromised. The labeling also cautions the user to hydrate the product only in room-temperature sterile saline solution.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Authentic Visual References RS
USP Bovine Acellular Dermal Matrix Reference Photomicrographs.[1]
These photomicrographs show the histological appearance of failed, cell-containing source material (Photomicrographs 1 and 2) and of passing, processed, decellularized material (Photomicrographs 3 and 4). The samples were prepared as directed in the test for *Histological Evaluation* in *Specific Tests*.

Add the following:

▲Scaffold Human Amniotic Membrane Allograft

DEFINITION

Scaffold Human Amniotic Membrane Allograft is derived from normal human placental amniotic tissue obtained from full-term pregnancies. The amniotic tissue is isolated and decellularized to remove cellular contaminants, resulting in an extracellular matrix with basement membrane. Scaffold Human Amniotic Membrane Allograft is composed predominantly of collagen and elastin. Tissues are obtained from sources that have passed applicable donor tissue eligibility requirements for relevant diseases, are processed, dehydrated, and terminally sterilized with e-beam irradiation. Scaffold Human Amniotic Membrane Allograft is supplied in a dry sheet form and is stored at ambient temperature.

IMPURITIES

• **ANALYSIS OF RESIDUAL SODIUM DEOXYCHOLATE**
Solution A: Purified water liquid chromatography–mass spectrometry (LC/MS) grade
Solution B: Acetonitrile LC/MS grade
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
8	25	75
9	2	98
13	95	5

Diluent 1: Acetonitrile and purified water, both LC/MS grade (50:50), and 0.1% formic acid
Diluent 2: Isopropyl alcohol and purified water (90:10)
Diluent 3: 50% ethanol in phosphate buffered saline (PBS)
Lock mass stock solution: Add 2.5 mL of purified LC/MS grade water to a 10-mg vial of leucine enkephalin acetate to obtain a 4-µg/µL stock solution.
Lock mass solution: 2 ng/µL prepared as follows. Transfer a 10-µL aliquot of the *Lock mass stock solution* to a 20-mL volumetric flask, and add *Diluent 1* to volume.
Calibration solution: 5 mM sodium formate in *Diluent 2*
Sodium deoxycholate stock solution 1: Weigh a 25 ± 0.5-mg portion of sodium deoxycholate, add to a 50-mL volumetric flask, and dissolve in *Diluent 3* to obtain a 500-µg/mL solution.
Sodium deoxycholate stock solution 2: Dilute *Sodium deoxycholate stock solution 1* volumetrically at a ratio of 1:50 in *Diluent 3* to obtain a 10-µg/mL solution.
Sodium deoxycholate standard solution: Dilute *Sodium deoxycholate stock solution 2* volumetrically at a ratio of 1:5 in *Diluent 3* to obtain a 2-µg/mL solution.
Lithocholic acid stock solution 1: Weigh a 25 ± 0.5-mg portion of lithocholic acid, add to a 50-mL volumetric flask, and dissolve in *Diluent 3* using a sonicator for approximately 30 min to obtain an approximate 500-µg/mL stock solution.
Lithocholic acid stock solution 2: Dilute *Lithocholic acid stock solution 1* volumetrically at a ratio of 1:50 in *Diluent 3* to obtain an approximate 10-µg/mL solution.
Lithocholic acid standard solution: Dilute *Lithocholic acid stock solution 2* volumetrically at a ratio of 1:5 in *Diluent 3* to obtain a 2-µg/mL solution.
Spiked internal standard solution 1: Dilute *Sodium deoxycholate standard solution* and *Lithocholic acid standard solution* in *Diluent 3* at a ratio of 1:1:18.
Spiked internal standard solution 2: Dilute *Sodium deoxycholate stock solution 2* and *Lithocholic acid stock solution 2* in *Diluent 3* at a ratio of 1:1:3.
Sample: 2 mg of Scaffold Human Amniotic Membrane Allograft. Subdivide the test and control sample(s) to facilitate transfer into 20-mL vials.
Spiked sample: Spike the sample with 60 µL of *Lithocholic acid standard solution*. Dry under a stream of nitrogen for 2 h.
Spiked control sample: Spike the sample with 60 µL of *Spiked internal standard solution 2*. Dry under a stream of nitrogen for 2 h.
Chromatographic system
Mode: UPLC
Detector: UV 190–400 nm
Column: 50-mm × 2.1-mm; 1.7-µm packing L7
Column temperature: 35°
Flow rate: 0.3 mL/min
Injection volume: 5 µL
Run time: 15 min
Mass spectrometry instrumental conditions
Mass analyzer: Quadrupole time of flight (QToF)
Mode of MS ionization: Electrospray ionization, negative ion mode
MS mode: Sensitivity
Spectral range: 50–1500 *m/z*
Detector: Time of flight (ToF)
Lock mass: 554.26 Da
Capillary: 2.6 kV
Sampling cone: 30 V
Extraction cone: 4 V
Temperatures
Source: 140°
Desolvation: 450°
System suitability
Sample: *Spiked internal standard solution 1*
Suitability requirements
Percent relative standard deviation (% RSD) detection limit: NMT 15%
Quality control check: 70%–130% of theoretical concentration
Relative response factor: 0.5–1.3
Deoxycholate–molecular mass (M–H): 391.2848 ± 0.02 Da
Internal standard–molecular mass (M–H): 375.2899 ± 0.02 Da
Analysis
Samples: *Spiked sample* and *Spiked control sample*
Sample extraction: Add *Diluent 3* to each sample vial containing *Spiked sample* or *Spiked control sample* using a ratio of 1.2 mL of *Diluent 3* per 2 mg of the sample. For each vial, extract the sample at 37 ± 2° for 120 ± 12 min. After the extraction is complete, remove the sample from each extraction vessel using a clean inert article, such as a disposable glass Pasteur pipette. Store the extract at 2°–8°.
Sample analysis: Analyze the extracts using instrument conditions described. Analyze 6 replicates of *Spiked internal standard solution 1* and *Diluent 3* as solvent blank as appropriate. Perform quality control (QC) checks at least every 10 samples using *Spiked internal standard solution 1*.
Analysis with mass spectrometer: Change the QToF mass spectrometer settings as follows.
Capillary: 2.6 kV
Sampling cone: 30.0 V
Extraction cone: 4 V
Temperatures
Source: 140°
Desolvation: 450°
Desolvation gas flow: 750 L/min
Use the *Calibration solution* to calibrate the mass spectrometer for a mass range of 50–1500 Da in sensitivity mode using tune page settings and without collision energy. Set up lock mass using *Lock mass solution* with a capillary voltage of 2.6 kV in negative mode of ionization at 554.26 Da (M–H).
Calculate the ratio of the deoxycholate peak area to the lithocholic acid peak area from the *Spiked sample* and the *Spiked control sample*.
Acceptance criteria: The ratio of the deoxycholate peak area to the lithocholic acid peak area from the *Spiked sample* must be less than that from the *Spiked control sample*.

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• **Residual Cell Analysis**

Harris hematoxylin stock solution: Dissolve hematoxylin[1] in alcohol and 20 mL of water. Add ammonium aluminum sulfate in water with heat until completely dissolved. Remove the ammonium aluminum sulfate solution from heat and mix with the hematoxylin solution. Bring the mixed solution to a rapid boil. Immediately remove from heat, add sodium iodate, and mix. Reheat the solution to boiling and boil for approximately 2–3 min. Remove from heat and plunge the solution container into an ice bath. Allow the solution to cool and pass through a grade 541 or comparable filter paper. Store the solution in a light-protected bottle at room temperature. This solution is stable for approximately 2 months.

Harris hematoxylin working solution: Add 4 mL of glacial acetic acid to 100 mL of filtered *Harris hematoxylin stock solution*.

Eosin Y stock solution (1%): Add 1.0 g of eosin Y to 100 mL of distilled water and mix well. [Note—This solution can be stored for up to 1 year at room temperature.]

Phloxine B stock solution: Add 1.0 g of phloxine B to 100 mL of distilled water and mix well. [Note—This solution can be stored for up to 1 year at room temperature.]

Eosin–phloxine B working solution: Add 100 mL of *Eosin Y stock solution (1%)* and 10 mL of *Phloxine B stock solution* to 780 mL of 95% alcohol, then add 4 mL of glacial acetic acid and mix well. [Note—This solution can be stored for up to 1 year at room temperature.]

Samples: 5-mm × 5-mm pieces of Scaffold Human Amniotic Membrane Allograft prepared as follows. Place each piece of trimmed tissue into a pre-labeled tissue cassette. Place the cassettes into a tissue processor cassette basket that is set into a container of 10% formalin.

Tissue processing: Tissue processing can be done using an automated tissue processor programmed using a stepwise schedule with all steps done under a vacuum, except the first 10% neutral formalin step. The stepwise procedure is provided in *Table 2*.

Table 2

Step Solution	Time	Retort Temperature
10% neutral formalin	10 min	ambient
70% alcohol	15 min	ambient
80% alcohol	15 min	ambient
95% alcohol	15 min	ambient
95% alcohol	20 min	ambient
Absolute alcohol	15 min	ambient
Absolute alcohol	15 min	ambient
Absolute alcohol	20 min	ambient
Xylene	15 min	ambient
Xylene	20 min	ambient
Paraffin	20 min	61°
Paraffin	20 min	61°
Paraffin	20 min	61°

Embedding: Processed cassettes are placed into the embedding cassette bath and are embedded in paraffin–polyisobutylene embedding medium[2] on an on-edge orientation.

Sectioning: Blocks are sectioned at 5 µm using a microtome. Sections are floated on a water bath containing distilled water at a temperature of approximately 47°. A single section is placed on a charged slide. Multiple slides may be generated for additional stains. Before staining, slides are dried for 15 ± 5 min at 60 ± 5° in the slide drying oven.

Staining: Fixed *Samples* on slides are stained using the following procedure.

1. Deparaffinize sections, 2 changes of xylene, 5 min each
2. Re-hydrate in 1 change of absolute alcohol for 3 min
3. Re-hydrate in 95% alcohol for 2 min
4. Wash in running water for 3 min
5. Stain in *Harris hematoxylin working solution* for 10 min
6. Wash in running tap water for 5 min
7. Differentiate in 1% acid alcohol and 30% water containing 1% hydrochloric acid (70% for 15 s)
8. Wash in running tap water for 5 min
9. Stain with blue in ammonia water [1% solution of ammonium hydroxide (28%–30%)] for 6 s
10. Wash in running tap water for 5 min
11. 95% alcohol for 6 s
12. Counterstain in alcoholic eosin (0.25% eosin in 80% alcohol and 20% water) for 5 min
13. Differentiate in 95% alcohol for 10 s
14. Dehydrate in absolute alcohol for 30 s
15. Dehydrate in 2 changes of absolute alcohol, 2 min each
16. Clear in 2 changes of xylene, 1 min each
17. Hold in xylene for 2 min

Coverslipping and mounting: Stained slides are removed from xylene and are coverslipped. The appropriate size coverglass is placed with a suitable mountant using a volume appropriate for the selected coverglass size. When complete, the slides are removed, placed into slide flats and allowed to dry.

Analysis: Scaffold Human Amniotic Membrane Allograft preparations are identified as thin strips, NMT 100 µm of eosinophilic membranous material. The structure of the tissue includes distinct amniotic basement membrane with the bulk of the preparation appearing as dense, eosinophillic acellular matrix. If any residual amnion cells are present, the cells align on the surface of the membrane. Identify the presence of cell nuclei and cytoplasm in the samples:

1. Cell nuclei: stain blue
2. Cytoplasm: stains pink
3. Erythrocytes: stain red

The presence of cells is noted for each tissue sample. Tissue samples are examined and the number of cells per 2.5-mm length of tissue is counted. [Note—*Samples* are sterilized with e-beam radiation with a certified dose sufficient to render any remaining eukaryotic cells non-viable.]

Acceptance criteria: NLT 99% cell free or NMT 64 cells/mm² or NMT 2 cells/*Sample*. No bacterial cells identified by abnormal blue inclusions can be detected in the observed field. No inflammatory inclusions (such as granuloma) can be present in the *Sample*. No malignant

[1] A suitable reagent can be obtained from VWR, catalog #95057-858, or use a suitable equivalent.

[2] A suitable embedding and infiltration medium can be obtained from McCormick, catalog #39501006, or use a suitable equivalent.

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cells, hyperplastic cells, benign tumor cells, or other cell debris can be present in the *Sample*.

SPECIFIC TESTS

• **HYDROXYPROLINE ASSAY FOR COLLAGEN CONTENT**

10X Assay buffer (10X acetate/citrate assay buffer): Weigh 50 g of citric acid, 120 g of sodium acetate trihydrate, and 34 g of sodium hydroxide. Add 890 mL of deionized or sterile water, 12 mL of glacial acetic acid, and 50 µL of toluene. Mix solution until it is free of visible particles, and then pass through a 0.22-µm filter. This buffer is stored at 2°–8° and is stable for at least 1 month.

1X Assay buffer (1X acetate/citrate assay buffer): Dilute 100 mL of *10X Assay buffer* with 900 mL of deionized or sterile water, and mix until solution is homogenous. Pass one time through a 0.22-µm filter. This buffer is stored at 2°–8° and is stable for at least 1 month.

Chloramine T reagent: Accurately weigh 4.23 g of chloramine T hydrate. Add 53.3 mL of *10X Assay buffer*, 20.7 mL of sterile water, and 26.0 mL of 1-propanol. Mix for approximately 1 h until solution is clear. This reagent is stored at room temperature and is stable for at least 12 h.

DMAB reagent: Weigh 12.0 g of 4-(dimethylamino) benzaldehyde (DMAB). Add 48 mL of 1-propanol and 20.7 mL of 70% perchloric acid, and then mix until the solution is free of visible particles. Solution is light sensitive and should be covered with foil until use. This reagent is stable at room temperature for at least 12 h after preparation.

System suitability solutions: Weigh 250 ± 5 mg of *trans*-4-hydroxy-L-proline. Calculate the volume of 6.0 N hydrochloride needed to create a 1000 µg/100 µL hydroxyproline solution and then, using the density of 6.0 N hydrochloride, which is 1.06 g/mL at 20°, determine the mass of that volume. Weigh the previously calculated mass of 6.0 N hydrochloride into an appropriate container, and then mix in the pre-weighed hydroxyproline reference material creating the 1000 µg/100 µL hydroxyproline solution.

High system suitability solution: 22.5 µg/200 µL prepared as follows. Weigh 121.476 g of 6.0 N hydrochloride and mix with 5.40 mL of 1000 µg/100 µL hydroxyproline solution.

Middle system suitability solution: 14.5 µg/200 µL prepared as follows. Weigh 123.511 g of 6.0 N hydrochloride and mix with 3.48 mL of 1000 µg/100 µL hydroxyproline solution.

Low system suitability solution: 6.5 µg/200 µL prepared as follows. Weigh 125.546 g of 6.0 N hydrochloride and mix with 1.56 mL of 1000 µg/100 µL hydroxyproline solution.

The *System suitability solutions* are stored in 2-mL aliquots at 2°–8° and are stable for at least 1 month.

Hydroxyproline stock solution: Purified hydroxyproline (1 mg/mL) in 0.01 N hydrochloride prepared as follows. Weigh 150 ± 5.0 mg of *trans*-4-hydroxy-L-proline reference material into a glass vial. Add a calculated volume of 0.01 N hydrochloride to create a 50-mg/mL hydroxyproline solution and mix until homogenous. Add 2.0 mL of 50-mg/mL hydroxyproline solution to a 100-mL volumetric flask, and add 0.01 N hydrochloride to volume. Invert the flask 7–10 times to ensure solution is homogenous. This solution is stored in 5.0-mL aliquots at 2°–8° and is stable for at least 1 month.

Standard solutions: Remove a single vial of the *Hydroxyproline stock solution* (1 mg/mL in 0.01 N hydrochloride) from storage and dilute 8 times in 0.01 N hydrochloride to a final concentration of 25 µg/200 µL. This working standard is the highest reference point in the hydroxyproline calibration curve. Prepare additional standard solutions by diluting the working standard with 0.01 N hydrochloride to final concentrations of 20, 16, 12, 8, and 4 µg per 200 µL. Include a 0.01 N hydrochloride blank in the assay as a background control.

Samples: Weigh NLT 3 samples, 5 ± 0.5 mg each, of Scaffold Human Amniotic Membrane Allograft. Place the samples in individual glass vials, and add 2 mL of 6.0 N hydrochloride to each vial so that the solution covers the material. Hydrolyze the samples by placing the vials on a dry heat block for 18 ± 1 h at 110°. After hydrolysis is complete, remove the vial caps and dry the samples uncovered at 110° for an additional 3–4 h. Resuspend the concentrated hydrolysate in 4.0 mL of 0.01 N hydrochloride for assay use.

System suitability requirements: The standard curve (R^2) must be NLT 0.98. The percent bias of the standard curve must be NMT 20% for 75% of all standard points (14 of 18). The percent coefficient value of the standard curve must be NMT 20% for 75% of all standard points. The percent bias of the *High system suitability solution*, *Middle system suitability solution*, and *Low system suitability solution* is NMT 20%. The percent coefficient value of the *High system suitability solution*, *Middle system suitability solution*, and *Low system suitability solution* is NMT 20%.

Analysis: Triplicate samples (100 µL) are added to individual glass vials labeled with the corresponding *Sample* weight (mg). Add 100 µL of 0.01 N hydrochloride to each *Sample* vial. To generate the hydroxyproline standard curve, 200 µL of each *Standard solution* is pipetted, in triplicate, into individual glass vials and 1.8 mL of *1X Assay buffer* is added to each *Sample* and hydroxyproline standard, bringing the final volumes to 2.0 mL. The hydroxyproline is oxidized by incubating the samples at room temperature for 30 min in 1.0 mL of *Chloramine T reagent*. At the end of this reaction, add 1.0 mL of *DMAB reagent* to each vial and allow to incubate at 60° for an additional 30 min. During this time, the *DMAB reagent* reacts with oxidized hydroxyproline residues, generating a colorimetric product that is directly proportional to the amount of hydroxyproline present in the test samples. At the conclusion of this reaction, transfer 200 µL of each *Standard solution* and *Sample* to individual wells of a clear, flat-bottom 96-well plate. Acquire data by spectrophotometer at an optical density (OD) of 550 nm. Hydroxyproline levels in test samples are determined by interpolation against the hydroxyproline standard curve using suitable software or by plotting.

Calculate the total amount of collagen:

$$\text{Result} = \{[(H_p/V_a) \times V_s]/F_1\}/F_2$$

H_p = hydroxyproline value interpolated from the standard curve (µg)
V_a = volume used in the assay (µL)
V_s = volume of 0.01 N hydrochloride used to resuspend the concentrated hydrolysate, 4000 µL
F_1 = standard factor, mass ratio of hydroxyproline residue in collagen, 0.125
F_2 = unit conversion factor, 1000 µg/mg

Calculate the percentage of collagen (%):

$$\text{Result} = (T/W) \times 100$$

T = total amount of collagen, previously calculated
W = *Sample* weight

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Acceptance criteria: Collagen content measured by hydroxyproline content is 74%–94%.

• **ELASTIN CONTENT**

Diluent: 200 mL of 85% (v/v) 1-propanol in water prepared as follows. Mix 30 mL of sterile water with 170 mL of 1-propanol.

Dye dissociation reagent: 3 M guanidine hydrochloride in *Diluent* prepared as follows. Weigh 28.7 ± 0.5 g of guanidine hydrochloride and add into 100 mL of *Diluent*. Stir at room temperature until completely dissolved (approximately 30 min). Wrap with aluminum foil. This reagent is stable for at least 1 month at room temperature.

Precipitating reagent: 15% (w/v) trichloroacetic acid in hydrochloride prepared as follows. Weigh 15 ± 1 g of trichloroacetic acid, add 60 mL of sterile water, and stir to dissolve. Add 20 mL of 0.06 N hydrochloride to make a solution with a pH of −0.3 ± 0.1. Add additional sterile water to 100 mL. Wrap the bottle with aluminum foil; store at 2°–8°. This reagent is stable for at least 3 months at a storage temperature of 2°–8°.

0.4 M phosphate buffer: Mix 320 mL of 1 M phosphate buffer stock solution, pH 7.4, with 480 mL of sterile water. This solution is stable for at least 3 months at a storage temperature of 2°–8°.

Saturated ammonium sulfate buffer: Dispense 200 mL of *0.4 M phosphate buffer* into an appropriate container. Add 150 ± 1 g of ammonium sulfate. Stir at room temperature for 30 min. The top solution phase is ready to use after undissolved ammonium sulfate settles completely. This solution is stable for at least 3 months at 2°–8°.

0.4 M phosphate buffer with 24% saturated ammonium sulfate: Prepare 600 mL as follows. Mix 144 mL of *Saturated ammonium sulfate buffer* with 456 mL of *0.4 M phosphate buffer*. This solution is stable for at least 3 months at 2°–8°.

Dye reagent: 0.5 mg/mL of 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine-*p*, *p*′,*p*″,*p*‴-tetrasulfonic acid tetrasulfonate (TPPS) in *0.4 M phosphate buffer with 24% saturated ammonium sulfate*. Weigh 0.250 ± 0.05 g of TPPS powder. Add the weighed powder into 500 mL of *0.4 M phosphate buffer with 24% saturated ammonium sulfate*. Stir at room temperature until completely dissolved (approximately 15 min). Pass TPPS dye solution through a 0.22-µm filter. Wrap the bottle with aluminum foil. This reagent is stable for at least 1 month at 2°–8°.

Assay diluent: 0.25 M oxalic acid prepared as follows. Mix 1 part 0.5 M oxalic acid with 1 part deionized water. Store at 15°–30°.

High system suitability solution: 25 µg/250 µL of tropoelastin in *Assay diluent*

Middle system suitability solution: 20 µg/250 µL of tropoelastin in *Assay diluent*

Low system suitability solution: 15 µg/250 µL of tropoelastin in *Assay diluent*

Tropoelastin standard stock solution: 0.200 mg/mL of human recombinant tropoelastin in 0.25 M oxalic acid prepared as follows. Weigh 11 ± 1 g of tropoelastin (combine vials as needed), and place in a labeled glass vial. Record the weight. Calculate the amount of *Assay diluent* needed to make 4 mg/mL with the amount of tropoelastin measured above (e.g., 2.5 mL of oxalic acid is needed for 10.0 mg of tropoelastin). Record the volume. Add the calculated amount of *Assay diluent* to the labeled glass vial using a calibrated pipettor. Mix 6 times on a vortex mixer for 20 s each time at 3000 rpm. Invert the vial 3 times between each vortex. Visually check if tropoelastin is completely dissolved. If it did not dissolve, repeat previous step. Further dilute with *Assay diluent* the reconstituted 4 mg/mL tropoelastin solution to 0.200 mg/mL using a volumetric flask. Store at −20 ± 2°.

Standard solutions: Remove a single 2.2-mL vial of the *Tropoelastin standard stock solution* (0.200 mg/mL in *Assay diluent*) from storage at −20 ± 2°, thaw at room temperature, and dilute in *Assay diluent* to the following concentrations: 30, 27, 22, 17, 12, 10, and 8 µg per 250 µL. A blank of *Assay diluent* is included in the assay as a non-specific background control.

Sample: Weigh NLT 3 samples, 5.0 ± 1.0 mg each, of Scaffold Human Amniotic Membrane Allograft and place into individual glass vials. Add 2.5 mL of *Assay diluent* to each vial so that the solution covers the sample. Hydrolyze the samples by placing the vials on a dry heat block for 30 ± 5 min at 100°. Centrifuge the samples, and remove and set aside the supernatant. Repeat the hydrolysis step a second time, and pool the resulting supernatants. Store the pooled hydrolysate at 2°–8° until processed in the assay.

System suitability requirements: The standard curve (R^2) must be NLT 0.98. The percent bias of the standard curve must be NMT 20% for 75% of all standard points. The percent bias of the *High system suitability solution*, *Middle system suitability solution*, and *Low system suitability solution* is NMT 30% of each level's target concentration. The percent coefficient value of the *High system suitability solution*, *Middle system suitability solution*, and *Low system suitability solution* is NMT 20%.

Analysis: To each of 3 individual, labeled, microcentrifuge tubes, add 65 µL of the pooled hydrolysate sample. Add 185 µL of *Assay diluent* to each sample tube. To generate the tropoelastin standard curve, pipette 250 µL of each *Standard solution*, in triplicate, into individual microcentrifuge tubes. Thaw the *Standard solutions*, and transfer 250 µL of each into individual microcentrifuge tubes, in triplicate. Add 250 µL of pre-chilled *Precipitating reagent* (1:1) to all test samples and standards, and incubate at room temperature for 10 min. Centrifuge all samples and standards, and discard the supernatants. Add 1 mL of elastin dye reagent (5,10,15,20-tetraphenyl-21,23-porphine tetra-sulfonate or TPPS), which complexes with solubilized elastin, to the test samples and standards. Mix all the vials vigorously on a vortex mixer, and incubate for 120 min on an orbital shaker at room temperature. Subsequently, centrifuge the samples and standards, and discard the supernatants. Add 250 µL of elastin *Dye dissociation reagent* to the pellet (to dissociate the dye from the solubilized elastin), and incubate for 10 min at room temperature. Centrifuge the samples and standards briefly to pull down all condensate off of the microcentrifuge tube walls, and then transfer the full 250 µL from each tube to a 96-well microplate. Data is acquired by spectrophotometer at an optical density (OD) of 513 nm. Determine the elastin levels in the test samples by interpolating against the tropoelastin standard curve, using an absorbance plate reader and associated software capable of reading absorbance. Additional data analysis is performed using statistical analysis software.

Calculate the total amount of elastin in mg:

$$\text{Result} = (M_E/V_S) \times V_T \times F$$

M_E = measured mass of elastin (µg)
V_S = volume of pooled hydrolysate *Sample*, 65 µL
V_T = total volume of elastin extract for each *Sample*, 5000 µL
F = conversion factor, 0.001 (µg to mg)

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Calculate the percentage of elastin (%):

$$\text{Result} = (E/S_W) \times 100$$

E = total amount of elastin (mg)
S_W = sample weight (mg)

Acceptance criteria: 9.0%–17.0% elastin content

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩
Sample: Prepare 2 cm × 3 cm of Scaffold Human Amniotic Membrane Allograft as follows. Extract the sample by immersing in endotoxin-free water and placing it on an orbital shaker in an incubator for NLT 60 min at 37°–40°.
Acceptance criteria: NMT 3.3 USP Endotoxin Units/*Sample*

• **STERILITY TESTS** ⟨71⟩
Sample: 1 cm × 2 cm of Scaffold Human Amniotic Membrane Allograft
Analysis: Test NLT 5 *Samples* in both *Soybean–Casein Digest Medium* and *Fluid Thioglycollate Medium* at an incubation temperature of 30°–35° and 20°–25°, respectively.
Acceptance criteria: Meets the requirements

• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*
Sample: Prepare 6 cm × 6 cm of Scaffold Human Amniotic Membrane Allograft as follows. Place the sample in a dry vial. Seal the vial with an aluminum seal and a cap and place it in a drying oven where the water content is thermally released and transferred to a titration vessel by means of a dried, inert purge gas.
Analysis: Proceed as directed in the chapter.
Acceptance criteria: NMT 20%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Scaffold Human Amniotic Membrane Allografts range in size from 1 cm × 2 cm to 6 cm × 6 cm per batch. Scaffold Human Amniotic Membrane Allografts are packaged in polyester-based heat-sealed laminate film peel pouches, supplied as a single-use, sterile, membrane sheet, and stored at room temperature.

• **LABELING:** The package label indicates the size of the Scaffold Human Amniotic Membrane Allograft, the lot number, the expiration date, the trade name, the required storage conditions, the dosage information, and manufacturer and/or distributor contact information. "Instructions for Use", which includes a summary of records used to make donor eligibility determination and necessary information for properly using the product for its intended use, is provided with each allograft.▲ 2S (USP41)

Scaffold Human Dermis

DEFINITION

Scaffold Human Dermis is derived from donated allograft human dermis that is processed to remove cells and freeze-dried to remove moisture while preserving biologic components and structure of the dermal matrix. It is biocompatible and supports remodeling by the recipient's own tissue. The matrix is composed of native human dermal architecture, consisting of collagen (mainly collagen Type I, with additional components of collagen Types III and IV), chondroitin sulfate and hyaluronic acid glycosaminoglycans, and elastin. The product can be provided in a sheet or powder form.

The donated human skin is processed in a manner that removes all cellular components, including the epidermal layer and dermal cells. The resulting product is then rendered into a particulate form (microsized), with a mean particle size of less than 100 µm, by processing with a freezer mill. Scaffold Human Dermis does not contain intact cells, cell nuclei, or chemically induced crosslinks. Human skin used to produce Scaffold Human Dermis is obtained from sources that have passed applicable donor eligibility requirements for relevant communicable diseases. Scaffold Human Dermis is manufactured using sterile solutions and equipment under aseptic conditions. The final product is inspected and tested to ensure that the product meets specifications.

SPECIFIC TESTS

• **HISTOLOGICAL EVALUATION**
Sucrose solution: Dissolve 20 g of sucrose in 100 mL of water.
Tissue preparation: Submerge a 1.4-cm or greater size piece of Scaffold Human Dermis in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Cut three 1.5-cm × 0.5-cm pieces of Scaffold Human Dermis, and place each piece from the same donor lot into a suitable histological tissue cassette for routine histology processing and paraffin embedding. Place the cassettes in 10% neutral buffered formalin[1] at room temperature for a minimum of 12 h. [NOTE—the cassettes may be kept at room temperature for 1 month.] From the same donor lot, cut a minimum of three 1.0-cm × 0.5-cm pieces of Scaffold Human Dermis for immunohistochemical (IHC) staining, and place in a vial containing the *Sucrose solution*. Store at between 2° and 8° for a minimum of 4 h, but the product can be held in the solution for up to 2 weeks.
Embedding and sectioning of histological samples: Place all cassettes to be processed into a suitable histological cassette basket including one cassette containing formalin-fixed normal skin to serve as a process control. The following steps can be performed using a vacuum infiltration tissue processor.[2] At room temperature (37° ± 2°), tissue samples that continue to fix in 10% neutral buffered formalin are dehydrated through graded reagent alcohol (70%, 80%, 95%, and 100%).[3] The tissue samples are cleared in a xylene substitute.[4]
The tissue is infiltrated with paraffin[5] at 60°. The following embedding procedure can be performed with the use of a histology tissue embedding unit, consisting of a thermal console, dispensing console, and cryo-console. Open the histological cassette. Partially fill embedding molds with molten paraffin (60°). While keeping the paraffin heated, position the three tissue pieces on edge in the paraffin so that cross-sections of the Scaffold Human Dermis, once they are completely embedded, can be cut. Rapidly cool the paraffin so that it partially solidifies. Completely fill the embedding mold with additional molten paraffin (60°), and cool until completely solidified.
Remove solidified paraffin blocks from embedding molds for sectioning. Sectioning of paraffin-embedded samples can be performed on a suitable microtome.[6] Clamp the tissue block into the holder of the microtome. Fill a histological water bath with fresh water. [NOTE—A quarter teaspoon of gelatin may be added to the water bath for

[1] 10% Neutral buffered formalin can be purchased through Statlab Medical Products, Inc. Lewisville, TX.
[2] A suitable processor can be the Tissue Tek V.I.P. Model #1000 vacuum infiltration tissue processor or the Leica ASP300 tissue processor.
[3] Graded alcohols: 70%, 80%, and 95% are prepared using 100% reagent alcohol, such as that purchased from Statlab Medical Products, Inc. Lewisville, TX.
[4] Such as a citrus clearing solvent from Richard-Allan Scientific, Kalamazoo, MI.
[5] Such as Paraplast, Extra, manufactured by McCormick Scientific, St. Louis, MO.
[6] Such as Leica RM 2155 or RM 2255 Rotary Microtome.

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section adhesion.] Maintain the water bath at 39° ± 2°. Cut sections to a thickness of 3–5 µm to form a ribbon, and gently lay the ribbon on the water bath. Place two consecutive sections on each glass microscope slide.

Embedding and sectioning of immunohistochemical samples: Fill histology embedding molds with a cryo-protectant embedding media. Transfer tissue pieces from the *Sucrose solution* to the embedding media in the mold, orient so that cross-sections can be cut, and let sit for 1–30 min. Freeze the molds with liquid nitrogen, and store at –80°. Remove the frozen blocks from the molds for sectioning. Cryo-section each block to 4–7 µm in thickness, and place the sections on glass microscope slides. Place at least two sections on each slide. Slides can be stored at between –70° and –85°.

Hematoxylin and eosin sample staining: [NOTE—Store all solutions at room temperature.]

1% Acid alcohol solution: Alcohol, hydrochloric acid, and water (1309:20:509)

95% Reagent alcohol solution: Alcohol and water (190:10)

80% Reagent alcohol solution: Alcohol and water (160:40)

Saturated lithium carbonate solution: Water, lithium carbonate (200:2), (v:w)

Analysis: The slide with affixed tissue (sections) is deparaffinized in three changes of xylene substitute, rehydrated in graded reagent alcohols (100% and 95%), and rinsed in tap water. The sections are stained with hematoxylin, rinsed, decolorized with *1% Acid alcohol solution*, and neutralized with *Saturated lithium carbonate solution*. The sections are counterstained with 1% alcoholic eosin Y, dehydrated through graded reagent alcohols (95% and 100%) and xylene substitute, and then coverslipped. A positive control is stained with each batch to ensure successful staining.

Acceptance criteria: Adequate staining is defined by a blue nuclear stain and a pink-red cytoplasm. Sections are assessed for tissue matrix integrity and are acceptable if they pass the following criteria: they must not show evidence of intact epidermal or dermal cells and must not show evidence of widespread collagen damage (broken, condensed, or distorted fibers) or unrecognizable papillary layer, as shown in the USP Scaffold Human Dermis Reference Photomicrographs[7] of products with acceptable appearance.

Immunohistochemical sample staining

0.5 M phosphate-buffered saline, pH 7.4 (PBS): Prepare by combining 8.50 g of sodium chloride, 0.85 g of dibasic sodium phosphate, and 0.54 g of monobasic potassium phosphate in 1 L water. Adjust the pH to 7.4 with 1.0–3.0 M sodium hydroxide. Store at room temperature.

Diluent PBS: Combine 0.250 g of bovine serum albumin, 25 mL of *PBS*, and 0.02–0.03 g of thimerosal. Store at between 2° and 8° for up to 1 year.

Primary and secondary antibodies: Perform a titer assay to determine the optimal dilution for each antibody when a new lot is used. Dilute in *Diluent PBS* as per titer assay.

Human monoclonal anti-type IV collagen:[8] 25- to 100-µL aliquots of concentrated antibody may be stored at between –70° and –85°.

Human monoclonal anti-MHC class I:[9] 100- to 200-µL aliquots of concentrated antibody may be stored at between –70° and –85°.

Human monoclonal anti-MHC class II:[10] 100- to 200-µL aliquots of concentrated antibody may be stored at between –70° and –85°.

Goat anti-mouse IgG (Fab-specific) peroxidase conjugate:[11] 100- to 200-µL aliquots of concentrated antibody may be stored at between –70° and –85°.

3,3-Diaminobenzidine staining kit or stable diaminobenzidine (DAB):[12] To make the working stain, consult kit instructions. Store the kit at between 2° and 8°. 3,3-Diaminobenzidine is light sensitive; minimum exposure to light is advisable. Stable DAB is stored at between 0° and –20°.

3% Hydrogen peroxide: Store at room temperature.

Acetone: Store at between –10° and –20°.

Analysis for monoclonal anti-MHC class I and class II: Frozen sections are obtained and slides are fixed in *Acetone*. Endogenous peroxides are blocked with *3% Hydrogen peroxide*. A cocktail of *Human monoclonal anti-MHC class I*, *Human monoclonal anti-MHC class II*, and bovine serum albumin are applied to the sections. Sections are rinsed with *PBS*, and the secondary antibody, *Goat anti-mouse IgG peroxidase conjugate*, is applied. Following the application of the secondary antibody, the sections are rinsed in *PBS*, and *DAB* is applied. The sections are then counterstained in hematoxylin, decolorized in *1% Acid alcohol solution*, neutralized in *Saturated lithium carbonate solution*, dried, and coverslipped.

Control slides: Normal skin samples are frozen and sectioned for control tissue. The positive control receives exactly the same treatment as the test samples. The negative control is the same tissue section as the positive. The difference is that one critical stain step is omitted, such as the application of the primary antibodies. All other stain steps are completed in accordance with the test slides. If acceptable control stains are not achieved, the stain is repeated.

Acceptance criteria: Adequate positive staining of sections will appear brown in color, focal, and cell-associated and will be clearly distinct from the background, as shown in the USP Scaffold Human Dermis Reference Photomicrographs of products with acceptable appearance for staining with MHC I and II antibodies. Negative stain results appear clear with no brown staining; only a light blue hematoxylin counterstain will be seen.

Analysis for monoclonal anti-type IV collagen: Frozen sections are obtained, and slides are fixed in *Acetone*. Endogenous peroxides are blocked with *3% Hydrogen peroxide*. *Human monoclonal anti-type IV collagen* along with bovine serum albumin are applied to the sections. Sections are rinsed with *PBS*, and the secondary antibody, *Goat anti-mouse IgG peroxidase conjugate*, is applied. Following the application of the secondary antibody, the sections are rinsed in *PBS*, and *DAB* is applied. The sections are then counterstained in hematoxylin, decolorized in *1% Acid alcohol solution*, neutralized in *Saturated lithium carbonate solution*, dried, and coverslipped.

Control slides: Normal skin samples are frozen and sectioned for control tissue. The positive control receives

[7] These photomicrographs are available as a CD from the USP Reference Standards collection, available to the reader through USP Customer Services. To order these and other Reference Standards, call 1-800-227-8772 (U.S. and Canada), +1-301-881-0666 or 00-800-4875-5555 (select Europe); or go online to www.usp.org. Order item number 1535813.

[8] A suitable primary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C1926.

[9] A suitable primary antibody is available from VMRD Inc., PO Box 502, Pullman, WA 99163, catalog #H58A.

[10] A suitable primary antibody is available from VMRD Inc., PO Box 502, Pullman, WA 99163, catalog #TH14B.

[11] A suitable secondary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103 cat #A9917.

[12] DAB can be sourced from Invitrogen/Zymed, Serotec, or Dako.

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exactly the same treatment as the test samples. The negative control is the same tissue section as the positive. The difference is that one critical stain step is omitted, such as the application of the primary antibodies. All other stain steps are completed in accordance with the test slides. If acceptable control stains are not achieved, the stain is repeated.

Acceptance criteria: Adequate positive stain results will reveal a brown stain in the location of the basement membrane, as shown in the USP Scaffold Human Dermis Reference Photomicrographs of products with acceptable appearance for staining with collagen type IV antibodies. Negative stain results appear clear with no brown staining; only a light blue hematoxylin counterstain will be seen.

• **BIOCHEMICAL ANALYSIS**

Sample solution: Submerge 20 mg of Scaffold Human Dermis in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Freeze the sample, and mill using a freezer mill.[13] Suspend in 10 mL of 4 M guanidine hydrochloride, 50 mM sodium acetate, 5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM *N*-ethylmaleimide (NEM), 0.2% Triton X-100, pH 5.8, and extract for 48 h at 4°, with agitation. Centrifuge for 10 min at 4° and 23,500 *g*. Remove the supernatant, and digest with 20 µL of chondroitinase ABC,[14] 1 milli-Unit/µL. Analyze for *Glycosaminoglycan (GAG) content* and *Immunochemical analysis of decorin* (below). For *Collagen content* analysis, wash the pellet three times with 10 mL of deionized water, centrifuging for 10 min at 4° and 23,500 *g* after each wash. Resuspend in a minimal amount of water, freeze at –80°, and lyophilize. Resuspend 20 mg of the lyophilized pellet in 20 mL of 0.5 M acetic acid containing 100 µg of pepsin per mg of tissue, and incubate overnight at 4° with agitation. Remove undigested material by centrifugation at 23,500 *g* for 20 min.

Collagen content

Sample buffer: Prepare as directed for *Sample Buffer 1* in *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩, *Electrophoretic Separation*.

Sample collagen solution: Add 20 µL of the supernatant from the *Sample solution* to 20 µL of *Sample buffer*, mix, and incubate at 60° for 15 min.

Control samples: Prepare suitable positive control samples (human placental collagen types I[15] and III[16]) similar to *Sample collagen solution*.

Gel electrophoresis: Load 40 µL of each of *Sample collagen solution* and the *Control samples* onto a 6% acrylamide gel, perform the electrophoretic separation, and stain with Coomassie brilliant blue as directed under *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩.

Acceptance criteria: The bands obtained from Scaffold Human Dermis are distinct and clearly visible at a mobility similar to that of the bands obtained from the collagen types I and III control samples. No smears corresponding to degraded collagen types I and III are found on the gel.

Glycosaminoglycan (GAG) content

Sample GAG solution: Use 15 µL of the supernatant prepared as directed for *Sample solution*.

DMMB reagent: Dissolve 16 mg of 1,9 dimethyl-methylene blue (DMMB) in 5 mL ethanol. Add 3.24 mL of formic acid and 2.94 mL of 10 M sodium hydroxide. Bring to 1000 mL with water.

50 mM Sodium acetate solution: Dissolve 1.03 g of sodium acetate in 250 mL of water.

Analysis: Add 15 µL of *Sample GAG solution* to each well of a 96-well microtiter plate. To each sample well, add 200 µL of *DMMB reagent* and 35 µL of *50 mM Sodium acetate solution*. Read the samples on a spectrophotometric microplate reader at 540 nm, using 595 nm as a reference. Perform the same procedure on a sample of bovine collagen type I[17] negative control and positive controls hyaluronic acid[18] and chondroitin sulfate,[19] using 15 µL of 3 mg/mL solutions from each of the controls.

Acceptance criteria: Scaffold Human Dermis contains glycosaminoglycans (GAG) as represented by positive control standards. GAG concentration can be determined by the preferential binding of DMMB dye to negatively charged ions such as sulfated GAGs.

Immunochemical analysis of decorin

Sample decorin solution: Submerge 100–200 mg of Scaffold Human Dermis in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Using a freezer mill as outlined above, extract the tissue in a solution of 4 M guanidine hydrochloride, 50 mM sodium acetate (pH 5.8) containing 5 mM EDTA, 0.1 mM PMSF, and 10 mM NEM for 24–48 h with agitation at 4°. Centrifuge the extracted tissue at 23,500 *g* for 10 min, and remove the supernatant.

Nitro blue tetrazolium (NBT): Dissolve 0.5 g in 10 mL of 70% dimethyl sulfoxide (DMSO). Store in the dark at room temperature.

5-Bromo-4-chloro-3-indolyl phosphate (BCIP): Dissolve 0.5 g in 10 mL of 100% DMSO. Store in the dark at room temperature.

Alkaline phosphatase buffer: 100 mM sodium chloride, 5 mM magnesium chloride, 100 mM Tris(hydroxymethyl) aminomethane hydrochloride, pH 9.5. Filter, and store at 4°.

Chromogenic substrate mixture: Mix 66 µL of *NBT* and 33 µL of *BCIP* in 10 mL of *Alkaline phosphatase buffer*.

Blotting buffer 1: 50 mM Tris buffered saline, pH 7.4, with 0.1% Tween 20 (TBST)

Blotting buffer 2: 5% blotting grade milk in TBST

Blotting buffer 3: 0.1% blotting grade milk in TBST

Analysis: Run an aliquot of *Sample decorin solution* on an 8% polyacrylamide gel at 100 V for approximately 60 min along with 100 µg of recombinant decorin[20] positive control, bovine type I collagen[21] negative control, and prestained molecular weight marker, and transfer to a polyvinylidene fluoride (PVDF) membrane according to the following procedure. In a shallow tray, soak two sheets of Whatman paper and two pieces of sponge in transfer buffer (200 mM glycine, 25 mM tris, and 20% methanol, pH 8.3). Soak the transfer membrane in methanol before soaking in the transfer buffer. Equilibrate the gel in transfer buffer for 10 min. Assemble the tank transfer apparatus according to manufacturer's instructions. Layer the following items within the apparatus in proper order: sponge, paper, gel, PVDF membrane, paper, and sponge.

[13] Such as SPEX/CertiPrep Freezer/Mill.

[14] Suitable chondroitinase is available from Seikagaku, Tokyo, Japan, catalog #100330.

[15] Suitable type I collagen is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C7774.

[16] Suitable type III collagen is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C4407.

[17] Suitable type I collagen is available from Invitrogen, 1600 Faraday Ave., PO Box 6482, Carlsbad, CA, 92008 catalog #100.

[18] Suitable hyaluronic acid is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #H1876.

[19] Suitable chondroitin sulfate is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C4384.

[20] Suitable decorin is available from R&D Systems, 614 McKinley Place NE, Minneapolis, MN 55413, catalog #143DE100.

[21] Suitable type I collagen is available from Invitrogen, 1600 Faraday Ave., PO Box 6482, Carlsbad, CA 92008.

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Clamp the apparatus closed. Connect the electrodes, and transfer the gel bands to the membrane at about 100 V at 4° for about 1 h. Using prestained protein molecular weight marker, and staining the gel after electro-blotting, allow for visual determination of transfer completion. When transfer is complete, disconnect the transfer apparatus, and carefully remove the membrane. Block the membrane for at least 1 h with *Blotting buffer 2*, cover, and shake gently for 30 min. Wash the membrane three times for 5 min each with *Blotting buffer 1*. Add rabbit anti-human decorin primary antibody,[22] diluted to 2 µg/mL in *Blotting buffer 3*, to the membrane, and incubate for 1 h with gentle shaking. Wash the membrane three times for 5 min with *Blotting buffer 1*. Add goat anti-rabbit IgG secondary antibody conjugated[23] to alkaline phosphatase, diluted 1:3000 in *Blotting buffer 3*, to the membrane, and incubate for 1 h with gentle shaking. Wash the membrane three times for 5 min with *Blotting buffer 1*. Add 10 mL of *Chromogenic substrate mixture*, and incubate until color has developed. When the bands are clearly visible, stop the development reaction by rinsing the membrane with water.

Acceptance criteria: The decorin band obtained from Scaffold Human Dermis is the only band on the gel and is clearly visible at a mobility similar to the band obtained from the control sample recombinant decorin.

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. Scaffold Human Dermis meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **PARTICLE SIZE ANALYSIS**

(See *Particulate Matter in Injections* ⟨788⟩.)

[NOTE—Test specific to the powder form]

Sample: 100 mg of powder form of Scaffold Human Dermis material

Analysis: Add the *Sample* to 25 mL of saline in a clean 50-mL conical tube. Rehydrate and disperse the particles by probe sonication at 20 watts for 60 s. Place an appropriate aliquot of sample suspension in an appropriate liquid particle counter (LPC) for analysis by laser light obscuration (light extinction).

Acceptance criteria: The mean particle size is NMT 100 µm.

ADDITIONAL REQUIREMENTS

- **LABELING:** The package label indicates the weight of the Scaffold Human Dermis material enclosed. The label also contains the lot number, the expiration date, the trade name, the required storage conditions, the dosage information, and manufacturer and/or distributor contact information. "Instructions for Use," which includes a summary of records used to make donor eligibility determination and necessary information for properly using the product for its intended use, is provided with each unit.
- **PACKAGING AND STORAGE:** Scaffold Human Dermis is aseptically packaged and supplied in a freeze-dried configuration in a double pouch configuration. It is supplied in various sizes and thicknesses. The powder configuration is supplied in a single-use delivery syringe packaged in a hermetically sealed pouch. Scaffold Human Dermis is stored between 1° and 10° and labeled with the expiry date.

[22] Suitable primary antibody is available from BioVision, 980 Linda Vista Ave., Mountain View, CA 94043, catalog #3645100.

[23] A suitable secondary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, cat #A9917.

- **USP AUTHENTIC VISUAL REFERENCES** ⟨11⟩

USP Scaffold Human Dermis Reference Photomicrographs: the samples were prepared as directed in the test for *Histological Evaluation*. These photomicrographs show the histological appearance of passing histological evaluation with no evidence of intact epidermal or dermal cells, and no evidence of collagen damage (photomicrographs 2–6) and of failed material with collagen damage and condensed papillary dermis or attached epidermal remnant (photomicrographs 7–10). Passing and failed material are compared to photomicrograph 1 showing the staining of unprocessed normal human skin with intact epidermal or dermal cells. Photomicrographs 11 and 12 are negative and positive controls for the analysis of immunostaining with MHC I and II antibodies. Photomicrographs 13 and 14 are negative and positive controls for the analysis of immunostaining with collagen type IV antibodies. For both types of staining, a passing positive stain will appear brown in color, focal, and cell-associated, whereas a failed negative stain will appear clear with no brown staining.

Scaffold Porcine Bladder

DEFINITION

Scaffold Porcine Bladder is derived from porcine urinary bladder walls obtained from a certified closed pathogen-free herd. The bladder walls are processed by both mechanical and chemical methods that result in a sterile, decellularized material retaining a basement membrane with type IV collagen. Scaffold Porcine Bladder is manufactured in single and multi-layered lyophilized sheets as well as powder forms.

IDENTIFICATION

[NOTE—Powdered and multi-layered product forms must meet the requirements for *Identification* as single sheets before conversion to the other product types.]

- **A. IMMUNOHISTOCHEMISTRY FOR TYPE IV COLLAGEN**

Diluent: Tris-buffered saline (TBS) containing suitable amounts of normal serum, surfactant, and a preservative[1]

Primary antibody: Rabbit anti-human collagen IV,[2] cross-reactive with the corresponding porcine protein, diluted 1:500 in *Diluent*

Negative control solution: Diluted normal rabbit serum[3]

Secondary antibody: About 25 µg/mL of polymerized alkaline phosphatase-labeled anti-rabbit IgG[4] in *Diluent*

Sample preparations: Formalin-fixed, paraffin-embedded tissue samples are prepared, one per device, and sectioned at a thickness of 5 µm using suitable, validated methods. [NOTE—See also *Preparation of Biological Specimens for Histologic and Immunohistochemical Analysis* ⟨1285⟩ for helpful but not mandatory guidance.] Positive controls include normal porcine bladder, which is known to contain collagen IV at the location of the epithelial basement membrane. An additional sample of Scaffold Porcine Bladder is used as a negative control and incubated with *Negative control solution* rather than *Primary antibody* in the *Analysis*. One negative and one positive control section are used per test group. Before staining, sections are dewaxed with a suitable solvent[5] for 5 min, immersed in 100%

[1] Included in Leica Biosystems kit catalog #AR9352 or a suitable alternative.

[2] Abcam catalog #ab6586 or a suitable alternative.

[3] Leica Biosystems catalog #PA0777 or a suitable alternative.

[4] A suitable antibody source is catalog #BA-1000, from Vector, Burlingame, CA.

[5] Leica Biosystems catalog #AR9222 or a suitable alternative.

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ethanol for 5 min, then washed after the alcohol step and between each subsequent immunohistochemical step in a suitable buffer[6] three times for 5 min each. Note that all immersions and washes are done at ambient temperatures.

Analysis: *Sample preparations* are then treated for 10 min with a suitable enzyme[7] to retrieve the type IV collagen antigen followed by rinsing as above. Except for the negative control sample, the *Primary antibody* is then added for 30 min followed by rinsing as described above. For the negative control sample, *Negative control solution* is added to the sample for this incubation, rather than *Primary antibody*. Next the *Secondary antibody* is added for 30 min followed by rinsing as described above and then a final rinse in deionized water for 5 min. A suitable enzyme[8] substrate is added for 5 min followed by three rinses of 5 min each in deionized water to stop the reaction. The tissue is then stained with a hematoxylin solution[9] for 5 min followed by two rinses of 5 min each in deionized water then one rinse in a suitable buffer[10] for 5 min. The tissue is then placed in a clarifying buffer[11] for 10 min, followed by three washes for 5 min each in a suitable buffer. The tissue sections are then dehydrated in 100% alcohol, followed by xylene, and then a coverslip is mounted on each section using a suitable mounting medium.[12] Sections are examined with a light microscope at 40× magnification.

System suitability criteria: Negative controls should not contain any magenta staining, and positive controls should have magenta staining.

Acceptance criteria: Intense magenta staining (intensity of magenta staining should be similar to that observed with the positive controls) for the basement membrane protein type IV collagen must be observed on the luminal surface of the Scaffold Porcine Bladder sample.

- **B. Periodic Acid-Schiff Stain for Basement Membrane Structure**

Sample preparations: Formalin-fixed, paraffin-embedded tissue samples are prepared, one per device, and sectioned at a thickness of 5 µm using suitable, validated methods. [Note—See also ⟨1285⟩ for helpful but not mandatory guidance.] Positive controls include normal porcine bladder and a canine positive tissue control. Two positive control sections are used per test group. Before staining, sections are dewaxed with a suitable solvent[13] for 5 min, then immersed in 100% ethanol for 5 min, followed by a graded series of alcohols of 5 min each, ending in distilled water for 5 min.

Analysis: *Sample preparations* are placed in a 0.5% periodic acid solution[14] for 5 min followed by several washes in distilled water. The sections are then placed in a room temperature Schiff reagent solution[15] for 15 min, followed by rinsing with running, lukewarm water for 10 min. The sections are then immersed in a hematoxylin solution[16] for about 1 min, followed by rinsing in distilled water for 30 s. Sections are then placed in a bluing reagent[17] for 1 min, followed by rinsing in distilled water for 30 s. The stained sections are then placed in 95% ethanol for 1 min, then 100% ethanol for 1 min, then three washes in xylene for 1 min each, followed by mounting a coverslip over each section using a suitable mounting medium.[18] Stained sections are examined with a light microscope at 40× magnification.

System suitability criteria: Positive controls should have dark purple staining.

Acceptance criteria: Intense dark purple staining (intensity of dark purple staining should be similar to that observed with the positive controls) for basement membrane must be observed on the luminal surface of a single layer of scaffold.

SPECIFIC TESTS

- **Tensile Strength of Sheet Forms**

[Note—Powdered products must meet the requirement for *Tensile Strength of Sheet Forms* before conversion to powder.]

Sample preparation: Cut the test specimens using a dogbone die template with a narrow width of 0.125 in and an overall length of 2.50 in, from a representative of the final product.

Analysis: Hydrate the material. Test using an Instron material testing system. Set the distance between the grips to 1 in and the crosshead speed to 0.5 in/min (12.7 mm/min). Secure the test sample in the bottom and top test grips. Start the test and run until sample failure.

Calculations: To determine strain, take the extension at failure (l_f), subtract the extension at pre-load (l_i), and divide the difference by extension at pre-load (l_i). Multiply by 100 to convert to a percentage.

Acceptance criteria: >10% strain

- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Bacterial Endotoxins Test** ⟨85⟩**:** It contains NMT 20 USP Endotoxin Units/device or per 1000 mg of powder.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Sheets are packaged in single-use, double barrier, peel-open pouches. Powder forms are stored in a glass vial or other container within a peel-open package. Store under clean, dry conditions, between 15° and 30°.
- **Labeling:** The package label indicates dimensions or mg, as applicable, of the Scaffold Porcine Bladder device, as well as the lot number, expiration date, and required storage conditions. The label is also marked "Sterile" followed by an "R" for irradiation method of sterilization.

Scaffold Porcine Small Intestinal Submucosa

DEFINITION

Scaffold Porcine Small Intestinal Submucosa is a collagen-based scaffold, translucent and off-white in color. It is obtained from the small intestinal submucosa layer of the domestic pig (*Sus scrofa* L.). This layer has been mechanically separated from the adjoining layers of the intestine to remove the serosal, mucosal, and muscular elements. The isolated submucosa is chemically cleaned, decellularized, freeze-dried, and terminally sterilized. Scaffold Porcine Small Intestinal Submucosa also undergoes a viral inactivation; the inactivation method is validated using parvovirus, reovirus, pseudorabies virus, and leukemia retrovirus as the test viruses. By dried weight, Scaffold Porcine Small Intestinal Submucosa consists of about 70% protein, about 20%

[6] Leica Biosystems catalog #AR9590 or a suitable alternative.
[7] Leica Biosystems catalog #AR9551 or a suitable alternative.
[8] Included in Leica Biosystems kit catalog #DS9390 or a suitable alternative.
[9] Dako catalog #CS700 or a suitable alternative.
[10] Leica Biosystems catalog #AR9590 or a suitable alternative.
[11] Leica Biosystems catalog #3802918 or a suitable alternative.
[12] ThermoScientific catalog #8312-4 or a suitable alternative.
[13] Leica Biosystems catalog #AR9222 or a suitable alternative.
[14] Richard Allen catalog #87007 or a suitable alternative.
[15] Richard Allen catalog #87007 or a suitable alternative.
[16] Richard Allen catalog #87007 or a suitable alternative.
[17] Richard Allen catalog #7301 or a suitable alternative.
[18] ThermoScientific catalog #8312-4 or a suitable alternative.

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carbohydrate, and about 7% lipid. The protein component is primarily collagen type I, with minor amounts of elastin and collagen type III, collagen type IV, and collagen type VI. In addition to these components, additional extracellular matrix components, such as glycosaminoglycans and fibroblast growth factor-2, are also retained.

SPECIFIC TESTS

- **FIBROBLAST GROWTH FACTOR-2 CONTENT**

Sterile PBS solution: 8.065 g/L of sodium chloride and 0.2 g/L of potassium chloride in 0.01 M sodium phosphate buffer, pH 7.4

Sample solution: Obtain a 1-cm^2 sample of Scaffold Porcine Small Intestinal Submucosa, weigh, and submerge in 400 µL of *Sterile PBS solution*. Pulverize the tissue for 90 s using a tissue grinder, intermittently checking to be sure that the tissue remains immersed in the *Sterile PBS solution* and becomes homogenized. Centrifuge at 12,000 × *g* for 5 min at 4°. Use immediately upon preparation.

Analysis: Examine duplicate aliquots of the *Sample solution* by a suitably sensitive ELISA method.[1]

System suitability: The analysis is considered valid if the ELISA kit generates a linear standard curve with the square of the correlation coefficient (r^2) NLT 0.95, and if the duplicate aliquots of the *Sample solution* yield results that are within 20% of each other.

Acceptance criteria: The average content of fibroblast growth factor-2 is NLT 10,000 pg/g of Scaffold Porcine Small Intestinal Submucosa.

- **GLYCOSAMINOGLYCAN CONTENT**

1,9-Dimethylmethylene blue solution: Mix 95 mL of 0.1 M hydrochloric acid in 500 mL of water. Add 16 mg of 1,9-dimethylmethylene blue, 3.04 g of aminoacetic acid, and 2.37 g of sodium chloride. Dilute with water to 1 L, and adjust to a pH of 3.0 with sterile solutions of either 1.0 M sodium hydroxide or 1.0 M hydrochloric acid. Store in low-actinic glassware.

Sterile PBS solution: 8.065 g/L of sodium chloride and 0.2 g/L of potassium chloride in 0.01 M sodium phosphate buffer, pH 7.4

Proteinase K solution: 600 Units/mL of *Tritirachium album* proteinase K

Stock heparin standard solution: 1 mg/mL of heparin

Heparin standard curve solutions: Use *Stock heparin standard solution* to prepare three solutions containing 20, 50, and 100 µg/mL of heparin.

Sample solution: Prepare samples in duplicate. Accurately weigh about 25 mg of Scaffold Porcine Small Intestinal Submucosa and cut into small pieces (roughly 2 mm × 2 mm). Transfer to a 1.5-mL microcentrifuge tube, and add 180 µL of *Sterile PBS solution* and 20 µL of *Proteinase K solution*. Mix, and incubate the sample at 56° for 15 min; during the incubation, mix intermittently on a vortex mixer. Cool the sample to room temperature. Dilute with water to obtain a concentration of 12.5 mg/mL of digested Scaffold Porcine Small Intestinal Submucosa.

Blank solution: Water

Collagen control solution: Accurately weigh about 25 mg of a bovine collagen, type I, that contains less than 1 µg of glycosaminoglycan/mg. Transfer to a 1.5-mL microcentrifuge tube, and add 180 µL of *Sterile PBS solution* and 20 µL of *Proteinase K solution*. Mix, and incubate the sample at 56° for 15 min; during the incubation, mix intermittently on a vortex mixer. Cool the sample to room temperature. Dilute with water to obtain a concentration of 12.5 mg/mL of digested bovine collagen.

Analysis

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Add 2.5 mL of *1,9-Dimethylmethylene blue solution* to triplicate 100-µL aliquots each of *Heparin standard curve solutions*, *Sample solution*, *Blank solution*, and *Collagen control solution*. Mix on a vortex mixer for 1 s and immediately read the absorbance at 525 nm. Generate a standard curve of absorbance versus concentration using the averages of each *Heparin standard curve solution*, correcting for the blank, and calculate the regression line and regression coefficient. The concentration of glycosaminoglycan in the *Sample solution* and the *Collagen control solution* is determined directly from the regression line. If the absorbance of the *Sample solution* is greater than the highest *Heparin standard curve solution*, then dilute the *Sample solution* appropriately, and repeat the *Analysis* detailed in this section.

System suitability: The test is considered valid if the regression curve has a square of the correlation coefficient (r^2) NLT 0.95; the triplicate aliquots of the *Sample solution* and *Collagen control solution* yield results that are within 20% of each other, respectively; and the average glycosaminoglycan content of the *Sample solution* is statistically greater than the *Collagen control solution* using one-tailed, unequal variances, *t*-test at $\alpha = 0.05$.

Acceptance criteria: The average glycosaminoglycan content of the *Sample solution* is NLT 2 µg/mg.

- **BIOACTIVITY**

[NOTE—Aseptic cell culture techniques must be used throughout the performance of this test.]

Modified RPMI-1640 culture medium: Prepare a sterile solution that contains the components included in *Table 1*.

Table 1

Component	Content (mg/L)
Calcium chloride	264.9
Ferric nitrate, nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate, heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic, monohydrate	125.0
Glucose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride, monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0

[1] A suitably sensitive ELISA test kit for the quantitation can be obtained from R&D Systems Inc., 614 McKinley Place N.E., Minneapolis, MN; product number DFB50.

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Table 1 *(continued)*

Component	Content (mg/L)
L-Tyrosine	72.0
L-Valine	93.6
L-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0
Sodium 1-heptanesulfonic acid	2383.0

Penicillin–streptomycin solution: 10,000 USP Penicillin Units of penicillin/mL and 10 mg of streptomycin/mL in a suitable buffer[2]

PC12 cell line culture medium: Mix 420 mL of *Modified RPMI-1640 culture medium*, 50 mL of horse serum,[3] 25 mL of fetal bovine serum,[4] and 5 mL of *Penicillin–streptomycin solution*. Sterilize by passing through a 0.22-µm filter.

Sterile PBS solution: 8.065 g/L of sodium chloride and 0.2 g/L of potassium chloride in 0.01 M sodium phosphate buffer, pH 7.4

Rat tail collagen solution: Prepare a suspension containing 0.2 mg/mL of rat tail collagen, type I, in sterile water.

Cell culture apparatus: Prepare by adding a sufficient volume of *Rat tail collagen solution* to completely cover the bottom of each well of a 12-well cell culture plate (dimension of each well is about 22–23 mm in diameter and about 17–18 mm in depth). Incubate under sterile conditions for 2 h at 37° or overnight at room temperature. Remove the *Rat tail collagen solution* by aspiration. Rinse with *Sterile PBS solution* that has been preheated to 37°.

PC12 cells: Use cultured rat pheochromocytoma cells (ATCC CRL-1721).

Cultivation of PC12 cells: Prewarm *PC12 cell line culture medium* to 37°. Add 15 mL of prewarmed *PC12 cell line culture medium* to a T-75 culture flask. Place a single vial containing the frozen *PC12 cells* in a 37° water bath with gentle agitation until they start to thaw (about 1 min). Complete the thawing procedure by slowly rotating the vial between the hands. Rinse the outside of the vial with 70% alcohol. Transfer the contents of the vial to the T-75 flask, and mix. Incubate the cells overnight at 37° in a 5% carbon dioxide atmosphere. Transfer the contents of the T-75 culture flask to a sterile centrifuge tube, centrifuge at 200 × *g* for 5 min at 37°, and discard the supernatant. Resuspend the cells in 15 mL of *PC12 cell line culture medium*, and transfer the contents back to the T-75 culture flask. Incubate the cells at 37° in a 5% carbon dioxide atmosphere for 3 days.

Cell feeding: At the end of 3 days, the cells will need to be fed for optimal growth. To feed the cells, remove a flask of cells from the incubator, tightening the cap in the process. Examine the T-75 flask under the microscope and check for microbial contamination and confluency. If there is microbial contamination, then discard the flask. If the cells appear confluent, follow the instructions below for perpetuating the PC12 cell line (see *Culture perpetuation*). Otherwise, harvest the cells from the flask by pipetting the contents of the flask across the bottom of the flask several times. Transfer the cell suspension to a sterile 50-mL centrifuge tube. Centrifuge the cells at 200 × *g* for 5 min at 37°, and discard the supernatant. Resuspend the cells in 13 mL of *PC12 cell line culture medium*, prewarmed to 37°. Transfer the cell suspension back to the T-75 flask, and mix. Loosen the cap of the flask, and return to the incubator; incubate the cells at 37° in a 5% carbon dioxide atmosphere for another 3–7 days.

Culture perpetuation: To perpetuate a line of *PC12 cells* for culture, examine under the microscope a T-75 flask containing cells and check for microbial contamination and confluency. If there is microbial contamination, discard the flask and use another. If the cells do not appear confluent, then follow the instructions above for feeding the PC12 cell line (see *Cell feeding*), beginning with "Otherwise, harvest the cells from the flask by pipetting the contents of the flask across the bottom of the flask several times." If the cells are confluent and there is no contamination, harvest the cells from the flask by pipetting the contents of the flask across the bottom of the flask several times to loosen up the cells from their attachment to the bottom of the flask and to break up cell clusters. Check under the microscope before proceeding to ensure that most of the cells have detached from the plastic. Transfer the cell suspension to a sterile 50-mL centrifuge tube, and centrifuge the cells at 200 × *g* for 5 min at 37°. Discard the supernatant and resuspend the cells with 10 mL of *PC12 cell line culture medium*, prewarmed to 37°. Dispense an equal amount of the cell suspension into each of 3–5 T-75 flasks, each flask containing 10 mL of *PC12 cell line culture medium*, prewarmed to 37°, and mix. Return the passaged cells to the incubator, being sure to loosen the cap of the flasks. Incubate the cells at 37° in a 5% carbon dioxide atmosphere. Feed the cells after 3 days as directed above, beginning with "To feed the cells, remove a flask of cells from the incubator, tightening the cap in the process." [NOTE—Do not use cells that have undergone more than 15 passages after obtaining them from ATCC.]

Positive control solution: Prepare a solution containing about 10 ng of fibroblast growth factor-2/mL of *PC12 cell line culture medium*.

Negative control solution: Use *PC12 cell line culture medium*.

Sample solution: Immerse 70 cm² of Scaffold Porcine Small Intestinal Submucosa in sterile water for 5 min. Remove the Scaffold Porcine Small Intestinal Submucosa, and blot excess water using sterile gauze. Weigh the rehydrated Scaffold Porcine Small Intestinal Submucosa to the nearest 0.1 g and add *Modified RPMI-1640 culture medium* at a ratio of 7.5 mL of *Modified RPMI-1640 culture medium* for each 1.0 g of Scaffold Porcine Small Intestinal Submucosa. Incubate for 24 h at 37° with constant shaking. Remove the Scaffold Porcine Small Intestinal Submucosa, and pass the solution through a 0.22-µm filter. Add sufficient quantities of sterile horse serum and sterile fetal bovine serum to concentrations of 10% and 5%, respectively, and add a sufficient quantity of *Penicillin–streptomycin solution* such that there are 100 USP Penicillin Units and 0.1 mg of streptomycin/mL. Adjust the pH of the *Sample solution* to 7.4, using a sterile solution of either 1.0 M sodium hydroxide or 1.0 M hydrochloric acid.

[2] A suitable buffered solution containing 10,000 USP Penicillin Units of penicillin/mL and 10 mg of streptomycin/mL can be obtained from Sigma-Aldrich Corp., St. Louis, MO.

[3] A suitable horse serum can be obtained from American Type Culture Collection, P.O. Box 1549, Manassas, VA (www.atcc.org).

[4] A suitable fetal bovine serum can be obtained from American Type Culture Collection, P.O. Box 1549, Manassas, VA (www.atcc.org).

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Analysis: Harvest a flask of confluent *PC12 cells* by pipetting the contents of the flask across the bottom of the flask to loosen the cells, then transfer the cell suspension to a centrifuge tube and centrifuge at 200 × *g* for 5 min. Remove the supernatant by aspiration, and resuspend the pellet to obtain a concentration of about 1 × 10^6 cells/mL of *PC12 cell line culture medium*. Add to each of three wells of the *Cell culture apparatus* 1.0 mL of *Negative control solution*. To a second set of three wells, add to each well 1.0 mL of *Positive control solution*, and to a third set of three wells, add to each well 1.0 mL of *Sample solution*. Add to each well about 20,000 cells, mix by gentle rocking, and incubate for 48 h at 37°. For each well, count three random microscopic fields of cells using a microscope with a 10× ocular lens and a 20× objective lens. Each field should have at least 20 cells; avoid large clumps of cells where individual cell bodies cannot be ascertained. Determine the total number of cells in the field and, using USP Cultured Rat Pheochromocytoma Reference Photomicrographs of normal and differentiated rat pheochromocytoma cells for comparison, determine the total number of cells that have formed at least one neurite-like extension at least twice the diameter of a normal, undifferentiated cell body. For each experimental group, record the total number of cells counted and the total number of cells differentiated across all three wells, and calculate the total percentage of cells that have differentiated.

System suitability: For a test to be valid, the following criteria must be met: (1) none of the wells are microbially contaminated; (2) the weighted percentage of differentiated cells across the *Negative control solution* wells is NMT 5%; (3) the weighted percentage of differentiated cells across the *Positive control solution* wells is NLT 6%; and (4) the weighted percentage of differentiated cells across the *Negative control solution* wells is statistically less than the weighted percentage of differentiated cells across the *Positive control solution* wells, using a one-sided, two-sample test for proportions at $\alpha = 0.05$.

Acceptance criteria: The weighted percentage of differentiated cells incubated in the *Sample solution* wells is statistically greater than those incubated in the *Negative control solution* wells, using a one-sided, two-sample test for proportions at $\alpha = 0.05$.

- **METABOLIC ACTIVITY ASSESSMENT**

Dulbecco's modified Eagle's tissue culture medium: Prepare a solution that contains the components included in *Table 2*.

Table 2

Component	Content (mg/L)
Calcium nitrate, tetrahydrate	100.0
Ferric nitrate, nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate, anhydrous	48.840
Sodium chloride	6,000.0
Sodium bicarbonate	1,500.0
Sodium phosphate, dibasic (anhydrous)	800.0
Glucose	4,500.0
Glutathione (reduced)	1.0
Phenol red	5.0
Sodium pyruvate	110.0
L-Arginine (free base)	200.0
L-Asparagine, monohydrate	56.620
L-Aspartic Acid	20.0
L-Cystine dihydrochloride	65.20
Aminoacetic acid	10.0
L-Histidine (free base)	15.0
Hydroxy-L-proline	20.0
L-Isoleucine	50.0
L-Leucine	50.0
L-Lysine hydrochloride	40.0
L-Methionine	15.0
L-Phenylalanine	15.0
L-Proline	20.0
L-Serine	30.0
L-Threonine	20.0
L-Tryptophan	5.0
L-Tyrosine, disodium, dihydrate	28.830
L-Valine	20.0
D-Biotin	0.20
D-Calcium pantothenate	2.50
Choline chloride	3.0
Folic acid	1.0
Inositol	35.0
Nicotinamide	1.0
p-Aminobenzoic acid	1.0
Pyridoxine hydrochloride	1.0
Riboflavin	0.20
Thiamine hydrochloride	1.0
Cyanocobalamine	0.0050

MTT reagent: A suitable solution of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide.[5]

Detergent reagent: A suitable sodium dodecyl sulfate detergent solution.[6]

Analysis: Remove three 12-mm diameter circular sections of Scaffold Porcine Small Intestinal Submucosa, using the appropriate size biopsy punch. Immerse each section into individual wells of a 12-well cell culture plate (dimension of each well is about 22–23 mm in diameter and about 17–18 mm in depth), each containing 1 mL of *Dulbecco's modified Eagle's tissue culture medium*. Prepare a positive control by harvesting a full-thickness section of porcine jejunum immediately following slaughter. Rinse the section of jejunum at 37° in isotonic sodium chloride solution for 5 min to remove intestinal debris. Using scissors, split open the section of jejunum to form a sheet. Remove three

[5] A suitable solution of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide can be obtained as part of the MTT Cell Proliferation Assay (Catalog # 30-1010K) from American Type Culture Collection, P.O. Box 1549, Manassas, VA (www.atcc.org) or equivalent.

[6] A suitable sodium dodecyl sulfate detergent reagent can be obtained as part of the MTT Cell Proliferation Assay (Catalog #30-1010K) from American Type Culture Collection, P.O. Box 1549, Manassas, VA (www.atcc.org) or equivalent.

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12-mm diameter circular sections of jejunum, using the appropriate size biopsy punch. Immerse each section into individual wells of a 12-well cell culture plate, each well containing 1 mL of *Dulbecco's modified Eagle's tissue culture medium*. Treat these positive control wells in the same manner as the sample wells. Prepare a *Blank solution* using 1 mL of *Dulbecco's modified Eagle's tissue culture medium*. Allow sections to hydrate for 5 min, add 50 µL of *MTT reagent* to each of the sections and the blank, and mix. Incubate for 3 h at 37° in an atmosphere containing 5% carbon dioxide. Add 100 µL of *Detergent reagent* to each well, and mix. Leave the samples at ambient temperature in the dark for 2 h. Measure the absorbance of the resulting solution at 570 nm, adjusting for the blank.

System suitability: The average absorbance in the positive control wells is greater than 0.100.

Acceptance criteria: The average absorbance reading for the Scaffold Porcine Small Intestinal Submucosa wells is less than 0.100.

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩

Sample: 70 cm^2 of Scaffold Porcine Small Intestinal Submucosa

Analysis: Immerse the *Sample* in 40 mL of LAL Reagent Water. Extract for 60 min at 37° with shaking. Remove a 100-µL aliquot to measure the amount of bacterial endotoxins.

Acceptance criteria: NMT 20.0 USP Endotoxin Units/70 cm^2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in single-use, peel-open pouches that are gas permeable for sterilization purposes. Store under clean, dry conditions at 25°, excursions permitted between 15° and 30°.
- **LABELING:** The package is labeled to indicate the dimensions of the enclosed Scaffold Porcine Small Intestinal Submucosa, the expiry date, required storage conditions, and the lot number. The label indicates that the Scaffold Porcine Small Intestinal Submucosa is sterile if the package is intact, and that the Scaffold Porcine Small Intestinal Submucosa is designed for single patient, one-time use.
- **USP REFERENCE STANDARDS,** *Authentic Visual References* ⟨11⟩

USP Cultured Rat Pheochromocytoma Reference Photomicrographs

These photomicrographs represent examples of normal and differentiated rat pheochromocytoma cells and are used to assist in ascertaining bioactivity.

Scaffold Silk Fibroin

DEFINITION

Scaffold Silk Fibroin is a surgical scaffold device made from the silk of the *Bombyx Mori* silkworm. Raw silk filaments are comprised of a fibroin protein core that is naturally coated with the globular protein sericin. The sericin is removed from the fiber by aqueous extraction leaving the fibroin protein behind, consisting of layers of anti-parallel beta sheets, which give the fiber strength. The fiber is then assembled into a three dimensional patterned scaffold. The scaffold is a mechanically strong, flexible, single use device that can be produced in a variety of shapes, sizes, and thicknesses, and is terminally sterilized.

IMPURITIES

- **NONPOLAR SOLVENT EXTRACTION**

Positive control: 20 mg of mineral oil[1]

Negative control: 45 mL of *n*-hexane

Sample: Scaffold Silk Fibroin

Analysis: [NOTE—Gloves should be worn throughout the procedure as natural skin oil may affect the results. Glassware preparation: Each sample or control requires one 60-mL bottle and cap and one glass Petri dish. Wash glass Petri dishes (10 cm × 2 cm) and a corresponding number of 60-mL glass jars with lids with liquid detergent and rinse well with tap water. Rinse again with deionized water. Transfer glassware in the chemical hood and rinse all glassware with hexane. Air-dry glassware in the chemical hood for at least 15 min. Transfer dried glassware to a desiccator for NLT 30 min before weighing.] Ensure the relative humidity of the room is between 30% and 80% before weighing or labeling bottles and petri dishes. Label and pre-weigh 60-mL bottles. For the *Positive control*, add 20 mg of mineral oil to a 60-mL bottle and for the *Negative control*, add 45 mL of hexane to a 60-mL bottle. For the *Sample*, use stainless steel tweezers to place one device per 60-mL bottle and cap. Re-weigh bottles and calculate the sample weight (SW) by subtracting the initial mass of the bottle from the mass of the sample-containing bottle. In the chemical hood, open the bottles and add 45 mL of *n*-hexane to each, then re-close making sure that the hexane does not come in contact with the caps. Prepare an ultrasonic water bath for sample processing by degassing it. For this, use the "degas" setting of the equipment for 10 min. Place all 60-mL bottles, prepared as above, in the water bath. Ensure that the water level is 0.5-cm below the lids of the bottles. Sonicate bottles for 5 min on the "sonication" setting (frequency of 40 KHz). Label Petri dishes so that each bottle has a correspondingly labeled Petri dish. Weigh each dish individually and record the mass ($m_{initial}$). Pour the hexane solution from each bottle into the corresponding pre-labeled Petri dish. Add 15 mL of *n*-hexane to each bottle, rinse then pour in the corresponding Petri dish. Repeat this rinsing step and collect the solution. The final hexane volume in each of the Petri dishes should be approximately 75 mL (45 mL from extraction + 15 mL from first rinse + 15 mL from second rinse). Leave Petri dishes uncovered in the chemical hood for 12 h to evaporate the *n*-hexane. Re-weigh each Petri dish after hexane evaporation and record mass (m_{final}). Calculate the recovery of the *Positive control* in percentage with the following formula:

$$\text{Result} = [(m_F - m_I)/W_O] \times 100$$

m_F = final mass of Petri dish after *n*-hexane evaporation in mg

m_I = initial mass of empty Petri dish in mg

W_O = weight of oil added, mg

Calculate the concentration of organic residuals, in ppm, in each tested device:

$$\text{Result} = [(m_F - m_I)/W_S] \times F$$

m_F = final mass of Petri dish after *n*-hexane evaporation in mg

m_I = initial mass of empty Petri dish in mg

[1] A suitable mineral oil can be obtained from Avantor Performance Materials, Inc. (Mallinckrodt Chemicals, Inc.), Center Valley, PA or equivalent.

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W_S = sample weight, g (calculated by subtracting the initial mass of the bottle from the mass of the sample-containing bottle)
F = conversion factor for kg to g, 1000

System suitability criteria: The determined m_F for the *Negative control* should be NMT 0.2 mg and the *Positive control* recovery should be 95%–105%.
Acceptance criteria: Hexane extractable residues should be NMT 1106 ppm per tested device.

• **ELEMENTAL IMPURITIES—LIMITS** ⟨232⟩ and **ELEMENTAL IMPURITIES—PROCEDURES** ⟨233⟩
Nitric acid solution: 2% (v/v) nitric acid in water
Sample solution: Weigh 3–4 g of Scaffold Silk Fibroin, record the mass, then place in a 50-mL conical tube. Add 25 mL of *Nitric acid solution* to the tube. Incubate for 16 h at room temperature.
Analysis: Transfer 5 mL of the *Sample solution* to a tube that is compatible for testing. The levels of chromium, cadmium, nickel, vanadium, lead, and arsenic are determined by an inductively coupled plasma–atomic emission spectroscopy (ICP-AES) (see *Plasma Spectrochemistry* ⟨730⟩) normalized to the mass of the tested device and reported in parts per million (ppm).
Acceptance criteria: Meets the requirements of the Parental Daily Dose PDE (µg/day) in *Elemental Impurities—Limits* ⟨232⟩

SPECIFIC TESTS

• **FIBROIN CONTENT**
Digestion solution: 6 N hydrochloric acid
Reconstitution solution: 0.02 N hydrochloric acid
Borate buffer: 0.2 M sodium borate, pH 8.8 with 5 mM calcium disodium ethylenediaminetetraacetic acid (EDTA). Prepare the buffer by dissolving 124 g boric acid in 80 mL of deionized water; add 187 mg calcium disodium EDTA then titrate with 1 N sodium hydroxide to a pH of 8.8. Add deionized water to a final volume of 100 mL.
Derivatization reagent: 10 mM of 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate in acetonitrile. Vortex for 10 s at 2700 rpm. Incubate the solution for 10 min at 55°, and vortex occasionally until the powder dissolves.
Preparation of hydrolysis sample vials and tubes: Clean glass acid hydrolysis sample vials (30 × 120 mm) and hydrolysis tubes (6 × 50 mm) used for sample preparation with a phosphate-free detergent[2], rinse with purified water then pyrolyze at 220° for 24 h. Allow vials to cool to room temperature then add 200 µL of constant boiling 6 N hydrochloric acid[3] to each hydrolysis tube along with a single phenol crystal.[4] Cap tubes with a polytetrafluoroethyl (PTFE) cap with a chlorotrifluoroethylene (CTFE) screw type on-off valve until needed.
System suitability solution: 25 µM in water from commercially available amino acid standard[5]
Fibroin solution: Bring 2 L of water to a boil in a glass beaker, then add 4.24 g of sodium carbonate. Add 5 g of raw silk yarn[6] and boil for 1 h. Remove yarn containing fibroin with a spatula and rinse in 2 L of water. Squeeze yarn with gloved hands to remove excess water. Rinse yarn 3 more times in 2 L of water for 20 min under agitation (use a stir bar for agitation and set the stir plate to 250 rpm). Squeeze yarn with gloved hands to remove excess water, place the fibroin yarn on a clean sheet of aluminum foil and dry for 16 h in a chemical hood. Transfer 1 mg of the fibroin yarn to a hydrolysis tube to obtain 5 mg/mL of fibroin in 6 N hydrochloric acid. Prepare three replicates. Incubate at 115° for 16 h. Allow the tubes to cool for 1 h at room temperature. Remove acid with a speed vacuum. Add 20 µL of *Reconstitution solution* to each tube and incubate at 50° for 10 min. Dilute each solution 5-fold (to 0.1 µg/µL) with *Reconstitution solution*.
Sericin solution: Add 1 mg of sericin[7] per hydrolysis tube to obtain 5 mg/mL sericin in 6 N hydrochloric acid. Prepare three replicates. Incubate at 115° for 16 h. Allow the tubes to cool for 1 h at room temperature. Remove acid with a speed vacuum. Add 20 µL of *Reconstitution solution* to each tube and incubate at 50° for 10 min. Dilute each solution 5-fold (to 0.1 g/µL) with *Reconstitution solution*.
Standard solutions: Prepare amino acid standards at the following concentrations: 600, 500, 400, 300, 200, 100, 50, 10, 5, and 1 µM in water from commercially available amino acid standard.[8]
Sample solutions: Add 1 mg of Scaffold Silk Fibroin per hydrolysis tube to obtain 5 mg/mL of Scaffold Silk Fibroin in 6 N hydrochloric acid. Prepare six replicates. Incubate at 115° for 16 h. Allow the tubes to cool for 1 h at room temperature. Remove acid with a speed vacuum. Add 20 µL of *Reconstitution solution* to each tube and incubate at 50° for 10 min. Dilute each solution 5-fold (to 0.1 µg/µL) with *Reconstitution solution*.
Amino acid derivatization: *Standard solutions* and reconstituted *Fibroin solution*, *Sericin solution*, and *Sample solutions* are derivatized. *Borate buffer* (70 µL) is added to 12- × 32-mm screw neck total recovery vials (the number of vials needed is equal to the number of Standards, samples, fibroin and sericin solutions, and one blank solution). An aliquot of 10 µL of each amino acid standard (one standard sample for each concentration) and each control (triplicates of *Fibroin solution* and *Sericin solution*) and sample (one sample per finished Scaffold Silk Fibroin) are added to the respective vials. The vials are vortexed for 5 s at 2700 rpm, followed by the addition of 20 µL of *Derivatization reagent*. The vials are capped and vortexed again for 5 s. The vials are then heated for 10 min at 55°. A blank is also prepared using 10 µL of *Reconstitution solution* instead of amino acid standard.
Solution A: Prepare a mixture of acetonitrile, formic acid, and 100 mM ammonium formate (10:6:84). Dilute this mixture with water (5:95).
Solution B: Acetonitrile and formic acid (98:2)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	99.9	0.1
0.54	99.9	0.1
5.74	90.9	9.1
7.74	78.8	21.2

[2] A suitable detergent can be obtained from EMD Biosciences, Inc., Darmstadt, Germany or equivalent.
[3] Suitable acid can be obtained from Pierce Biotechnology, Inc., Rockford, IL or equivalent.
[4] Suitable phenol crystals can be obtained from Sigma-Aldrich, St. Louis, MO or equivalent.
[5] Suitable amino acid standards can be obtained from Pierce Biotechnology, Inc., Rockford, IL or equivalent.
[6] Suitable raw silk yarn can be obtained from Bratac S.A, Londrina, Brazil or equivalent.
[7] A suitable sericin standard/reference can be obtained from Silk Biochemical Co., Ltd., Zhejiang, China or equivalent.
[8] Suitable amino acid standards can be obtained from Pierce Biotechnology, Inc., Rockford, IL or equivalent.

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
8.04	40.4	59.6
8.64	40.4	59.6
8.73	99.9	0.1
9.5	99.9	0.1

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 2.1-mm × 10-cm; L1 with particle size 1.7 µm and pore size 130 Å
Column temperature: 55°
Flow rate: 0.7 mL/min
Injection volume: 1 µL
Run time: 10 min
System suitability
Sample: *System suitability solution*
Suitability requirements
Plate count: NLT 2000 for all the amino acids
Tailing factor: NMT 2
Resolution: NLT 2
Signal-to-noise ratio: NLT 10 for all the amino acids
Analysis
Samples: *Fibroin solution*, *Sericin solution*, *Standard solutions*, and *Sample solutions*
Create a calibration curve for each amino acid by integrating the peak area obtained from the chromatograph for each concentration of *Standard solution* then plot the integrated peak area as a function of concentration. Run each replicate of *Fibroin solution* and *Sericin solution*. Perform one run for each of the six *Sample solutions*. Determine the concentration of each constituent amino acid of each control solution and *Sample solution* from the calibration curves of the amino acid standards.
A mathematical algorithm development software is used to set up a matrix based calculation for fibroin purity.[9] The algorithm uses 7 out of the 16 detected amino acids: glycine (Gly), alanine (Ala), tyrosine (Tyr), serine (Ser), asparagine (Asx), glutamate/glutamic acid (Glx), and threonine (Thr). [NOTE—The 7 amino acids were selected because the magnitude difference in weight percent of each found in sericin compared to fibroin is at least 2-fold.]
Convert the fibroin and sericin reference data into a [7 × 2] matrix (*Matrix A*), with 7 (columns) representing each of the selected amino acids and 2 (rows) representing the number of Reference Standards analyzed. Represent the amino acid composition of the silk samples being analyzed as a [7 × 6] matrix (*Matrix B*), where 7 (columns) represent each of the selected amino acids and 6 (rows) for each of the total number of samples analyzed. Specifically,

$$\text{Matrix A} = \begin{bmatrix} Gly & Ala & Tyr & Ser & Glx & Asx & Thr \\ Gly & Ala & Tyr & Ser & Glx & Asx & Thr \end{bmatrix}$$

Where:
- the first row is the sericin reference amino acid average from the three runs
- the second row is the fibroin reference amino acid average from the three runs

$$\text{Matrix B(1 to 6)} = \begin{bmatrix} Gly_1 & Ala_1 & Tyr_1 & Ser_1 & Glx_1 & Asx_1 & Thr_1 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ Gly_6 & Ala_6 & Tyr_6 & Ser_6 & Glx_6 & Asx_6 & Thr_6 \end{bmatrix}$$

Where:
- the rows indicated the amino acid averages for the six samples analyzed

The mass proportion of fibroin and sericin from each *Sample solution* is calculated by performing the matrix operation $B \times (A^{-1})$. The percent weight compositions of fibroin and sericin for each analyzed *Sample solution* are calculated by the software from the mass proportions.
Acceptance criteria: NLT 95% for fibroin content and NMT 5% for sericin content

- **DIMENSIONAL ANALYSIS**
Sample: Scaffold Silk Fibroin
Analysis: Measure the length and width of intact Scaffold Fibroin Silk devices with a NIST traceable caliper with an accuracy of at least 0.02 mm.[10] Take three measurements at the top, the center, and the bottom of the device for each parameter. Measure the length of the scaffold in the longest direction. Measure the width of the scaffold along the shortest direction. Measure the thickness of the sample (single measurement) using a NIST traceable thickness gauge with accuracy of at least 0.01 mm.[11] Place the sample (either intact scaffold or sectioned piece) between the gauge feelers taking care to avoid creases and folds. Close the feelers and take the measurement. [NOTE—Test between 13 and 50 devices.]
Acceptance criteria: Each device is NLT 15 ± 1.5 cm in length and NLT 5 ± 0.5 cm wide, with a thickness range of 0.6–1.0 mm.

- **DENSITY DETERMINATION**
Sample: Scaffold Silk Fibroin
Analysis: Determine the mass of the sample by a single measurement using a scientific calibrated balance that can be read to at least 0.1 mg.[12] The average length and width is determined by averaging the three measurements from the *Dimensional Analysis*. The thickness is measured as described in the *Analysis* of the *Dimensional Analysis*. Calculate the density, mg/mm ^{3} of each sample by using the equation:

$$\text{Result} = M/(L \times W \times T)$$

M = mass, mg
L = average length, mm
W = average width, mm
T = thickness, mm

Acceptance criteria: NLT 0.14 mg/mm ^{3} and NMT 0.18 mg/mm ^{3}

- **PORE CHARACTERIZATION**
Sample: Scaffold Silk Fibroin
Analysis: The assembled scaffold has a lattice-like appearance, with open spaces (pores) between the length and width constituent fibers that run perpendicular to each other. Measure the pore dimensions of the scaffold by using a stereo microscope with sufficient magnification and

[9] A suitable software can be obtained from MathWorks, Natick, MA or equivalent.

[10] A suitable ruler can be obtained from Mitutoyo American Corporation, Aurora, IL or equivalent.
[11] A suitable thickness gauge can be obtained from Käfer Messuhrenfabrik GmbH & Co. KG, Villingen-Schwenningen, Germany or equivalent.
[12] A suitable balance can be obtained from Denver Instrument, Bohemia, NY or equivalent.

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image capture capability. Select a magnification based upon the resolution of the pore in the scaffold pattern being examined (typical range is 0.5–2×). Take images under magnification. Randomly select eight pore images, determine the pore area and average the results. Measure pore size and analyze with a suitable image analysis software package.

Acceptance criteria: The pore area of each device is NLT 1 × 10^4 µm ^{2}.

- **BALL BURST TESTING**

0.01 M phosphate buffered saline: Dissolve 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of monobasic sodium phosphate, and 0.24 g of dibasic potassium phosphate in 800 mL of deionized water. Adjust with 1 N hydrochloric acid to a pH of 7.4, add deionized water to a final volume of 1000 mL.

Sample: Sections (40 × 40 mm) that are cut from the full sized, Scaffold Silk Fibroin. [NOTE—Test one section per device. Test between 13 and 50 devices.]

Analysis: Submerge the *Sample* fully in *0.01 M phosphate buffered saline* and incubate at 37° for 2 h. Use a suitable mechanical testing instrument and test frame.[13] Compress each device sample tested between the two circular fixation brackets (inner diameter of 18.6 mm) of the mechanical testing instrument. Secure the *Sample* with a torque wrench to a value of 0.2 ± 0.1 N/m.[14] Ensure that the lattice structure of the *Sample* is evenly distributed across the inner fixture diameter and not skewed or sheared (the *Sample* should remain taut within the fixation brackets with even distribution of tension). Attach the ball burst fixture (10 mm diameter) to the mechanical testing instrument with a calibrated load cell. Insert the fixture ball through the center of the fixation brackets at a constant rate of 60 mm/min until the *Sample* fails. The resulting instrument output represents the maximum load sustained by the test sample. Calculate the ultimate burst stress of the *Sample* in MPa:

$$\text{Result} = [B/(E_A)] \times F$$

B = maximum burst load, N
E_A = exposed area, m^2
F = conversion factor, 10^{-6}

The exposed area is the circular area of the test article covering the radius (r) of the inner fixture diameter and is calculated using the equation below:

$$\text{Exposed area} = \pi r^2$$

An additional sample parameter, burst stiffness that is reflective of elastic properties and indicative of the scaffold's capacity to withstand anatomical forces before failure is calculated by determining the slope of the middle of the linear region of the compressive load versus extension curve.

Acceptance criteria: The ultimate burst stress for each tested sample is NLT 0.5 MPa. The burst stiffness for each tested sample is NLT 30 N/mm.

- **TENSILE TESTING**

Sample: Cut two sample sections of 10 × 60 mm from each Scaffold Silk Fibroin tested, one in length direction and one in the width direction. Fully immerse samples in phosphate buffered saline and incubate at 37° for 2 h.

Analysis: Use a suitable mechanical testing instrument.[15] The tensile testing is done following the same principles as outlined in *Tensile Strength* ⟨881⟩, but modified as specified below. Clamp device samples in the mechanical test frame. Mount the upper clamp to the load cell, which is attached to the actuator. Mount the lower clamp to the support plate. Align the upper clamp to make the faces of both clamps parallel to each other. Adjust the height of the mechanical equipment crosshead so that the actuator is positioned to allow for a defined amount of upward movement and a specific sample gauge length resides between the upper and lower sample clamps.

Given a device sample of 60-mm long by 10-mm wide, load the sample by clamping the first 10 mm length of the *Sample* into the upper clamp and allowing the remainder of the *Sample* to fall unrestrained into the bottom clamp opening. Fix the last 10 mm of the *Sample* with the bottom clamp. Avoid pre-straining the *Sample*. Once the *Sample* is clamped, adjust the actuator height so that the *Sample* has a preload of 2 Newtons. Adjust the actuator position to achieve a 40-mm gauge length then reset to the zero-position. Strain the test sample at a rate of 2400 mm/min until it experiences ultimate tensile failure. The instrument output will provide maximum load value that was sustained by the *Sample*. [NOTE—The straining rate represents 100% of the gauge length/s and ensures that the *Sample* fails within 1 s (40 mm × 60 s = 2400 mm/min). Depending on the manufactured lot size, test between 13 and 50 devices. Use the maximum load value to calculate the ultimate tensile stress and percent elongation at break.]

Calculate the ultimate tensile stress in MPa using the equation:

$$\text{Result} = [M_L/(S_W \times S_T)] \times F$$

M_L = maximum load, N
S_W = sample width, m
S_T = sample thickness, m
F = conversion factor mm ^{2} to m ^{2}, 10^{-6}

The sample width (S_W) is equal to 10 mm, and the sample thickness is determined as described in the *Analysis* section of the *Dimensional Analysis*.

The elongation at break in percentage is determined using the equation:

$$\text{Result} = [(L_B - O_L)/O_L] \times 100$$

L_B = length at break, mm
O_L = original length, mm

The numerator (length at break – original length) is measured and indicated by the instrument length = 40 mm (60 mm total length – 10 mm held by the upper clamp – 10 mm held by the lower clamp). Tensile stiffness is calculated by determining the slope of the trend line of the linear portion of the tensile load vs. elongation curve bound by an upper and lower tensile load.

Acceptance criteria: The maximum load for each tested sample along the scaffold length is NLT 31 N. The ultimate tensile stress (the force at which the device fails) for each tested sample is NLT 5 MPa. The percent elongation at break for each tested sample is NLT 35% in the direction of length. In the same direction, the tensile stiffness for each tested sample is NLT 0.8 N/mm.

[13] Suitable mechanical testing instrument can be obtained from Instron Corporation, Norwood, MA or equivalent.
[14] A suitable torque wrench can be obtained from CDI Torque Products, City of Industry, CA or equivalent.
[15] A suitable mechanical testing instrument can be obtained from Instron Corporation, Norwood, MA or equivalent.

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• **SUTURE RETENTION FORCE**

Sample: Cut two sample sections of 20 × 40 mm from each Scaffold Silk Fibroin tested, one in the length direction and one in the width direction. Rehydrate all samples by completely submerging in phosphate buffered saline and incubating for 2 h at 37°.

Analysis: Use a suitable mechanical testing instrument.[16] Insert the upper part of the *Sample* into the upper clamp with the top 10 mm of the *Sample* gripped by the clamp with a pressure of 85 psi. Assess the overall suture retention strength of the samples in both orientations. Using 2-0 polypropylene,[17] guide the first suture 10 mm from both sides of the *Sample* and approximately 3 mm from the lower edge. Loop the suture through a pore. Guide two more polypropylene sutures through the same way, but at a distance of 6 mm to the left and right of the center suture loop. Lower the upper actuator to maintain a distance of 10 mm between bottom edge of the *Sample* and lower clamp. Clamp the ends of the three sutures into the bottom clamp with a pressure of 85 psi. The created gauge length is 27 mm. Preload the *Sample* with 1 Newton. Strain the *Sample* at a constant rate of 1620 mm/min until the device sample breaks from the sutures or the sutures pull through the *Sample*. The instrument output represents the maximum load at failure. [NOTE—The straining rate represents 100% of the gauge length/s (27 mm × 60 s = 1620 mm/min) and ensures that the *Sample* fails within 1 s. Depending on the manufactured lot size, test between 13 and 50 devices.]

Calculate the average suture retention strength using the equation:

$$\text{Average Maximum Strength per Suture} = \frac{Maximum\,Load\,[N]}{3\,[suture]}$$

Acceptance criteria: The average suture retention force is NLT 13 N/suture in both vertical and horizontal orientations.

• **TEAR TESTING**

Sample: Cut two sample sections of 20 × 40 mm from each Scaffold Silk Fibroin tested, one in the length direction, and one in the width direction. Make a small cut one fourth the size of the test sample width in the center of the sample perpendicular to the length. Submerge all samples completely and incubate for 2 h in phosphate buffered saline at 37°.

Analysis: Use a material characterization mechanical testing instrument.[18] Affix clamps to the test frame of the mechanical testing equipment with tear testing capability. Mount the upper clamp to the actuator and the lower clamp to the load cell that is attached to the base support plate. Align the two clamps parallel to each other. Adjust the height of the mechanical equipment crosshead to position the actuator to allow for a defined amount of upward movement (minimum of 57 mm) and to ensure that a sample gauge length of 40 mm resides between the upper and lower clamps. Place the device sample in the upper clamp. The clamp should cover the top 10 mm of the *Sample*. Align the *Sample* perpendicular with the clamp before the clamp is closed. Allow the bottom portion of the *Sample* to fall unrestrained into the bottom clamp opening. Close the clamp and preload the *Sample* with 2 Newtons. Strain the *Sample* at a constant rate 2400 mm/min until it tears at the cut point. The maximum tear resistance load is calculated by the software of the mechanical instrument.[19]

[NOTE—The strain rate represents 100% of the gauge length/s and was chosen to ensure that the *Sample* fails within 1 s (40 mm × 60 s = 2400 mm/min). Depending on the manufactured lot size, test between 13 and 50 samples.]

Acceptance criteria: The tear strength for each sample is NLT 109 N.

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩

Sample: Scaffold Silk Fibroin

Analysis: Using depyrogenated forceps, place a 70-cm ^{2} sample of Scaffold Silk Fibroin in a depyrogenated 250-mL Erlenmeyer flask. Add 27 mL of limulus amebocyte lysate reagent water and 3 mL of tetrahydrofuran to the flask and cover with parafilm. Place on an orbital shaker at 250 rotations/min for 1 h at room temperature. Collect 0.5-mL samples from each flask and further dilute 20-fold with limulus amebocyte lysate reagent water. Analyze each sample in duplicate for bacterial endotoxin following *Turbidimetric Technique* in the chapter.

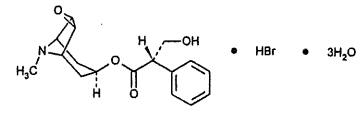
Acceptance criteria: Each sample should contain NMT 20.0 USP Endotoxin Units/device.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in single-use, peel-open pouches that are gas permeable for sterilization purposes. Store under clean, dry conditions at room temperature, between 15° and 25°.

• **LABELING:** The package label indicates the dimensions of the enclosed Scaffold Silk Fibroin, the lot number, the expiry date, the required storage conditions, the trade name, the product manufacturer, and the manufacturer's contact information. In addition, the label indicates the product has been sterilized by EO and provides a date when the sterilization has taken place. It also lists some precautionary statements such as the product should not be reused, it should not be re-sterilized, or used if the package is damaged or open upon receipt. The instructions for use are included with each unit.

Scopolamine Hydrobromide



$C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$ 438.31

Benzeneacetic acid, α-(hydroxymethyl)-, 9-methyl-3-oxa-9-azatricyclo[3.3.1.0.^{2,4}]non-7-yl ester, hydrobromide, trihydrate, [7(*S*)-(1α,2β,4β,5α,7β)]-;

6β,7β-Epoxy-1α*H*,5α*H*-tropan-3α-ol (–)-tropate (ester) hydrobromide trihydrate [6533-68-2].

Anhydrous 384.27

[114-49-8].

DEFINITION

Scopolamine Hydrobromide contains NLT 98.0% and NMT 102.0% of scopolamine hydrobromide ($C_{17}H_{21}NO_4 \cdot HBr$), calculated on the anhydrous basis.

[16] Suitable mechanical testing equipment and data processing software can be obtained from Instron Corporation, Norwood, MA or equivalent.

[17] A suitable suture (2-0 FiberWire, cat # AR-7220) can be obtained from Arthrex, Inc., Naples, FL or equivalent.

[18] Suitable mechanical testing equipment can be obtained from Instron Corporation, Norwood, MA or equivalent.

[19] Suitable data processing software can be obtained from Instron Corporation, Norwood, MA or equivalent.

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[**CAUTION**—Handle scopolamine hydrobromide with exceptional care, because it is highly potent.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Analysis: Dissolve 3 mg in 1 mL of alcohol, and evaporate the solution on a steam bath to dryness. Dissolve the residue in 0.5 mL of chloroform, add 200 mg of potassium bromide, previously dried at 105° for 30 min, and stir frequently for 5 min. Allow the chloroform to evaporate to dryness, and stir frequently to obtain a flowing powder residue. Dry the residue on a steam bath for 5 min, and then immediately compress the residue to a disk.
Acceptance criteria: Meets the requirements

Change to read:

• **B.**
Sample solution: 50 mg/mL ▲in water▲ (ERR 1-Apr-2019)
Analysis: To 1 mL of *Sample solution* add a few drops of chlorine TS, and shake the mixture with 1 mL of chloroform.
Acceptance criteria: Brownish color in chloroform layer
• **C.** The retention time of the scopolamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer solution: 7.0 g/L of monobasic potassium phosphate in water. Adjust with 0.05 M phosphoric acid to a pH of 3.3.
Solution A: Dissolve 3.5 g of sodium dodecyl sulfate in 606 mL of *Buffer solution*. Add 320 mL of acetonitrile and mix.
Solution B: acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2.0	95	5
20.0	70	30
20.1	95	5
25.0	95	5

Standard solution: 0.2 mg/mL of USP Scopolamine Hydrobromide RS in *Solution A*
Sample solution: 0.2 mg/mL of Scopolamine Hydrobromide in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of scopolamine hydrobromide ($C_{17}H_{21}NO_4 \cdot HBr$) in the portion of Scopolamine Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of scopolamine from the *Sample solution*
r_S = peak response of scopolamine from the *Standard solution*
C_S = concentration of USP Scopolamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Scopolamine Hydrobromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 1 g
Acceptance criteria: NMT 0.1%
• **ORGANIC IMPURITIES**
Buffer solution, Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.2 mg/mL of USP Scopolamine Hydrobromide RS and 0.001 mg/mL of USP Norscopolamine RS in *Solution A*
Standard solution: 0.002 mg/mL of USP Scopolamine Hydrobromide RS in *Solution A*
Sample solution: 2.0 mg/mL of Scopolamine Hydrobromide in *Solution A*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the norscopolamine and scopolamine peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Scopolamine Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of scopolamine from the *Standard solution*
C_S = concentration of USP Scopolamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Scopolamine Hydrobromide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%. Disregard any peak due to the bromide ion at RRT 0.1 that appears close to the solvent peak.

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Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tropic acid[a]	0.19	2.4	0.20
Norscopolamine	0.89	1.4	0.5
Scopolamine	1.0	—	—
Hyoscyamine[b]	1.3	1.3	0.20
Apohyoscine[c]	1.8	2.2	0.20
Apoatropine[d]	2.4	2.3	0.20
Any other individual impurity	—	—	0.10
Total impurities	—	—	0.7

[a] 3-Hydroxy-2-phenylpropanoic acid.
[b] (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl (*S*)-3-hydroxy-2-phenylpropanoate.
[c] (1*R*,2*R*,4*S*,5*S*,7*s*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-7-yl 2-phenylacrylate.
[d] (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-phenylacrylate.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 50 mg/mL of anhydrous Scopolamine Hydrobromide in *water*
  Acceptance criteria: −24° to −26°
- **pH** ⟨791⟩
  Sample solution: 50 mg/mL of Scopolamine Hydrobromide in *water*
  Acceptance criteria: 4.0–5.5
- **WATER DETERMINATION** ⟨921⟩, *Method III*
  Analysis: Dry in two stages (see *Loss on Drying* ⟨731⟩); first at 80° for 2 h, and then at 105° for an additional 3 h.
  Acceptance criteria: NMT 13.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Norscopolamine RS
  (1*R*,2*R*,4*S*,5*S*,7*s*)-3-Oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-7-yl (*S*)-3-hydroxy-2-phenylpropanoate.
  $C_{16}H_{19}NO_4$ 289.33
  USP Scopolamine Hydrobromide RS

Delete the following:

▲Scopolamine Hydrobromide Injection

» Scopolamine Hydrobromide Injection is a sterile solution of Scopolamine Hydrobromide in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$.

Packaging and Storage—Preserve in light-resistant, single-dose or multiple-dose containers, preferably of Type I glass.
USP Reference Standards ⟨ 11 ⟩ —
USP Scopolamine Hydrobromide RS

Identification—

A: Transfer a volume of Injection, equivalent to about 3 mg of scopolamine hydrobromide, to a 50-mL separator, dilute with water, if necessary, to 10 mL, add 0.2 mL of ammonium hydroxide, and extract with 25 mL of chloroform. Add 50 mL of ether to the chloroform solution, and pass the mixture through a 25- × 250-mm chromatographic tube fitted with a pledget of glass wool at the base and packed with 2 g of purified siliceous earth that previously has been mixed with 1 mL of 0.2 N phosphoric acid saturated with sodium bromide. Discard the eluate, and pass 25 mL of water-saturated ether through the column and discard. Elute with 100 mL of water-saturated chloroform, collect the eluate in a suitable receiver, and evaporate just to dryness. Dissolve the residue in 1 mL of alcohol, and proceed as directed in *Identification* test *A* under *Scopolamine Hydrobromide*, beginning with "and evaporate the solution on a steam bath to dryness."

B: Add to the Injection silver nitrate TS: a yellowish white precipitate, insoluble in nitric acid but slightly soluble in 6 N ammonium hydroxide, is formed.

Bacterial Endotoxins ⟨ 85 ⟩ —It contains not more than 555.0 USP Endotoxin Units per mg of scopolamine hydrobromide.

pH ⟨ 791 ⟩ : between 3.5 and 6.5.

Other Requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

pH 9.0 Buffer—Dissolve 34.8 g of dibasic potassium phosphate in 900 mL of water, and adjust with 3 N hydrochloric acid or 1 N sodium hydroxide, as necessary, to a pH of 9.0, determined electrometrically, and mix.

Internal standard solution—Transfer about 25 mg of homatropine hydrobromide to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Prepare fresh daily.

Standard stock solution—Transfer about 10 mg of USP Scopolamine Hydrobromide RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Prepare fresh daily.

Standard preparation—Pipet 10 mL of the *Standard stock solution* into a separator, add 2.0 mL of *Internal standard solution* and 5.0 mL of *pH 9.0 Buffer*, and carefully adjust the solution with 1 N sodium hydroxide to a pH of 9.0, avoiding any excess. Immediately extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under nitrogen to approximately 2.0 mL.

Assay solution—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of scopolamine hydrobromide, to a 100-mL volumetric flask. Dilute with water to volume, and mix.

Assay preparation—Pipet 10 mL of the *Assay solution* into a separator, and proceed as directed for *Standard preparation*, beginning with "add 2.0 mL of *Internal standard solution*."

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph contains a 2-mm × 1.8-m glass column packed with 3% liquid phase G3 on support S1AB. The carrier gas is nitrogen, flowing at a rate of 25 mL per minute. The column temperature is maintained at 225°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution factor, *R*, between homatropine and scopolamine is not less than 5; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

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Procedure—Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the ratio, A_U, of the area of the scopolamine hydrobromide peak to the area of the internal standard peak in the chromatogram from the *Assay preparation*, and similarly calculate the ratio, A_S, in the chromatogram from the *Standard preparation*. Calculate the quantity, in mg, of scopolamine hydrobromide ($C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$) in the volume of Injection taken by the formula:

$$1.141W(A_U/A_S),$$

in which 1.141 is the ratio of the molecular weight of scopolamine hydrobromide trihydrate to that of anhydrous scopolamine hydrobromide; W is the weight, in mg, of USP Scopolamine Hydrobromide RS in the *Standard preparation*; and A_U and A_S are as calculated above. ▲ (USP 1-May-2020)

Delete the following:

▲Scopolamine Hydrobromide Ophthalmic Solution

» Scopolamine Hydrobromide Ophthalmic Solution is a sterile, buffered, aqueous solution of Scopolamine Hydrobromide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$. It may contain suitable antimicrobial agents and stabilizers, and may contain suitable additives for the purpose of increasing its viscosity.

Packaging and Storage—Preserve in tight containers.
USP Reference Standards 〈 11 〉 —
USP Scopolamine Hydrobromide RS

Identification—
A: A volume of Ophthalmic Solution, equivalent to about 3 mg of scopolamine hydrobromide, responds to *Identification* test *A* under *Scopolamine Hydrobromide Injection*.
B: Add to the Ophthalmic Solution silver nitrate TS: a yellowish white precipitate, insoluble in nitric acid but slightly soluble in 6 N ammonium hydroxide, is formed.
Sterility Tests 〈 71 〉 **:** meets the requirements.
pH 〈 791 〉 **:** between 4.0 and 6.0
Assay—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of scopolamine hydrobromide, to a 100-mL volumetric flask, dilute with water to volume, and mix. Using this as the *Assay solution*, proceed as directed in the *Assay* under *Scopolamine Hydrobromide Injection*. Calculate the quantity, in mg, of $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$ in the volume of Ophthalmic Solution taken by the formula given therein. ▲ (USP 1-May-2020)

Delete the following:

▲Scopolamine Hydrobromide Tablets

» Scopolamine Hydrobromide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$.

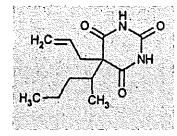
Packaging and Storage—Preserve in tight, light-resistant containers.
USP Reference Standards 〈 11 〉 —
USP Scopolamine Hydrobromide RS
Identification—Place an amount of powdered Tablets, equivalent to about 3 mg of scopolamine hydrobromide, in a 50-mL separator, add 10 mL of water, and shake for 2 minutes. Proceed as directed in *Identification* test *A* under *Scopolamine Hydrobromide Injection*, beginning with "add 0.2 mL of ammonium hydroxide."
Disintegration 〈 701 〉 : 15 minutes, the use of disks being omitted.
Uniformity of Dosage Units 〈 905 〉 : meet the requirements.
Assay—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1.0 mg of scopolamine hydrobromide, to a separator containing 5 mL of *pH 9.0 buffer*, and add, by pipet, 2.0 mL of *Internal standard solution* (prepared as directed in the *Assay* under *Scopolamine Hydrobromide Injection*). Adjust with 1 N sodium hydroxide to a pH of 9.0, extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under nitrogen to approximately 2.0 mL. Using this as the *Assay preparation*, proceed as directed in the *Assay* under *Scopolamine Hydrobromide Injection*. Calculate the quantity, in mg, of $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$ in the portion of Tablets taken by the formula:

$$1.141(W/10)(A_U/A_S)$$

in which W is the weight, in mg, of USP Scopolamine Hydrobromide RS in the *Standard solution*; and 1.141 is the ratio of the molecular weight of scopolamine hydrobromide trihydrate to that of anhydrous scopolamine hydrobromide and A_U and A_S are as defined therein. ▲ (USP 1-May-2020)

Delete the following:

▲Secobarbital



$C_{12}H_{18}N_2O_3$ 238.28
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-(1-methylbutyl)-5- (2-propenyl)-.
5-Allyl-5-(1-methylbutyl)barbituric acid [76-73-3].

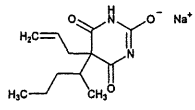
USP Monographs

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» Secobarbital contains not less than 97.5 percent and not more than 100.5 percent of $C_{12}H_{18}N_2O_3$, calculated on the dried basis.

Packaging and Storage—Preserve in tight containers.
USP Reference Standards ⟨ 11 ⟩ —
USP Secobarbital RS
Identification, *Infrared Absorption* ⟨ 197M ⟩ .
Loss on Drying ⟨ 731 ⟩ —Dry it over silica gel for 18 hours: it loses not more than 1.0% of its weight.
Residue on Ignition ⟨ 281 ⟩ : not more than 0.1%.
Isomer content—Dissolve about 300 ± 5 mg in 5 mL of sodium hydroxide solution (1 in 100), add a solution of 300 ± 5 mg of *p*-nitrobenzyl bromide in 10 mL of alcohol, reflux for 30 minutes, cool, collect the precipitate on a small filter, and wash with water: the precipitate, recrystallized from 25 mL of alcohol and dried at 105° for 30 minutes, melts between 156° and 161°.
Assay—Add about 450 mg of Secobarbital, accurately weighed, to 60 mL of dimethylformamide in a 125-mL conical flask. Add 4 drops of thymol blue TS, and titrate with 0.1 N sodium methoxide VS, using a magnetic stirrer and taking precautions against the absorption of atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 23.83 mg of $C_{12}H_{18}N_2O_3$. ▲ (USP 1-May-2020)

Secobarbital Sodium



$C_{12}H_{17}N_2NaO_3$ 260.26
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-(1-methylbutyl)-5-(2-propenyl)-, monosodium salt.
Sodium 5-allyl-5-(1-methylbutyl)barbiturate [309-43-3].

» Secobarbital Sodium contains not less than 98.0 percent and not more than 102.0 percent of $C_{12}H_{17}N_2NaO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Secobarbital RS
Completeness of solution—Mix 1.0 g with 10 mL of carbon dioxide-free water: after 1 minute, the solution is clear and free from undissolved solid.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)—
Test specimen: Dissolve about 500 mg of Secobarbital Sodium, accurately weighed, in 15 mL of water in a separator. To the solution, add 2 mL of hydrochloric acid, shake, and extract the liberated secobarbital with eight 25-mL portions of dichloromethane. Test for completeness of extraction by extracting with an additional 10-mL portion of dichloromethane and evaporating the solvent: not more than 0.5 mg of residue remains. Filter the extracts into a tared beaker, and finally rinse the separator and the filter with several small portions of dichloromethane. Evaporate the combined filtrate and washings on a steam bath with the aid of a current of air just to dryness. Dissolve the residue in 2 mL of dehydrated alcohol, and evaporate to dryness. Repeat the dissolution and evaporation with 2 mL of dehydrated alcohol, and dry the residue at 100° for 2 hours. Use a suitable amount of the residue for examination.
B: Ignite about 500 mg: the residue effervesces with acids, and meets the requirements of the tests for *Sodium* ⟨191⟩.
C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
pH ⟨791⟩: between 9.7 and 10.5, in the solution prepared in the test for *Completeness of solution*.
Loss on drying ⟨731⟩—Dry it at 80° for 5 hours: it loses not more than 3.0% of its weight.

Related compounds—
Buffer, *Mobile phase*, and *Standard preparation*—Prepare as directed in the *Assay*.
Standard solution—Quantitatively dilute with *Mobile phase* a suitable volume of the *Standard preparation* to obtain a final solution having a known concentration of about 5 µg per mL of USP Secobarbital RS.
Test solution—Use the *Assay stock preparation*.
Chromatographic system—Prepare as directed for *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the tailing factor for secobarbital is not more than 2.0; and the relative standard deviation for five replicate injections for secobarbital is not more than 6.0%.
Procedure—Separately inject a volume (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms. Identify the peaks due to the impurities using the relative retention times given in *Table 1*. Calculate the percentage of each impurity in the portion of Secobarbital Sodium taken by the formula:

$$100(r_i/r_s)(C_s/C_u)(1/F)$$

in which r_i is the response of the impurity peak obtained from the *Test solution;* r_s is the response of secobarbital obtained from the *Standard solution;* C_s is the concentration of USP Secobarbital RS, in mg per mL, in the *Standard solution*; C_u is the nominal concentration of Secobarbital Sodium, in mg per mL, in the *Test solution;* and *F* is the relative response factor given in *Table 1*. The impurities meet the limits given in *Table 1*.

Table 1

Compound	Relative Retention Time (RRT)	Relative Response Factor (*F*)	Limit (%)
Imino impurity[1]	0.33	1.7	NMT 1.0
2-Hydroxy propyl impurity[2]	0.38	0.95	NMT 0.25
Secobarbital	1.0	1.0	—
MIBK impurity[3]	1.61	0.96	NMT 0.55
Any individual unspecified impurity	—	1.0	NMT 0.10

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Table 1 *(continued)*

Compound	Relative Retention Time (RRT)	Relative Response Factor (*F*)	Limit (%)
Total impurities	—	—	NMT 1.5

[1] 5-Allyl-4-imino-5-(1-methylbutyl) barbituric acid.
[2] 5-(2-Hydroxypropyl)-5-(1-methylbutyl) barbituric acid.
[3] 5-Allyl-5-(1-3-dimethylbutyl) barbituric acid.

Other requirements—Where the label states that Secobarbital Sodium is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and for *Bacterial endotoxins* under *Secobarbital Sodium for Injection*. Where the label states that Secobarbital Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Secobarbital Sodium for Injection*.

Assay—

Buffer—Dissolve 1.36 g of monobasic potassium phosphate in 1000 mL of water.

Diluted phosphoric acid—Dilute orthophosphoric acid with water (4:1).

Mobile phase—Prepare a mixture of *Buffer* and acetonitrile (13:7). Adjust with *Diluted phosphoric acid* to a pH of 3.5.

Standard preparation—In a suitable volumetric flask, dissolve an accurately weighed quantity of USP Secobarbital RS in *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL of secobarbital.

Assay stock preparation—Transfer about 100 mg of Secobarbital Sodium into a 100-mL volumetric flask. Add 60 mL of *Mobile phase,* and sonicate to dissolve. Dilute with *Mobile phase* to volume, and mix.

Assay preparation—Quantitatively dilute with *Mobile phase* the *Assay stock preparation* to obtain a nominal concentration of 0.4 mg per mL of secobarbital sodium.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15.0-cm column containing 3.5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for secobarbital is not more than 2.0; and the relative standard deviation from replicate injections for secobarbital is not more than 2.0%.

Procedure—Separately inject a volume (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and record the chromatograms. Identify the peaks due to the impurities using the relative retention times given in *Table 1*. Calculate the percentage of $C_{12}H_{17}N_2NaO_3$, in the portion of Secobarbital Sodium taken by the formula:

$$100(C_S/C_U)(r_U/r_S)(Mr_1/Mr_2)$$

in which C_S is the concentration, in mg per mL, of USP Secobarbital RS in the *Standard preparation;* C_U is the nominal concentration of Secobarbital Sodium, in mg per mL, in the *Assay preparation;* r_U is the response of secobarbital peak obtained from the *Assay preparation;* r_S is the response of secobarbital peak obtained from the *Standard preparation;* Mr_1 is the molecular weight of secobarbital sodium, 260.27; and Mr_2 is the molecular weight of secobarbital, 238.28.

Secobarbital Sodium Capsules

DEFINITION

Secobarbital Sodium Capsules contain NLT 92.5% and NMT 107.5% of the labeled amount of secobarbital sodium ($C_{12}H_{17}N_2NaO_3$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)

Sample solution: Nominally 5 mg/mL of secobarbital sodium in water from the contents of Capsules

Analysis: Transfer 10 mL of the filtered *Sample solution* to a separator. Add 2 mL of 3 N hydrochloric acid. Extract the liberated secobarbital with 20 mL of chloroform, and evaporate the extract to dryness. Dissolve the residue in chloroform to prepare a 5-mg/mL solution.

Acceptance criteria: The *Sample solution* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Secobarbital RS.

- **B.** The retention time of the secobarbital peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Phosphoric acid and water (80:20)

Buffer: 1.4 g/L of monobasic potassium phosphate in water adjusted with *Solution A* to a pH of 3.5

Mobile phase: Acetonitrile and *Buffer* (35:65)

Diluent: Acetonitrile and water (35:65)

Standard solution: 0.4 mg/mL of USP Secobarbital RS in *Diluent*. Sonication may be used to aid in dissolution.

Sample solution: Nominally 0.4 mg/mL of secobarbital sodium in a solution prepared as follows. Transfer the equivalent of 44 mg of secobarbital sodium from the contents of NLT 20 Capsules to a 100-mL volumetric flask. Dilute with *Diluent* to volume. Shaking and sonication may be used to aid in dissolution. Centrifuge a portion of this solution, and use the supernatant.

Chromatographic system
(see *Chromatography* ⟨621⟩, *System Suitability*)
Mode: LC
Detector: 215 nm
Column: 4.6-mm × 15.0-cm; 3.5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of secobarbital

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of secobarbital sodium ($C_{12}H_{17}N_2NaO_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Secobarbital RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of secobarbital sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of secobarbital sodium, 260.26
M_{r2} = molecular weight of secobarbital, 238.28

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 60 min
Standard solution: USP Secobarbital RS in 0.1 N sodium hydroxide
Sample solution: Solution under test, mixed with sufficient sodium hydroxide to provide a concentration of 0.1 N sodium hydroxide, and diluted with *Medium*, as needed, so that the concentration is similar to that of the *Standard solution*
Instrumental conditions
Mode: UV
Analytical wavelength: 243 nm
Tolerances: NLT 75% (*Q*) of the labeled amount of secobarbital sodium ($C_{12}H_{17}N_2NaO_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Buffer, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.005 mg/mL of USP Secobarbital RS in *Diluent*. Sonication may be used to aid in dissolution.
Sample solution: Nominally 1 mg/mL of secobarbital sodium in a solution prepared as follows. Transfer the equivalent of 100 mg of secobarbital sodium from NLT 20 Capsules to a 100-mL volumetric flask. Dilute with *Mobile phase* to 50% of the final flask volume. Sonicate and shake. Dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size.
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 6.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual unspecified degradant in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any individual unspecified degradant from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Secobarbital RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of secobarbital sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of secobarbital sodium, 260.26
M_{r2} = molecular weight of secobarbital, 238.28

Acceptance criteria: See *Table 1*.

Table 1

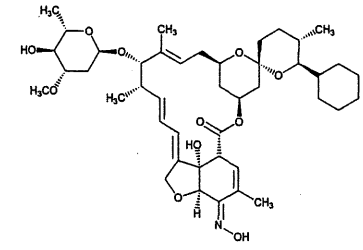
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Iminosecobarbital[a]	0.33	1.0
2-Hydroxypropyl analog[b]	0.38	0.24
Secobarbital	1.0	—
1,3-Dimethylbutyl analog[c]	1.61	0.54
Any individual unspecified degradant	—	0.10
Total impurities	—	1.5

[a] 5-Allyl-4-imino-5-(1-methylbutyl) barbituric acid.
[b] 5-(2-Hydroxypropyl)-5-(1-methylbutyl) barbituric acid.
[c] 5-Allyl-5-(1-3-dimethylbutyl) barbituric acid.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Secobarbital RS

Selamectin



$C_{43}H_{63}NO_{11}$ 769.96
25-Cyclohexyl-4′-*O*-de(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-5-demethoxy-25-de(1-methylpropyl)-22,23-dihydro-5-(hydroxyimino)-avermectin A1a; (2a*E*,4*E*,5′*S*,6*S*,6′*S*,7*S*,8*E*,11*R*,13*R*,15*S*,17a*R*,20a*R*,20b*S*)-6′-Cyclohexyl-7-[(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)oxy]-3′,4′,5′,6,6′,7,10,11,14,15,20a,20b-dodecahydro-20b-hydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17,20(17a*H*)-dione 20-oxime [220119-17-5].

DEFINITION

Selamectin contains NLT 96.0% and NMT 102.0% of selamectin ($C_{43}H_{63}NO_{11}$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Acetonitrile and water (80:20)
Standard solution: 0.2 mg/mL of USP Selamectin RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Selamectin in *Mobile phase*

USP Monographs

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 243 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of selamectin ($C_{43}H_{63}NO_{11}$) in the portion of Selamectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Selamectin RS in the *Standard solution* (mg/mL)
C_U = concentration of Selamectin in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩
Sample: 1.0 g
Acceptance criteria: NMT 0.1%
• **Organic Impurities**
Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	40	60
28	40	60
45	20	80

Diluent: Acetonitrile and water (60:40)
Standard solution: 2.5 µg/mL of USP Selamectin RS in *Diluent*
Sample solution: 500 µg/mL of Selamectin in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 243 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Column temperature: 30°
Flow rate: 2.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.6 for the selamectin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Selamectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of selamectin from the *Standard solution*
C_S = concentration of USP Selamectin RS in the *Standard solution* (µg/mL)
C_U = concentration of Selamectin in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold for impurities is 0.2%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Hydroxyselamectin[a]	0.2	1.0	2.0
Didehydroselamectin[b]	0.4	1.0	2.0
Selamectin aglycone[c]	0.5	1.2	1.5
Selamectin	1.0	—	—
α-Oleandrosyl selamectin[d]	1.7	0.67	1.5
Any other individual impurity	—	1.0	1.0
Total impurities	—	—	4.0

[a] (2a*E*,2′*R*,4*E*,5′*S*,6*S*,6′*S*,7*S*,8*E*,11*R*,13*R*,15*S*,17a*R*,20a*R*,20b*S*)-6′-Cyclohexyl-7-[(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)oxy]-3′,4′,5′,6,6′,7,10,11,14,15,20a,20b-dodecahydro-4′,20b-dihydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17,20(17a*H*)-dione 20-oxime.
[b] (2a*E*,4*E*,5′*S*,6*S*,6′*S*,7*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20a*R*,20b*S*)-6′-Cyclohexyl-7-[(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)oxy]-5′,6,6′,7,10,11,14,15,20a,20b-decahydro-20b-hydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17,20(17a*H*)-dione 20-oxime.
[c] (2a*E*,4*E*,5′*S*,6*S*,6′*S*,7*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20a*R*,20b*S*)-6′-Cyclohexyl-5′,6,6′,7,10,11,14,15,20a,20b-decahydro-7,20b-dihydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17,20(17a*H*)-dione 20-oxime.
[d] (2a*E*,4*E*,5′*S*,6*S*,6′*S*,7*S*,8*E*,11*R*,13*R*,15*S*,17a*R*,20a*R*,20b*S*)-6′-Cyclohexyl-7-[(4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)oxy]-3′,4′,5′,6,6′,7,10,11,14,15,20a,20b-dodecahydro-20b-hydroxy-5′,6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-17,20(17a*H*)-dione 20-oxime.

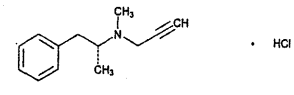
SPECIFIC TESTS

• **Water Determination** ⟨921⟩, *Method I, Method Ia*
Sample: 0.20 g
Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in a tight container.
• **Labeling:** Label it to indicate that it is for veterinary use only.
• **USP Reference Standards** ⟨11⟩
USP Selamectin RS

Selegiline Hydrochloride



$C_{13}H_{17}N \cdot HCl$ 223.74

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Benzeneethanamine, *N*,α-dimethyl-*N*-2-propynyl-, hydrochloride, (*R*)-.
(–)-(*R*)-*N*,α-Dimethyl-*N*-2-propynylphenethylamine hydrochloride [14611-52-0].

» Selegiline Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of $C_{13}H_{17}N \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Selegiline Hydrochloride RS
USP Methamphetamine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 0.5 mg per mL.
Medium: water.
C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay.*
D: It responds to the tests for *Chloride* ⟨191⟩.
Melting range ⟨741⟩: not greater than 2°, within the limits of 141° and 145°.
Specific rotation ⟨781S⟩: between –10.0° and –12.0°.
Test solution: 100 mg per mL, in water.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

Chromatographic purity—
Buffer solution, Mobile phase, and *System suitability solution*—Proceed as directed in the *Assay.*
Standard solution—Transfer 10.0 mL of the *System suitability solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 50 mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Test solution—Transfer 50 mg of Selegiline Hydrochloride to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Chromatographic system—Proceed as directed in the *Assay.* Inject about 20 µL of the *Standard solution,* and record the peak responses as directed in the *Procedure:* the resolution, *R,* between the methamphetamine and selegiline peaks is not less than 3, and the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than three times the retention time of selegiline. Record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Selegiline Hydrochloride taken by the formula:

$$5000(C/W)(r_i/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Selegiline Hydrochloride RS in the *Standard solution, W* is the weight, in mg, of Selegiline Hydrochloride taken to prepare the *Test solution,* r_i is the peak response for each impurity in the chromatogram of the *Test solution,* and r_S is the peak response for selegiline in the chromatogram of the *Standard solution.* Not more than 0.2% of any individual impurity is found, and the sum of all impurities is not more than 1.0%.

Assay—
Buffer solution—Prepare a solution of 0.1 M monobasic ammonium phosphate, adjust with phosphoric acid to a pH of 3.1, and mix.
Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Selegiline Hydrochloride RS, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.
System suitability solution—Dissolve accurately weighed quantities of USP Methamphetamine Hydrochloride RS and USP Selegiline Hydrochloride RS in *Mobile phase* to obtain a solution containing 0.1 mg per mL of each Reference Standard.
Assay preparation—Transfer an accurately weighed quantity, about 50 mg of Selegiline Hydrochloride, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the methamphetamine and selegiline peaks is not less than 3, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{17}N \cdot HCl$ in the portion of Selegiline Hydrochloride taken by the formula:

$$500C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Selegiline Hydrochloride RS in the *Standard preparation,* and r_U and r_S are the selegiline peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Selegiline Hydrochloride Capsules

DEFINITION
Selegiline Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of selegiline hydrochloride ($C_{13}H_{17}N \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

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ASSAY

• **PROCEDURE**

Buffer: 11.5 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.1.

Mobile phase: Acetonitrile and *Buffer* (20:80)

System suitability solution: 0.1 mg/mL each of USP Selegiline Hydrochloride RS and USP Methamphetamine Hydrochloride RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Selegiline Hydrochloride RS in *Mobile phase*

Sample stock solution: Nominally, 1 mg/mL of selegiline hydrochloride from the contents of NLT 20 Capsules, prepared as follows. To the weighed portion of the sample in a suitable volumetric flask add *Mobile phase* to fill 80% of the final flask volume. Sonicate for at least 10 min. Dilute with *Mobile phase* to volume, and centrifuge a portion of the solution for at least 10 min.

Sample solution: Nominally, 0.1 mg/mL of selegiline hydrochloride, by diluting a portion of the supernatant from the *Sample stock solution* with *Mobile phase*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 1.5 times the retention time of selegiline

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for methamphetamine and selegiline are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between the methamphetamine and selegiline peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of selegiline hydrochloride ($C_{13}H_{17}N \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of selegiline from the *Sample solution*

r_S = peak response of selegiline from the *Standard solution*

C_S = concentration of USP Selegiline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of selegiline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL

Apparatus 1: 50 rpm

Time: 20 min

Buffer, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of USP Selegiline Hydrochloride RS in *Mobile phase*

Sample solution: Pass a portion of the solution under test through a suitable filter.

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of selegiline hydrochloride ($C_{13}H_{17}N \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Selegiline Hydrochloride RS in the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of selegiline hydrochloride ($C_{13}H_{17}N \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.

Standard stock solution A: 0.2 mg/mL each of USP Selegiline Hydrochloride RS and USP Selegiline Related Compound D RS in *Mobile phase*

Standard stock solution B: 0.2 mg/mL of USP Methamphetamine Hydrochloride RS in *Mobile phase*

Standard solution: 2 µg/mL each of USP Selegiline Hydrochloride RS and USP Selegiline Related Compound D RS, and 20 µg/mL of USP Methamphetamine Hydrochloride RS in *Mobile phase* from *Standard stock solution A* and *Standard stock solution B*, respectively

Sample solution: Nominally, 1 mg/mL of selegiline hydrochloride in *Mobile phase* from the contents of NLT 20 Capsules, prepared as follows. To the weighed portion of sample in a suitable volumetric flask add *Mobile phase* to fill 60% of the final flask volume. Sonicate for at least 10 min, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system: Proceed as directed in the *Assay* except use a run time of 3 times the retention time of selegiline.

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 6.0 between methamphetamine and selegiline related compound D; NLT 3.2 between selegiline related compound D and selegiline

Relative standard deviation: NMT 10.0% for each of the selegiline related compound D and selegiline peaks; NMT 5.0% for the methamphetamine peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methamphetamine and selegiline related compound D in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methamphetamine or selegiline related compound D from the *Sample solution*

r_S = peak response of the corresponding Reference Standard from the *Standard solution*

C_S = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)

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C_U = nominal concentration of selegiline hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of USP Selegiline Hydrochloride RS from the *Standard solution*
C_S = concentration of USP Selegiline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of selegiline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak below 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methamphetamine[a]	0.62	2.0
Selegiline related compound D[b]	0.85	0.50
Selegiline	1.0	--
Any individual unspecified degradation product	—	0.50
Total impurities[c]	—	2.0

[a] Benzeneethanamine, *N*,α-dimethyl-, (*S*)-.
[b] (*R*)-*N*-(1-Phenylpropan-2-yl)prop-2-yn-1-amine.
[c] Does not include methamphetamine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Methamphetamine Hydrochloride RS
  USP Selegiline Hydrochloride RS
  USP Selegiline Related Compound D RS
  (*R*)-*N*-(1-Phenylpropan-2-yl)prop-2-yn-1-amine hydrochloride.
  $C_{12}H_{15}N \cdot HCl$ 209.72

Selegiline Hydrochloride Tablets

» Selegiline Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{13}H_{17}N \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Selegiline Hydrochloride RS
USP Methamphetamine Hydrochloride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 1: 50 rpm.
Time: 20 minutes.

Determine the amount of $C_{13}H_{17}N \cdot HCl$ dissolved using the following method.

Monobasic ammonium phosphate solution—Dissolve 11.5 g of monobasic ammonium phosphate in 1000 mL of water. Adjust with 85% phosphoric acid to a pH of 3.1.

Mobile phase—Prepare a filtered and degassed mixture of *Monobasic ammonium phosphate solution* and acetonitrile (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Transfer 25.0 mg, accurately weighed, of USP Selegiline Hydrochloride RS to a 50-mL volumetric flask. Dissolve in and dilute with water to volume, and mix. Pipet 2.0 mL of this solution into a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution—At 20 minutes withdraw a 10-mL portion of the solution under test and centrifuge for 10 minutes at 3500 rpm.

Chromatographic system—The liquid chromatograph is equipped with a 205-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is 1.0 mL/min. Chromatograph the *Standard solution*, and record the peak responses. The relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of $C_{13}H_{17}N \cdot HCl$ dissolved.

Tolerances—Not less than 80% (*Q*) of the labeled amount of $C_{13}H_{17}N \cdot HCl$ is dissolved in 20 minutes.

Chromatographic purity—

Buffer solution , *Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the test for *Chromatographic purity* under *Selegiline Hydrochloride*.

Standard solution—Transfer 10.0 mL of the *System suitability solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 20-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Test solution—Use a portion of the supernatant obtained from the *Assay preparation*.

Procedure—Proceed as directed in the test for *Chromatographic purity* under *Selegiline Hydrochloride*. Calculate the percentage of each impurity, excluding methamphetamine hydrochloride, in the portion of Tablets taken by the formula:

$$5000(C/W)(r_i/r_s)$$

in which W is the weight, in mg, of the labeled content of selegiline hydrochloride in the portion of Tablets taken to prepare the *Test solution*, and the other terms are as defined therein. Not more than 0.5% of any individual impurity is found, and the sum of all impurities, excluding methamphetamine hydrochloride, is not more than 2.0%.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Limit of methamphetamine hydrochloride—

Buffer solution , *Mobile phase*, *System suitability solution*, *Test solution*, and *Chromatographic system*—Proceed as directed in the test for *Chromatographic purity* under *Selegiline Hydrochloride*.

Standard solution—Transfer 10.0 mL of the *System suitability solution* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed in the test for *Chromatographic purity* under *Selegiline Hydrochloride*.

USP Monographs

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Calculate the percentage of methamphetamine hydrochloride in the portion of Tablets taken by the formula:

$$5000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Methamphetamine Hydrochloride RS in the *Standard solution*, W is the weight, in mg, of the labeled content of selegiline hydrochloride in the portion of Tablets taken to prepare the *Test solution*, and r_U and r_S are the methamphetamine peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 2.0% of methamphetamine hydrochloride is found.

Assay—

Buffer solution , *Mobile phase*, *Standard preparation*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the test for *Assay* under *Selegiline Hydrochloride*.

Assay preparation—Weigh and powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder equivalent to about 50 mg of selegiline hydrochloride to a 50-mL volumetric flask. Add 40 mL of *Mobile phase*, sonicate for 10 minutes, dilute with *Mobile phase* to volume, and mix. Centrifuge a 25-mL aliquot of this solution at 3500 rpm for 10 minutes. [NOTE—Retain a portion of the supernatant for the *Chromatographic purity* and *Limit of methamphetamine hydrochloride* tests.] Transfer 10.0 mL of the supernatant to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Selegiline Hydrochloride*. Calculate the quantity, in mg, of $C_{13}H_{17}N \cdot HCl$ in the portion of Tablets taken by the formula:

$$500C(r_U/r_S)$$

in which the terms are as defined therein.

Selegiline Hydrochloride Compounded Topical Gel

DEFINITION

Selegiline Hydrochloride Compounded Topical Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of selegiline hydrochloride ($C_{13}H_{17}N \cdot HCl$).

Prepare Selegiline Hydrochloride Compounded Topical Gel 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩). For this preparation, a soy lecithin–isopropyl palmitate mixture and a poloxamer gel need to be separately prepared.

Sorbic Acid	0.2 g
Soy lecithin	50 g
Isopropyl Palmitate	50 g

Prepare a soy lecithin–isopropyl palmitate mixture by mixing 0.2 g of *Sorbic Acid*, 50 g of *Soy lecithin*, and 50 g of *Isopropyl Palmitate* together. Allow the mixture to set overnight, and mix again.

Sorbic Acid	0.2 g
Poloxamer 407	30 g
Purified Water, a sufficient quantity to make	100 mL

Separately, prepare a 30% poloxamer gel by mixing 0.2 g of *Sorbic Acid*, 30 g of *Poloxamer 407*, and a sufficient amount of *Purified Water* to bring to a final volume of 100 mL. Place the gel in the refrigerator overnight, and mix again.

Selegiline hydrochloride powder	1 g
Purified Water	5 mL
Soy lecithin–isopropyl palmitate mixture	22 mL
Poloxamer gel (30%), a sufficient quantity to make	100 mL

Dissolve 1 g of *Selegiline hydrochloride powder* in 5 mL of *Purified Water* in a suitable mortar. Add 22 mL of the previously prepared soy lecithin–isopropyl palmitate mixture to the mortar, and mix well. Add a sufficient amount of the previously prepared 30% poloxamer gel to bring to final volume, and mix using high-shear methods.

ASSAY

• **PROCEDURE**

Solution A: 0.1 M monobasic ammonium phosphate adjusted with phosphoric acid to a pH of 3.1

Mobile phase: Acetonitrile and *Solution A* (20:80). Filter, and degas.

System suitability solution: 0.4 mg/mL each of USP Selegiline Hydrochloride RS and USP Methamphetamine Hydrochloride RS in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Selegiline Hydrochloride RS in *Mobile phase*

Sample solution: Shake thoroughly each container of Topical Gel. Transfer 0.4 mL of Topical Gel into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 8 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for methamphetamine hydrochloride and selegiline hydrochloride are about 0.63 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

Resolution: NLT 8.0 between selegiline and methamphetamine, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of selegiline hydrochloride ($C_{13}H_{17}N \cdot HCl$) in the portion of Topical Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of selegiline hydrochloride from the *Sample solution*

r_S = peak response of selegiline hydrochloride from the *Standard solution*

C_S = concentration of selegiline hydrochloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of selegiline hydrochloride in the *Sample solution* (mg/mL)

USP Monographs

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Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.6–5.6

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in a tight, light-resistant suitable calibrated dispenser. Store at 2°–8° or at controlled room temperature.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature
- **Labeling:** Label it to indicate that it is for external use only, keep out of the reach of children, use only as directed, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Methamphetamine Hydrochloride RS
  USP Selegiline Hydrochloride RS

Selenious Acid

H_2SeO_3 128.97
Selenium dioxide, monohydrated;
Selenious acid [7783-00-8].

DEFINITION
Selenious Acid contains NLT 93.0% and NMT 101.0% of H_2SeO_3.

IDENTIFICATION
- **A.**
  Sample: 50 mg
  Analysis: Dissolve the *Sample* in 5 mL of water. Add 100 mg of sodium bicarbonate, and mix.
  Acceptance criteria: Gas bubbles develop.
- **B.**
  Sample: 50 mg
  Analysis: Dissolve the *Sample* in 5 mL of 0.1 N hydrochloric acid. Add 50 mg of stannous chloride.
  Acceptance criteria: A curdy tan-orange precipitate is formed.

ASSAY
- **Procedure**
  Sample solution: Transfer 100 mg to a suitable glass-stoppered flask, and dissolve in 50 mL of water.
  Analysis: Add 10 mL of a 300-mg/mL potassium iodide solution and 5 mL of hydrochloric acid to the *Sample solution,* and mix. Insert the stopper in the flask, and allow to stand for 10 min. Add 50 mL of water and 3 mL of starch TS. Titrate with 0.1 N sodium thiosulfate VS until the solution is colorless, then titrate with 0.1 N iodine VS to a blue endpoint. Subtract the volume of 0.1 N iodine from the volume of 0.1 N sodium thiosulfate to obtain the volume of 0.1 N sodium thiosulfate equivalent to selenious acid. Each mL of 0.1 N sodium thiosulfate is equivalent to 3.225 mg of H_2SeO_3.
  Acceptance criteria: 93.0%–101.0%

IMPURITIES
- **Residue on Ignition** ⟨281⟩
  Sample: 10 g
  Acceptance criteria: NMT 1.0 mg (0.01%)
- **Insoluble Matter**
  Sample: 1 g
  Analysis: Dissolve the *Sample* in 5 mL of water.
  Acceptance criteria: It dissolves completely, and the solution is clear.
- **Selenate and Sulfate**
  Sample solution: Dissolve 0.5 g in 10 mL of water.
  Analysis: Add 0.1 mL of hydrochloric acid and 1 mL of barium chloride TS to the *Sample solution,* and mix.
  Acceptance criteria: No turbidity or precipitate is formed in 10 min.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.

Selenious Acid Injection

DEFINITION
Selenious Acid Injection is a sterile solution in Water for Injection of Selenious Acid or of selenium dissolved in nitric acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of selenium (Se).

IDENTIFICATION
- **A.** The *Sample solution,* prepared as directed in the *Assay,* exhibits an absorption maximum at about 196 nm, when tested as directed in the *Assay.*

ASSAY
- **Procedure**
  [**Caution**—Selenium is toxic; handle it with care.]
  Standard stock solution: 1000 µg/mL of selenium, prepared as follows. Dissolve about 1 g of metallic selenium in a minimum volume of nitric acid. Evaporate to dryness, add 2 mL of water, and evaporate to dryness. Repeat the addition of water and the evaporation to dryness three times. Dissolve the residue in 3 N hydrochloric acid, transfer to a 1000-mL volumetric flask, dilute with 3 N hydrochloric acid to volume, and mix.
  Standard solution A: 30 µg/mL of selenium from the *Standard stock solution* diluted with water
  Standard solution B: 40 µg/mL of selenium from the *Standard stock solution* diluted with water
  Standard solution C: 50 µg/mL of selenium from the *Standard stock solution* diluted with water
  Sample solution: 40 µg/mL of selenium from Injection diluted with water
  Instrumental conditions
  (See *Atomic Absorption Spectroscopy* ⟨852⟩.)
  Mode: Atomic absorption spectrophotometer
  Lamp: Selenium electrodeless discharge
  Flame: Air–acetylene
  Analytical wavelength: Selenium emission line at 196 nm
  Blank: Water
  Analysis
  Samples: *Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*
  Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of selenium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C_S, in µg/mL, of selenium in the *Sample solution.*
  Calculate the percentage of the labeled amount of selenium (Se) in the portion of Injection taken:

$$\text{Result} = (C_S/C_U) \times 100$$

C_S = concentration of selenium in the *Sample solution* determined from the calibration graph (µg/mL)
C_U = nominal concentration of selenium in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

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SPECIFIC TESTS
- **pH** ⟨791⟩: 1.8–2.4
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 3.5 USP Endotoxin Units/µg of selenium
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.
- **Labeling:** Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid before administration.

Selenium Sulfide

SeS_2 143.09
Selenium sulfide (SeS_2).
Selenium sulfide (SeS_2) [7488-56-4].

» Selenium Sulfide contains not less than 52.0 percent and not more than 55.5 percent of selenium (Se).

Packaging and storage—Preserve in well-closed containers.

Identification—
A: Filter 20 mL of the solution of Selenium Sulfide prepared as directed in the *Assay*, and to 10 mL of the filtrate add 5 mL of water and 5 g of urea. Heat to boiling, cool, and add 2 mL of potassium iodide solution (1 in 10): a yellowish orange to orange color is produced, and it darkens rapidly (*presence of selenium*).
B: Allow the solution obtained in *Identification* test *A* to stand for 10 minutes, filter, and to the filtrate add 10 mL of barium chloride TS: the solution becomes turbid (*presence of sulfur*).
Residue on ignition ⟨281⟩: not more than 0.2%.

Soluble selenium compounds—
Test solution—Mix 10.0 g of Selenium Sulfide with 100.0 mL of water in a 250-mL flask, allow to stand for 1 hour, with frequent agitation, and filter. To 10.0 mL of the filtrate add 2 mL of 2.5 M formic acid, dilute with water to 50 mL, mix, and adjust, if necessary, to a pH of 2.5 ± 0.5. Add 2 mL of freshly prepared 3,3′-diaminobenzidine hydrochloride solution (1 in 200), mix, allow to stand for 45 minutes, and adjust with 6 N ammonium hydroxide to a pH of 6.5 ± 0.5. Transfer to a separator, add 10.0 mL of toluene, shake vigorously for 1 minute, allow the layers to separate, and discard the aqueous phase.
Standard solution—Using 10.0 mL of a solution of selenious acid containing 0.5 µg of selenium per mL, prepare a solution as directed under *Test solution*, beginning with "add 2 mL of 2.5 M formic acid."
Procedure—Concomitantly determine the absorbances of the toluene layers of the *Test solution* and the *Standard solution* in 1-cm cells at 420 nm, with a suitable spectrophotometer, using a blank consisting of the same quantities of the same reagents treated in the same manner as the *Test solution:* the absorbance of the *Test solution* is not greater than that of the *Standard solution* (5 ppm).

Assay—Place about 100 mg of Selenium Sulfide, accurately weighed, in a suitable container, add 25 mL of fuming nitric acid, and digest over gentle heat until no further solution occurs. Cool, transfer the solution to a 250-mL volumetric flask containing 100 mL of water, cool again, dilute with water to volume, and mix. Pipet 50 mL of the solution into a suitable flask, add 25 mL of water and 10 g of urea, and heat to boiling. Cool, add 3 mL of starch TS, then add 10 mL of potassium iodide solution (1 in 10), and immediately titrate with 0.05 N sodium thiosulfate VS. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N sodium thiosulfate is equivalent to 987.0 µg of Se.

Selenium Sulfide Topical Suspension

» Selenium Sulfide Topical Suspension is an aqueous, stabilized suspension of Selenium Sulfide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of SeS_2. It contains suitable buffering and dispersing agents.
[NOTE—Where labeled for use as a shampoo, it contains a detergent. Where labeled for other uses, it may contain a detergent.]

Packaging and storage—Preserve in tight containers.
Identification—Digest about 2 g with 5 mL of nitric acid over gentle heat for 1 hour, dilute with water to about 50 mL, and filter: the solution responds to *Identification* test *A* under *Selenium Sulfide,* when tested as directed, beginning with "to 10 mL of the filtrate add 5 mL of water."
pH ⟨791⟩: between 2.0 and 6.0.
Assay—Place a portion of well-mixed Topical Suspension, equivalent to about 100 mg of selenium sulfide and accurately weighed, in a suitable flask. Cautiously digest with 25 mL of fuming nitric acid over gentle heat for 2 hours, and proceed as directed in the *Assay* under *Selenium Sulfide,* beginning with "Cool, transfer the solution to a 250-mL volumetric flask." Each mL of 0.05 N sodium thiosulfate is equivalent to 1.789 mg of SeS_2. Where the Topical Suspension is labeled in terms of percentage (w/v) or of the amount of SeS_2 in a given volume of Topical Suspension, determine the density of the Topical Suspension as follows: Using a tared, 100-mL volumetric flask, weigh 100 mL of Topical Suspension that previously has been shaken to ensure homogeneity, allowed to stand until the entrapped air rises, and finally inverted carefully just prior to transfer to the volumetric flask. From the observed weight of 100 mL of the Topical Suspension, calculate the quantity of SeS_2 in each 100 mL.

Senna Leaf

DEFINITION
Senna Leaf consists of the dried leaflet of *Senna alexandrina* Mill., also known as *Cassia acutifolia* Delile (Alexandrian senna) or *C. angustifolia* Vahl (Tinnevelly senna), (Fam. Fabaceae). Senna Leaf contains NLT 2.5% of anthraquinone glucosides, calculated as sennosides, on the dried basis.

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IDENTIFICATION

• **A.**

Potassium hydroxide solution: 100 mg/mL of potassium hydroxide in alcohol

Sample: 500 mg of finely powdered Senna Leaf

Analysis: Add 10 mL of *Potassium hydroxide solution* to the *Sample*. Boil for 2 min, dilute with 10 mL of water, and filter. Acidify the filtrate with hydrochloric acid. Shake with ether, remove the ether layer, and shake with 5 mL of 6 N ammonium hydroxide.

Acceptance criteria: The aqueous layer is orange or bluish red.

ASSAY

• **PROCEDURE**

[NOTE—Conduct all sample preparations with minimal exposure to subdued light, and use low-actinic glassware to protect solutions from light.]

Ferric chloride solution: 105 mg/mL of ferric chloride

Methanolic magnesium acetate solution: 5 mg/mL of magnesium acetate in methanol

Sodium bicarbonate solution: 5 mg/mL of sodium bicarbonate

Standard solution: 0.13 mg/mL of USP Sennosides RS in *Sodium bicarbonate solution*

Sample solution: Weigh and pulverize 10 g of Senna Leaf. Transfer 0.15 g to a 100-mL round-bottom flask. Add 30 mL of water. Mix, weigh, attach a condenser, and reflux in a water bath for 15 min. Cool to room temperature, weigh, and adjust to the original weight with water. Centrifuge, and transfer 20.0 mL of the supernatant to a 150-mL separatory funnel. Add 0.1 mL of diluted hydrochloric acid, and shake with three 15-mL quantities of chloroform. Allow to separate, and discard the chloroform layer after each addition. Add about 0.1 g of sodium bicarbonate, shake for 3 min, and centrifuge. Use the supernatant as the *Sample solution*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Wavelength: 515 nm

Cell: Quartz

Blank: Methanol

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer 10.0 mL each of the *Standard solution* and the *Sample solution* to separate 100-mL round-bottom flasks equipped with condensers. Add 20 mL of *Ferric chloride solution*, and mix. Reflux in a water bath for 20 min. Add 1 mL of hydrochloric acid, and reflux for an additional 20 min, with frequent shaking, to dissolve the precipitates. Cool to room temperature, transfer the mixtures to separate 100-mL separatory funnels, and shake with three 25-mL quantities of ether previously used to rinse the flasks. Combine the ether extracts, mix, and wash with two 15-mL quantities of water. Transfer the ether layers to separate 100-mL volumetric flasks. Dilute with ether to volume, and mix. Evaporate 10.0 mL of the ether extracts to dryness, and dissolve the residue in 10.0 mL of *Methanolic magnesium acetate solution*. Determine the absorbance of the resulting solution from the *Standard solution* and *Sample solution*, with a suitable spectrophotometer fitted with matched quartz cells, using the *Blank*.

Calculate the percentage of sennosides in the portion of Senna Leaf taken:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/W) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Sennosides RS in the *Standard solution* (mg/mL)

V = volume of water to which powdered Senna Leaf was added, 30 mL

W = weight of powdered Senna Leaf (mg)

Acceptance criteria: NLT 2.5% of anthraquinone glucosides, calculated as sennosides, on the dried basis

CONTAMINANTS

• **MICROBIAL ENUMERATION TESTS** ⟨2021⟩**:** The total bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^3 cfu/g.

• **ABSENCE OF SPECIFIED MICROORGANISMS** ⟨2022⟩, *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli***:** Meets the requirements

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis***:** Meets the requirements

SPECIFIC TESTS

• **BOTANICAL CHARACTERISTICS**

Macroscopic

Unground Alexandrian senna leaf: Inequilaterally lanceolate or lance-ovate leaflets, frequently broken; 1.5–3.5 cm in length and 5–10 mm in width, unequal at the base, with very short, stout petiolules. The leaflets are acutely cuspidate, entire, brittle, and subcoriaceous, with short and somewhat appressed hairs, few on the upper surface, more numerous on the lower surface, where they occur spreading on the midrib, especially on its lower part. The color is weak yellow to light grayish green to pale olive. The odor is characteristic.

Unground Tinnevelly senna leaf: Usually unbroken leaflets, 2–5 cm in length and 6–15 mm in width; acute at the apex; and slightly hairy. The color of the leaves is weak yellow to pale olive.

Powdered Senna Leaf: Dusky greenish yellow to light olive brown

Microscopic

Transverse section: Senna Leaf shows polygonal epidermal cells with straight walls and frequently containing mucilage; numerous, broadly elliptical stomata mostly 20–35 µm in length, usually bordered by two neighbor-cells with their long axes parallel to that of the stoma, and rarely, though more frequently in Alexandrian senna leaf, a third epidermal cell at the end of the stoma. The hairs are nonglandular, one-celled, conical, often curved, with thick papillose walls, 100–350 µm in length. Palisade cells in a single layer underlie both surfaces except in the midrib region where they occur only beneath the upper epidermis. A meristele occurs in the midrib composed of several radially arranged fibrovascular bundles, the latter separated by narrow vascular rays and supported above and below by arcs of lignified pericyclic fibers. Calcium oxalate occurs in rosette aggregates in the spongy parenchyma and in six- to eight-sided prisms in the crystal fibers, which lie on the outer surface of each group of pericyclic fibers.

Powdered Senna Leaf: Fragments of veins bearing lignified vessels, tracheids, and crystal fibers, isolated hairs, masses of palisade and spongy parenchyma, fragments of epidermis with stomata, free calcium oxalate rosette aggregates, and prisms 10–20 µm in length. In powdered Alexandrian senna leaf, the hairs are more numerous than in powdered Tinnevelly senna leaf.

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Foreign Organic Matter***:** The amount of senna stems is NMT

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8.0%, and the amount of senna pods or other foreign organic matter is NMT 2.0%.
- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Total Ash*: NMT 12.0%
- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*: NMT 3.0%
- **LOSS ON DRYING** ⟨731⟩

Sample: 1.0 g of finely powdered Senna Leaf
Analysis: Dry the *Sample* at 105° for 2 h.
Acceptance criteria: It loses NMT 12.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve against attack by insects and rodents. Store protected from light and moisture at room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the part(s) of the plant contained in the article.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sennosides RS

Senna Fluidextract

DEFINITION
Prepare Senna Fluidextract as follows. Mix 1000 g of Senna, in coarse powder, with a sufficient quantity (600–800 mL) of menstruum consisting of a mixture of 1 volume of alcohol and 2 volumes of water to make it evenly and distinctly damp. After 15 min, pack the mixture firmly into a suitable percolator, and cover the drug with additional menstruum. Macerate for 24 h, then percolate at a moderate rate, adding fresh menstruum, until the drug is practically exhausted of its active principals. Reserve the first 800 mL of percolate, and use it to dissolve the residue from the additional percolate that has been concentrated to a soft extract at a temperature not to exceed 60°. Add water and alcohol to make the product measure 1000 mL.

OTHER COMPONENTS
- **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩: 23.0%–27.0% of C_2H_5OH

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

Senna Pods

DEFINITION
Senna Pods are the dried ripe fruits of *Senna alexandrina* Mill., also known as *Cassia acutifolia* Delile (Alexandrian senna) or *C. angustifolia* Vahl (Tinnevelly senna), (Fam. Fabaceae). Senna Pods contain NLT 3.4% (Alexandrian senna) and NLT 2.2% (Tinnevelly senna) of anthraquinone glucosides, calculated as sennosides, on the dried basis.

IDENTIFICATION
- **A.**

Potassium hydroxide solution: 100 mg/mL of potassium hydroxide in alcohol
Sample: 500 mg of finely powdered Senna Pods
Analysis: Add 10 mL of *Potassium hydroxide solution* to the *Sample*. Boil for 2 min, dilute with 10 mL of water, and filter. Acidify the filtrate with hydrochloric acid. Shake it with ether, remove the ether layer, and shake it with 5 mL of 6 N ammonium hydroxide.
Acceptance criteria: The aqueous layer is orange or bluish red.

ASSAY
- **PROCEDURE**

[NOTE—Conduct all sample preparations with minimal exposure to subdued light, and use low-actinic glassware to protect solutions from light.]

Ferric chloride solution: 105 mg/mL of ferric chloride
Methanolic magnesium acetate solution: 5 mg/mL of magnesium acetate in methanol
Sodium bicarbonate solution: 5 mg/mL of sodium bicarbonate
Standard solution: 0.13 mg/mL of USP Sennosides RS in *Sodium bicarbonate solution*
Sample solution: Weigh and pulverize 10 g of Senna Pods. Transfer 0.15 g to a 100-mL round-bottom flask. Add 30 mL of water. Mix, weigh, attach a condenser, and reflux in a water bath for 15 min. Cool to room temperature, weigh, and adjust to the original weight with water. Centrifuge, and transfer 20.0 mL of the supernatant to a 150-mL separatory funnel. Add 0.1 mL of diluted hydrochloric acid, and shake with three 15-mL quantities of chloroform. Allow to separate, and discard the chloroform layer after each addition. Add about 0.1 g of sodium bicarbonate, shake for 3 min, and centrifuge. Use the supernatant as the *Sample solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Wavelength: 515 nm
Cell: Quartz
Blank: Methanol
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Transfer 10.0 mL each of the *Standard solution* and the *Sample solution* to separate 100-mL round-bottom flasks equipped with condensers. Add 20 mL of *Ferric chloride solution*, and mix. Reflux in a water bath for 20 min. Add 1 mL of hydrochloric acid, and reflux for an additional 20 min, with frequent shaking, to dissolve the precipitates. Cool to room temperature, transfer the mixtures to separate 100-mL separatory funnels, and shake with three 25-mL quantities of ether previously used to rinse the flasks. Combine the ether extracts, mix, and wash with two 15-mL quantities of water. Transfer the ether layers to separate 100-mL volumetric flasks. Dilute with ether to volume, and mix. Evaporate 10.0 mL of the ether extracts to dryness, and dissolve the residue in 10.0 mL of *Methanolic magnesium acetate solution*. Determine the absorbance of the resulting solution from the *Standard solution* and the *Sample solution*, with a suitable spectrophotometer fitted with matched quartz cells, using the *Blank*.
Calculate the percentage of sennosides in the portion of Senna Pods taken:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/W) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Sennosides RS in the *Standard solution* (mg/mL)
V = volume of water to prepare the *Sample solution*, 30 mL
W = weight of powdered Senna Pods taken (mg)

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Acceptance criteria: NLT 3.4% (Alexandrian senna) and NLT 2.2% (Tinnevelly senna) of anthraquinone glucosides, calculated as sennosides, on the dried basis

CONTAMINANTS

- **MICROBIAL ENUMERATION TESTS** ⟨2021⟩: The total bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS** ⟨2022⟩, *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meet the requirements
- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meet the requirements

SPECIFIC TESTS

- **BOTANICAL CHARACTERISTICS**

Macroscopic

Unground Alexandrian senna pods: Occur as flattened, reniform, membranous, leathery pods; green to greenish brown with brown patches at the positions corresponding to the seeds; 40–50 mm in length and at least 20 mm wide; at one end is a stylar point and at the other a short stalk. The pods contain six or seven flattened and obovate seeds, green to pale brown, with a continuous network of prominent ridges on the testa.

Unground Tinnevelly senna pods: Occur as flattened, slightly reniform, membranous, leathery pods; brown to yellowish brown with brown patches at the positions corresponding to the seeds; slightly longer but narrower than Alexandrian senna pods, 35–60 mm in length and 14–18 mm wide; at one end is a stylar point and at the other a short stalk. The pods contain 5–8 flattened and obovate seeds, green to pale brown, with incomplete, wavy, transverse ridges on the testa.

Powdered Senna Pods: Brown powder

Microscopic

Senna Pods: Senna Pods show epicarp with very thick cuticulized polygonal cells, occasional anomocytic or paracytic stomata, and very few nonglandular, one-celled, conical, often curved hairs, with thick papillose walls, 100–350 µm in length; mesocarp consists of parenchymatous tissue containing a layer of calcium oxalate prisms and vascular bundles partially enclosed by fibers; endocarp with two crossed layers of fibers; seeds with a subepidermal layer of palisade cells with thick outer walls and endosperm of polyhedral cells with mucilaginous walls.

Powdered Senna Pods: Polygonal cells with occasional small numbers of nonglandular hairs and anomocytic or paracytic stomata, fibers in two crossed layers accompanied by a crystal sheath of calcium oxalate prisms, isolated hairs, masses of palisade cells of the seeds, clusters, and prisms of calcium oxalate.

- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*: NMT 1.0%
- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Total Ash*: NMT 9.0%
- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*: NMT 2.0%
- **LOSS ON DRYING** ⟨731⟩

Sample: 1.0 g of finely powdered Senna Pods
Analysis: Dry the *Sample* at 105° for 2 h.
Acceptance criteria: It loses NMT 12.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve against attack by insects and rodents. Store protected from light and moisture at room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the part(s) of the plant contained in the article.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sennosides RS

Senna Oral Solution

DEFINITION

Prepare Senna Oral Solution as follows.

Senna Fluidextract	250 mL
Suitable essential oil(s)	
Sucrose	635 g
Purified Water, a sufficient quantity to make	1000 mL

Mix the oil(s) with the *Senna Fluidextract*, and gradually add 330 mL of *Purified Water*. Allow the mixture to stand for 24 h in a cool place, with occasional agitation, then filter, and pass enough *Purified Water* through the filter to obtain 580 mL of filtrate. Dissolve the *Sucrose* in this liquid, and add sufficient *Purified Water* to make the product measure 1000 mL. Mix, and strain.

OTHER COMPONENTS

- **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩: 90.0%–110.0% of the labeled amount of C_2H_5OH

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, at a temperature not exceeding 25°.

Sennosides

DEFINITION

Change to read:

Sennosides is a partially purified natural complex of anthraquinone glucosides, isolated from senna leaflets and/or senna pods, *Senna alexandrina* Mill. [syn. *Cassia acutifolia* ▲Delile▲ 2S (USP41) or *C. angustifolia* ▲Vahl] (Family Fabaceae),▲ 2S (USP41) as calcium salts. It contains NLT 90.0% and NMT 110.0% of the labeled amount of sennosides. The labeled amount is NLT 60.0% (w/w), calculated on the dried basis.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Solvent: Ethyl acetate, *n*-propyl alcohol, and water (1:1:1). Shake well, and discard the upper layer.
Standard solution: 1 mg/mL of USP Sennosides RS in *Solvent*
Sample solution: 1 mg/mL of Sennosides in *Solvent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Ethyl acetate, *n*-propyl alcohol, and water (4:4:3)

USP Monographs

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Analysis: Apply the solutions, as 1-cm bands, on a line 2.5 cm from the bottom edge of a thin-layer chromatographic plate. Develop and dry. Examine the plate under long-wavelength UV light. Expose the plate to ammonium hydroxide vapor until color develops (about 5 min). Cover the plate with a piece of glass, and heat at 120° for 5 min.

Acceptance criteria: The two most prominent spots of the *Sample solution* correspond in color and position to those of the *Standard solution*.

COMPOSITION

• **CONTENT OF TOTAL SENNOSIDES**

Buffer: Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of the dibasic sodium phosphate solution. Adjust, if necessary, with the dibasic sodium phosphate solution to a pH of 7.0.

Borate solution: 37.9 g/L of sodium borate in water

Sodium dithionite solution: 15 g/L of sodium dithionite in water

Standard solution: 1 mg/mL of USP Sennosides RS in *Buffer*. Dissolve with the aid of an ultrasonic bath.

Sample solution: 1 mg/mL of Sennosides in *Buffer*

Instrumental conditions

(See *Fluorescence Spectroscopy* ⟨853⟩.)

Mode: Fluorescence

Excitation wavelength: 392 nm

Emission wavelength: 505 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Pipet 1-mL portions of the *Standard solution* and the *Sample solution* into separate 100-mL volumetric flasks, and dilute with *Borate solution* to volume. Transfer 5.0-mL portions of each of the resulting solutions to separate, low-actinic glass, 50-mL volumetric flasks. Add 15.0 mL of *Borate solution* and 15.0 mL of *Sodium dithionite solution*. Pass nitrogen through the solutions, seal the flasks with nitrogen-filled balloons, and heat in a water bath for 30 min. Cool the flasks for 15 min in a water bath thermostatically controlled at 20°. Dilute the solutions with *Borate solution* to volume. Determine, without delay, the fluorescence intensities of the resulting solutions, for which the time elapsed between the addition of *Sodium dithionite solution* and the measurement is the same.

Calculate the percentage of the labeled amount of sennosides in the portion of the *Sample* taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times (100/L)$$

I_U = fluorescence value observed in the *Sample solution*

I_S = fluorescence value observed in the *Standard solution*

C_S = concentration of USP Sennosides RS in the *Standard solution*, corrected for loss on drying (mg/mL)

C_U = concentration of Sennosides in the *Sample solution* (mg/mL)

L = labeled amount of total sennosides (mg/mg)

Acceptance criteria: 90.0%–110.0% of the labeled amount of sennosides

Change to read:

• **CONTENT OF SENNOSIDES A AND B**

Solvent: 1% sodium acetate in water

Buffer solution: Dissolve 3.6 g of dibasic sodium phosphate dodecahydrate in 50 mL of water, add to a solution of 6.2 g monobasic sodium phosphate dihydrate in 200 mL of water, mix, and adjust the pH to 5.0. Dilute the final solution (1:10) in water.

Solution A: Use a filtered and degassed mixture of *Buffer solution* and acetonitrile (1:1), containing 0.5% of benzyldimethylstearylammonium chloride.

Solution B: Use filtered and degassed acetonitrile.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Elution
0–35	100	0	Isocratic
35–40	100→30	0→70	Linear gradient
40–50	30	70	Isocratic
50–55	30→100	70→0	Linear gradient
55–60	100	0	Isocratic

Standard solution A: 0.1 mg/mL of USP Sennoside A RS in *Solvent*. Pass through a membrane filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

Standard solution B: 0.1 mg/mL of USP Sennoside B RS in *Solvent*. Pass through a membrane filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

▲**Standard solution C:** 0.3 mg/mL of USP Sennosides RS in *Solvent*. Pass through a membrane filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.▲ 2S (USP41)

Sample solution: 0.3 mg/mL of Sennosides in *Solvent*. Pass through a membrane filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: About 1 mL/min, adjusted so the retention time of the sennoside B peak is 30 min

Injection volume: 10 µL

System suitability

Sample: ▲*Standard solution C*▲ 2S (USP41)

Suitability requirements

Resolution: ▲NLT 1.5 between the sennoside B and preceding peaks, between the sennoside B and sennoside A peaks, and between the sennoside A and subsequent peaks▲ 2S (USP41)

Relative standard deviation: NMT 2.0% determined for the sum of the areas of the sennoside A and sennoside B peaks in replicate injections

▲**Chromatogram similarity:** The chromatogram obtained is similar to the reference chromatogram provided with the lot of USP Sennosides RS being used.▲ 2S (USP41)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of sennosides A and B in the portion of Sennosides taken:

$$\text{Result} = (r_U/r_S) \times (C \times V/W) \times 100 \times F$$

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r_U = peak area of relevant sennoside in the *Sample solution*
r_S = peak area of relevant sennoside in the corresponding *Standard solution*
C = concentration of relevant sennoside in the corresponding *Standard solution* (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Sennosides, corrected for the loss on drying, taken to prepare the *Sample solution* (mg)
F = conversion factor for the molecular weights of sennoside A or sennoside B to the corresponding calcium salt, 1.044

Calculate the total percentage of sennoside A and sennoside B relative to the labeled amount of total sennosides:

$$\text{Result} = [(A + B)/L] \times 100$$

A = percentage of sennoside A
B = percentage of sennoside B
L = labeled amount of total sennosides (%)

Acceptance criteria: The total percentage of sennosides A and B is NLT 60% of the labeled amount of total sennosides, calculated on the dried basis.

CONTAMINANTS

Delete the following:

▲• **HEAVY METALS,** *Method II* ⟨231⟩**:** 60 µg/g▲ (Official 1-Jan-2018)

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 6.3–7.3, in a 100-mg/mL solution
- **RESIDUE ON IGNITION** ⟨281⟩**:** 5.0%–8.0%, ignited at 800 ± 25°, the use of sulfuric acid being omitted
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 100° to constant weight.
Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store protected from light and moisture, at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sennoside A RS
USP Sennoside B RS
USP Sennosides RS

Sennosides Tablets

DEFINITION

Sennosides Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of Sennosides.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**

Solvent: Ethyl acetate, *n*-propyl alcohol, and water (1:1:1). Shake well, and discard the upper layer.
Standard solution: 1 mg/mL of USP Sennosides RS in *Solvent*
Sample solution: Shake a portion of finely powdered Tablets equivalent to 20 mg of sennosides with 20 mL of *Solvent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Ethyl acetate, *n*-propyl alcohol, and water (4:4:3)
Analysis: Proceed as directed in the chapter. Apply the solutions, as 1-cm streaks, on a line 2.5 cm from the bottom edge of a thin-layer chromatographic plate. Examine the plate under long-wavelength UV light. Expose the plate to ammonium hydroxide vapor until color develops (about 5 min). Cover the plate with a piece of glass, and heat at 120° for 5 min.
Acceptance criteria: The two most prominent spots from the *Sample solution* correspond in color and mobility to those from the *Standard solution*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of the dibasic sodium phosphate solution. Adjust, if necessary, to a pH of 7.0 with the dibasic sodium phosphate solution.
Borate solution: 37.9 g/L of sodium borate in water
Sodium dithionite solution: 15 g/L of sodium dithionite in water
Standard solution: 1 mg/mL of USP Sennosides RS in *Buffer*. Dissolve with the aid of an ultrasonic bath.
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 25 mg of sennosides, to a 25-mL volumetric flask. Add 20 mL of *Buffer*, and sonicate to dissolve. Add additional *Buffer* to volume. Centrifuge the resulting suspension for 15 min at 3500 rpm. Use the supernatant.
Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)
Mode: Fluorescence
Excitation wavelength: 392 nm
Emission wavelength: 505 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Pipet 1-mL portions of the *Standard solution* and the *Sample solution* into separate 100-mL volumetric flasks, and dilute with *Borate solution* to volume. Transfer 5.0-mL portions of each of the resulting solutions to separate low-actinic glass, 50-mL volumetric flasks, and add 15 mL of *Borate solution* and 15.0 mL of *Sodium dithionite solution*. Pass nitrogen through the solutions, seal the flasks with nitrogen-filled balloons, and heat in a water bath for 30 min. Cool the flasks for 15 min in a water bath thermostatically controlled at 20°. Dilute the solutions with *Borate solution* to volume. Determine without delay the fluorescence intensities of the resulting solutions, for which the time elapsed between the addition of *Sodium dithionite solution* and the measurement is the same.
Calculate the percentage of the labeled amount of sennosides in the portion of Tablets taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence value observed in the *Sample solution*
I_S = fluorescence value observed in the *Standard solution*
C_S = concentration of USP Sennosides RS in the *Standard solution* (mg/mL)

USP Monographs

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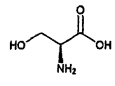
C_U = nominal concentration of sennosides in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 1: 100 rpm
  Time: 120 min
  Analysis: Determine the amount of sennosides dissolved, using the procedure set forth in the *Assay*, making any necessary volumetric adjustments.
  Tolerances: NLT 75% (*Q*) of the labeled amount of sennosides is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sennosides RS

Serine



$C_3H_7NO_3$ 105.09
L-Serine [56-45-1].

DEFINITION
Serine contains NLT 98.5% and NMT 101.5% of L-serine ($C_3H_7NO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample: 100 mg of Serine
  Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Endpoint detection: Potentiometric
  Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the *Blank* determination.
  Calculate the percentage of serine ($C_3H_7NO_3$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 105.1 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

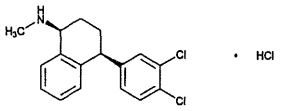
IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Standard solution: 0.50 mL of 0.020 N hydrochloric acid
  Sample: 0.73 g of Serine
  Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Standard solution: 0.10 mL of 0.020 N sulfuric acid
  Sample: 0.33 g of Serine
  Acceptance criteria: NMT 0.03%
- **IRON** ⟨241⟩: NMT 30 ppm
- **RELATED COMPOUNDS**
  System suitability solution: 0.4 mg/mL each of USP L-Serine RS and USP L-Methionine RS in 0.1 N hydrochloric acid
  Standard solution: 0.05 mg/mL of USP L-Serine RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to about 0.5% of that of the *Sample solution*.]
  Sample solution: 10 mg/mL of Serine in 0.1 N hydrochloric acid
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)
  Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
  System suitability
  Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.
  Analysis
  Samples: *System suitability solution*, *Standard solution*, and *Sample solution*.
  After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
  Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
  Individual impurities: NMT 0.5%
  Total impurities: NMT 2.0%

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 100 mg/mL in 2 N hydrochloric acid
  Acceptance criteria: +14.0° to +15.6°
- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 3 h: it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Methionine RS
  USP L-Serine RS

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Sertraline Hydrochloride



$C_{17}H_{17}Cl_2N \cdot HCl$ 342.69

1-Naphthalenamine, 4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-, hydrochloride, (1*S*-*cis*)-;
(1*S*,4*S*)-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthylamine hydrochloride [79559-97-0].

DEFINITION

Sertraline Hydrochloride contains NLT 97.0% and NMT 102.0% of sertraline hydrochloride ($C_{17}H_{17}Cl_2N \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of sertraline hydrochloride from the *System suitability solution*, as obtained in the test for *Limit of (R,R) Sertraline Hydrochloride*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

Sample solution: 1 mg/mL of Sertraline Hydrochloride in a mixture of dehydrated alcohol and water (1:1), prepared as follows. Dissolve 10 mg of Sertraline Hydrochloride in 5 mL of dehydrated alcohol. Add 5 mL of water to the solution.

Acceptance criteria: Meets the requirements of test *A*

ASSAY

- **PROCEDURE**

Buffer: To 28.6 mL of glacial acetic acid, slowly add, while stirring, 34.8 mL of triethylamine, and dilute with water to 100 mL. Dilute 10 mL of the resulting solution with water to 1 L.

Mobile phase: Acetonitrile, methanol, and *Buffer* (45:15:40)

Standard solution: 0.05 mg/mL of USP Sertraline Hydrochloride RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Sertraline Hydrochloride in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Column temperature: 30°
Flow rate: 1.8 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of sertraline

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sertraline hydrochloride ($C_{17}H_{17}Cl_2N \cdot HCl$) in the portion of Sertraline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Sertraline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.3%
- **LIMIT OF (*R,R*) SERTRALINE HYDROCHLORIDE**

Mobile phase: Hexane, 2-propanol, and diethylamine (960: 40: 1.5)

System suitability solution: Transfer 10 mg of USP Sertraline Hydrochloride Racemic Mixture RS into a 20-mL volumetric flask. Add 4 mL of diluted ammonia water (1 in 10) and 10 mL of hexane. Shake well until the organic phase is clear. Wait for phase separation, transfer about 2.0 mL from the top layer into a 20-mL volumetric flask, and dilute with hexane to volume.

Standard solution: Transfer 10 mg of USP Sertraline Hydrochloride RS into a 20-mL volumetric flask. Add 4 mL of diluted ammonia water (1 in 10) and 10 mL of hexane. Shake well until the organic phase is clear. Wait for phase separation, transfer 1.0 mL from the top layer into a 10-mL volumetric flask, and dilute with hexane to volume. Further dilute quantitatively and stepwise, if necessary, to obtain a solution having a known concentration of 0.01 mg/mL.

Sample solution: Transfer 20 mg of Sertraline Hydrochloride to a 20-mL volumetric flask. Add 4 mL of diluted ammonia water (1 in 10) and 10 mL of hexane. Shake well until the organic phase is clear. Wait for phase separation, and use the top layer.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L40
Column temperature: 5°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for sertraline and (*R,R*) sertraline are about 1.0 and 1.16, respectively.]
Suitability requirements
Resolution: NLT 2.8 between sertraline and (*R,R*) sertraline
Relative standard deviation: NMT 10.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of (*R,R*) sertraline hydrochloride in the portion of Sertraline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for (*R,R*) sertraline from the *Sample solution*
r_S = peak response for sertraline from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Sertraline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.5%

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• **ORGANIC IMPURITIES, PROCEDURE 1**

If sertralone is a known process impurity, then the tests for *Organic Impurities, Procedure 2*, and *Limit of Mandelic Acid* are recommended.

Buffer: 5.8 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 4.2.

Mobile phase: Methanol and *Buffer* (48:52)

System suitability solution: 0.5 mg/mL each of USP Sertraline Hydrochloride RS and USP Sertraline Hydrochloride Related Compound A RS in *Mobile phase*

Standard solution: 0.5 µg/mL of USP Sertraline Hydrochloride RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Sertraline Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm × 25-cm; 5-µm packing L45

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.2 between sertraline hydrochloride related compound A (trans-*R,S* isomer) and sertraline, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in sertraline hydrochloride ($C_{17}H_{17}Cl_2N \cdot HCl$) in the portion of Sertraline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity in the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Sertraline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,3-Isosertraline[a]	0.32	0.15
4-Deschlorosertraline[b]	0.42	0.20
3-Deschlorosertraline[c]	0.60	0.20
Mandelic acid	0.70	0.10
Sertraline	1.0	—
Sertraline related compound A (trans-*R,S* isomer)	1.19	0.10
Sertraline related compound A (trans-*S,R* isomer)	1.64	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] (1*RS*,4*RS*)-4-(2,3-Dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine.

[b] (1*RS*,4*RS*)-4-(3-Chlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine.

[c] (1*RS*,4*RS*)-4-(4-Chlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine. [NOTE—Disregard any peak at or below 0.05%.]

• **ORGANIC IMPURITIES, PROCEDURE 2**

System suitability solution: Dissolve 50 mg of USP Sertraline Hydrochloride RS and 0.5 mg of USP Sertraline Hydrochloride Related Compound A RS in 2 mL of methanol in a stoppered centrifuge tube. Add 0.2 mL of 25% potassium carbonate solution, and mix in a vortex mixer for 30 s. Add 8 mL of methylene chloride. Stopper the tube, and mix in a vortex mixer for 60 s. Add 1 g of anhydrous sodium sulfate. Mix well, and centrifuge for 5 min.

Sample solution: Dissolve 250 mg of Sertraline Hydrochloride in 2 mL of methanol in a stoppered centrifuge tube. Add 0.2 mL of 25% potassium carbonate solution, and mix in a vortex mixer for 30 s. Add 8 mL of methylene chloride. Stopper the tube, and mix in a vortex mixer for 60 s. Add 1 g of anhydrous sodium sulfate. Mix well, and centrifuge for 5 min.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame-ionization

Column: 30-m × 0.53-mm fused silica column coated with a 1.0-µm film of phase G3

Temperatures

Injection port: 250°

Detector: 280°

Column: See *Table 2* for the column temperature program.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
200	0	200	1
200	2	260	8

Carrier gas: Helium

Flow rate: 9 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 1:10

System suitability

Sample: *System suitability solution*

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the sertraline related compound A peak to the height of the valley between the sertraline related compound A peak and the sertraline peak is NLT 15.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Sertraline Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

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r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the peak responses for all the peaks from the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3,4-Deschlorosertraline[a]	0.5	0.2
3-Deschlorosertraline[b] and 4-Deschlorosertraline[c]	0.7	0.8
Sertraline	1.0	—
Sertraline related compound A	1.05	0.2
Sertralone[d]	1.1	0.2
Any individual unspecified impurity	—	0.10
Total impurities[e]	—	1.5

[a] (1*RS*,4*RS*)-4-Phenyl-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine.
[b] (1*RS*,4*RS*)-4-(4-Chlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine.
[c] (1*RS*,4*RS*)-4-(3-Chlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine.
[d] 4-(3,4-Dichlorophenyl)-3,4-dihydronaphthalen-1(2*H*)-one.
[e] Does not include mandelic acid and 1*R*,4*R*-sertraline hydrochloride. [NOTE—Disregard any peak at or below 0.05%.]

• **LIMIT OF MANDELIC ACID**
Perform this test only if *Organic Impurities, Procedure 2*, is used.
Solution A: Dissolve 1 g of sodium dodecylsulfate in 800 mL of water. Add 200 mL of acetonitrile and 1 mL of phosphoric acid.
Solution B: Dissolve 1 g of sodium dodecylsulfate in 100 mL of water. Add 900 mL of acetonitrile and 1 mL of phosphoric acid.
Diluent: *Solution A* and *Solution B* (50:50)
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	60	40
8	60	40
9	10	90
16	10	90
16.1	60	40
20	60	40

System suitability solution: 0.005 mg/mL each of USP Benzoic Acid RS and USP Mandelic Acid RS in *Diluent*
Standard solution: 0.002 mg/mL of USP Mandelic Acid RS in *Diluent*
Sample solution: 1 mg/mL of Sertraline Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for mandelic acid, benzoic acid, and sertraline are 0.2, 0.3, and 1.0 respectively.]
Suitability requirements
Resolution: NLT 5 between benzoic acid and mandelic acid, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of mandelic acid in the portion of Sertraline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mandelic acid from the *Sample solution*
r_S = peak response of mandelic acid from the *Standard solution*
C_S = concentration of USP Mandelic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Sertraline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 0.50%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature NMT 40°.
• **LABELING:** Label to indicate the *Organic Impurities* procedure with which the article complies, if *Organic Impurities, Procedure 1*, is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzoic Acid RS
USP Mandelic Acid RS
USP Sertraline Hydrochloride RS
USP Sertraline Hydrochloride Racemic Mixture RS
(1*RS*,4*RS*)-4-(3,4-Dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydro-1-naphthylamine hydrochloride.
$C_{17}H_{17}Cl_2 \cdot HCl$ 342.69
USP Sertraline Hydrochloride Related Compound A RS
(1*RS*,4*SR*)-4-(3,4-Dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydro-1-naphthylamine hydrochloride.
$C_{17}H_{17}Cl_2N \cdot HCl$ 342.69

Sertraline Hydrochloride Oral Solution

DEFINITION
Sertraline Hydrochloride Oral Solution contains an amount of sertraline hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of sertraline ($C_{17}H_{17}Cl_2N$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 0.8 g/L of ammonium acetate in water. To each L of this solution add 10 mL of triethylamine, and adjust with phosphoric acid to a pH of 5.0.
Mobile phase: Acetonitrile and *Buffer* (30:70)

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Diluent: Methanol and water (50:50)
Standard solution: 0.1 mg/mL of USP Sertraline Hydrochloride RS in *Diluent*
Sample solution: Nominally, 0.1 mg/mL of sertraline in *Diluent*, from a portion of Oral Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: 1.3 times the retention time of sertraline
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.7
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sertraline ($C_{17}H_{17}Cl_2N$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sertraline from the *Sample solution*
r_S = peak response of sertraline from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sertraline in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sertraline free base, 306.23
M_{r2} = molecular weight of sertraline hydrochloride, 342.69

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Deliverable Volume** ⟨698⟩**:** Meets the requirement

IMPURITIES
• **Organic Impurities**
Buffer: Dissolve 5.7 g of β-cyclodextrin hydrate and 8 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 6.5 ± 0.1.
Solvent mixture: Acetonitrile and methanol (55:45)
Mobile phase: *Solvent mixture* and *Buffer* (40:60)
Diluent: Acetonitrile and water (70:30)
Standard solution: 2.3 µg/mL of USP Sertraline Hydrochloride RS in *Diluent*
Sample solution: Nominally, 2 mg/mL of sertraline in *Diluent*, from a portion of Oral Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 3 times the retention time of sertraline
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of sertraline from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sertraline in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sertraline free base, 306.23
M_{r2} = molecular weight of sertraline hydrochloride, 342.69

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sertraline	1.0	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 4.5–6.0
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL. The total molds and yeasts count does not exceed 10^1 cfu/mL. It meets the requirement of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in light-resistant containers. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Sertraline Hydrochloride RS

Sertraline Hydrochloride Tablets

DEFINITION
Sertraline Hydrochloride Tablets contain an amount of sertraline hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of sertraline free base ($C_{17}H_{17}Cl_2N$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Methanol and 0.1% (v/v) phosphoric acid (1:1)
Standard solution: 0.05 mg/mL of USP Sertraline Hydrochloride RS in *Mobile phase*
Sample stock solution: 0.5 mg/mL of sertraline free base prepared as follows. Transfer NLT 10 Tablets to a suitable

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volumetric flask. Dissolve in 0.1% phosphoric acid equivalent to 50% of the flask volume. Sonicate for 15 min with intermittent shaking to disperse the Tablets. Add an amount of methanol equivalent to 40% of the flask volume, and continue to sonicate for an additional 10 min. Cool the solution, and dilute with methanol to volume.

Sample solution: Nominally 0.05 mg/mL of sertraline free base in *Mobile phase* from the *Sample stock solution*. Pass a portion of this solution through a nylon filter of 0.45-µm or finer pore size, discard the first few mL, and collect the rest of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: Twice the retention time of sertraline

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sertraline free base ($C_{17}H_{17}Cl_2N$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sertraline free base in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sertraline, 306.23
M_{r2} = molecular weight of sertraline hydrochloride, 342.69

Acceptance criteria: 90.0%–110.0% of sertraline free base

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Test 1
Medium: Acetate buffer (3.0 g/L of sodium acetate trihydrate and 1.6 mL/L of glacial acetic acid; adjust with glacial acetic acid to a pH of 4.5); 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Standard stock solution: 0.56 mg/mL of USP Sertraline Hydrochloride RS in *Medium*. A small volume of methanol, not exceeding 5% of the final volume, may be used to help solubilize sertraline.
Standard solution
For Tablets labeled to contain 50, 100, 150, or 200 mg: 0.056 mg/mL of USP Sertraline Hydrochloride RS in *Medium* from the *Standard stock solution*
For Tablets labeled to contain 25 mg: 0.028 mg/mL of USP Sertraline Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium*, if necessary.
Mobile phase: Acetonitrile and 0.1% (v/v) phosphoric acid (1:3)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume
For Tablets labeled to contain 50, 100, 150, or 200 mg: 10 µL
For Tablets labeled to contain 25 mg: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sertraline free base ($C_{17}H_{17}Cl_2N$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times D \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of sertraline, 306.23
M_{r2} = molecular weight of sertraline hydrochloride, 342.69
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of sertraline free base ($C_{17}H_{17}Cl_2N$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: pH 4.5 acetate buffer (6.8 g/L of sodium acetate trihydrate and 32 mL/L of 2 N acetic acid; adjust with 2 N acetic acid to a pH of 4.5); 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Buffer: 3 mL/L of glacial acetic acid and 7 mL/L of triethylamine in water
Mobile phase: Acetonitrile, methanol, and *Buffer* (10:4:8)
Standard solution: (L/800) mg/mL of USP Sertraline Hydrochloride RS in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 273 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sertraline free base ($C_{17}H_{17}Cl_2N$) dissolved:

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$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times D \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of sertraline, 306.23
M_{r2} = molecular weight of sertraline hydrochloride, 342.69
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of sertraline free base ($C_{17}H_{17}Cl_2N$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 4.5 acetate buffer (6.8 g/L of sodium acetate trihydrate; adjust with 2 N acetic acid to a pH of 4.5); 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: Dissolve 0.8 g/L of ammonium acetate in water and add 10 mL of triethylamine. Adjust with phosphoric acid to a pH of 5.0 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard stock solution: 0.6 mg/mL of USP Sertraline Hydrochloride RS in *Medium*, prepared as follows. Place an appropriate amount of USP Sertraline Hydrochloride RS into a suitable volumetric flask and add 70% of the final flask volume of *Medium*. Sonicate to dissolve and dilute with *Medium* to volume. Pass through a suitable filter of 0.45-µm pore size.
Standard solution: (L/800) mg/mL of USP Sertraline Hydrochloride RS in *Medium* from *Standard stock solution*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 2.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sertraline free base ($C_{17}H_{17}Cl_2N$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of sertraline, 306.23
M_{r2} = molecular weight of sertraline hydrochloride, 342.69
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of sertraline free base ($C_{17}H_{17}Cl_2N$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
[Note—Use freshly prepared samples.]
Buffer: 2.72 g/L of monobasic potassium phosphate. Adjust with triethylamine to a pH of 7.0.
Mobile phase: Methanol, acetonitrile, and *Buffer* (6:3:11). Adjust with triethylamine to a pH of 8.0.
System suitability solution: 5 µg/mL of USP Sertraline Hydrochloride Racemic Mixture RS and 0.5 mg/mL of USP Sertraline Hydrochloride RS in *Mobile phase*
Standard solution: 2.5 µg/mL of USP Sertraline Hydrochloride RS in *Mobile phase*
Sample solution: [Note—Sonicate for about 10 min with shaking to disperse the Tablets.] Prepare a solution of 0.5 mg/mL of sertraline in *Mobile phase* from NLT 20 powdered Tablets. Pass a portion of this solution through a nylon filter of 0.45-µm or finer pore size, discard the first few mL, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.0-mm × 25-cm; 5-µm packing L45
Flow rate: 0.7 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for the 1*R*, 4*R*-*cis*-isomer of sertraline and sertraline are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between sertraline and the 1*R*, 4*R*-*cis*-isomer of sertraline, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*
r_S = peak response of sertraline from the *Standard solution*
C_S = concentration of USP Sertraline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sertraline in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sertraline, 306.23
M_{r2} = molecular weight of sertraline hydrochloride, 342.69

Acceptance criteria
Disregard any peak below 0.1%. Disregard the peak due to the process impurity 1*R*,4*R*-*cis*-isomer of sertraline.
Individual degradation product: NMT 0.2%
Total degradation products: NMT 2.0%, excluding the 1*R*,4*R*-*cis*-isomer of sertraline

ADDITIONAL REQUIREMENTS

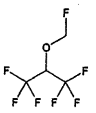
• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.

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• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sertraline Hydrochloride RS
USP Sertraline Hydrochloride Racemic Mixture RS
(1*RS*,4*RS*)-4-(3,4-Dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydro-1-naphthylamine hydrochloride.
$C_{17}H_{17}Cl_2N \cdot HCl$ 342.69

Sevoflurane



$C_4H_3F_7O$ 200.05

Propane, 1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)-; Fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl)ethyl ether [28523-86-6].

DEFINITION
Sevoflurane contains NLT 99.97% and NMT 100.00% of sevoflurane ($C_4H_3F_7O$).

IDENTIFICATION
• **A. INFRARED ABSORPTION**
Acceptance criteria: The IR absorption spectrum of Sevoflurane, obtained using a gas cell, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Sevoflurane RS.

ASSAY
• **PROCEDURE**
Analysis: Using the results from the test for *Organic Impurities*, calculate the percentage of sevoflurane ($C_4H_3F_7O$) in the volume of Sevoflurane taken by subtracting the sum of percentages for all impurities found from 100.00%.
Acceptance criteria: 99.97%–100.00%

IMPURITIES
• **LIMIT OF FLUORIDE**
[NOTE—Use plastic utensils throughout this test.]
Buffer solution: Transfer 110 g of sodium chloride and 1 g of sodium citrate to a 2000-mL volumetric flask. Dissolve in 700 mL of water. Carefully add 150 g of sodium hydroxide, and shake to dissolve. Cool to room temperature, and carefully add 450 mL of glacial acetic acid while stirring. Cool, add 600 mL of isopropyl alcohol, and dilute with water to volume. [NOTE—The pH of this solution is 5.0–5.5. This solution may be used for 6 weeks when stored at room temperature.]
Solution A: Transfer 221 mg of sodium fluoride, previously dried at 150° for 4 h, to a 100-mL volumetric flask. Add about 20 mL of water, and mix to dissolve. Add 1.0 mL of 0.01 N sodium hydroxide, and dilute with water to volume. Each mL of this solution contains 1 mg of fluoride. Store in a tightly closed, plastic container. [NOTE—This solution may be used for 2 weeks when stored in a refrigerator.]
Standard stock solution 1: 0.2 µg/mL of fluoride from *Solution A* in water
Standard stock solution 2: 0.5 µg/mL of fluoride from *Solution A* in water
Standard stock solution 3: 2 µg/mL of fluoride from *Solution A* in water
Standard stock solution 4: 5 µg/mL of fluoride from *Solution A* in water
Standard solution 1: 0.10 µg/mL of fluoride from *Standard stock solution 1* in *Buffer solution*
Standard solution 2: 0.25 µg/mL of fluoride from *Standard stocksolution 2* in *Buffer solution*
Standard solution 3: 1.0 µg/mL of fluoride from *Standard stocksolution 3* in *Buffer solution*
Standard solution 4: 2.5 µg/mL of fluoride from *Standard stocksolution 4* in *Buffer solution*
Sample solution: Pipet 50.0 mL of Sevoflurane and 50.0 mL of water into a separatory funnel, shake vigorously for 3 min, and allow the liquids to separate completely. Transfer 25.0 mL of the aqueous top layer to a 50-mL volumetric flask, and dilute with *Buffer solution* to volume.
Analysis
Samples: *Standard solutions 1–4* and *Sample solution*
Concomitantly measure the potentials, in mV, of *Standard solutions 1–4* and the *Sample solution* with a pH meter (see *pH* ⟨791⟩) capable of a minimum reproducibility of ±0.2 mV, and equipped with a fluoride-specific ion-indicating electrode and a glass-sleeved calomel reference electrode. When taking measurements, transfer the solution under test to a 100-mL beaker containing a polytef-coated stirring bar, and immerse the electrodes. Allow to stir on a magnetic stirrer having an insulated top until equilibrium is attained in about 2–3 min, and record the potential. Rinse the electrodes with the *Buffer solution*, and dry, taking care to avoid damaging the crystal of the specific-ion electrode. A satisfactory response is achieved if the difference between the potentials obtained with *Standard solution 4* and *Standard solution 2* is in the range between 50 and 60 mV. Plot the logarithms of the fluoride concentrations, in µg/mL, of *Standard solutions 1–4* versus potentials, in mV. From the graph and the measured potential of the *Sample solution*, determine the concentration, *C* (µg/mL) of fluoride in the *Sample solution*. Multiply *C* by 2 to obtain the concentration (µg/mL) of fluoride in the portion of Sevoflurane taken.
Acceptance criteria: NMT 2 µg/mL is found.

• **LIMIT OF NONVOLATILE RESIDUE**
Analysis: Transfer 10.0 mL of Sevoflurane to an evaporating dish, evaporate to dryness on a steam bath, and dry the residue at 105° for 2 h.
Acceptance criteria: The weight of the residue does not exceed 1.0 mg.

• **ORGANIC IMPURITIES**
Internal standard solution: Use dimethoxymethane.
Ethylene dichloride identification solution: Transfer 2.0 mL of Sevoflurane to a vial, and seal with a cap and septum. Using a microsyringe, add 20 µL of ethylene dichloride through the septum of the vial, and mix thoroughly.
Sevoflurane related compounds stock solution: Transfer 20 mL of Sevoflurane to a 40-mL vial with a septum lid. Add 20 µL each of USP Sevoflurane Related Compound A RS, USP Sevoflurane Related Compound B RS, and USP Sevoflurane Related Compound C RS to the vial, and mix thoroughly.
Related compounds identification solution: Transfer 1.0 mL of *Ethylene dichloride identification solution* to a 10-mL volumetric flask, and dilute with Sevoflurane to volume. Transfer 2 mL of this solution and 5 mL of *Sevoflurane related compounds stock solution* to a 50-mL volumetric flask, dilute with Sevoflurane to volume, and mix thoroughly.
Standard solutions: Prepare in duplicate, proceeding for each as follows. Transfer 2.0 mL of ethylene dichloride to a

USP Monographs

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screw-capped vial, immediately seal with a cap and septum, and place on a balance. Using a microsyringe, transfer about 20 µL of USP Sevoflurane RS to the vial by inserting the syringe needle through the septum. Record the quantity, in mg, of USP Sevoflurane RS added. Using the same method, transfer about 20 µL of the *Internal standard solution* to the vial, and record the quantity, in mg, of the solution added.

Control standard solution: Place a 40-mL vial with a septum lid on an analytical balance, and tare out the weight. Add 30 mL of ethylene dichloride to the vial, and seal tightly. Record the weight of the ethylene dichloride, and tare. Using a microsyringe, add 20 µL of the USP Sevoflurane RS through the septum of the vial, record the weight, and mix thoroughly. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with ethylene dichloride to volume.

Sample solution: Transfer 20.0 mL of Sevoflurane to a vial, and insert the stopper. Using a microsyringe, add 5 µL of the *Internal standard solution* to the vial.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m fused-silica capillary column coated with a 3.0-µm film of liquid phase G43

Temperatures

Injection port: 200°

Detector: 225°

Column: See *Table 1*. Before use, condition the column overnight at a temperature of 250°.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	10
40	10	200	14

Carrier gas: Helium

Flow rate: 1 mL/min. [NOTE—The make-up gas flow rate is 20 mL/min.]

Injection volume: 2 µL

Injection type: Split ratio 1:20

System suitability

Samples: *Related compounds identification solution* and one of the *Standard solutions*

Suitability requirements

[NOTE—Identify the peaks using the relative retention times given in *Table 2*.]

Resolution: NLT 2.0 between sevoflurane related compound C and ethylene dichloride, *Related compounds identification solution*

Column efficiency: NLT 6000 theoretical plates for the sevoflurane peak, *Standard solution*

Relative standard deviation: NMT 3.0% from the peak area ratio of sevoflurane to the internal standard, *Standard solution*

Analysis

Samples: *Ethylene dichloride identification solution*, *Standard solutions*, *Control standard solution*, and *Sample solution*

Calculate the response factor for each of the *Standard solutions*:

$$\text{Result} = (W_I/W_S) \times R_S$$

W_I = weight of the internal standard in the *Standard solutions* (mg)

W_S = weight of USP Sevoflurane RS in the *Standard solutions* (mg)

R_S = peak area response ratio of sevoflurane to that of the internal standard from the *Standard solutions*

The response factors for the duplicate *Standard solutions* do not differ by more than 3.0% from their average.

Calculate the quantity, in µg/g, of each impurity in the portion of Sevoflurane ($C_4H_3F_7O$) taken:

$$\text{Result} = (S_1/S_2) \times (R_I/F_R) \times (1/F) \times 250$$

S_1 = specific gravity of the internal standard, 0.859

S_2 = specific gravity of sevoflurane, 1.525

R_I = peak area response ratio of the impurity to that of the internal standard from the *Sample solution*

F_R = average response factor obtained as directed above

F = respective relative response factor for the impurities (see *Table 2*)

Acceptance criteria

[NOTE—Do not include sevoflurane, the internal standard, or any peak identified as solvent carryover (ethylene dichloride). Also, disregard any peak with an area less than 30% of the average area of the principal peak from the *Control standard solution*.]

Individual impurities: NMT 25 µg/g of sevoflurane related compound A and NMT 100 µg/g of any other single impurity

Total impurities: NMT 300 µg/g

Table 2

Name	Relative Retention Time	Relative Response Factor
Sevoflurane related compound A	0.78	1.0
Sevoflurane related compound B	0.83	1.0
Sevoflurane	1.0	—
Internal standard (dimethoxymethane)	1.35	—
Ethylene dichloride	2.28	—
Sevoflurane related compound C	2.31	0.46
Unknown impurities	—	1

SPECIFIC TESTS

- **REFRACTIVE INDEX** ⟨831⟩: 1.2745–1.2760 at 20°
- **ACIDITY OR ALKALINITY:** Transfer 20.0 mL of Sevoflurane and 20.0 mL of carbon dioxide-free water to a separatory funnel, shake for 3 min, and allow the layers to separate.

  Acceptance criteria: The aqueous layer requires NMT 0.10 mL of 0.010 N sodium hydroxide or NMT 0.60 mL of 0.010 N hydrochloric acid for neutralization, bromocresol purple TS being used as the indicator.
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature. Replace the cap securely after each use.
- **USP REFERENCE STANDARDS** ⟨11⟩

  USP Sevoflurane RS

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USP Sevoflurane Related Compound A RS
1,1,1,3,3-Pentafluoroisopropenyl fluoromethyl ether.
$C_4H_2F_6O$ 180.05
USP Sevoflurane Related Compound B RS
1,1,1,3,3,3-Hexafluoro-2-methoxy-propane.
$C_4H_4F_6O$ 182.06
USP Sevoflurane Related Compound C RS
1,1,1,3,3,3-Hexafluoro-2-propanol.
$C_3H_2F_6O$ 168.04

Sildenafil Tablets

DEFINITION
Sildenafil Tablets contain sildenafil citrate equivalent to NLT 90% and NMT 110% of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Solution A: Ammonium hydroxide and water (10:90)
Standard solution: 1.4 mg/mL of USP Sildenafil Citrate RS in *Solution A*
Sample solution: Grind 1 Tablet and add a sufficient amount of *Solution A* to obtain nominally 1 mg/mL of sildenafil. Sonicate for 2 min, shake well, and centrifuge. Use the supernatant.
Analysis: For each of the *Standard solution* and the *Sample solution*, prewash a 6-cc C18 solid phase extraction cartridge with 10 mL of methanol followed by 10 mL of *Solution A*, discarding both washings. Apply 5 mL each of the *Standard solution* and the *Sample solution* to separate prewashed cartridges and draw each solution through the cartridge. Wash each cartridge with 10 mL of water and allow the cartridge to dry under vacuum. Elute the sildenafil from each cartridge with 5 mL of methanol, collecting the eluant in a suitable container. Add about 200 mg of potassium bromide to each container, mix well, and evaporate to dryness. To about 70 mg of each dried mixture, add about 140 mg of potassium bromide and mix.
Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Buffer: Dilute 7 mL of triethylamine with water to 1 L. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: *Buffer*, methanol, and acetonitrile (58:25:17)
Diluent: Acetonitrile and water (90:10)
Standard solution: 0.028 mg/mL of USP Sildenafil Citrate RS in *Mobile phase*
Sample stock solution: Disperse 1 Tablet in at least 5 mL of *Diluent* with the aid of sonication. Once the Tablet is fully dispersed, dilute with *Mobile phase* to 250.0 mL while swirling. Centrifuge and use the supernatant.
Sample solution: Nominally 0.02 mg/mL of sildenafil prepared by diluting a suitable portion of the *Sample stock solution* with *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of sildenafil
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.3
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sildenafil from the *Sample solution*
r_S = peak response of sildenafil from the *Standard solution*
C_S = concentration of USP Sildenafil Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sildenafil in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sildenafil, 474.58
M_{r2} = molecular weight of sildenafil citrate, 666.70

Acceptance criteria: 90%–110%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 15 min
Standard solution: 0.03 mg/mL of USP Sildenafil Citrate RS in *Medium*
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*, if necessary
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: Maximum at about 290 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Sildenafil Citrate RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M_{r1} = molecular weight of sildenafil, 474.58
M_{r2} = molecular weight of sildenafil citrate, 666.70

Tolerances: NLT 80% (Q) of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**
Buffer, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*, except for *Run time*.

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Run time: NLT 3 times the retention time of sildenafil
System suitability solution: A mixture of sildenafil and sildenafil *N*-oxide in *Mobile phase*, prepared as follows. Dissolve 70 mg of USP Sildenafil Citrate RS in 1 mL of a solution of hydrogen peroxide and formic acid (2:1), allow to stand for NMT 10 min, and then dilute with *Mobile phase* to 250.0 mL.
Standard solution: 0.0014 mg/mL of USP Sildenafil Citrate RS in *Mobile phase*
Sensitivity solution: 0.00035 mg/mL of USP Sildenafil Citrate RS in *Mobile phase* from the *Standard solution*
Sample stock solution: Transfer 5 Tablets to a 250-mL volumetric flask and disperse in 25 mL of *Diluent* with the aid of sonication. Dilute with *Mobile phase* to volume. Sonicate, if necessary. Centrifuge and use the supernatant.
Sample solution: Nominally 0.5 mg/mL of sildenafil prepared by diluting a suitable portion of the *Sample stock solution* with *Mobile phase*
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.6 between sildenafil *N*-oxide and sildenafil, *System suitability solution*
Tailing factor: NMT 1.3, *Standard solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*
r_S = peak response of sildenafil from the *Standard solution*
C_S = concentration of USP Sildenafil Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sildenafil in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sildenafil, 474.58
M_{r2} = molecular weight of sildenafil citrate, 666.70

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sildenafil	1.0	—
Sildenafil *N*-oxide[a]	1.2	0.20
Any individual degradation product	—	0.20
Total degradation products	—	0.50

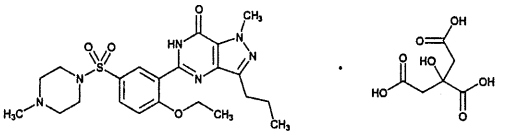
[a] 1-{[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl}-4-methylpiperazine N^4-oxide.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sildenafil Citrate RS

Sildenafil Citrate



$C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$ 666.70
Piperazine, 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1); 1-[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate (1:1) [171599-83-0].
Sildenafil

$C_{22}H_{30}N_6O_4S$ 474.58
[139755-83-2].

DEFINITION

Sildenafil Citrate contains NLT 98.0% and NMT 102.0% of sildenafil citrate ($C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**
Buffer: Dilute 7 mL of triethylamine with water to 1 L. Stir, and adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Mobile phase: *Buffer*, methanol, and acetonitrile (58:25:17)
Standard solution: 0.028 mg/mL of USP Sildenafil Citrate RS in *Mobile phase*
Sample solution: 0.028 mg/mL of Sildenafil Citrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.85% for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sildenafil citrate ($C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sildenafil from the *Sample solution*

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r_S = peak response of sildenafil from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: NLT 0.5 g
Acceptance criteria: NMT 0.1%

• **LIMIT OF IMIDAZOLE**
Diluent: Methanol, water, and ammonium hydroxide (15:5:1)
Standard solution 1: 0.035 mg/mL of USP Imidazole RS in *Diluent*
Standard solution 2: 0.0175 mg/mL of USP Imidazole RS in *Diluent* from *Standard solution 1*
Sample solution: 17.5 mg/mL of Sildenafil Citrate in *Diluent*
System suitability solution: Mix equal volumes of *Sample solution* and *Standard solution 1*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.2-mm layer of chromatographic silica gel mixture with a particle size of 2–10 µm (HPTLC plates)
Application volume: 10 µL. [NOTE—Apply as 6-mm bands.]
Developing solvent system: Methylene chloride, ethyl acetate, alcohol, and ammonium hydroxide (50:30:20:1)
System suitability
Sample: *System suitability solution*
Suitability requirements: The chromatogram shows two clearly separated zones.
Analysis:
Samples: *Standard solution 2* and *Sample solution*
Develop the plate over a distance of about two-thirds of the length of the plate. Dry at 100° for about 15 min, and cool. Expose the plate to iodine vapor until the plate is light brown, and examine the plate under UV light at 254 nm. [NOTE—The retardation factors for citrate, imidazole, and sildenafil are about 0, 0.25, and 0.4, respectively.]
Acceptance criteria: Any spot corresponding to imidazole in the *Sample solution* is not more intense than the principal spot from *Standard solution 2* (0.1%).

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*, except to run the chromatograph for 3 times the retention time of sildenafil.
Identification solution: 7.5 µg/mL of USP Sildenafil Related Compound A RS in *Mobile phase*
System suitability solution: Dissolve 70 mg of Sildenafil Citrate in 1 mL of a solution of hydrogen peroxide and anhydrous formic acid (2:1). Allow to stand for at least 10 min to generate sildenafil *N*-oxide, and then dilute with *Mobile phase* to 250 mL.
Sample solution: 0.7 mg/mL of Sildenafil Citrate in *Mobile phase*
Diluted sample solution: 1.4 µg/mL of sildenafil citrate in *Mobile phase* from the *Sample solution*
Sensitivity solution: 0.35 µg/mL of sildenafil citrate in *Mobile phase* from the *Diluted sample solution*
System suitability
Samples: *Diluted sample solution, Sensitivity solution,* and *System suitability solution*
[NOTE—The relative retention times for sildenafil, sildenafil *N*-oxide, and sildenafil related compound A are about 1.0, 1.2, and 1.7, respectively.]
Suitability requirements
Resolution: NLT 2.5 between sildenafil *N*-oxide and sildenafil, *System suitability solution*
Tailing factor: NMT 1.5 for the sildenafil peak, *Diluted sample solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Identification solution, Diluted sample solution,* and *Sample solution*
[NOTE—Identify sildenafil related compound A from the *Identification solution*.]
Calculate the percentage of sildenafil related compound A and any other unspecified individual impurity in the portion of Sildenafil Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sildenafil related compound A or any other unspecifed impurity from the *Sample solution*
r_S = peak response of sildenafil from the *Diluted sample solution*
C_S = concentration of the *Diluted sample solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria
Sildenafil related compound A: NMT 0.3%
Any other unspecified individual impurity: NMT 0.10%
Total unspecified impurities: NMT 0.3%
Total impurities: NMT 0.5%. Disregard any peak less than 0.05%.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in air-tight containers, and store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Imidazole RS
$C_3H_4N_2$ 68.08
USP Sildenafil Citrate RS
USP Sildenafil Related Compound A RS
5-[2-Ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl]-1-methyl-3-(2-methylpropyl)-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one.
$C_{23}H_{32}N_6O_4S$ 488.60

Sildenafil Compounded Oral Suspension

DEFINITION

Sildenafil Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$).

Prepare Sildenafil Compounded Oral Suspension containing 2.5 mg/mL of sildenafil as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Sildenafil Citrate tablets[a] equivalent to	250 mg of sildenafil

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Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Viagra 25-mg tablets, Pfizer Inc., New York, NY.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Sildenafil Citrate tablets* in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a sildenafil liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 0.2 M ammonium acetate (50:50). Pass through a nylon 66 filter of 0.45-µm pore size, and degas.

Standard stock solution: 2.5 mg/mL of sildenafil prepared from USP Sildenafil Citrate RS in *Mobile phase*

Standard solution: 0.25 mg/mL of sildenafil prepared from *Standard stock solution* and *Mobile phase*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.25 mg/mL of sildenafil from Oral Suspension and *Mobile phase*, and centrifuge.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 3.0-mm × 15-cm; 5-µm packing L1

Column temperature: 25°

Flow rate: 0.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for sildenafil is about 7.1 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sildenafil ($C_{22}H_{30}N_6O_4S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of sildenafil in the *Standard solution* (mg/mL)
C_U = nominal concentration of sildenafil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.9–4.9

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sildenafil Citrate RS

Silver Nitrate

$AgNO_3$ 169.87

Nitric acid silver(1+) salt;
Silver(1+) nitrate [7761-88-8].

DEFINITION

Silver Nitrate, powdered and then dried in the dark over silica gel for 4 h, contains NLT 99.8% and NMT 100.5% of silver nitrate ($AgNO_3$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Silver* ⟨191⟩

Sample: 20 mg/mL

Acceptance criteria: Meets the requirements

• **B.**

Sample solution: 100 mg/mL

Analysis: Add 1 drop of diphenylamine TS to the *Sample solution*, and then carefully superimpose it upon sulfuric acid.

Acceptance criteria: A deep blue color appears at the zone of contact.

ASSAY

• **PROCEDURE**

Sample solution: Powder about 1 g of Silver Nitrate, and dry in the dark over silica gel for 4 h. Dissolve about 700 mg of this dried salt in 50 mL of water, add 2 mL of nitric acid and 2 mL of ferric ammonium sulfate TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N ammonium thiocyanate VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant*. Each mL of 0.1 N ammonium thiocyanate is equivalent to 16.99 mg of silver nitrate ($AgNO_3$.)

Acceptance criteria: 99.8%–100.5%

SPECIFIC TESTS

• **CLARITY AND COLOR OF SOLUTION**

Sample solution: 100 mg/mL

Acceptance criteria: The solution is clear and colorless.

• **COPPER**

Sample solution: 100 mg/mL

Analysis: To 5 mL of the *Sample solution* add 6 N ammonium hydroxide, dropwise, until a precipitate first formed is just dissolved.

Acceptance criteria: No blue color is produced.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Silver Nitrate Ophthalmic Solution

DEFINITION

Silver Nitrate Ophthalmic Solution is a solution of Silver Nitrate in a water medium. It contains NLT 0.95% and NMT 1.05% of silver nitrate ($AgNO_3$). The solution may be buffered by the addition of Sodium Acetate.

USP Monographs

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IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Silver* ⟨191⟩and*Nitrate* ⟨191⟩**:** Meets the requirements

ASSAY
• **PROCEDURE**
Sample solution: Place 5 mL of Ophthalmic Solution in a conical flask, dilute with 20 mL of water, and add 1 mL of nitric acid and 1 mL of ferric ammonium sulfate TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.02 N ammonium thiocyanate VS
Endpoint detection: Visual
Analysis: Titrate the *Sample solution* with *Titrant*. Each mL of 0.02 N ammonium thiocyanate is equivalent to 3.397 mg of silver nitrate ($AgNO_3$).
Acceptance criteria: 0.95%–1.05%

SPECIFIC TESTS
• **CLARITY AND COLOR OF SOLUTION:** Clear and colorless
• **pH** ⟨791⟩**:** 4.5–6.0
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve it protected from light, in inert, collapsible capsules or in other suitable single-dose containers.

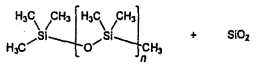
Toughened Silver Nitrate

» Toughened Silver Nitrate contains not less than 94.5 percent of $AgNO_3$, the remainder consisting of silver chloride (AgCl).

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—
A: A solution (1 in 50) responds to the tests for *Silver* ⟨191⟩.
B: Mix a solution (1 in 10) in a test tube with 1 drop of diphenylamine TS, then carefully superimpose it upon sulfuric acid: a deep blue color appears at the zone of contact.
Copper—A solution (1 in 10) shows no trace of blue coloration when treated with an excess of 6 N ammonium hydroxide.
Assay—Add about 700 mg of Toughened Silver Nitrate, accurately weighed, to 50 mL of water, and when the silver nitrate has dissolved, filter the solution. Thoroughly wash the filter and sediment with water, add 2 mL of nitric acid and 2 mL of ferric ammonium sulfate TS to the combined filtrate and washings, and titrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N ammonium thiocyanate is equivalent to 16.99 mg of $AgNO_3$.

Simethicone



Simethicone;
α-(Trimethylsilyl)-ω-methylpoly[oxy(dimethylsilylene)], mixture with silicon dioxide [8050-81-5].

DEFINITION
Simethicone is a mixture of fully methylated linear siloxane polymers containing repeating units of the formula $[-(CH_3)_2SiO-]_n$, stabilized with trimethylsiloxy end-blocking units of the formula $[-(CH_3)_3SiO-]$, and silicon dioxide. It contains NLT 90.5% and NMT 99.0% of polydimethylsiloxane ($[-(CH_3)_2SiO-]_n$), and NLT 4.0% and NMT 7.0% of silicon dioxide.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩**,** *Infrared Spectroscopy*: **197F.▲** (CN 1-May-2020) Meets the requirements

ASSAY
• **PROCEDURE**
Standard stock solution: 2 mg/mL of USP Polydimethylsiloxane RS in toluene
Standard solution: Add 25.0 mL of *Standard stock solution* to a round, narrow-mouth, screw-capped, 120-mL bottle. Add 50 mL of dilute hydrochloric acid (2 in 5), close the bottle securely with a cap having an inert liner, and shake for 5 min, accurately timed, on a reciprocating shaker at a suitable rate (e.g., about 200 oscillations/min and a stroke of 38 ± 2 mm). Transfer the mixture to a 125-mL separator, and remove 5 mL of the upper organic (toluene) layer to a 15-mL, screw-capped test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.
Sample stock solution: Add 50 mg of Simethicone to a round, narrow-mouth, screw-capped, 120-mL bottle. Add 25.0 mL of toluene, and swirl to disperse.
Sample solution: Using the *Sample stock solution*, proceed as directed in the *Standard solution*, beginning with "Add 50 mL of dilute hydrochloric acid (2 in 5)".
Blank: Add 25 mL of toluene to a round, narrow-mouth, screw-capped, 120-mL bottle. Proceed as directed in the *Standard solution*, beginning with "Add 50 mL of dilute hydrochloric acid (2 in 5)".
Instrumental conditions
Mode: IR
Analytical wavelength: Wavelength of maximum absorbance at about 7.9 µm
Cell: 0.5 mm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the absorbance of the *Samples*, using the *Blank* to set the instrument.
Calculate the percentage of polydimethylsiloxane $[-(CH_3)_2SiO-]_n$ in the portion of Simethicone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Polydimethylsiloxane RS in the *Standard stock solution* (mg/mL)
C_U = concentration of Simethicone in the *Sample stock solution* (mg/mL)

Acceptance criteria: 90.5%–99.0%

OTHER COMPONENTS
• **CONTENT OF SILICON DIOXIDE**
Standard solution: Transfer 3.00 g of USP Simethicone RS to a screw-capped bottle, add 10.0 mL of *n*-hexane, and mix by shaking.

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Sample solution: Transfer 3.00 g of Simethicone to a screw-capped bottle, add 10.0 mL of *n*-hexane, and mix by shaking.
Dimethicone solution: Transfer 3.00 g of dimethicone having a viscosity of 500 centistokes to a screw-capped bottle, add 10.0 mL of *n*-hexane, and mix by shaking.
Instrumental conditions
Mode: IR
Analytical wavelength: 7–9 µm
Cell: 0.1 mm
Blank: *n*-Hexane
Analysis
Samples: *Standard solution*, *Sample solution*, and *Dimethicone solution*
Record the absorbance spectra of the *Samples* between 7 and 9 µm. Determine the absorbances of the *Samples* at the wavelength of minimum absorbance at about 8.2 µm, observed in the spectrum obtained from the *Dimethicone solution*.
Calculate the percentage of silicon dioxide in the portion of Simethicone taken:

$$\text{Result} = [(A_U - A_D)/(A_S - A_D)] \times C$$

A_U = absorbance of the *Sample solution*
A_D = absorbance of the *Dimethicone solution*
A_S = absorbance of the *Standard solution*
C = designated percentage of silicon dioxide in USP Simethicone RS

Acceptance criteria: 4.0%–7.0%

SPECIFIC TESTS

• **Loss on Heating**
Sample: 15 g
Analysis: Heat the *Sample* in an open, tared vessel having a diameter of 5.5 ± 0.5 cm and a wall height of 2.5 ± 1.0 cm at 200° in a circulating air oven for 4 h, and allow to come to room temperature in a desiccator before weighing.
Acceptance criteria: NMT 18.0%
• **Defoaming Activity**
Foaming solution: 1 g of octoxynol 9 in 100 mL of water
Sample solution: Transfer 200 mg of Simethicone to a 60-mL bottle, add 50 mL of tertiary butyl alcohol, cap the bottle, and shake vigorously. Warm slightly, if necessary, to dissolve.
Analysis
Samples: *Foaming solution* and *Sample solution*
For each test use a clean, unused, 250-mL glass jar. Add, dropwise, 500 µL of the *Sample solution* to the 250-mL glass jar, fitted with a 50-mm cap, containing 100 mL of the *Foaming solution*. Cap the jar, and clamp it in an upright position on a wrist-action shaker. Using a radius of 13.3 ± 0.4 cm (measured from the center of the shaft to the center of the bottle), shake for 10 s through an arc of 10° at a frequency of 300 ± 30 strokes/min. Record the time required for the foam to collapse. The time, in s, for foam collapse is determined at the instant the first portion of foam-free liquid surface appears, measured from the end of the shaking period.
Acceptance criteria: The defoaming activity time does not exceed 15 s.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Polydimethylsiloxane RS
USP Simethicone RS

Simethicone Capsules

DEFINITION

Simethicone Capsules contain an amount of polydimethylsiloxane ($[-(CH_3)_2\ SiO-]_n$) that is NLT 85.0% and NMT 115.0% of the labeled amount of simethicone.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **1975**▲ (CN 1-May-2020)
Standard solution, Sample solution, and **Blank:** Prepare as directed in the *Assay*.
Cell: 0.5 mm
Acceptance criteria: Meet the requirements

ASSAY

• **Procedure**
Perform this procedure on at least 3 individual Capsules. The mean of the assay values obtained is the assay value.
Standard solution: Prepare similarly to the *Sample solution*, except use 2.5 mg/mL of USP Polydimethylsiloxane RS in toluene.
Sample solution: Transfer 1 Capsule to a round, narrow-mouth, screw-capped, 120-mL bottle. Add 20 mL of 6 N hydrochloric acid, and allow to stand, with frequent swirling, until the Capsule has dissolved. Add 10.0 mL of toluene for each 25 mg of the labeled amount of simethicone in the Capsule, close the bottle securely with a cap having an inert liner, and shake by mechanical means for 5 min. Allow the phases to separate, draw off 10 mL of the upper organic (toluene) layer to a test tube containing 0.5 g of anhydrous sodium sulfate, agitate vigorously for 30 s, and allow the mixture to settle. If necessary, centrifuge the mixture to obtain a clear solution.
Blank: Prepare similarly to the *Sample solution*, except use 10 mL of toluene.
Analysis
Samples: *Standard solution* and *Sample solution*
Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution* in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9 µm, with a suitable IR spectrophotometer, using the *Blank* to set the instrument.
Calculate the percentage of polydimethylsiloxane ($[-(CH_3)_2\ SiO-]_n$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Polydimethylsiloxane RS in the *Standard solution* (mg/mL)
V = volume of toluene used to prepare the *Sample solution* (mL)
L = labeled amount of simethicone (mg/Capsule)

Acceptance criteria: 85.0%–115.0%

PERFORMANCE TESTS

• **Disintegration** ⟨701⟩
For hard gelatin capsules
Time: 30 min, determined using water
For soft gelatin capsules
Time: 30 min, determined using 1.0% octoxynol-9 (w/w) in water, using disks
Acceptance criteria: Meet the requirements

USP Monographs

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- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Polydimethylsiloxane RS

Simethicone Emulsion

DEFINITION
Simethicone Emulsion is a water-dispersible form of Simethicone composed of Simethicone, suitable emulsifiers, preservatives, and water. It may contain suitable viscosity-increasing agents. It contains an amount of polydimethylsiloxane ($[-(CH_3)_2SiO-]_n$) that is NLT 85.0% and NMT 110.0% of the labeled amount of simethicone.

IDENTIFICATION

Change to read:

- **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)

Standard solution and **Sample solution:** Prepare as directed in the *Assay*.

Analysis: Place a suitable amount of the *Sample solution* in a suitable container, and dry it with a stream of nitrogen or other appropriate means to evaporate the solvent. Similarly proceed with the *Standard solution*. Record the spectra of the residues using ⟨197A⟩ or ⟨197F⟩, and examine the spectra in the range between 1400 cm^{-1} and 700 cm^{-1}.

Acceptance criteria

For spectra obtained using ⟨197A⟩: The spectrum from the *Sample solution* shows strong bands at 1260 ± 10 cm^{-1}, 1015 ± 15 cm^{-1}, and 795 ± 15 cm^{-1}, and a broad shoulder at 1085 ± 15 cm^{-1}, similar to the spectrum from the *Standard solution*. [NOTE—The exact maxima of the broad shoulder near 1085 cm^{-1} may differ by up to 15 cm^{-1} for the spectra from the *Sample solution* and the *Standard solution*.]

For spectra obtained using ⟨197F⟩: The spectrum from the *Sample solution* shows a strong band at 1260 ± 10 cm^{-1} and partially resolved bands near 1095 ± 15 cm^{-1}, 1020 ± 15 cm^{-1}, and 800 ± 15 cm^{-1}, similar to the spectrum from the *Standard solution*.

ASSAY
- **PROCEDURE**

Use glass apparatus only; avoid use of plasticware. Proper mixing of the Emulsion before sampling and testing is crucial.

Standard solution: 2 mg/mL of USP Polydimethylsiloxane RS in toluene. Treat a 25.0-mL aliquot of this solution in the same manner as the *Blank*, beginning with "add 50 mL of dilute hydrochloric acid (2 in 5)...".

Sample: Equivalent to 50 mg of simethicone from the Emulsion

Sample solution: To the *Sample* add 25.0 mL of toluene. Treat in the same manner as the *Blank*, beginning with "add 50 mL of dilute hydrochloric acid (2 in 5)...".

Instrumental conditions
(See *Mid-Infrared Spectroscopy* ⟨854⟩.)
Mode: Infrared
Cell: 0.5 mm
Analytical wavelength: Maximum absorbance at about 7.9 µm (1259 cm^{-1})

Blank: To 25.0 mL of toluene add 50 mL of dilute hydrochloric acid (2 in 5), close the bottle securely with a cap having an inert liner, vigorously shake by hand for 5 s, then shake for 5–20 min on a suitable shaker. [NOTE—The following shakers were found suitable: (1) a reciprocating shaker at a rate of about 200 oscillations/min and a stroke of 38 ± 2 mm or (2) a wrist-action shaker with a radius of 13.3 ± 0.4 cm (measured from center of shaft to center of bottle) that uses an arc of 10° at a frequency of 300 ± 30 strokes/min. The recommended shaking time is about 20 min, which can be adjusted as needed.] Centrifuge the mixture. [NOTE—The following centrifuging condition was found suitable: 1500 × g for 30–40 min.] Remove from the centrifuge, and immediately transfer about 5 mL of the upper organic (toluene) layer to a 15-mL screw-capped test tube containing about 1 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained. [NOTE—The following centrifuging condition was found suitable: 1500 × g for about 10 min.]

Analysis
[NOTE—Wash the cell with copious amounts of toluene to ensure that there is no carryover or residue.]

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of polydimethylsiloxane ($[-(CH_3)_2SiO-]_n$) in the labeled amount of simethicone in the Emulsion:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Polydimethylsiloxane RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of simethicone in the *Sample solution* (mg/mL)

Acceptance criteria: 85.0%–110.0%

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩: The total aerobic microbial count does not exceed 10^2 cfu/g.
- **DEFOAMING ACTIVITY**

Foaming solution: 10 mg/mL of octoxynol 9 in water

Sample solution: Transfer a quantity of Emulsion, equivalent to 300 mg of simethicone, to a 60-mL bottle. Dilute with water to 30 g, cap the bottle, and shake vigorously.

Analysis
[NOTE—For each test, use a clean, unused, 250-mL glass jar.]
Add, dropwise, 500 µL of the *Sample solution* to a clean, unused, cylindrical 250-mL glass jar, fitted with a 50-mm cap and containing 100 mL of the *Foaming solution*. Cap the jar, and clamp it in an upright position on a wrist-action shaker. Using a radius of 13.3 ± 0.4 cm (measured from the center of the shaft to the center of the bottle), shake for 10 s through an arc of 10° at a frequency of 300 ± 30 strokes/min. Record the time, in s, required for the foam to collapse. The time for foam collapse is determined at the instant the first portion of foam-free liquid surface appears, measured from the end of the shaking period.

Acceptance criteria: The defoaming activity time does not exceed 15 s.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Polydimethylsiloxane RS

Simethicone Oral Suspension

DEFINITION
Simethicone Oral Suspension is a suspension of Simethicone in Water. It contains an amount of polydimethylsiloxane ($[-(CH_3)_2SiO-]_n$) that is NLT 85.0% and NMT 115.0% of the labeled amount of simethicone.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
Standard solution, Sample solution, and **Blank:** Prepare as directed in the *Assay*.
Cell: 0.5 mm
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Standard solution: 2.4 mg/mL of USP Polydimethylsiloxane RS, prepared as follows. Transfer 60 mg of USP Polydimethylsiloxane RS to a 25-mL volumetric flask, add 15 mL of hexanes, and sonicate for 3 min. Allow to cool, dilute with hexanes to volume, and mix. Transfer 10 mL of this solution to a capped test tube, add 1 g of anhydrous sodium sulfate, mix for about 1 min, centrifuge, and use the clear supernatant.
Sample solution: Transfer an quantity of Oral Suspension, equivalent to about 240 mg of simethicone, to a glass-stoppered centrifuge tube. Add 5 mL of methanol, and mix for about 15 s. Add 30.0 mL of hexanes, and mix for about 10 s. Loosen the stopper, and heat the tube for about 10 min in a water bath at 65° ± 1°. Mix for 1 min, and centrifuge. Using a glass syringe, transfer the upper hexanes layer to a 100-mL volumetric flask. Repeat the extraction with two 30.0-mL portions of hexanes, combining the hexanes extracts in the 100-mL volumetric flask. If an emulsion forms during any of the extractions, as much as 2 mL of methanol may be added to disperse the emulsion. Allow the combined extracts to cool, dilute with hexanes to volume, and mix. Transfer 10 mL of this solution to a capped test tube, add 1 g of anhydrous sodium sulfate, mix for 1 min, centrifuge, and use the clear supernatant.
Blank: Mix 100 mL of hexanes and 10 g of anhydrous sodium sulfate, allow to settle, centrifuge, and use the clear supernatant.
Analysis
Samples: *Standard solution* and *Sample solution*
Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution* at the wavelength of maximum absorbance at about 7.9 µm, with a suitable IR spectrophotometer, using the *Blank* to set the instrument.
Calculate the percentage of polydimethylsiloxane ($[-(CH_3)_2SiO-]_n$) in the portion of the Oral Suspension taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Polydimethylsiloxane RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of simethicone in the *Sample solution* (mg/mL)

Acceptance criteria: 85.0%–115.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.5–4.6

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Polydimethylsiloxane RS

Simethicone Tablets

» Simethicone Tablets contain an amount of polydimethylsiloxane ($[-(CH_3)_2SiO-]_n$) that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

Packaging and storage—Preserve in well-closed containers.
Labeling—Tablets that are gelatin-coated are so labeled.
USP Reference standards ⟨11⟩—
USP Polydimethylsiloxane RS

Change to read:

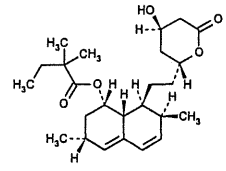
Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197S*▲ (CN 1-May-2020)
[NOTE—Use the procedural blank, prepared as directed in the *Assay*, to set the instrument.]
Test solution—Prepare as directed for the *Assay preparation* in the *Assay*.
Standard solution—Prepare as directed for the *Standard preparation* in the *Assay*.
Cell size: 0.5 mm.
Disintegration ⟨701⟩: 30 minutes in water; 60 minutes in water for plain-coated Tablets; and 45 minutes in simulated gastric fluid for Tablets labeled as gelatin-coated.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of simethicone, to a round, narrow-mouth, screw-capped, 120-mL bottle, and proceed as directed in the *Assay* under *Simethicone*, beginning with "add 25.0 mL of toluene," except that for Tablets labeled as gelatin-coated, shake for 30 minutes instead of 5 minutes. Calculate the quantity, in mg, of $[-(CH_3)_2SiO-]_n$ in the portion of Tablets taken by the formula:

$$25C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Polydimethylsiloxane RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

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Simvastatin



$C_{25}H_{38}O_5$ 418.57

Butanoic acid, 2,2-dimethyl-, 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)ethyl]-1-naphthalenyl ester, [1*S*-[1α,3α,7β,8β(2*S**,4*S**),8aβ]].

2,2-Dimethylbutyric acid, 8-ester with (4*R*,6*R*)-6-2-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one [79902-63-9].

» Simvastatin contains not less than 98.0 percent and not more than 102.0 percent of $C_{25}H_{38}O_5$, calculated on the dried basis. It may contain a suitable antioxidant.

Packaging and storage—Preserve in well-closed containers. Store between 15° and 30°, or under refrigeration.

USP Reference standards ⟨11⟩—
USP Lovastatin RS
USP Simvastatin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Specific rotation ⟨781S⟩**:** between +285° and +298°.
Test solution: 5 mg per mL, in acetonitrile.

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Chromatographic purity— [NOTE—The Simvastatin solutions are stable for up to 3 days when stored at 4°. Without refrigeration, they should be injected immediately after preparation.]

Mobile phase, Diluent, and *Chromatographic system*—Proceed as directed in the *Assay.*

Test solution—Use the *Assay preparation.*

Procedure—Inject about 5 µL of the *Test solution* into the chromatograph, record the chromatogram, identify the specified impurities listed in *Table 1,* and measure the areas for all the peaks. Calculate the percentage of each impurity in the portion of Simvastatin taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak area for each impurity; and r_s is the sum of the areas of all the peaks. Reporting level for impurities is 0.05%.

Table 1

Name	Relative Retention Time	Limit %
Simvastatin hydroxyacid[1]	0.45	0.4
Epilovastatin[2] and Lovastatin	0.60	1.0[3]
Methylene simvastatin[4]	0.80	0.4
Simvastatin	1.0	n/a
Acetyl simvastatin[5]	2.38	0.4
Anhydro simvastatin[6]	2.42	0.4
Simvastatin dimer[7]	3.80	0.4
Any other individual impurity	—	0.1
Total impurities other than lovastatin and epilovastatin	—	1.0

[1] (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-Dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid.

[2] (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*R*)-2-methylbutanoate.

[3] If present, lovastatin and epilovastatin may not be completely resolved by the method. These peaks are integrated together to determine conformance.

[4] (1*S*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbut-3-enoate.

[5] (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-(Acetyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

[6] (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-Dimethyl-8-[2-[(2*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl]-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

[7] (2*R*,4*R*)-2-[[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-Dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]-6-oxotetrahydro-2*H*-pyran-4-yl (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate.

Assay— [NOTE—The Simvastatin solutions are stable for up to 3 days when stored at 4°. Without refrigeration, they should be injected immediately after preparation.]

Dilute phosphoric acid—Transfer 1 mL of phosphoric acid to a 1-L volumetric flask, and dilute with water to volume.

Solution A—Prepare a mixture of acetonitrile and *Dilute phosphoric acid* (50:50).

Solution B—Transfer 1 mL of phosphoric acid to a 1-L volumetric flask, and dilute with acetonitrile to volume.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B,* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Buffer solution—Prepare a solution containing 1.4 g of monobasic potassium phosphate per L, and adjust with phosphoric acid to a pH of 4.0.

Diluent—Prepare a mixture of acetonitrile and *Buffer solution* (3:2).

System suitability preparation—Dissolve accurately weighed quantities of USP Simvastatin RS and USP Lovastatin RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 1.5 mg per mL of USP Simvastatin RS and 0.015 mg per mL of USP Lovastatin RS.

Standard preparation—Dissolve an accurately weighed quantity of USP Simvastatin RS in *Diluent* to obtain a solution having a known concentration of about 1.5 mg per mL.

Assay preparation—Transfer about 75 mg of Simvastatin, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩—The liquid chromatograph is equipped with a 238-nm detector and a 4.6- × 33-mm column that contains packing L1. The flow rate is about 3.0 mL per minute. The chromatograph is programmed as follows.

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Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–4.5	100	0	isocratic
4.5–4.6	100→95	0→5	linear gradient
4.6–8.0	95→25	5→75	linear gradient
8.0–11.5	25	75	isocratic
11.5–11.6	25→100	75→0	linear gradient
11.6–13	100	0	re-equilibration

Chromatograph the *System suitability preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.60 for lovastatin and 1.0 for simvastatin; and the resolution, *R,* between simvastatin and lovastatin is greater than 3. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{25}H_{38}O_5$ in the portion of Simvastatin taken by the formula:

$$VC(r_U/r_S)$$

in which V is the volume, in mL, of the *Assay preparation;* C is the concentration, in mg per mL, of USP Simvastatin RS in the *Standard preparation;* and r_U and r_S are the responses of the simvastatin peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Simvastatin Tablets

DEFINITION

Simvastatin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of simvastatin ($C_{25}H_{38}O_5$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer solution: Dissolve 3.9 g of monobasic sodium phosphate in 900 mL of water. Adjust, if necessary, with either sodium hydroxide or phosphoric acid to a pH of 4.5. Dilute with water to 1000 mL and mix.

Mobile phase: Acetonitrile and *Buffer solution* (65:35)

Solution A: Add 3.0 mL of glacial acetic acid to 900 mL of water. Adjust with 5 N sodium hydroxide to a pH of 4.0 and dilute with water to 1 L.

Diluent: Acetonitrile and *Solution A* (8:2)

Standard solution: 0.1 mg/mL of USP Simvastatin RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of simvastatin from Tablets in *Diluent* as follows. Crush NLT 20 Tablets into a fine powder and transfer the powder equivalent to 100 mg of simvastatin to a suitable volumetric flask. Add *Diluent* to fill 70% of the volume of the flask, and sonicate with intermittent swirling for 10 min. Equilibrate to room temperature and dilute with *Diluent* to volume. Centrifuge a portion of the mixture, and pass the clear supernatant through a filter of 0.45-µm pore size. Discard the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 238 nm. For *Identification B,* use a diode array detector in the range of 190–300 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of simvastatin ($C_{25}H_{38}O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of simvastatin from the *Sample solution*

r_S = peak area of simvastatin from the *Standard solution*

C_S = concentration of USP Simvastatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of simvastatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Prepare a pH 7.0 buffer solution containing 0.5% sodium dodecyl sulfate in 0.01 M sodium phosphate as follows. Dissolve 30 g of sodium dodecyl sulfate and 8.28 g of monobasic sodium phosphate in 6000 mL of water and adjust with 50% (w/v) sodium hydroxide solution to a pH of 7.0; 900 mL.

Apparatus 2: 50 rpm

Time: 30 min

Prewashed manganese dioxide: Transfer 10 g of manganese dioxide to a suitable container, and treat as follows. Add 50 mL of *Medium,* and shake vigorously for 5 min. Centrifuge, decant the supernatant layer, and discard. Repeat twice, first with *Medium* and then with water. Dry the solid at 100° for 1 h before use.

Standard solution: USP Simvastatin RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Transfer a portion of the solution to a centrifuge tube containing about 10 mg of *Prewashed manganese dioxide* per milliliter of transferred solution under test, and mix. Allow the mixture to stand for 30 min with occasional shaking, centrifuge, and use a portion of the clear supernatant.

Instrumental conditions

Mode: UV

Analytical wavelengths: 247 and 257 nm

Blank: Prepare as directed for the *Sample solution,* except use the *Medium.*

Analysis: Calculate the percentage of the labeled amount of simvastatin ($C_{25}H_{38}O_5$) dissolved from the difference between the UV absorbances at the wavelengths of maximum and minimum absorbances at about 247 and 257 nm, respectively, of the *Sample solution,* in comparison with the *Standard solution.*

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Tolerances: NLT 75% (*Q*) of the labeled amount of simvastatin ($C_{25}H_{38}O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Prepare a mixture of acetonitrile and dilute phosphoric acid (1 mL in 1 L of water) (45:55).

Solution B: Prepare a mixture of acetonitrile and dilute phosphoric acid (1 mL in 1 L of water) (90:10).

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
38	100	0
40	65	35
50	65	35
70	0	100
90	0	100
92	100	0
100	100	0

Solution C: 1.4 g/L of monobasic potassium phosphate in water. Adjust with diluted ammonia solution to a pH of 7.0.

Diluent: Acetonitrile and *Solution C* (60:40)

System suitability stock solution: 0.2 mg/mL each of USP Simvastatin RS and USP Lovastatin RS in *Diluent*. Sonicate if necessary.

System suitability solution: 0.02 mg/mL each of USP Simvastatin RS and USP Lovastatin RS in *Diluent* from *System suitability stock solution*

Standard solution: 7.5 µg/mL of USP Simvastatin RS and equivalent to 0.015 mg/mL of tenivastatin from USP Tenivastatin Calcium RS in *Diluent*

Sample solution: Nominally 1.5 mg/mL of simvastatin in *Diluent* prepared as follows. Transfer a suitable amount of powdered Tablets (NLT 20) to a volumetric flask. Add *Diluent* to fill 60% of the volume of the flask, and sonicate with intermittent swirling for 5 min. Dilute with *Diluent* to volume. Pass the solution through a filter of 0.45-µm pore size. Discard the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 238 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Temperatures

Autosampler: 10°

Column: 30°

Flow rate: 2 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of simvastatin

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 8.0 between simvastatin and lovastatin, *System suitability solution*

Tailing factor: NMT 1.5 for simvastatin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tenivastatin in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tenivastatin from the *Sample solution*

r_S = peak response of tenivastatin from the *Standard solution*

C_S = concentration of USP Tenivastatin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of simvastatin in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of simvastatin from the *Standard solution*

C_S = concentration of USP Simvastatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of simvastatin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tenivastatin[a]	0.43	1.0
Lovastatin[b, c]	0.63	—
Epilovastatin[b, d]	0.66	—
Methylene simvastatin[b, e]	0.80	—
Simvastatin	1.00	—
Tenivastatin methyl ester[b, f]	1.14	—
Acetyl simvastatin[b, g]	1.54	—
Anhydro simvastatin[b, h]	1.59	—
Simvastatin related compound D[b, i]	2.30	—
Individual unspecified impurity	—	0.5

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Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities[i]	—	2.0

[a] (3*R*,5*R*)-7-{((1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2)-Dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-3,5-dihydroxyheptanoic acid.
[b] Process impurity included in the table for identification purposes only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.
[c] (*S*)-(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate.
[d] (*R*)-(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate.
[e] (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbut-3enoate.
[f] Methyl (3*R*,5*R*)-7-{(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl}-3,5-dihydroxyheptanoate.
[g] (1*S*,3*R*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-Acetoxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.
[h] (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-Dimethyl-8-{2-[(*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl}-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.
[i] (3*R*,5*R*)-(2*R*,4*R*)-2-(2-{(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-Dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl}ethyl)-6-oxotetrahydro-2*H*-pyran-4-yl 7-{(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl}-3,5-dihydroxyheptanoate.
[j] Excluding lovastatin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Lovastatin RS
(*S*)-(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate.
$C_{24}H_{36}O_5$ 404.54

USP Simvastatin RS

USP Tenivastatin Calcium RS
Calcium (3*R*,5*R*)-7-{(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl}-3,5-dihydroxyheptanoate (1:2) monohydrate.
$C_{50}H_{78}CaO_{12} \cdot H_2O$ 929.24

Sipuleucel-T

DEFINITION

Sipuleucel-T is an autologous cellular immunotherapy. The active components of sipuleucel-T are autologous antigen presenting cells (APCs) and a recombinant protein, PAP-GM-CSF that consists of human prostatic acid phosphatase (PAP) fused through the C-terminus to the N-terminus of human granulocyte-macrophage colony-stimulating factor (GM-CSF) by a Gly-Ser linker. PAP is an antigen expressed in prostate cancer tissue and GM-CSF is an immune cell activator. Minimal residual levels of the intact PAP-GM-CSF are detectable in the final product.

Sipuleucel-T is prepared using a patient's own peripheral blood mononuclear cells obtained via a 1.5–2.0 blood volume leukapheresis approximately 3 days prior to the infusion date. The cells obtained are further processed and then cultured for 36–44 h in a nutrient-rich medium in the presence of PAP-GM-CSF. The cells are then aseptically harvested, washed, formulated, and packaged for intravenous infusion. Sipuleucel-T is a sterile, live cell suspension for autologous use only. Each dose contains a minimum of 50 million autologous CD54+ cells activated with PAP-GM-CSF, suspended in 250 mL of Lactated Ringer's Injection, *USP*.

IDENTIFICATION

- **A.** Meets the requirements in the *Assay*

ASSAY

- **PROCEDURE**

[NOTE—To determine potency by flow cytometry, both an in-process sample taken prior to culture with PAP-GM-CSF and a sipuleucel-T final product sample are tested.]

Phosphate buffered saline (PBS): Use Dulbecco's phosphate-buffered saline without calcium or magnesium, pH 7.0–7.2.

Analysis: Stain one 30-µL aliquot of cells (containing 20 × 10^6) with 40 µL of monoclonal anti-CD54 antibody conjugated 1:1 with phycoerythrin (PE) and a second aliquot with 40 µL of isotype control antibody conjugated 1:1 with PE.[1] Incubate for 15–25 min in the dark, then wash away unbound antibody with *PBS* by centrifugation. Resuspend cells in 400 µL of *PBS* containing 1 µg/mL of laser dye styryl–751 (LDS-751),[2] stain for 5 min, and then add an equal volume of *PBS* to each sample. Reconstitute PE-conjugated flow cytometry bead standards[3] in 300 µL of *PBS* containing 0.5 µg/mL of LDS-751.

Data acquisition: Acquire the flow cytometry bead standards on a multi-color flow cytometer using an acquisition plot with forward scatter (FSC) vs. side scatter (SSC) axes and a gate to encompass approximately 10,000 bead singlet events. Use a second acquisition plot gated on the bead singlet events to graph only the events in the singlet gate by PE fluorescence vs. event count. Use histogram region markers to calculate the geometric mean fluorescence intensity (MFI) of each of the labeled bead populations. The number of PE molecules reported by the manufacturer is used to generate a PE vs. MFI standard curve from which the number of PE molecules on the surface of the test sample population can be calculated.

Acquire approximately 10,000 events for the CD54-PE and isotype control-stained test samples using an acquisition plot with LDS-751 fluorescence vs. FSC and an inclusion gate set on LDS+ leukocytes. This gate allows the user to remove nonspecific signal from debris and/or erythrocytes from the evaluation. Precise gating and therefore removal (from data analysis) of these two smaller product subcomponents is not critical due to the use of a second acquisition plot that focuses solely on the analysis of CD54+ large cell events.

Calculating number of CD54+ cells: LDS fluorescence vs. FSC is evaluated for both the anti-CD54-PE (1:1)-stained and the isotype control-stained samples. The second analysis plot, which includes only the LDS+ leukocyte populations in both the test and control samples, evaluates PE fluorescence vs. FSC, and is used to derive statistics for each sample. A pair of stacked regions allow the user to set a threshold (lower region) based on the isotype control-stained cells, while placing the upper region to encompass the large CD54+ leukocytes. The latter region provides the results for both MFI and the

[1] Suitable anti-CD54-PE (1:1) and isotype control-PE (1:1) antibodies can be obtained from Becton Dickinson, catalog# 332811.
[2] Suitable laser dye styryl–751 (LDS-751) can be obtained from Molecular Probes, catalog# L-7595.
[3] Suitable flow cytometry standards, QuantiBRITE-PE™ Beads, can be obtained from Becton Dickinson, catalog# 340495.

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percentage of large CD54+ cells present in each test sample. The percentage of large CD54+ cells is multiplied by both the total leukocyte concentration (determined using an automated hematology analyzer) and the percent of viable cells to obtain the number of CD54+ cells in the product.

Calculating CD54 upregulation: To determine CD54 upregulation, stain and acquire preculture samples and final product samples as described above. For each sample, calculate the MFI for the large CD54+ cells for both the isotype control and the anti-CD54-PE (1:1)-stained sample. Using the corresponding bead standard PE vs. MFI standard curves, convert the test MFI results to the number of PE molecules bound per cell. To correct for nonspecific staining, subtract the isotype control result from the corresponding result for the anti-CD54-PE (1:1)-stained sample to obtain the number of PE molecules specifically bound. Divide the final product number of PE molecules specifically bound by the preculture number to obtain the CD54 upregulation ratio.

Acceptance criteria: The preculture material must contain NLT 165 × 10^6 CD54+ cells to meet in-process acceptance criteria. Sipuleucel-T final product must contain NLT 50 × 10^6 CD54+ cells and must demonstrate NLT a 2.6-fold CD54 upregulation.

SPECIFIC TESTS

- **ELISA ASSAY**

[NOTE—To ensure that the cells have been treated with the appropriate antigen, an ELISA test is used to measure residual intact PAP-GM-CSF in the final product.]

Phosphate buffered saline (PBS): Prepare as directed in the *Assay*.

Capture antibody: Prepare a solution containing 5 µg of monoclonal anti-PAP antibody/mL[4] of *PBS*.

Assay diluent: Prepare a solution of 10% blocking solution[5] in Lactated Ringer's Injection, *USP*.

Secondary antibody: Prepare a solution containing anti-GM-CSF antibody[6] conjugated with horseradish peroxidase (HRP) diluted in blocking solution.

2X positive control: Prepare a solution containing 500 pg of PAP-GM-CSF/mL of *Assay diluent*.

Wash buffer: Dilute concentrated wash buffer[7] to the recommended working strength.

Test sample: A 2-mL test sample is removed from the final product formulation. A 1-mL aliquot of this sample is centrifuged to pellet the cells, and then 900 µL of supernatant is combined with 100 µL of blocking solution in preparation for loading on the plate.

Analysis: Prepare assay plates by coating microtiter plate wells with *Capture antibody* for 120 min and then blocking with undiluted blocking solution. Load separate wells with either 50 µL of *Assay diluent* (in triplicate), 50 µL of *Test sample*, or 50 µL of a 1:1 mixture of *Test sample* with *2X positive control*. The triplicate *Assay diluent* wells act as a negative control for the establishment of a background signal. Add 50 µL of *Secondary antibody* to each well, and incubate for 90 min at room temperature. Wash the plate with *Wash buffer*, and add 100 µL of TMB Chromogen.[8] After 30 min, stop the reaction with 100 µL of stop solution[9] and, using a plate reader, determine the absorbance of the well contents with an optical density of 450 nm, with a reference filter set to 570 nm.

Calculations and system suitability: The mean and standard deviation of the negative control sample replicates (*Assay diluent*) are calculated. The mean of the *Test sample* results is also calculated. The threshold value is calculated as the mean absorbance of the negative control plus three times the standard deviation. The mean absorbance of the negative control must be NMT 0.061.

Acceptance criteria: If the *Test sample* mean absorbance is greater than the threshold value, then the *Test sample* is identified as sipuleucel-T that has been exposed to PAP-GM-CSF.

- **TOTAL NUCLEATED CELL COUNT ASSAY**

Test sample: 2 mL of Sipuleucel-T. If required, dilute the sample with *PBS* to obtain a result within the linear range of the method being used.

Analysis: Use a commercially available hematology analyzer or a manual counting procedure to determine the white blood cell concentration on triplicate analyses of neat sample derived from the appropriate process step. Proceed as directed in the instrument operating manual or manual procedure.

Calculation: Multiply the white blood cell concentration by the net volume and the viability of the sample to determine the total nucleated cell count.

Acceptance criteria: NLT 5 × 10^7 cells in the final product. The total nucleated cell result for the final product formulation is used to determine the number of CD54+ cells.

- **TRYPAN BLUE EXCLUSION (VIABILITY) ASSAY**

Test sample: 2 mL of Sipuleucel-T

Analysis: Based on the cell concentration (which can be determined manually, or using an automated hematology analyzer), dilute duplicate aliquots of the *Test sample* with *PBS* to yield total counts of 100–400 cells per hemacytometer chamber. Mix equal volumes of each *Test sample* with the same volume of 0.4% isotonic trypan blue. Load the samples in a hemacytometer, and count the cells in the four large corner squares in each chamber.

Calculation: Calculate the percentage of viable cells (unstained cells) relative to the total number of cells (stained and unstained cells).

Acceptance criteria: NLT 80% viability in both the incoming leukapheresis component and in the sipuleucel-T final product.

- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Unit/mL of final product. A 2-mL sample is removed from the final product formulation and 0.45-mL of this sample is mixed with 0.45 mL of LAL Reagent Water, then 0.10-mL duplicate samples are analyzed.

ADDITIONAL REQUIREMENTS

- **LABELING:** The final product is labeled to indicate the identity of the patient, the expiration time and date, the required storage conditions (refrigerated at 2–8°), and the lot number. The label indicates that the product is for autologous use only, and that the product is not routinely tested for transmissible infectious diseases.
- **PACKAGING AND STORAGE:** Sipuleucel-T is supplied in a sealed infusion bag labeled for the specific recipient. The product is shipped in a cardboard shipping box with a

[4] A suitable antibody can be produced with the hybridoma cell line, ATCC catalog No. HB-8526, or Aragen Bioscience, special order.

[5] A suitable blocking solution can be obtained from Thermo Scientific, catalog# 337516.

[6] A suitable antibody can be obtained from Anogen, catalog# MO-C40090T.

[7] A suitable wash buffer concentrate can be obtained from Invitrogen, catalog# WB01.

[8] A suitable TMB Chromogen can be obtained from Invitrogen, catalog# SB01.

[9] A suitable stop solution can be obtained from Invitrogen, catalog# SS01.

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special insulated polyurethane container inside. The insulated container and gel packs within the container maintain the appropriate transportation and storage temperature of sipuleucel-T until infusion. The infusion bag must remain within the insulated polyurethane container and shipping box until the time of administration.

Sitagliptin Tablets

DEFINITION

Sitagliptin Tablets contain an amount of sitagliptin phosphate ($C_{16}H_{15}F_6N_5O \cdot H_3PO_4 \cdot H_2O$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of sitagliptin ($C_{16}H_{15}F_6N_5O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020) or ⟨197A⟩

  Sample: Grind a Tablet, and use a suitable amount of the powdered Tablet to prepare a specimen.

  Analysis: Record the spectra of the *Sample* and the Standard using ⟨197A⟩ or ⟨197K⟩, and examine the spectra in the range between 1800 cm^{-1} and 650 cm^{-1}.

  Acceptance criteria: The spectrum obtained from the *Sample* shows bands at approximately 1669, 1513, 1425, 1207, 880, and 844 cm^{-1}, similar to the spectrum from the Standard similarly obtained. [NOTE—Peak positions may vary slightly between instruments (within ±10 cm^{-1}). Other peaks may be present in the spectra that do not appear in this list.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

  Buffer: 1.36 g/L of monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 2.0

  Mobile phase: Acetonitrile and *Buffer* (15:85)

  Dilute phosphoric acid: Transfer 1 mL of phosphoric acid to a 1-L volumetric flask, and dilute with water to volume.

  Diluent: Acetonitrile and *Dilute phosphoric acid* (5:95)

  Standard solution: 0.1 mg/mL of USP Sitagliptin Phosphate RS in *Diluent*

  Sample stock solution: Nominally 1.0 mg/mL of sitagliptin, prepared as follows. Place 10 Tablets in a suitable volumetric flask, and dilute with *Diluent* to volume. Add a stir bar and stir vigorously for 1 h, ensuring that a vortex is achieved during stirring.

  Sample solution: Nominally 0.08 mg/mL of sitagliptin, prepared as follows. Transfer 8.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min or until the solution is clear, and use the supernatant.

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *System Suitability*.)

  Mode: LC

  Detector: UV 205 nm

  Column: 4.6-mm × 15-cm; 5-µm packing L10

  Column temperature: 30°

  Flow rate: 1.0 mL/min

  Injection volume: 20 µL

  System suitability

  Sample: *Standard solution*

  Suitability requirements

  Relative standard deviation: NMT 2.0%

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of the labeled amount of sitagliptin ($C_{16}H_{15}F_6N_5O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

  r_U = peak area from the *Sample solution*

  r_S = peak area from the *Standard solution*

  C_S = concentration of USP Sitagliptin Phosphate RS in the *Standard solution* (mg/mL)

  C_U = nominal concentration of sitagliptin in the *Sample solution* (mg/mL)

  M_{r1} = molecular weight of sitagliptin, 407.32

  M_{r2} = molecular weight of anhydrous sitagliptin phosphate, 505.31

  Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩

  Time: 5 min

  Acceptance criteria: Meet the requirements
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

  Buffer, Mobile phase, Dilute phosphoric acid, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

  System suitability solution (***Procedure 1*** may be used for Tablets containing sodium stearyl fumarate as an excipient in the formulation, and ***Procedure 2*** may be used for all products.)

  Procedure 1: Place 1 Tablet of any potency into a vial, add 1 mL of water, and tightly seal the vial. Heat at 80° for about 30 h to generate a fumarate adduct of sitagliptin. Transfer the content of the vial into a 100-mL volumetric flask, using a small amount of *Diluent*, and dilute with *Diluent* to volume. Mix well by stirring for 1 h. If needed, further dilute with *Diluent* to obtain a solution with a concentration of 0.08 mg/mL of sitagliptin, based on the label claim of the Tablets. Centrifuge a portion of the solution for 10 min or until the solution is clear, and use the supernatant.

  Procedure 2: Place 10 mg of USP Sitagliptin Phosphate RS and 1 mg of sodium stearyl fumarate into a vial, add 1 mL of water, and tightly seal the vial. Heat at 80° for about 30 h to generate a fumarate adduct of sitagliptin. Transfer the content of the vial into a 100-mL volumetric flask, using a small amount of *Diluent*, and dilute with *Diluent* to volume. Mix well by stirring for 1 h. Centrifuge a portion of the solution for 10 min or until the solution is clear, and use the supernatant.

  Standard solution: 0.0002 mg/mL of USP Sitagliptin Phosphate RS in *Diluent*

  Sensitivity solution: 0.0001 mg/mL of USP Sitagliptin Phosphate RS in *Diluent* from the *Standard solution*

  System suitability

  Samples: *System suitability solution* and *Sensitivity solution*

  [NOTE—See *Table 1* for relative retention times.]

  Suitability requirements

  Resolution: NLT 1.5 between sitagliptin and fumarate adduct, *System suitability solution*

  Signal-to-noise ratio: NLT 10, *Sensitivity solution*

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Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of sitagliptin from the *Standard solution*
C_S = concentration of USP Sitagliptin Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sitagliptin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)
M_{r1} = molecular weight of sitagliptin, 407.32
M_{r2} = molecular weight of anhydrous sitagliptin phosphate, 505.31

Acceptance criteria: See *Table 1*. Reporting level for impurities: 0.1%

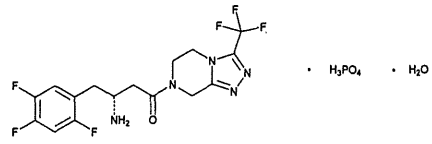
Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sitagliptin acid[a]	0.55	0.65	0.2
Sitagliptin	1.0	—	—
Fumarate adduct (if present)[b, c]	1.2	1.0	0.2
Sitagliptin triazecine analog[d]	1.8	1.0	0.2
Sitagliptin styrylacetyl analog[e]	4.1	1.0	0.2
Sitagliptin phenylcrotonyl analog[f]	4.7	2.1	0.2
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	0.6

[a] (*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoic acid.
[b] 2-[[(*R*)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-yl]amino]succinic acid.
[c] This degradation product may be present only for Tablets containing sodium stearyl fumarate as an excipient in the formulation.
[d] 10-(2,4,5-Trifluorobenzyl)-3-(trifluoromethyl)-6,7,10,11-tetrahydro-[1,2,4]triazolo[3,4-*c*][1,4,7]triazecine-8,12(5*H*,9*H*)-dione.
[e] (*E*)-1-{3-(Trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl}-4-(2,4,5-trifluorophenyl)but-3-en-1-one.
[f] (*E*)-1-{3-(Trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl}-4-(2,4,5-trifluorophenyl)but-2-en-1-one.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sitagliptin Phosphate RS

Sitagliptin Phosphate



$C_{16}H_{15}F_6N_5O \cdot H_3PO_4 \cdot H_2O$ 523.32

$C_{16}H_{15}F_6N_5O \cdot H_3PO_4$ 505.31

1,2,4-Triazolo[4,3-*a*]pyrazine, 7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-, phosphate (1:1) monohydrate;
7-[(*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(trifluoromethyl)-5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*] pyrazine monophosphate monohydrate;
(3*R*)-3-Amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4] triazolo[4,3-*a*]pyrazin-7(8*H*)-yl-4-(2,4,5-trifluorophenyl) butan-1-one phosphate monohydrate [654671-77-9].

DEFINITION
Sitagliptin Phosphate contains NLT 98.0% and NMT 102.0% of sitagliptin phosphate ($C_{16}H_{15}F_6N_5O \cdot H_3PO_4$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
  [NOTE—If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in dehydrated alcohol, evaporate to dryness, and record new spectra using the residues.]
- **B.** Meets the requirements of the test for *Enantiomeric Purity*
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Phosphate*: A solution containing about 40 mg/mL in water meets the requirements of test *A* of Orthophosphates.

ASSAY
- **PROCEDURE**

Buffer: 1.36 g/L of monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 2.0
Mobile phase: Acetonitrile and *Buffer* (15:85)
Dilute phosphoric acid: Transfer 1 mL of phosphoric acid to a 1-L volumetric flask, and dilute with water to volume.
Diluent: Acetonitrile and *Dilute phosphoric acid* (5:95)
Standard solution: 0.1 mg/mL of USP Sitagliptin Phosphate RS in *Diluent*
Sample solution: 0.1 mg/mL of Sitagliptin Phosphate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of sitagliptin phosphate ($C_{16}H_{15}F_6N_5O \cdot H_3PO_4$) in the portion of Sitagliptin Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Sitagliptin Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Sitagliptin Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Dilute phosphoric acid, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: Place 10 mg of Sitagliptin Phosphate and 1 mg of sodium stearyl fumarate into a vial, add 1 mL of water, and tightly seal the vial. Heat at 80° for about 30 h to generate a fumarate adduct of sitagliptin. [NOTE—The chemical name of fumarate adduct of sitagliptin is 2-[[(*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-yl]amino]succinic acid.] Transfer the content of the vial into a 100-mL volumetric flask using a small amount of *Diluent*, and dilute with *Diluent* to volume. Mix well by stirring for 1 h. Centrifuge a portion of the solution for 10 min or until the solution is clear, and use the supernatant.

Standard solution: 0.0001 mg/mL of USP Sitagliptin Phosphate RS in *Diluent*

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for sitagliptin and fumarate adduct of sitagliptin are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.5 between sitagliptin and fumarate adduct of sitagliptin

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Sitagliptin Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of sitagliptin from the *Standard solution*
C_S = concentration of USP Sitagliptin Phosphate in the *Standard solution* (mg/mL)
C_U = concentration of Sitagliptin Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: Disregard any peak below 0.05%.
Any individual impurity: NMT 0.10%
Total impurities: NMT 0.5%

• **ENANTIOMERIC PURITY**

Mobile phase: Dehydrated alcohol, chromatographic *n*-heptane, diethylamine, and water (600:400:1:1)

Diluent: Methanol and water (9:1)

System suitability solution: 8 mg/mL of USP Sitagliptin System Suitability Mixture RS in *Diluent*

Sample solution: 8 mg/mL of Sitagliptin Phosphate in *Diluent*

Sensitivity solution: 8 µg/mL of Sitagliptin Phosphate in *Diluent* from the *Sample solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: UV 268 nm
Column: 4.6-mm × 25-cm; 5-µm packing L51
Column temperature: 35°
Flow rate: 0.8 mL/min
Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times for sitagliptin, which is the *R*-enantiomer, and the *S*-enantiomer are 1.0 and 0.9, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the *S*-enantiomer and sitagliptin, *System suitability solution*

Signal-to-noise ratio: NLT 10 for the sitagliptin peak, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of *S*-enantiomer in the portion of Sitagliptin Phosphate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the *S*-enantiomer from the *Sample solution*
r_T = sum of the peak responses of the *S*-enantiomer and sitagliptin from the *Sample solution*

Acceptance criteria: NMT 0.5% of the *S*-enantiomer

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** 3.3%–3.7%

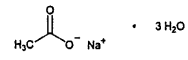
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sitagliptin Phosphate RS
USP Sitagliptin System Suitability Mixture RS
Sitagliptin Phosphate containing *S*-enantiomer.

Sodium Acetate



$C_2H_3NaO_2 \cdot 3H_2O$ 136.08
$C_2H_3NaO_2$ 82.03

Acetic acid, sodium salt, trihydrate;
Sodium acetate trihydrate [6131-90-4].
Anhydrous [127-09-3].

DEFINITION

Sodium Acetate contains three molecules of water of hydration, or is anhydrous. It contains NLT 99.0% and NMT 101.0% of sodium acetate ($C_2H_3NaO_2$), calculated on the dried basis.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements

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• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Acetate*
Sample solution : 10 mg/mL of Sodium Acetate in water. Adjust with 0.1 N sodium hydroxide VS to a slightly alkaline pH.
Acceptance criteria: Meets the requirements in test *A*

ASSAY
• **PROCEDURE**
Sample solution: Equivalent to 200 mg of anhydrous Sodium Acetate in 50 mL of glacial acetic acid. Heat gently on a steam bath for about 5 min to dissolve. Cover the solution and stir while cooling.
Blank: 50 mL of glacial acetic acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis: Titrate the *Sample solution* with *Titrant*. Perform a blank determination, and make any necessary correction. Calculate the percentage of sodium acetate ($C_2H_3NaO_2$) in the portion of Sodium Acetate taken:

$$\text{Result} = [(V_S - V_B) \times N_A \times F \times 100]/W$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N_A = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 82.03 mg/mEq
W = equivalent weight of anhydrous Sodium Acetate in the *Sample solution* (mg)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES
• **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*
Sample: Equivalent to 1.0 g of anhydrous Sodium Acetate
Acceptance criteria: Shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid VS (350 ppm).
• **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*
Sample: Equivalent to 10 g of anhydrous Sodium Acetate
Acceptance criteria: Shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid VS (50 ppm).
• **CALCIUM AND MAGNESIUM**
Sample solution: Equivalent to 10 mg/mL of anhydrous Sodium Acetate
Analysis: To 20 mL of *Sample solution*, add 2 mL each of 6 N ammonium hydroxide, ammonium oxalate TS, and dibasic sodium phosphate TS.
Acceptance criteria: No turbidity is produced within 5 min.
• **POTASSIUM**
Sample solution: Dissolve the equivalent of 3 g of anhydrous Sodium Acetate in 5 mL of water.
Analysis: To the *Sample solution*, add 1 N acetic acid dropwise until the solution is slightly acidic, and then add 5 drops of sodium cobaltinitrite TS.
Acceptance criteria: No precipitate is formed.
• **ALUMINUM** ⟨206⟩ (where it is labeled as intended for use in hemodialysis)
Test preparation: Prepare as directed in the chapter, using a portion equivalent to 10 g of sodium acetate trihydrate.
Acceptance criteria: 0.2 µg/g

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: Equivalent to 30 mg/mL of anhydrous Sodium Acetate in carbon dioxide-free water
Acceptance criteria: 7.5–9.2
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 120° to constant weight.
Acceptance criteria
Anhydrous: NMT 1.0%
Trihydrate: 38.0%–41.0%
• **INSOLUBLE MATTER**
Sample: Equivalent to 20 g of anhydrous Sodium Acetate
Analysis: Dissolve the *Sample* in 150 mL of water, heat to boiling, and digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°.
Acceptance criteria: The weight of the residue is NMT 10 mg (0.05%).

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Label it to indicate whether it is the trihydrate or is anhydrous. Where Sodium Acetate is intended for use in hemodialysis, it is so labeled.

Sodium Acetate Injection

» Sodium Acetate Injection is a sterile solution of Sodium Acetate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of CH_3COONa.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
Labeling—The label states the sodium acetate content in terms of weight and of milliequivalents in a given volume. Label the Injection to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Acetate* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.90 USP Endotoxin Units per mEq.
pH ⟨791⟩: between 6.0 and 7.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements under *Small-volume Injections*.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Standard stock solution—Dissolve 570.0 mg of sodium chloride, previously dried at 105° for 2 hours, in 100 mL of water, transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 224 µg of sodium, equivalent to 800 µg of anhydrous sodium acetate.
Standard preparations—Transfer to each of four 100-mL volumetric flasks 10 mL of a nonionic wetting agent (1 in 500). Dilute the contents of one of the flasks with water to volume to provide a blank. To the remaining flasks add, respectively,

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5.0, 10.0, and 15.0 mL of *Standard stock solution*, dilute with water to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 800 mg of anhydrous sodium acetate, to a 1000-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask containing 10 mL of a nonionic wetting agent (1 in 500), dilute with water to volume, and mix.

Standard graph—Set a flame photometer for maximum transmittance at a wavelength of about 589 nm. Adjust the instrument to zero transmittance with the blank, and to 100% transmittance with the most concentrated of the *Standard preparations*. Read the transmittances of the other *Standard preparations*, and plot transmittances versus equivalent concentration of sodium acetate.

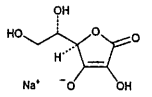
Procedure—Adjust the instrument as directed under *Standard graph*, read the transmittance of the *Assay preparation*, and calculate the sodium acetate content, in mg per mL, of Injection.

Sodium Acetate Solution

» Sodium Acetate Solution is an aqueous solution of Sodium Acetate. It contains not less than 97.0 percent and not more than 103.0 percent (w/w) of the labeled amount of $C_2H_3NaO_2$.

Packaging and storage—Preserve in tight containers.
Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Acetate* ⟨191⟩.
pH ⟨791⟩: between 7.5 and 9.2, when diluted with carbon dioxide-free water to contain 5% of solids.
Insoluble matter—Dilute a quantity of Solution, equivalent to 20 g of anhydrous sodium acetate, with water to 150 mL, heat to boiling, and digest in a covered beaker on a steam bath for 1 hour. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°: the weight of the residue does not exceed 1 mg (0.005%).
Chloride ⟨221⟩—A quantity of Solution, equivalent to 1.0 g of anhydrous sodium acetate, shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).
Sulfate ⟨221⟩—A quantity of Solution, equivalent to 10 g of anhydrous sodium acetate, shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid (0.005%).
Calcium and magnesium—Dilute a quantity of Solution, equivalent to 1.0 g of anhydrous sodium acetate, to 100 mL with water. To 20 mL of this solution add 2 mL each of 6 N ammonium hydroxide, ammonium oxalate TS, and sodium phosphate TS: no turbidity is produced within 5 minutes.
Potassium—To a quantity of Solution, equivalent to 3.0 g of anhydrous sodium acetate, add 0.2 mL of sodium bitartrate TS: no turbidity is produced within 5 minutes.
Assay—Weigh accurately about 1 g of Solution into a 250-mL conical flask, cautiously add (in a fume hood) 2.6 mL of acetic anhydride, mix, and allow to stand for 5 minutes. Add 25 mL of glacial acetic acid and 2 drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, using 0.5 mL of water, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 8.203 mg of $C_2H_3NaO_2$.

Sodium Ascorbate



$C_6H_7NaO_6$ 198.11
L-Ascorbic acid, monosodium salt;
Monosodium L-ascorbate [134-03-2].

DEFINITION
Sodium Ascorbate contains NLT 99.0% and NMT 101.0% of sodium ascorbate ($C_6H_7NaO_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
  Sample: Use undried Sodium Ascorbate.
  Acceptance criteria: Meets the requirements
- **B. REDUCING CAPACITY**
  Sample solution: 20 mg/mL
  Analysis: Add 1 mL of 0.1 N hydrochloric acid to 4 mL of the *Sample solution*, and add alkaline cupric tartrate TS.
  Acceptance criteria: The solution reduces alkaline cupric tartrate TS slowly at room temperature, but more readily upon heating.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩: A 20-mg/mL solution meets the requirements.

ASSAY
- **PROCEDURE**
  Sample: 400 mg of Sodium Ascorbate
  Blank: 100 mL of carbon dioxide-free water and 25 mL of 2 N sulfuric acid
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N iodine VS
  Endpoint detection: Visual
  Analysis: Dissolve the *Sample* in a mixture of 100 mL of carbon dioxide-free water and 25 mL of 2 N sulfuric acid. Titrate immediately with the *Titrant*, and add 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.
  Calculate the percentage of sodium ascorbate ($C_6H_7NaO_6$) in the portion of Sodium Ascorbate taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 99.05 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 99.0%–101.0% on the dried basis

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 100 mg/mL in carbon dioxide-free water. Use the solution immediately after preparation.
  Acceptance criteria: +103° to +108°
- **pH** ⟨791⟩
  Sample solution: 100-mg/mL solution
  Acceptance criteria: 7.0–8.0

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• **Loss on Drying** ⟨731⟩: Dry a sample in a suitable vacuum drying tube, using phosphorus pentoxide as the desiccant, at 60° for 4 h: it loses NMT 0.25% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Sodium Ascorbate RS

Add the following:

▲Sodium Benzoate Compounded Oral Solution

DEFINITION
Sodium Benzoate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of sodium benzoate ($C_7H_5NaO_2$).
Prepare Sodium Benzoate Compounded Oral Solution 100 mg/mL and 300 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Sodium Benzoate Compounded Oral Solution 100 mg/mL

Sodium Benzoate powder	10 g
Purified Water, a sufficient quantity to make	100 mL

Sodium Benzoate Compounded Oral Solution 300 mg/mL

Sodium Benzoate powder	30 g
Purified Water, a sufficient quantity to make	100 mL

In an appropriately size calibrated container, dissolve the *Sodium Benzoate powder* in about 80 mL of *Purified Water*. Stir until dissolved. Bring to final volume with *Purified Water* and mix well.

ASSAY
• **Procedure**
Solution A: 30 mM of potassium phosphate monobasic buffer adjusted with phosphoric acid to a pH of 3
Mobile phase: Methanol and *Solution A* (50:50)
Standard solution: 0.1 mg/mL of USP Sodium Benzoate RS in *Mobile phase*
Sample solution
For Oral Solution 100 mg/mL: Transfer 1 mL of Oral Solution into a 100-mL volumetric flask, add approximately 80 mL of *Mobile phase*, and vortex. Dilute with *Mobile phase* to volume. Transfer 1 mL of this solution to a 10-mL volumetric flask, add approximately 8 mL of *Mobile phase*, and vortex. Dilute with *Mobile phase* to volume.
For Oral Solution 300 mg/mL: Transfer 1 mL of Oral Solution into a 100-mL volumetric flask, add approximately 80 mL of *Mobile phase*, and vortex. Dilute with *Mobile phase* to volume. Transfer 7 mL of this solution to a 200-mL volumetric flask, add approximately 150 mL of *Mobile phase*, and vortex. Dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*)
Mode: LC
Detector: UV 230 nm
Column: 2-mm × 15-cm; 3-µm packing L1
Flow rate: 0.2 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
[Note—The retention time for sodium benzoate is about 6.5 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium benzoate ($C_7H_5NaO_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sodium benzoate from the *Sample solution*
r_S = peak response of sodium benzoate from the *Standard solution*
C_S = concentration of USP Sodium Benzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium benzoate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩
For Oral Solution 100 mg/mL: 6.7–7.7
For Oral Solution 300 mg/mL: 7.3–8.3

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
• **Beyond-Use Date:** NMT 90 days from the date on which it was prepared when stored at controlled room temperature or in a refrigerator.
• **Labeling:** Label it to state the *Beyond-Use Date*.
• **USP Reference Standards** ⟨11⟩
USP Sodium Benzoate RS▲ (USP 1-May-2020)

Sodium Bicarbonate

$NaHCO_3$ 84.01
Carbonic acid monosodium salt;
Monosodium carbonate [144-55-8].

DEFINITION
Sodium Bicarbonate contains NLT 99.0% and NMT 100.5% of sodium bicarbonate ($NaHCO_3$), calculated on the dried basis.

IDENTIFICATION
• **A. Identification Tests—General** ⟨191⟩, *Sodium*: Meets the requirements
• **B. Identification Tests—General** ⟨191⟩, *Bicarbonate*: Meets the requirements

ASSAY
• **Procedure**
Sample: 3 g

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Analysis: Mix the *Sample* with 100 mL of water, add methyl red TS, and titrate with 1 N hydrochloric acid. Add the acid slowly, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the faint pink color no longer fades after boiling. Each mL of 1 N hydrochloric acid is equivalent to 84.01 mg of sodium bicarbonate.

Acceptance criteria: 99.0%–100.5% on the dried basis

Change to read:

IMPURITIES

- **INSOLUBLE SUBSTANCES**

Sample solution: Dissolve 1 g in 20 mL of water.

Acceptance criteria: The resulting solution is complete and clear.

- **CARBONATE** (where it is labeled as intended for use in hemodialysis)

Apparatus: The apparatus (see *Figure 1*) consists of a 50-mL flask with a side arm connected to a source of carbon dioxide humidified by bubbling through a saturated solution of sodium bicarbonate. The flask is equipped with a top-mounted stopper fitted with an exit tube. The exit tube is connected via a T-tube to a system vent and a leveling buret and reservoir.

Reagents

Saturated sodium bicarbonate solution: Mix 20 g of sodium bicarbonate in 100 mL of water, and allow any undissolved crystals to settle. Use the clear supernatant.

Displacement solution: Dissolve 100 g of sodium chloride in 350 mL of water, and add 1 g of sodium bicarbonate and 1 mL of methyl orange TS. After the sodium bicarbonate has dissolved, add 6 N sulfuric acid until the solution turns pink. Use this solution to fill the reservoir of the apparatus.

Analysis: Add 25 mL of *Saturated sodium bicarbonate solution* to the 50-mL flask, and flush the system by allowing humidified carbon dioxide to enter through the side arm. Close the carbon dioxide inlet and the system vent, and stir the *Saturated sodium bicarbonate solution* until no further carbon dioxide absorption is noted from successive buret readings. Maintain atmospheric pressure in the apparatus by adjusting the *Displacement solution* to the same level in both the reservoir and the buret, noting the buret reading. Open the system vent, and reintroduce humidified carbon dioxide through the side arm of the flask. Close the carbon dioxide inlet and the system vent, and stir the *Saturated sodium bicarbonate solution* vigorously until no further carbon dioxide absorption is noted. Repeat the carbon dioxide absorption procedure starting with "Open the system vent" until NMT a 0.2-mL change in buret reading is noted. Discontinue stirring, reintroduce humidified carbon dioxide through the side arm of the flask, remove the top-mounted stopper from the flask briefly, and promptly add 10 g of ▲Sodium Bicarbonate▲ (ERR 1-Jul-2019) to the flask. Replace the stopper, continue the flow of humidified carbon dioxide for about 30 s, and then close the carbon dioxide inlet and the system vent. Stir the solution in the flask vigorously until carbon dioxide absorption ceases, noting the volume absorbed from the buret reading. Restore atmospheric pressure in the apparatus by leveling the *Displacement solution* in the reservoir and the buret, and discontinue stirring. Open the system vent, and flush humidified carbon dioxide through the system. Close the carbon dioxide inlet and the system vent, and stir the solution in the flask vigorously until carbon dioxide absorption ceases.

Determine the total volume, *V*, in mL, of carbon dioxide absorbed after the addition of the specimen to the flask, and calculate the percentage of carbonate in the portion of specimen tested:

$$\text{Result} = 273V \times (6001P)/[22{,}400 \times (273 + T) \times (760W)]$$

V = total volume of carbon dioxide absorbed (mL)
P = ambient atmospheric pressure (mm of mercury)
T = ambient temperature
W = weight of Sodium Bicarbonate taken (g)

[NOTE—Maintain a constant temperature during the measurement of the volume of carbon dioxide absorbed.]

Acceptance criteria: NMT 0.23%

- **NORMAL CARBONATE**

Sample: 1 g

Analysis: Dissolve the *Sample* in 20 mL of water with very gentle swirling at a temperature not exceeding 15°. Add 2.0 mL of 0.10 N hydrochloric acid and 2 drops of phenolphthalein TS.

Acceptance criteria: The solution does not assume more than a faint pink color immediately.

- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*

Sample: 0.35 g

Acceptance criteria: No more chloride than corresponds to 1.5 mL of 0.0010 N hydrochloric acid (NMT 0.015%)

- **LIMIT OF SULFUR COMPOUNDS**

Sample: 2.0 g

Standard solution: To 0.30 mL of 0.02 N sulfuric acid add 1 mL of 0.06 N hydrochloric acid, and dilute with water to 20 mL.

Sample solution: Dissolve the *Sample* in 20 mL of water, and evaporate to 5 mL by boiling. Add 1 mL of bromine TS, evaporate to dryness, and cool. Dissolve the residue in 10 mL of 3 N hydrochloric acid, evaporate to dryness, and cool. Dissolve the residue in 5 mL of 3 N hydrochloric acid, evaporate to dryness, and cool. Dissolve the residue in 10 mL of water, and adjust with 3 N hydrochloric acid or 6 N ammonium hydroxide to a pH of 2. If necessary to obtain a clear solution, filter the solution, washing the filter with two 2-mL portions of water. Dilute with water to 20 mL.

Analysis: Add 1 mL of barium chloride TS to each of the *Standard solution* and the *Sample solution*. Mix, and allow to stand for 30 min.

Acceptance criteria: Any turbidity produced in the *Sample solution* is not more intense than that produced in the *Standard solution* (NMT 0.015%).

- **ALUMINUM** ⟨206⟩ (where it is labeled as intended for use in hemodialysis)

Test preparation: Transfer 1.0 g of Sodium Bicarbonate to a 100-mL plastic volumetric flask. Carefully add 4 mL of nitric acid. Sonicate for 30 min, and dilute with water to volume.

Acceptance criteria: NMT 2 µg/g

- **ARSENIC** ⟨211⟩, *Method I*

Test preparation: Dissolve 1.5 g of Sodium Bicarbonate in 20 mL of 7 N sulfuric acid, and add 35 mL of water.

Analysis: Proceed as directed in the chapter, omitting the addition of 20 mL of 7 N sulfuric acid.

Acceptance criteria: NMT 2 ppm

- **CALCIUM** (where it is labeled as intended for use in hemodialysis)

[NOTE—The *Standard solutions* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

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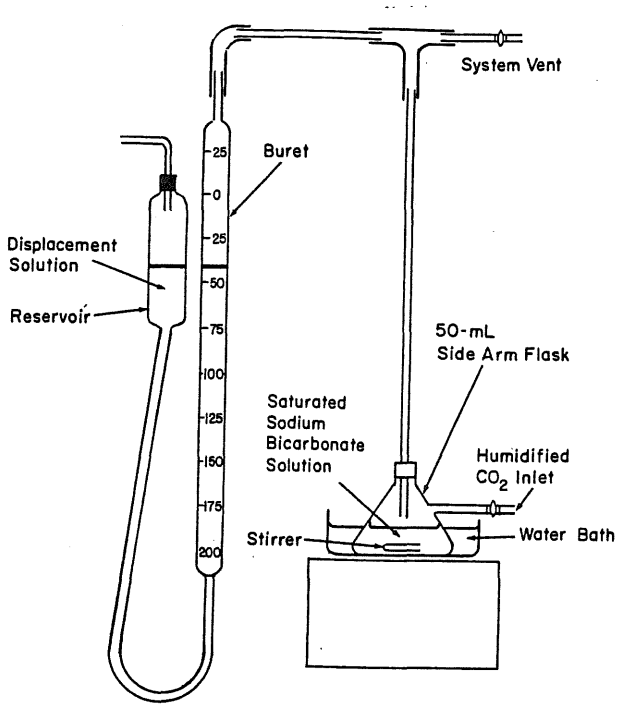


Figure 1. Carbonate apparatus. (Sodium Bicarbonate)

Potassium chloride solution: 10 mg/mL of potassium chloride in 0.36 N hydrochloric acid

Standard solutions: Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in 6 mL of 6 N hydrochloric acid, add 1 g of potassium chloride, and dilute with water to volume. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with *Potassium chloride solution* to volume. This solution contains 100 µg/mL of calcium. Transfer 2.0-, 3.0-, 4.0-, and 5.0-mL portions of this solution to separate 100-mL volumetric flasks (each containing 6 mL of 6 N hydrochloric acid), and dilute with *Potassium chloride solution* to volume. These *Standard solutions* contain 2.0, 3.0, 4.0, and 5.0 µg/mL of calcium, respectively.

Sample solution: Transfer 3.0 g of Sodium Bicarbonate to a 100-mL volumetric flask. Add 6 mL of 6 N hydrochloric acid and 1 g of potassium chloride, and dilute with water to volume.

Blank: *Potassium chloride solution*

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Nitrous oxide–acetylene

Analysis

Samples: *Standard solutions* and *Sample solution*

Plot the absorbances of the *Standard solutions* versus their contents of calcium, in µg/mL, by drawing a straight line best fitting the four plotted points. From the graph so obtained determine the concentration, *C*, in µg/mL, of calcium in the *Sample solution*.

Calculate the percentage of calcium in the portion of Sodium Bicarbonate taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of calcium in the *Sample solution* (µg/mL)

C_U = concentration of Sodium Bicarbonate in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.01%

• **MAGNESIUM** (where it is labeled as intended for use in hemodialysis)

[NOTE—The *Standard solutions* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

Potassium chloride solution, Sample solution, and **Blank:** Proceed as directed in the test for *Calcium*.

Standard solutions: To 1.000 g of magnesium in a 250-mL beaker containing 20 mL of water, carefully add 20 mL of hydrochloric acid, warming if necessary to complete the reaction. Transfer this solution to a 1000-mL volumetric flask containing 10 g of potassium chloride, and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask containing 1 g of potassium chloride, and dilute with water to volume. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with *Potassium chloride solution* to volume. This solution contains 10.0 µg/mL of magnesium. Transfer 2.0-, 3.0-, 4.0-, and 5.0-mL portions of this solution to separate 100-mL volumetric flasks (each containing 6 mL of 6 N hydrochloric acid), and dilute with *Potassium chloride solution* to volume. These *Standard solutions* contain 0.2, 0.3, 0.4, and 0.5 µg/mL of magnesium, respectively.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Magnesium emission line at 285.2 nm

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Lamp: Magnesium hollow-cathode
Flame: Reducing air–acetylene
Analysis
Samples: *Sample solution* and *Standard solutions*
Plot the absorbances of the *Standard solutions* versus their contents of magnesium, in µg/mL, by drawing a straight line best fitting the four plotted points. From the graph so obtained determine the concentration, *C*, in µg/mL, of magnesium in the *Sample solution*.
Calculate the percentage of magnesium in the portion of Sodium Bicarbonate taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium in the *Sample solution* (µg/mL)
C_U = concentration of Sodium Bicarbonate in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.004%

• **COPPER** (where it is labeled as intended for use in hemodialysis)
[NOTE—The *Standard solution* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]
Diluent: Dilute 40 mL of nitric acid with water to 1000 mL.
Standard solution: 10.0 µg/mL of copper, prepared as follows. Transfer 1.000 g of copper to a 1000-mL volumetric flask. Dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. Transfer 10.0 mL of this solution to a second 1000-mL volumetric flask, and dilute with 0.2 N nitric acid to volume. Store in a polyethylene bottle.
Sample solution: Transfer 5.0 g of Sodium Bicarbonate to a 100-mL plastic volumetric flask, and carefully add 4 mL of nitric acid. Sonicate for 30 min, and dilute with water to volume.
Spiked sample solution: To 10.0 mL of the *Sample solution* add 20 µL of the *Standard solution*. This solution contains 0.02 µg/mL of added copper.
Blank: *Diluent*
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Copper emission line at 324.7 nm
Lamp: Copper hollow-cathode
Flame: Flameless electrically heated furnace
Analysis
Samples: *Sample solution* and *Spiked sample solution*
Plot the absorbances of the *Sample solution* and the *Spiked sample solution* versus their contents of added copper, in µg/mL. Draw a line connecting the two points, and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the concentration, *C*, in µg/mL, of copper in the *Sample solution*.
Calculate the amount, in ppm, of copper in the portion of Sodium Bicarbonate taken:

$$\text{Result} = (C/C_U) \times 10^6$$

C = concentration of copper in the *Sample solution* (µg/mL)
C_U = concentration of Sodium Bicarbonate in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1 ppm

• **IRON** ⟨241⟩ (where it is labeled as intended for use in hemodialysis)
Sample solution: Place 2.0 g of Sodium Bicarbonate in a beaker, and neutralize with hydrochloric acid, noting the volume of acid consumed. Transfer this solution to a 25-mL volumetric flask with the aid of water.
Standard solution: Transfer 1.0 mL of *Standard Iron Solution* to a 25-mL volumetric flask. Add the same volume of hydrochloric acid as used to prepare the *Sample solution*.
Blank: Use the same volume of hydrochloric acid in a 25-mL volumetric flask as used to prepare the *Sample solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 480 nm
Analysis
Samples: *Sample solution*, *Standard solution*, and *Blank*
To each of the flasks containing the *Standard solution*, the *Sample solution*, and the *Blank* add 50 mg of ammonium peroxydisulfate crystals and 2 mL of *Ammonium Thiocyanate Solution*. Dilute with water to volume. Concomitantly determine the absorbances of the solutions from the *Standard solution* and the *Sample solution*.
Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 5 ppm).

• **LIMIT OF AMMONIA**
Use water with a resistivity NLT 18 megohm-cm to prepare all of the solutions.
Solution A: 100 mM methanesulfonic acid in water
Solution B: Water
Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	7	93
26	7	93
26.1	70	30
34	70	30

Alternatively, *Mobile phase* can be generated electrolytically using an automatic eluant generator.
System suitability stock solution A: 10 mg/mL of sodium bicarbonate in water
System suitability stock solution B: 3 µg/mL of ammonium chloride (equivalent to 1 µg/mL of ammonium ions) in water. [NOTE—This solution may also be prepared as containing 1 µg/mL of ammonium ions from commercially available NIST traceable ion chromatography ammonium ion standard solution.]
System suitability solution: 1 mg/mL of Sodium Bicarbonate and 0.02 µg/mL of ammonium ions from suitable volumes of *System suitability stock solution A* and *System suitability stock solution B* in water
Standard solution: 0.02 µg/mL of ammonium ions from commercially available NIST traceable ion chromatography ammonium ion standard solution
Sample solution: 1 mg/mL of Sodium Bicarbonate in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with suppression. [NOTE—A 2-mm suppressor is recommended for better performance.]
Columns
Guard: 3-mm × 5-cm; 5-µm packing L84

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Analytical: 3-mm × 25-cm; 5-µm packing L84
Temperatures
Detector: 30°
Column: 40°
Flow rate: 0.43 mL/min
Injection volume: 25 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for sodium and ammonium ions are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 4.0 between sodium and ammonium ions
Tailing factor: NMT 1.5 for ammonium ion
Signal-to-noise ratio: NLT 20 for ammonium ion
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The response due to the ammonium ion in the *Sample solution* is NMT the response due to the ammonium ion in the *Standard solution* (NMT 20 ppm).

• **LIMIT OF ORGANICS** (where it is labeled as intended for use in hemodialysis)
Silver sulfate solution: 11 g/L of silver sulfate in sulfuric acid
Indicator solution: 14.85 mg/mL of 1,10-phenanthroline and 6.95 mg/mL of ferrous sulfate in water
Standard solution: Transfer 850.3 mg of potassium biphthalate, previously crushed lightly and dried at 120° for 2 h, to a 1000-mL volumetric flask. Dilute with water to volume. Transfer 6.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume. It contains the equivalent of 0.06 mg/mL of organic equivalents. Transfer 40.0 mL of this solution to a 500-mL reflux flask.
Sample solution: Transfer 20 g of Sodium Bicarbonate to a 500-mL reflux flask. Add 20 mL of water, and swirl. Cautiously add 20 mL of sulfuric acid, and swirl. [**CAUTION**—Perform this operation under a hood.]
Blank: Add 40 mL of water to a 500-mL reflux flask.
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Concomitantly treat the *Samples* as follows. To each flask add 1 g of mercuric sulfate and about five glass beads. Cool the flask in an ice bath, and add 5 mL of *Silver sulfate solution*. While gently swirling the flask in the ice bath, add 25.0 mL of 0.025 N potassium dichromate VS and, slowly, 70 mL of *Silver sulfate solution*. Fit a cold water condenser on the reflux flask, and reflux for 2 h. Allow the contents of the flask to cool for 10 min, and wash the condenser with 50 mL of water, collecting the washings in the flask. Add water to the flask to obtain a volume of about 350 mL. Add 3 drops of *Indicator solution*, and titrate at room temperature with 0.07 N ferrous ammonium sulfate VS until the solution changes from greenish blue to reddish brown.
To verify the suitability of the system, calculate the amount, in mg, of organic equivalents in the *Standard solution*:

$$\text{Result} = (V_B - V_S) \times 8N$$

V_B = volume of 0.07 N ferrous ammonium sulfate VS consumed by the *Blank* (mL)
V_S = volume of 0.07 N ferrous ammonium sulfate VS consumed by the *Standard solution* (mL)
N = normality of ferrous ammonium sulfate VS

Suitability criteria: 2.328–2.424 mg
Calculate the amount, in mg, of organic equivalents in the portion of Sodium Bicarbonate taken:

$$\text{Result} = (V_B - V_U) \times 8N$$

V_B = volume of 0.07 N ferrous ammonium sulfate VS consumed by the *Blank* (mL)
V_U = volume of 0.07 N ferrous ammonium sulfate VS consumed by the *Sample solution* (mL)
N = normality of ferrous ammonium sulfate VS

Acceptance criteria: NMT 0.01%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Sample: 4 g
Analysis: Dry the *Sample* over silica gel for 4 h.
Acceptance criteria: NMT 0.25%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Where Sodium Bicarbonate is intended for use in hemodialysis, it is so labeled.

Sodium Bicarbonate Injection

» Sodium Bicarbonate Injection is a sterile solution of Sodium Bicarbonate in Water for Injection, the pH of which may be adjusted by means of added Carbon Dioxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $NaHCO_3$.
[NOTE—Do not use the Injection if it contains a precipitate.]

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I glass. Store at controlled room temperature.
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Bicarbonate* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.0 USP Endotoxin Units per mEq.
pH ⟨791⟩: between 7.0 and 8.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Measure accurately a volume of Injection, equivalent to about 3 g of sodium bicarbonate, add methyl red TS, and titrate with 1 N hydrochloric acid VS. Add the acid slowly, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the faint pink color no longer fades after boiling. Each mL of 1 N hydrochloric acid is equivalent to 84.01 mg of $NaHCO_3$.

USP Monographs

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Sodium Bicarbonate Compounded Injection

DEFINITION

Sodium Bicarbonate Compounded Injection contains NLT 95.0% and NMT 105.0% of the labeled amount of sodium bicarbonate ($NaHCO_3$). It contains no bacteriostat or other preservatives.

Prepare Sodium Bicarbonate Compounded Injection 8.4% (1 mEq/mL) as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Sodium Bicarbonate	8.4 g
Sterile Water for Injection, a sufficient quantity to make	100 mL

Dissolve the *Sodium Bicarbonate* in sufficient *Sterile Water for Injection* and bring to final volume. Pass through a sterile filter of 0.22-µm pore size into a sterile container.

ASSAY

- **PROCEDURE FOR SODIUM**

Mobile phase: 8 mM methanesulfonic acid

Standard solution: 0.025 mg/mL of sodium prepared with USP Sodium Chloride RS and water

Sample solution: Transfer 1 mL of Injection into a 100-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Conductivity

Column: 4.6-mm × 25-cm; packing L97

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for sodium is about 3.6 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sodium from the *Sample solution*

r_S = peak response of sodium from the *Standard solution*

C_S = concentration of sodium in the *Standard solution* (mg/mL)

C_U = nominal concentration of sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

Change to read:

- **PROCEDURE FOR SODIUM BICARBONATE**

Sample solution: A volume of Injection, equivalent to about 3 g of sodium bicarbonate

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 1 N hydrochloric acid VS

Endpoint detection: Visual

Analysis: Add methyl red TS to the *Sample solution*. Add *Titrant* slowly, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the faint pink color no longer fades after boiling. Perform a blank determination, and make any necessary correction.

Calculate the percentage of the labeled amount of sodium bicarbonate ($NaHCO_3$) in the portion of Injection taken:

$$\text{Result} = [(V_S - V_B) \times N_A \times {}^{\blacktriangle}F \times 100]/W_{\blacktriangle}$$ (ERR 1-May-2019)

V_S = *Titrant* volume consumed by the *Sample solution* (mL)

V_B = *Titrant* volume consumed by the blank (mL)

N_A = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 84.01 mg/$^{\blacktriangle}$mEq

W = sample weight (mg)$_{\blacktriangle}$ (ERR 1-May-2019)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 7.0–8.5
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 5.0 USP Endotoxin Units/mEq of sodium bicarbonate
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in single-dose glass containers. Store at controlled room temperature.
- **BEYOND-USE DATE:** In the absence of passing a sterility and endotoxin test, the storage conditions for high-risk compounded sterile preparations (CSPs) in *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩ apply. After successful completion of sterility and endotoxin testing, NMT 120 days after the date on which it was compounded when stored at controlled room temperature.
- **LABELING:** Label it to state the *Beyond-Use Date*. The label states the total osmolar concentration in mOsmol/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Chloride RS

Sodium Bicarbonate Oral Powder

» Sodium Bicarbonate Oral Powder contains Sodium Bicarbonate and suitable added substances. It contains not less than 98.5 percent and not more than 100.5 percent of $NaHCO_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label Oral Powder to indicate that it is for oral use only.

Other requirements—It meets the requirements for *Identification* and *Loss on drying* under *Sodium Bicarbonate*.

Assay—Proceed with Oral Powder as directed in the *Assay* under *Sodium Bicarbonate*.

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Sodium Bicarbonate Tablets

» Sodium Bicarbonate Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $NaHCO_3$.

Packaging and storage—Preserve in well-closed containers.
Identification—A solution of Tablets responds to the tests for *Sodium* ⟨191⟩ and for *Bicarbonate* ⟨191⟩.
Disintegration ⟨701⟩: 30 minutes, simulated gastric fluid TS being substituted for water in the test.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 2 g of sodium bicarbonate, dissolve in 100 mL of water, add methyl red TS, and titrate with 1 N hydrochloric acid VS. Add the acid slowly, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the pink color no longer fades after boiling. Each mL of 1 N hydrochloric acid is equivalent to 84.01 mg of $NaHCO_3$.

Sodium Bromide

NaBr 102.89
Sodium bromide.
Sodium bromide [7647-15-6].

» Sodium Bromide contains not less than 98.0 percent and not more than 100.5 percent of NaBr, calculated on the dried basis. It contains no added substances.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.
Appearance of solution: clear and colorless.
Test solution—Dissolve 10.0 g in carbon dioxide-free water, and dilute with the same solvent to 100 mL.

Identification—
A: A solution containing 4.0 mg of sodium bromide responds to the test for *Bromide* ⟨191⟩.
B: It responds to the test for *Sodium* ⟨191⟩.
Acidity or alkalinity—To 10 mL of the solution prepared for the test for *Appearance of solution,* add 0.1 mL of bromothymol blue TS: not more than 0.5 mL of 0.01 N hydrochloric acid or 0.01 N sodium hydroxide is required to change the color of this solution.
Loss on drying ⟨731⟩—Dry it at 100° to 105° for 3 hours: it loses not more than 3.0% of its weight.

Bromates—
Starch–mercuric iodide solution—Triturate 1.0 g of soluble starch with 5 mL of water, and pour the mixture into 100 mL of boiling water containing 10 mg of mercuric iodide.
Procedure—To 10 mL of the solution prepared for the test for *Appearance of solution* add 1 mL of *Starch–mercuric iodide solution,* 0.1 mL of a 100 g per L solution of potassium iodide, and 0.25 mL of 0.5 M sulfuric acid. Allow to stand protected from light for 5 minutes. No blue or violet color develops.
Limit of chlorine: not more than 0.6%.
Nitric acid solution and *Ferric ammonium sulfate solution*—Proceed as directed in the *Assay.*
Procedure—Dissolve 1.000 g Sodium Bromide in 20 mL of *Nitric acid solution* in a conical flask, add and mix 5 mL of 30 percent hydrogen peroxide, and heat in a water bath until the solution is colorless. Rinse the sides of the flask with a small quantity of water, and heat in a water bath for 15 minutes. Allow to cool, dilute with water to 50 mL, and add 5.0 mL of silver nitrate VS and 1 mL of dibutyl phthalate. Mix, and back titrate the excess silver nitrate with ammonium thiocyanate VS (see *Titrimetry* ⟨541⟩), using 5 mL of *Ferric ammonium sulfate solution* as the indicator. Perform a blank titration. Not more than 1.7 mL of silver nitrate VS is used.
Iodides—To 5 mL of the solution prepared for the test for *Appearance of solution* add 0.15 mL of a 10.5 g per 100 mL ferric chloride solution, and 2 mL of dichloromethane. Shake, and allow to separate. The lower layer is colorless.
Sulfates ⟨221⟩—A 2.0-g portion shows no more sulfate than corresponds to 0.2 mL of 0.020 N sulfuric acid (0.01%).
Limit of iron: not more than 20 ppm.
Citric acid solution—Prepare a 200 mg citric acid per mL solution.
Iron standard solution—Transfer 0.863 g of ferric ammonium sulfate to a 500-mL volumetric flask, and dissolve in 25 mL of dilute sulfuric acid. Dilute with water to volume. Transfer 1.0 mL of the resulting solution to a 10-mL volumetric flask, and dilute with water to volume. Transfer 2.5 mL of this resulting solution to a 50-mL volumetric flask, and dilute with water to volume.
[NOTE—Prepare immediately before use.]
Test solution—Transfer 5 mL of the solution prepared for the test for *Appearance of solution* to a 10-mL volumetric flask, and dilute with water to volume.
Procedure—To 10 mL each of the *Iron standard solution* and the *Test solution* add 2.0 mL of the *Citric acid solution* and 0.1 mL of thioglycolic acid. Make alkaline to litmus with ammonia water, and dilute with water to 20 mL. After 5 minutes, any pink color in the *Test solution* is not more intense than that in the *Iron standard solution.*
Magnesium and alkaline-earth metals—To 200 mL of water add 0.1 g of hydroxylamine hydrochloride, 10 mL of pH 10.0 ammonia–ammonium chloride buffer (prepared by dissolving 5.4 g of ammonium chloride in 20 mL of water, adding 20 mL of ammonium hydroxide, and diluting to 100 mL), 1 mL of 0.1 M zinc sulfate, and about 0.2 g of eriochrome black T trituration. Heat to about 40°. Titrate this solution (see *Titrimetry* ⟨541⟩) with 0.01 M edetate disodium VS until the violet color changes to deep blue. To this solution add 10.0 g of Sodium Bromide dissolved in 100 mL of water. If the color changes to violet, titrate the solution with 0.01 M edetate disodium VS to a deep blue endpoint. The volume of 0.01 M edetate disodium consumed in the second titration does not exceed 5.0 mL (0.02%, calculated as Ca).

Assay—
Nitric acid solution—Dilute 14 mL of nitric acid with water to 100 mL.
Ferric ammonium sulfate solution—Transfer 10 g of ferric ammonium sulfate to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.
Procedure—Dissolve 2.000 g of Sodium Bromide in water, and dilute with water to 100.0 mL. To 10.0 mL of the solution add 50 mL of water, 5 mL of *Nitric acid solution,* 25.0 mL of silver nitrate VS, and 2 mL of dibutyl phthalate. Mix, and back titrate the excess silver nitrate with ammonium thiocyanate VS (see *Titrimetry* ⟨541⟩), using 2 mL of *Ferric ammonium sulfate solution* as the indicator, shaking vigorously towards the endpoint. Each mL of 0.1 M silver nitrate is equivalent to 10.29 mg of NaBr. Calculate the percent content of Sodium Bromide, corrected for the chloride content, by the formula:

$$a - 2.902b$$

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in which *a* is the percent content of NaBr and NaCl obtained, calculated as NaBr; and *b* is the percent content of chlorides.

Sodium Bromide Compounded Injection, Veterinary

DEFINITION

Sodium Bromide Compounded Injection, Veterinary contains NLT 27 mg and NMT 33 mg of Sodium Bromide (NaBr) per mL, equivalent to NLT 21.0 mg and NMT 25.6 mg of bromide (Br^-) per mL.

Prepare Sodium Bromide Compounded Injection, Veterinary as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Sodium Bromide	3.0 g
Sterile Water for Injection, *USP*, a sufficient quantity to make	100 mL

Dissolve the *Sodium Bromide* in *Sterile Water for Injection* with mixing. Sterilize by a suitable means such as sterile filtration or autoclaving.

ASSAY

• **PROCEDURE**

TCA solution: 20% (w/v) trichloroacetic acid in water

Gold chloride solution: 5 mg/mL of gold chloride in water

Standard stock solution: Dissolve USP Sodium Bromide RS in water to obtain a solution with a nominal concentration of 20 mg/mL of bromide.

Standard solutions: Prepare four solutions of known concentrations of about 2.0, 1.0, 0.5, and 0.25 mg/mL of bromide from *Standard stock solution* and water.

Sample solution: Dilute Injection, Veterinary quantitatively with water (1:19).

Blank: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Analytical wavelength: 440 nm

System suitability

Samples: *Standard solutions* and *Blank*

Suitability requirements

Correlation coefficient: NLT 0.99, linear regression of the *Standard solutions*

Analysis

Samples: *Sample solution* and *Blank*

To 750-µL aliquots of each *Sample* add 500 µL of *TCA solution* and 250 µL of *Gold chloride solution*. Mix on a vortex mixer, and immediately read the absorbance of each *Sample*.

Calculate the concentration of bromide (Br^-), in mg/mL, in the portion of Injection, Veterinary taken:

$$\text{Result} = C \times D$$

C = concentration of the *Sample solution* (mg/mL) calculated from the standard curve

D = dilution factor of the *Sample solution* (20)

Acceptance criteria: 21.0–25.6 mg/mL of bromide (Br^-)

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.03 USP Endotoxin Units/mg of sodium bromide

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in single-dose containers, preferably of Type I glass, and store in a refrigerator.

Change to read:

• **BEYOND-USE DATE:** ▲In the absence of passing a sterility test and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 180 days after the date on which it was compounded when stored in a refrigerator.▲ (CN 1-May-2020)

• **LABELING:** Label to state the *Beyond-Use Date*. Label to state that it is for infusion only at a rate not to exceed 150 mg of sodium bromide per kg of body weight per hour. Label to state that it is for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Bromide RS

Sodium Bromide Compounded Oral Solution, Veterinary

DEFINITION

Sodium Bromide Compounded Oral Solution, Veterinary contains NLT 194.4 mg and NMT 237.6 mg of sodium bromide (NaBr) per mL equivalent to NLT 151 mg and NMT 185 mg of bromide (Br^-) per mL.

Prepare Sodium Bromide Compounded Oral Solution, Veterinary as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Sodium Bromide	21.6 g
Purified Water	60 mL
Corn Syrup, *FCC*, a sufficient quantity to make	100 mL

Dissolve the *Sodium Bromide* in *Purified Water*. Add *Corn Syrup* to bring the preparation to final volume with mixing.

ASSAY

• **PROCEDURE**

TCA solution: 20% (w/v) trichloroacetic acid in water

Gold chloride solution: 5 mg/mL of gold chloride in water

Standard stock solution: Dissolve USP Sodium Bromide RS in water to obtain a solution with a nominal concentration of 20 mg/mL of bromide.

Standard solutions: Prepare four solutions of known concentrations of about 2.0, 1.0, 0.5, and 0.25 mg/mL of bromide from *Standard stock solution* in water.

Sample solution: Dilute Oral Solution, Veterinary quantitatively with water (1:99).

Blank: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Analytical wavelength: 440 nm

System suitability

Samples: *Standard solutions* and *Blank*

Suitability requirements

Correlation coefficient: NLT 0.99, linear regression of the *Standard solutions*

Analysis

Samples: *Sample solution* and *Blank*

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To 750-µL aliquots of each *Sample* add 500 µL of *TCA solution* and 250 µL of *Gold chloride solution*. Mix on a vortex mixer, and immediately read the absorbance of each *Sample*.
Calculate the concentration of bromide (Br^-), in mg/mL, in the portion of Oral Solution, Veterinary taken:

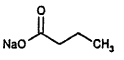
$$\text{Result} = C \times D$$

C = concentration of the *Sample solution* (mg/mL) calculated from the standard curve
D = dilution factor of the *Sample solution* (100)

Acceptance criteria: 151–185 mg/mL of bromide (Br^-)

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in a tight container, and store in a refrigerator.
- **BEYOND-USE DATE:** NMT 180 days after the date on which it was compounded when stored in a refrigerator
- **LABELING:** Label to state the *Beyond-Use Date*. Label to state that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Sodium Bromide RS

Sodium Butyrate



$C_4H_7NaO_2$ 110.09
Butyric acid, sodium salt.
Sodium butyrate [156-54-7].

» Sodium Butyrate contains not less than 98.0 percent and not more than 101.0 percent of $C_4H_7NaO_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
Labeling—Label it to indicate that it is intended for use in compounding dosage forms for rectal use only.
USP Reference standards ⟨11⟩—
USP Sodium Butyrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020), on the undried specimen.
B: A solution (1 in 10) meets the requirements of the tests for *Sodium* ⟨191⟩.
Alkalinity—Dissolve 2.0 g in 20 mL of water, and add 1 drop of phenolphthalein TS: if a pink color is produced, it is discharged by 0.50 mL of 0.10 N sulfuric acid.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%.
Assay—Dissolve about 200 mg of Sodium Butyrate, accurately weighed, in 50 mL of glacial acetic acid. Add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 11.01 mg of $C_4H_7NaO_2$.

Sodium Chloride

Portions of this monograph that are national *USP* text, and are not part of the harmonized text, are marked with symbols (• •) to specify this fact.

NaCl 58.44
Sodium Chloride [7647-14-5].

DEFINITION
Sodium Chloride contains NLT 99.0% and NMT 100.5% of sodium chloride (NaCl), calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*: Meets the requirements
- **B. CHLORIDE**
Sample solution: 3 mg of Sodium Chloride in 2 mL of water
Analysis: Acidify the *Sample solution* with diluted nitric acid, and add 0.4 mL of silver nitrate TS. Shake, and allow to stand. A curdled, white precipitate is formed. Centrifuge, wash the precipitate with three 1-mL portions of water, and discard the washings. Carry out this operation rapidly in subdued light, disregarding the fact that the supernatant may not become perfectly clear. Suspend the precipitate in 2 mL of water, and add 1.5 mL of 10 N ammonium hydroxide.
Acceptance criteria: The precipitate dissolves easily with the possible exception of a few large particles, which dissolve more slowly.

ASSAY
- **PROCEDURE**
Sample: 50 mg of Sodium Chloride
Analysis: Dissolve the *Sample* in 50 mL of water, and titrate with 0.1 N silver nitrate VS (see *Titrimetry* ⟨541⟩), determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).
Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES
- **ALUMINUM** (where it is labeled as intended for use in the manufacture of peritoneal dialysis solutions, hemodialysis solutions, or hemofiltration solutions)
Buffer: Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 250 mL.
Aluminum standard solution: To 352 mg of aluminum potassium sulfate in a 100-mL volumetric flask add a few mL of water, and swirl to dissolve. Add 20 mL of diluted sulfuric acid, dilute with water to volume, and mix. Immediately before use, transfer 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Dissolve 20.0 g of Sodium Chloride in 100 mL of water, and add 10 mL of *Buffer*. Extract this solution with successive portions of 20, 20, and 10 mL of a 0.5% solution of 8-hydroxyquinoline in chloroform, combining the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.
Standard solution: Prepare a mixture of 2.0 mL of *Aluminum standard solution*, 10.0 mL of *Buffer*, and 98 mL of water. Extract this mixture as described for the *Sample solution*, and dilute the combined extracts with chloroform to volume.
Blank solution: Prepare a mixture of 10 mL of *Buffer* and 100 mL of water. Extract this mixture as described for *Sample solution*, and dilute the combined extracts with chloroform to volume.
Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)

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Mode: Fluorescence
Excitation wavelength: 392 nm
Emission wavelength: 518 nm
Analysis
Samples: *Sample solution, Standard solution,* and *Blank solution.* Use the *Blank solution* to set the instrument to zero.
Determine the fluorescence intensities of the *Sample solution* and *Standard solution.*
Acceptance criteria: The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (0.2 ppm).

• ‘ **ARSENIC** ⟨211⟩, *Method I*: NMT 1 ppm.

• **BARIUM**
Analysis: To 5 mL of the solution prepared for the test for *Appearance of Solution* add 2 mL of 2 N sulfuric acid and 5 mL of water, as test solution. To another 5 mL of the solution prepared for the test for *Appearance of Solution* add 7 mL of water, as reference solution. Let stand for 2 h.
Acceptance criteria: Any opalescence in the test solution is not more intense than that in the reference solution.

• **FERROCYANIDES**
Sample solution: Dissolve 2.0 g in 6 mL of water.
Analysis: To the *Sample solution* add 0.5 mL of a mixture of 5 mL of ferric ammonium sulfate solution (10 mg/mL in 2.5 g/L (0.05 N) of sulfuric acid) and 95 mL of ferrous sulfate solution (10 mg/mL in water).
Acceptance criteria: No blue color develops in 10 min.

• **IODIDES**
Sample: 5 g of Sodium Chloride
Analysis: Moisten the *Sample* by the dropwise addition of a freshly prepared mixture of 0.15 mL of sodium nitrite solution (100 mg/mL), 2 mL of 1 N sulfuric acid, 25 mL of iodide-free starch TS, and 25 mL of water. After 5 min, examine the substance in natural light.
Acceptance criteria: No blue color is observed.

• **IRON**
Sample solution: Use a 10-mL portion of the solution prepared for the test for *Appearance of Solution.*
Standard solution: Immediately before use, dilute *Standard Iron Solution* (see *Iron* ⟨241⟩) 1 to 10 with water. This solution contains the equivalent of 1 µg/mL of iron. Combine 4 mL of this solution and 6 mL of water.
Analysis: To each of the solutions add 2 mL of a 200-mg/mL solution of citric acid and 0.1 mL of thioglycolic acid. Mix, make alkaline with stronger ammonia water, and dilute with water to 20 mL.
Acceptance criteria: After 5 min, any pink color in the *Sample solution* is not more intense than that from the *Standard solution* (NMT 2 ppm).

• **LIMIT OF BROMIDES**
[NOTE—Prepare the *Sample solution* and the *Standard solution* concomitantly.]
Standard solution: To 5.0 mL of a solution containing 3 µg/mL of potassium bromide add 2.0 mL of pH 4.7 phenol red TS and 1.0 mL of chloramine T solution (0.1 mg/mL), and mix immediately. After 2 min, add 0.15 mL of 0.1 N sodium thiosulfate, mix, and dilute with water to 10.0 mL.
Sample solution: To 0.5 mL of the solution prepared for the test for *Appearance of Solution* add 4.0 mL of water, 2.0 mL of pH 4.7 phenol red TS, and 1.0 mL of chloramine T solution (0.1 mg/mL), and mix immediately. After 2 min, add 0.15 mL of 0.1 N sodium thiosulfate, mix, and dilute with water to 10.0 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 590 nm
Comparison liquid: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The absorbance of the *Sample solution* is not greater than that of the *Standard solution* (NMT 100 ppm).

• **LIMIT OF PHOSPHATES**
Sulfomolybdic acid solution: Dissolve with heating 2.5 g of ammonium molybdate in 20 mL of water. Dilute 28 mL of sulfuric acid in 50 mL of water, then cool. Mix the two solutions, and dilute with water to 100 mL.
Phosphate standard stock solution: 0.716 mg/mL of monobasic potassium phosphate in water
Phosphate standard solution: 7.16 µg/mL from *Phosphate standard stock solution* in water. [NOTE—Prepare this solution fresh.]
Standard solution: Dilute 2 mL of the *Phosphate standard solution* with water to 100 mL.
Sample solution: Dilute 2 mL of the solution prepared in the test for *Appearance of Solution* with water to 100 mL.
Analysis: To the *Standard solution* and the *Sample solution* add 4 mL of *Sulfomolybdic acid solution,* and add 0.1 mL of a mixture of 1 mL of stronger acid stannous chloride TS and 10 mL of 2 N hydrochloric acid. After 10 min, compare the colors of 20 mL of each solution.
Acceptance criteria: Any color in the *Sample solution* is not more intense than that in the *Standard solution* (NMT 25 ppm).

• **LIMIT OF POTASSIUM** (where it is labeled as intended for use in the manufacture of injectable dosage forms, peritoneal dialysis solutions, hemodialysis solutions, or hemofiltration solutions)
[NOTE—The *Standard solution* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]
Standard solutions: Dissolve 1.144 g of potassium chloride, previously dried at 105° for 3 h, in water. Dilute with water to 1000 mL, and mix. This solution contains the equivalent of 600 µg/mL of potassium. Dilute as required to obtain NLT 3 solutions at concentrations that span the expected value in the *Sample solution.*
Sample solution: Transfer 1.00 g of Sodium Chloride to a 100-mL volumetric flask. Add water and swirl to dissolve, dilute with water to volume, and mix.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic emission spectroscopy
Analytical wavelength: 766.5 nm
Flame: Air–acetylene
Analysis
Samples: *Standard solutions* and *Sample solution*
Measure, at least three times, the emission intensity of the *Sample solution* and the *Standard solutions.* Prepare a calibration curve from the mean of the readings obtained with the *Standard solutions,* and determine the concentration of potassium in the *Sample solution.*
Acceptance criteria: NMT 500 ppm

• **MAGNESIUM AND ALKALINE-EARTH METALS**
Buffer: Prepare pH 10.0 ammonia–ammonium chloride buffer by dissolving 5.4 g of ammonium chloride in 20 mL of water, adding 20 mL of ammonium hydroxide, and diluting with water to 100 mL.
Analysis: To 200 mL of water add 0.1 g of hydroxylamine hydrochloride, 10 mL of *Buffer,* 1 mL of 0.1 M zinc sulfate, and 0.15 g of eriochrome black T trituration. Heat to 40°. Titrate this solution with 0.01 M edetate disodium VS until the violet color changes to deep blue. To this solution add 10.0 g of Sodium Chloride dissolved in 100 mL of water. If

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the color changes to violet, titrate the solution with 0.01 M edetate disodium VS to a deep blue endpoint.
Acceptance criteria: The volume of 0.01 M edetate disodium consumed in the second titration does not exceed 2.5 mL (NMT 100 ppm, calculated as Ca).

• **NITRITES**
Sample solution: To 10 mL of the solution prepared in the test for *Appearance of Solution* add 10 mL of water.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 354 nm
Cell: 1 cm
Analysis
Sample: *Sample solution*
Acceptance criteria: Absorbance is NMT 0.01.

• **SULFATE**
[NOTE—All solutions used for this test must be prepared with distilled water.]
Sulfate standard solution A: To 181 mg of potassium sulfate in a 100-mL volumetric flask add a few mL of 30% alcohol. Swirl to dissolve, dilute with 30% alcohol to volume, and mix. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, and dilute with 30% alcohol to volume (10 µg/mL of sulfate).
Sulfate standard solution B: To 181 mg of potassium sulfate in a 100-mL volumetric flask add a few mL of water. Swirl to dissolve, dilute with water to volume, and mix. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume (10 µg/mL of sulfate).
Sodium chloride solution: 50 mg/mL of Sodium Chloride in water
Barium chloride solution: 250 mg/mL of barium chloride in water
Standard solution: To 1.5 mL of *Sulfate standard solution A* add 1 mL of *Barium chloride solution*. Shake, and allow to stand for 1 min. To 2.5 mL of the resulting suspension add 15 mL of *Sulfate standard solution B* and 0.5 mL of 5 N acetic acid, and mix.
Sample solution: To 1.5 mL of *Sulfate standard solution A* add 1 mL of *Barium chloride solution*. Shake, and allow to stand for 1 min. To 2.5 mL of the resulting suspension add 15 mL of *Sodium chloride solution* and 0.5 mL of 5 N acetic acid, and mix.
Acceptance criteria: Any opalescence produced in the *Sample solution* after 5 min standing is NMT that produced in the *Standard solution* (200 ppm).

SPECIFIC TESTS

• **‘APPEARANCE OF SOLUTION:** Dissolve 20.0 g of Sodium Chloride in carbon dioxide-free water, and dilute with the same solvent to 100.0 mL. This solution is clear and colorless.◆

• **ACIDITY OR ALKALINITY**
Analysis: To 20 mL of the solution prepared for the test for *Appearance of Solution* add 0.1 mL of bromothymol blue TS.
Acceptance criteria: NMT 0.5 mL of 0.01 N hydrochloric acid or 0.01 N sodium hydroxide is required to change the color of this solution.

• **LOSS ON DRYING** ⟨731⟩
Sample: 1.000 g
Analysis: Dry the *Sample* in an oven at 105° for 2 h.
Acceptance criteria: NMT 0.5%

• ‘ **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: The level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Sodium Chloride is used can be met. Where the label states that Sodium Chloride must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Sodium Chloride is used can be met.◆

• ‘ **STERILITY TESTS** ⟨71⟩: Where the label states that Sodium Chloride is sterile, it meets the requirements for *Sterility* under the relevant dosage form monograph(s) in which Sodium Chloride is used.◆

ADDITIONAL REQUIREMENTS

• ‘**PACKAGING AND STORAGE:** Preserve in well-closed containers.◆

• ‘**LABELING:** Where Sodium Chloride is intended for use in the manufacture of injectable dosage forms, peritoneal dialysis solutions, hemodialysis solutions, or hemofiltration solutions, it is so labeled. Where Sodium Chloride must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled. Where Sodium Chloride is sterile, it is so labeled.◆

Sodium Chloride Injection

DEFINITION

Sodium Chloride Injection is a sterile solution of Sodium Chloride in Water for Injection. It contains no antimicrobial agents. It contains NLT 95.0% and NMT 105.0% of the labeled amount of sodium chloride (NaCl).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩ and *Chloride* ⟨191⟩: Meets the requirements

ASSAY

• **PROCEDURE**
Sample solution: Transfer a volume of Injection, equivalent to 90 mg of sodium chloride, into a conical flask. Add water, if necessary, to bring the volume to 10 mL, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.
Analysis: Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).
Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **IRON** ⟨241⟩
Test preparation: Dilute 5.0 mL of Injection with water to 45 mL, and add 2 mL of hydrochloric acid.
Acceptance criteria: NMT 2 ppm

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.5–7.0

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Unit/mL where the labeled amount of sodium chloride in the Injection is between 0.5% and 0.9%, and NMT 3.6 USP Endotoxin Units/mL where the labeled amount of sodium chloride in the Injection is between 3.0% and 24.3%

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct

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injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Bacteriostatic Sodium Chloride Injection

DEFINITION

Bacteriostatic Sodium Chloride Injection is a sterile, isotonic solution of Sodium Chloride in Water for Injection, and it contains one or more suitable antimicrobial agents. It contains NLT 0.85% and NMT 0.95% of NaCl.

[NOTE—Use Bacteriostatic Sodium Chloride Injection with due regard for the compatibility of the antimicrobial agent or agents it contains with the particular medicinal substance that is to be dissolved or diluted.]

IDENTIFICATION

- **IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩ and *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Sample solution: Equivalent to 90 mg of sodium chloride from the volume of Injection. Add water, if necessary, to bring the volume to 10 mL, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Analysis: Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

Acceptance criteria: 0.85%–0.95%

IMPURITIES

INORGANIC IMPURITIES

- **Iron** ⟨241⟩

Test preparation: Dilute 5.0 mL of Injection with water to 45 mL, and add 2 mL of hydrochloric acid.

Acceptance criteria: NMT 2 ppm

SPECIFIC TESTS

- **ANTIMICROBIAL AGENT(S):** It meets the requirements under *Antimicrobial Effectiveness Testing* ⟨51⟩, and meets the labeled claim for content of the antimicrobial agent(s) as determined by the method set forth under *Antimicrobial Agents—Content* ⟨341⟩, except use the following procedure when methylparaben and propylparaben are used as the antimicrobial agents.

Mobile phase: Methanol and water (7:3)

Standard stock solution: 1.2 mg/mL of USP Methylparaben RS and 0.12 mg/mL of USP Propylparaben RS in methanol

Standard solution: Pipet 5 mL of the *Standard stock solution* into a 50-mL volumetric flask, add by pipet 30 mL of methanol, and dilute with water to volume.

Sample solution: Pipet 1 mL of Injection into a 10-mL volumetric flask, add by pipet 7.0 mL of methanol, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection size: 12 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: 0.52 for methylparaben and 1.05 for propylparaben

Resolution: NLT 2.0 between the methylparaben and propylparaben peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, in mg/mL, of methylparaben or propylparaben in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak height from the *Sample solution*

r_S = peak height from the *Standard solution*

C_S = concentration of USP Methylparaben RS or USP Propylparaben RS in the *Standard solution* (mg/mL)

- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **pH** ⟨791⟩**:** 4.5–7.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 1.0 USP Endotoxin Unit/mL
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, of not larger than 30-mL size, preferably of Type I or Type II glass.
- **LABELING:** Label it to indicate the name(s) and proportion(s) of the added antimicrobial agent(s). Label it also to include the statement: NOT FOR USE IN NEWBORNS, in boldface capital letters, on the label immediately under the official name, printed in a contrasting color, preferably red. Alternatively, the statement may be placed prominently elsewhere on the label if the statement is enclosed within a box. Label it also to include the statement: NOT FOR INHALATION.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Methylparaben RS

USP Propylparaben RS

Sodium Chloride Irrigation

» Sodium Chloride Irrigation is Sodium Chloride Injection that has been suitably packaged, and it contains no antimicrobial agents. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of NaCl.

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass. The container may be designed to empty rapidly and may contain a volume of more than 1 L.

Labeling—The designation "not for injection" appears prominently on the label.

Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Chloride* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.5 USP Endotoxin Unit per mL.

Sterility Tests ⟨71⟩: meets the requirements.

Other requirements—It meets the requirements for *pH*, *Iron*, and *Assay* under *Sodium Chloride Injection*.

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Sodium Chloride Ophthalmic Ointment

DEFINITION
Sodium Chloride Ophthalmic Ointment is Sodium Chloride in a suitable ophthalmic ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of sodium chloride (NaCl). It is sterile.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*
Sample solution: Transfer a quantity of Ophthalmic Ointment, equivalent to about 200 mg of sodium chloride, to a separator containing 25 mL of ether, and extract with 5 mL of water. Use the aqueous extract.
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
Sample solution: Use the *Sample solution* from *Identification A.*
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Sample solution: Transfer a quantity of Ophthalmic Ointment, equivalent to about 100 mg of sodium chloride, to a separator containing 50 mL of ether, and extract with four 20-mL portions of water. Combine the aqueous extracts in a conical flask, evaporate to a volume of about 10 mL, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis: Titrate, with shaking, with *Titrant* to a pink endpoint.
Calculate the percentage of the labeled amount of sodium chloride in the portion of Ophthalmic Ointment taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the sample (mL)
N = *Titrant* normality (mEq/mL)
F = equivalent weight of sodium chloride, 58.44 mg/mEq
W = nominal amount of sodium chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
• **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter*and*Container Contents.*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature. Store away from heat. Protect from freezing.

Sodium Chloride Inhalation Solution

» Sodium Chloride Inhalation Solution is a sterile solution of Sodium Chloride in water purified by distillation or by reverse osmosis and rendered sterile. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of NaCl. It contains no antimicrobial agents or other added substances.

Packaging and storage—Preserve in single-dose containers.
Identification—It responds to the test for *Sodium* ⟨191⟩ and for *Chloride* ⟨191⟩.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 4.5 and 7.0.
Assay—Pipet a volume of Inhalation Solution, equivalent to about 90 mg of sodium chloride, into a conical flask, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

Sodium Chloride Ophthalmic Solution

» Sodium Chloride Ophthalmic Solution is a sterile solution of Sodium Chloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of sodium chloride. It may contain suitable antimicrobial and stabilizing agents. It contains a buffer.

Packaging and storage—Preserve in tight containers.
Identification—Heat a portion of Ophthalmic Solution to boiling, and filter while hot. After cooling, the filtrate responds to the tests for *Sodium* ⟨191⟩ and for *Chloride* ⟨191⟩.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 6.0 and 8.0.
Assay—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 90 mg of sodium chloride, to a conical flask, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

Sodium Chloride Tablets

» Sodium Chloride Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of NaCl.

Packaging and storage—Preserve in well-closed containers.
Identification—A filtered extract of Tablets responds to the tests for *Sodium* ⟨191⟩ and for *Chloride* ⟨191⟩.
Disintegration ⟨701⟩: 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Iodide or bromide—Digest 2.0 g of powdered Tablets with 25 mL of warm alcohol for 3 hours, cool, and filter. Evaporate the filtrate to dryness, dissolve the residue in 5 mL of water, filter if necessary, and add 1 mL of chloroform. Cautiously introduce, dropwise, with constant agitation, 5 drops of dilute chlorine TS (1 in 3): the chloroform does not acquire a violet, yellow, or orange color.

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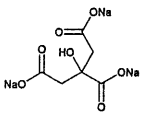
Barium—Digest 4.0 g of powdered Tablets with 20 mL of water, filter, and divide the solution into two equal portions. To one portion add 2 mL of 2 N sulfuric acid and to the other add 2 mL of water: the solutions are equally clear after standing for 2 hours.
Calcium and magnesium—Digest 1 g of powdered Tablets with 50 mL of water, and filter. Add 4 mL of 6 N ammonium hydroxide to the filtrate, and divide the mixture into two equal portions. Treat one portion with 1 mL of ammonium oxalate TS and the other portion with 1 mL of dibasic sodium phosphate TS: neither mixture becomes turbid within 5 minutes.
Assay—Dissolve a counted number of not less than 20 Tablets in about 100 mL of water, filter into a 500-mL volumetric flask, and wash the original container and the filter with 100 mL of water in divided portions, adding the washings to the original filtrate. Dilute with water to volume. Pipet a volume of the solution, equivalent to about 90 mg of sodium chloride, to a conical flask, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

Sodium Chloride Tablets for Solution

» Sodium Chloride Tablets for Solution are composed of Sodium Chloride in compressed form, containing no added substance. Sodium Chloride Tablets for Solution contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of NaCl.

Other requirements—The Sodium Chloride Tablets for Solution respond to the *Identification* test and meet the requirements for *Packaging and storage, Iodide or bromide, Barium, Calcium and magnesium, Disintegration, Uniformity of dosage units*, and *Assay* under *Sodium Chloride Tablets*.

Sodium Citrate



$C_6H_5Na_3O_7$ 258.07
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, trisodium salt; Trisodium citrate (anhydrous) [68-04-2].

$C_6H_5Na_3O_7 \cdot 2H_2O$ 294.10
Trisodium citrate dihydrate [6132-04-3].

DEFINITION
Sodium Citrate is anhydrous or contains two molecules of water of hydration. It contains NLT 99.0% and NMT 100.5% of $C_6H_5Na_3O_7$, calculated on the anhydrous basis.

IDENTIFICATION
• **A. Identification Tests—General,** *Sodium* ⟨191⟩
Sample solution: 50 mg/mL
Acceptance criteria: Meets the requirements
• **B. Identification Tests—General,** *Citrate* ⟨191⟩
Sample solution: 50 mg/mL
Acceptance criteria: Meets the requirements
• **C.** Upon ignition, it yields an alkaline residue that effervesces when treated with 3 N hydrochloric acid.

ASSAY
• **Procedure**
Sample: Add 100 mL of glacial acetic acid to 350 mg of Sodium Citrate (previously dried at 180° for 18 h) in a 250-mL beaker. Stir until completely dissolved.
Analysis: Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 8.602 mg of $C_6H_5Na_3O_7$.
Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES
• **Tartrate**
Analysis: To a solution of 1 g in 2 mL of water in a test tube add 1 mL of potassium acetate TS and 1 mL of 6 N acetic acid. Rub the wall of the tube with a glass rod.
Acceptance criteria: No crystalline precipitate is formed.

SPECIFIC TESTS
• **Alkalinity**
Sample solution: 1.0 g in 20 mL of water
Acceptance criteria: The *Sample solution* is alkaline to litmus paper, but after the addition of 0.20 mL of 0.10 N sulfuric acid, no pink color is produced by 1 drop of phenolphthalein TS.
• **Water Determination,** *Method III* ⟨921⟩**:** Dry a sample at 180° for 18 h: the anhydrous form loses NMT 1.0% of its weight; the hydrous form loses 10.0%–13.0% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **Labeling:** Label it to indicate whether it is anhydrous or hydrous.

Sodium Citrate and Citric Acid Oral Solution

DEFINITION
Sodium Citrate and Citric Acid Oral Solution is a solution of Sodium Citrate and Citric Acid in a suitable aqueous medium. It contains, in each 100 mL, NLT 2.23 g and NMT 2.46 g of sodium (Na), and NLT 6.11 g and NMT 6.75 g of citrate ($C_6H_5O_7$), equivalent to NLT 9.5 g and NMT 10.5 g of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), and NLT 6.34 g and NMT 7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$).

IDENTIFICATION
• **A.** Sodium Citrate and Citric Acid Oral Solution imparts an intense yellow color to a nonluminous flame.
• **B.**
Sample solution: 2 mL of Oral Solution
Analysis: Add 2 mL of 15% potassium carbonate TS to the *Sample solution*, boil, and cool. Add 4 mL of potassium pyroantimonate TS.
Acceptance criteria: A dense precipitate is formed (indicates the presence of sodium).
• **C.**
Sample solution: A dilution of Oral Solution (1 in 20)
Analysis: Add 5 mL of sodium cobaltinitrite TS to 2 mL of *Sample solution*.
Acceptance criteria: A yellow precipitate is not formed immediately (indicates the absence of potassium).

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• **D. Identification Tests—General** ⟨191⟩, *Chemical Identification Tests, Citrate*
Sample solution: 3–5 drops of Oral Solution
Analysis: Add 20 mL of a mixture of pyridine and acetic anhydride (3:1) to the *Sample solution.*
Acceptance criteria: A light red color is produced.

ASSAY

• **Sodium**
Sodium stock solution: 58.44 mg/mL of sodium chloride prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.
Diluent: 1.04 mg/mL of lithium nitrate in water (equivalent to 15 mEq per 1000 mL of lithium) with a suitable nonionic surfactant
Standard stock solution: Pipet 50 mL of the *Sodium stock solution* into a 500-mL volumetric flask and dilute with water to volume.
Standard solution: Dilute 50 µL of the *Standard stock solution* with *Diluent* to 10 mL.
Sample stock solution: Transfer a volume of Oral Solution, equivalent to 1 g of sodium citrate dihydrate, to a 100-mL volumetric flask and dilute with water to volume.
Sample solution: Dilute 50 µL of the *Sample stock solution* with *Diluent* to 10 mL.
Blank: *Diluent*
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: Sodium emission line at 589 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Adjust the flame photometer to read zero with the *Diluent.* Concomitantly determine the sodium flame emission readings for the *Standard solution* and the *Sample solution.*
Calculate the quantity (g) of sodium (Na) in 100 mL of Oral Solution taken:

$$\text{Result} = (R_{U,\,Na}/R_{S,\,Na}) \times (W/V) \times F \times (A_r/M_r) \times F_1$$

$R_{U,\,Na}$ = sodium emission reading of the *Sample solution*
$R_{S,\,Na}$ = sodium emission reading of the *Standard solution*
W = weight of sodium chloride in the *Sodium stock solution* (g)
V = volume of Oral Solution taken (mL)
F = ratio of the dilution factor of the *Sample solution* to the *Standard solution*, 0.04
A_r = atomic weight of sodium, 22.99
M_r = molecular weight of sodium chloride, 58.44
F_1 = conversion factor for each 100 mL of Oral Solution, 100 mL

Acceptance criteria: 2.23–2.46 g of sodium (Na) in each 100 mL of Oral Solution

• **Sodium Citrate**
Cation-exchange column: Mix 10 g of styrene–divinylbenzene cation-exchange resin with 50 mL of water in a suitable beaker. Allow the resin to settle and decant the supernatant until a slurry of resin remains. Pour the slurry into a 15-mm × 30-cm glass chromatographic tube (having a sealed-in, coarse-porosity fritted disk and fitted with a stopcock), and allow to settle as a homogeneous bed. Wash the resin bed with about 100 mL of water, closing the stopcock when the water level is about 2 mm above the resin bed.
Sample solution: Transfer a volume of Oral Solution, equivalent to 1 g of sodium citrate dihydrate, to a 100-mL volumetric flask and dilute with water to volume.
Titrimetric system
Mode: Direct titration
Titrant: 0.02 N sodium hydroxide VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Pipet 5 mL of the *Sample solution* carefully onto the top of the resin bed in the *Cation-exchange column.* Place a 250-mL conical flask below the column, open the stopcock, and allow to flow until the solution has entered the resin bed. Elute the column with 60 mL of water at a flow rate of about 5 mL/min, collecting about 65 mL of the eluate. Add 5 drops of phenolphthalein TS to the eluate, swirl the flask, and titrate with *Titrant.* Record the buret reading and calculate the volume of *Titrant* consumed.
Calculate the quantity (g) of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in 100 mL of the Oral Solution taken:

$$\text{Result} = \{[V \times N \times F \times (D/V_S)] - [(M_{r1}/M_{r2}) \times C]\} \times F_1 \times F_2$$

V = volume of *Titrant* consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 98.05 mg/mEq for sodium citrate dihydrate
D = dilution factor of the *Sample solution*, 20
V_S = volume of Oral Solution taken (mL)
M_{r1} = molecular weight of sodium citrate dihydrate, 294.10
M_{r2} = molecular weight of citric acid monohydrate, 210.14
C = concentration of citric acid monohydrate in the *Sample solution*, as obtained in the *Assay* for *Citric Acid* (mg/mL)
F_1 = conversion factor from mg to g, 0.001
F_2 = conversion factor for each 100 mL of Oral Solution, 100 mL

Acceptance criteria: 9.5–10.5 g of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in each 100 mL of Oral Solution

• **Citric Acid**
Sample solution: Transfer a volume of Oral Solution, equivalent to 0.67 g of citric acid monohydrate, to a 100-mL volumetric flask and dilute with water to volume.
Titrimetric system
Mode: Direct titration
Titrant: 0.02 N sodium hydroxide VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Transfer 5 mL of the *Sample solution* to a suitable flask. Add 25 mL of water and 5 drops of phenolphthalein TS. Titrate with *Titrant* to a pink endpoint. Record the buret reading and calculate the volume of *Titrant* consumed.
Calculate the quantity (g) of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in 100 mL of Oral Solution taken:

$$\text{Result} = V \times N \times F \times (D/V_S) \times F_1 \times F_2$$

V = volume of *Titrant* consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 70.05 mg/mEq for citric acid monohydrate
D = dilution factor of the *Sample solution*, 20
V_S = volume of Oral Solution taken (mL)
F_1 = conversion factor from mg to g, 0.001

USP Monographs

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F_2 = conversion factor for each 100 mL of Oral Solution, 100 mL

Acceptance criteria: 6.34–7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in each 100 mL of Oral Solution

PERFORMANCE TESTS
- **DELIVERABLE VOLUME** ⟨698⟩
  For multiple-unit containers
  Acceptance criteria: Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  For single-unit containers
  Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 4.0–4.4

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

Sodium Fluoride

NaF 41.99
Sodium fluoride [7681-49-4].

DEFINITION
Sodium Fluoride contains NLT 98.0% and NMT 102.0% of NaF, calculated on the dried basis.

IDENTIFICATION
- **A. FLUORIDE**
  Sample: 1 g
  Analysis: Place the *Sample* in a platinum crucible in a well-ventilated hood, add 15 mL of sulfuric acid, and cover the crucible with a piece of clear, polished glass. Heat the crucible on a steam bath for 1 h, remove the glass cover, rinse it in water, and wipe dry.
  Acceptance criteria: The surface of the glass is etched.
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩
  Sample solution: 1 in 25
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Sample solution: To 80.0 mg add a mixture of 5 mL of acetic anhydride and 20 mL of glacial acetic acid, and heat to dissolve. Allow to cool. [NOTE—The heating step to dissolve the sodium fluoride in the acetic acid mixture is critical. It is recommended to heat for a minimum of 30 min and use sonication, if necessary, to make sure dissolution is complete prior to the titration step.]
  Analysis: Add 20 mL of dioxane to the *Sample solution*. Add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 4.199 mg of NaF.
  Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **CHLORIDE**
  Sample solution: 300 mg in 20 mL of water
  Analysis: To the *Sample solution* add 200 mg of boric acid, 1 mL of nitric acid, and 1 mL of 0.1 N silver nitrate.
  Acceptance criteria: Any turbidity produced is NMT that of a blank to which has been added 1.0 mL of 0.0010 N hydrochloric acid (NMT 0.012%).

SPECIFIC TESTS
- **ACIDITY OR ALKALINITY**
  Sample: 2.0 g
  Analysis: Dissolve the *Sample* in 40 mL of water in a platinum dish. Add 10 mL of a saturated solution of potassium nitrate, cool the solution to 0°, and add 3 drops of phenolphthalein TS.
  Acceptance criteria: If no color appears, a pink color persisting for 15 s is produced by NMT 2.0 mL of 0.10 N sodium hydroxide. If the solution is colored pink by the addition of phenolphthalein TS, it is rendered colorless by NMT 0.50 mL of 0.10 N sulfuric acid. [NOTE—Save the neutralized solution for the test for *Fluosilicate*.]
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 150° for 4 h: it loses NMT 1.0% of its weight.
- **FLUOSILICATE**
  Analysis: After the solution from the test for *Acidity or Alkalinity* has been neutralized, heat to boiling, and titrate while hot with 0.10 N sodium hydroxide until a permanent pink color is obtained.
  Acceptance criteria: NMT 1.5 mL of 0.10 N sodium hydroxide is required.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Sodium Fluoride Gel

DEFINITION
Sodium Fluoride Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of sodium fluoride (NaF), in an aqueous medium containing a suitable viscosity-inducing agent. It may contain a suitable buffering agent.
[NOTE—This monograph is only applicable to *Preventive treatment gels* and is not applicable to *Dentifrices* as defined under 21 CFR §355.3.]

IDENTIFICATION

Change to read:

- **▲A.▲** (USP 1-May-2020)
  Sample: A suitable quantity of Gel, equivalent to 500 mg of fluoride ion
  Analysis: Place the *Sample* in a platinum crucible in a well-ventilated hood, add 15 mL of sulfuric acid, and cover the crucible with a piece of clear, polished glass. Heat the crucible on a steam bath for 1 h, remove the glass cover, rinse it in water, and dry.
  Acceptance criteria: The surface of the glass is etched.

Add the following:

▲• B. The retention time of the fluoride peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

- **PROCEDURE**
  ▲[NOTE—Store all solutions in plastic containers. It is recommended to use plastic HPLC vials. Use water with a resistivity of NLT 18 megohm-cm to prepare the solutions.]

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Mobile phase: 15 mM potassium hydroxide in water.
[NOTE—*Mobile phase* can be generated electrolytically using an automatic eluant generator.]
System suitability solution: 2.0 µg/mL of USP Sodium Fluoride RS and 1.0 µg/mL of USP Sodium Acetate RS in water
Standard solution: 2.0 µg/mL of USP Sodium Fluoride RS in water
Sample solution: Nominally 2.0 µg/mL of sodium fluoride from a portion of Gel in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with suppression
Columns
Guard: 4.0-mm × 0.5-cm; 4.6-µm packing L91
Analytical: 4.0-mm × 25-cm; 4.6-µm packing L91
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of fluoride
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for the fluoride and acetate ions are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the fluoride and acetate ions, *System suitability solution*
Tailing factor: NMT 2.0 for the fluoride ion, *Standard solution*
Relative standard deviation: NMT 2.0% for the fluoride ion, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium fluoride (NaF) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the fluoride ion from the *Sample solution*
r_S = peak response of the fluoride ion from the *Standard solution*
C_S = concentration of USP Sodium Fluoride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of sodium fluoride in the *Sample solution* (µg/mL) ▲(USP 1-May-2020)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **PH** ⟨791⟩
Sample: About 40 mL
Analysis: Place the *Sample* in a plastic beaker, and determine the pH using a suitable electrode system.
Acceptance criteria: 6.0–8.0

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight, plastic containers. ▲Store at controlled room temperature.▲ (USP 1-May-2020)
• **LABELING:** Label the Gel in terms of the content of sodium fluoride (NaF) and in terms of the content of fluoride ion.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Sodium Acetate RS▲ (USP 1-May-2020)
USP Sodium Fluoride RS

Sodium Fluoride Oral Solution

DEFINITION
Sodium Fluoride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of sodium fluoride (NaF).

IDENTIFICATION
• **A.** The retention time of the fluoride peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*
Sample solution: 10 mg/mL of sodium in Oral Solution. If necessary, reduce the volume of a portion of Oral Solution by heating on a steam bath.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
[NOTE—Use water with a resistivity of NLT 18 megohm-cm to prepare the solutions.]
Mobile phase: 150 mg/L of anhydrous sodium carbonate and 1.0 mL/L of 1 N sodium hydroxide in water. Pass through a suitable filter of 0.45-µm pore size.
System suitability solution: 1.0 µg/mL of USP Sodium Fluoride RS and 0.5 µg/mL of USP Sodium Chloride RS in water
Standard solution: 1.1 µg/mL of USP Sodium Fluoride RS in water
Sample solution: Nominally 1.1 µg/mL of sodium fluoride from a portion of Oral Solution in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with suppression
Columns
Guard: 4.0-mm × 5-cm; 10-µm packing L46
Analytical: 4.0-mm × 25-cm; 10-µm packing L46
Flow rate: 1.0 mL/min
Injection volume: 20 µL
[NOTE—It is recommended to use polymethylpentene HPLC vials.]
Run time: NLT 2 times the retention time of the fluoride peak
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for fluoride and chloride ions are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.5 between fluoride and chloride ions
Tailing factor: NMT 2.0 for fluoride ion
Relative standard deviation: NMT 2.0% for fluoride ion
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium fluoride (NaF) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluoride from the *Sample solution*

USP Monographs

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r_S = peak response of fluoride from the *Standard solution*
C_S = concentration of USP Sodium Fluoride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of sodium fluoride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers; use plastic containers for Oral Solution having a pH below 7.5.
- **LABELING:** Label the Oral Solution in terms of the content of sodium fluoride (NaF) and in terms of the content of fluoride ion.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sodium Chloride RS
  USP Sodium Fluoride RS

Sodium Fluoride Tablets

DEFINITION

Sodium Fluoride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of sodium fluoride (NaF).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*

Sample solution: Disperse 20 finely powdered Tablets in 25 mL of water, shake, and filter. Use the filtrate.
Acceptance criteria: The filtrate meets the requirements.

Change to read:

- **B.** ▲The retention time of the fluoride peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

- **PROCEDURE**

▲[NOTE—Store all solutions in plastic containers. It is recommended to use plastic HPLC vials. Use water with a resistivity of NLT 18 megohm-cm to prepare the solutions.]
Solution A: 100 mM of potassium hydroxide in water
Solution B: Water
Mobile phase: See *Table 1*. Alternatively, *Mobile phase* can be generated electrolytically using an automatic eluant generator.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	15	85
7.0	15	85
8.0	80	20
15.0	80	20
15.1	15	85
25.0	15	85

System suitability solution: 2.0 µg/mL of USP Sodium Fluoride RS and 1.0 µg/mL of USP Sodium Acetate RS in water
Standard solution: 2.0 µg/mL of USP Sodium Fluoride RS in water
Sample stock solution: Nominally 20 µg/mL of sodium fluoride prepared as follows. Finely powder NLT 20 Tablets and transfer an appropriate portion of the powder to a suitable volumetric flask. Add about 40% of the final volume of water. Shake for about 30 min and dilute with water to volume.
Sample solution: Nominally 2.0 µg/mL of sodium fluoride in water from the *Sample stock solution* prepared as follows. Transfer an appropriate amount of well-mixed *Sample stock solution* to a suitable volumetric flask. Dilute with water to volume and mix well. Centrifuge a portion of the solution and use the clear supernatant. [NOTE—Centrifuging at a speed of 8000 rpm for 10 min may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with suppression
Columns
Guard: 4.0-mm × 0.5-cm; 4.6-µm packing L91
Analytical: 4.0-mm × 25-cm; 4.6-µm packing L91
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for fluoride and acetate ions are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 1.5 between fluoride and acetate ions, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium fluoride (NaF) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluoride ion from the *Sample solution*
r_S = peak response of fluoride ion from the *Standard solution*
C_S = concentration of USP Sodium Fluoride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of sodium fluoride in the *Sample solution* (µg/mL)▲ (USP 1-May-2020)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩: NMT 15 min
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Store at controlled room temperature.▲ (USP 1-May-2020)
- **LABELING:** Label the Tablets in terms of the content of sodium fluoride (NaF) and in terms of the content of

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fluoride ion. Tablets that are to be chewed may be labeled as Sodium Fluoride Chewable Tablets.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Sodium Acetate RS▲ (USP 1-May-2020)
USP Sodium Fluoride RS

Sodium Fluoride and Acidulated Phosphate Topical Solution

DEFINITION

Sodium Fluoride and Acidulated Phosphate Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of fluoride ion.

IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the fluoride peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 1S (USP41)

Change to read:

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Phosphate*
▲**Sample:** A portion of Topical Solution
[NOTE—If the *Sample* contains hydrogen peroxide, use of a platinum-coated disc to neutralize hydrogen peroxide is recommended prior to performing the test.]
▲ 1S (USP41)
Acceptance criteria: Meets the requirements

ASSAY

Delete the following:

▲• **PROCEDURE 1**
[NOTE—Store all solutions, except *Buffer*, in plastic containers.]
Buffer: Dissolve 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of (1,2-cyclohexylenedinitrilo)tetraacetic acid in 500 mL of water. Adjust with 5 N sodium hydroxide to a pH of 5.25 ± 0.25, and dilute with water to 1000 mL.
Standard solution A: 420 µg/mL of USP Sodium Fluoride RS in water, equivalent to 190 µg/mL of fluoride ion (10^{-2} M)
Standard solution B: 19 µg/mL of fluoride ion (10^{-3} M) in water, from *Standard solution A*
Standard solution C: 1.9 µg/mL of fluoride ion (10^{-4} M) in water, from *Standard solution B*
Sample solution: Nominally 20 µg/mL of fluoride ion from Topical Solution in water
Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*
Pipet 20 mL of *Standard solution A*, *Standard solution B*, *Standard solution C* and *Sample solution* into separate plastic beakers, each containing a plastic-coated stirring bar. Pipet 20 mL of *Buffer* into each beaker. Concomitantly measure the potentials (see *pH* ⟨791⟩), in mV, of each solution with a pH meter capable of a minimum reproducibility of ± 0.2 mV and equipped with a fluoride-specific ion-indicating electrode and a suitable reference electrode.
[NOTE—When taking measurements, immerse the electrodes in the solution, stir on a magnetic stirrer having an insulated top until equilibrium is attained (1–2 min), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.]
Plot the logarithms of the fluoride-ion concentrations, in µg/mL, of *Standard solution A*, *Standard solution B*, and *Standard solution C* versus potential, in mV. From the measured potential of the *Sample solution* and the standard response line, determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*.
Calculate the percentage of the labeled amount of fluoride ion in the Topical Solution taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of fluoride in the *Sample solution* (µg/mL)
C_U = nominal concentration of fluoride ion in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%▲ 1S (USP41)

Change to read:

• **PROCEDURE**▲▲ 1S (USP41)
[NOTE—Use water with a resistivity of NLT 18 megohm-cm to prepare the solutions.]
Mobile phase: 150 mg/L of anhydrous sodium carbonate and 1.0 mL/L of 1 N sodium hydroxide in water. Pass through a suitable filter of 0.45-µm pore size.
▲**System suitability solution:** 1.1 µg/mL of USP Sodium Fluoride RS and 0.5 µg/mL of USP Sodium Chloride RS in water▲ 1S (USP41)
Standard solution: ▲▲ 1S (USP41) 1.1 µg/mL of USP Sodium Fluoride RS in water. Pass through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally ▲0.5 µg/mL of fluoride ion▲ (ERR 1-Aug-2018) from a portion of Topical Solution in water. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with suppression
Columns
Guard: 4.6-mm × 5-cm; ▲7-µm packing▲ 1S (USP41) L46
Analytical: 4.6-mm × 25-cm; ▲7-µm packing▲ 1S (USP41) L46
Flow rate: 1.0 mL/min
Injection volume: 20 µL
[NOTE—Use of polymethylpentene HPLC vials is recommended.]
▲**Run time:** NLT 2 times the retention time of the fluoride peak▲ 1S (USP41)
System suitability
▲**Sample:** *System suitability solution*
[NOTE—The relative retention times for fluoride and chloride ions are 1.0 and 1.2, respectively.▲ 1S (USP41)]
Suitability requirements
Resolution: NLT 1.5 between fluoride and chloride ions ▲▲ 1S (USP41)
Tailing factor: NMT 2.0 for fluoride ion
Relative standard deviation: NMT 2.0% for fluoride ion

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluoride ion in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (A_r/M_r) \times 100$$

r_U = peak response of fluoride from the *Sample solution*
r_S = peak response of fluoride from the *Standard solution*
C_S = concentration of USP Sodium Fluoride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ▲fluoride ion▲ (ERR 1-Aug-2018) in the *Sample solution* (µg/mL)
A_r = atomic weight of fluoride, 19.00
M_r = molecular weight of sodium fluoride, 41.99

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩
Analysis: Place about 40 mL in a plastic beaker, and determine the pH using a suitable electrode system.
Acceptance criteria: 3.0–4.5

Delete the following:

▲• **OTHER REQUIREMENTS:** It responds to the *Identification* tests under *Sodium Fluoride and Phosphoric Acid Gel.* ▲1S (USP41)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, plastic containers.

Change to read:

• **LABELING:** Label the Topical Solution in terms of the content of sodium fluoride (NaF) and in terms of the content of fluoride ion.▲1S (USP41)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Sodium Chloride RS▲1S (USP41)
USP Sodium Fluoride RS

Sodium Fluoride and Phosphoric Acid Gel

DEFINITION
Sodium Fluoride and Phosphoric Acid Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of fluoride ion, in an aqueous medium containing a suitable viscosity-inducing agent.

IDENTIFICATION
• **A.**
Sample: A suitable quantity of Gel nominally equivalent to about 500 mg of fluoride ion
Analysis: Place the *Sample* in a platinum crucible in a well-ventilated hood, and add 15 mL of *sulfuric acid.* Cover the crucible with a piece of clear, polished glass, and heat on a steam bath for 1 h. Remove the glass cover, rinse it in water, and dry.
Acceptance criteria: The glass surface exposed to vapors from the crucible is etched.
• **B.** *Identification Tests—General* ⟨191⟩, *Chemical Identification Tests, Phosphate*: Meets the requirements

ASSAY
• **PROCEDURE**
[NOTE—Store all solutions except the *Buffer solution* in plastic containers.]
Buffer solution: Dissolve 57 mL of glacial acetic acid, 58 g of *sodium chloride,* and 4 g of (1,2-cyclohexylenedinitrilo) tetraacetic acid in 500 mL of water. Adjust with 5 N *sodium hydroxide* to a pH of 5.25 ± 0.25, and dilute with water to 1000 mL.
Standard solution A: 420 µg/mL of USP Sodium Fluoride RS in water, equivalent to 190 µg/mL (10^{-2} M) of fluoride ion
Standard solution B: 19 µg/mL (10^{-3} M) of fluoride ion in water from *Standard solution A*
Standard solution C: 1.9 µg/mL (10^{-4} M) of fluoride ion in water from *Standard solution B*
Sample solution: Nominally 20 µg/mL of fluoride ion from Gel in water
Analysis
Samples: *Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*
Transfer 20 mL of *Standard solution A, Standard solution B, Standard solution C,* and *Sample solution* into separate plastic beakers, each containing a plastic-coated stirring bar. Transfer 20 mL of *Buffer solution* into each beaker. Concomitantly measure the potentials (see *pH* ⟨791⟩), in millivolts, of each solution with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride-specific ion-indicating electrode and a suitable reference electrode.
[NOTE—When taking measurements, immerse the electrodes in the solution, stir on a magnetic stirrer having an insulated top until equilibrium is attained (1–2 min), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.]
Plot the logarithms of the fluoride-ion concentrations, in µg/mL, of *Standard solution A, Standard solution B,* and *Standard solution C* versus potential, in millivolts. From the measured potential of the *Sample solution* and the standard response line, determine the concentration, *C,* in µg/mL, of fluoride ion in the *Sample solution.*
Calculate the percentage of the labeled amount of fluoride ion in the portion of Gel taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of fluoride in the *Sample solution* (µg/mL)
C_U = nominal concentration of fluoride ion in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample: About 40 mL
Analysis: Place the *Sample* in a plastic beaker, and determine the pH using a suitable electrode system.
Acceptance criteria: 3.0–4.0
• **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩
Sample: A portion of Gel
Analysis: Transfer the *Sample* to a suitable plastic container, insert the stopper securely, and allow to stand until the *Sample* is free from air bubbles. Place it in a water bath

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maintained at a temperature of 25 ± 0.5° until it adjusts to the temperature of the water bath (30 min or longer). Do not stir the *Sample* while it is in the bath. Remove the *Sample* from the bath, stir the *Sample* gently for 5 s, and without delay, using a rotational viscometer, determine the viscosity using the appropriate spindle to obtain a scale reading between 10% and 90% of full scale at a speed of 60 rpm or 30 rpm.

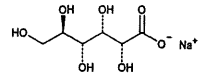
Calculate the viscosity, in centipoises, by multiplying the scale reading by the constant for the spindle and speed used.

Acceptance criteria: 7,000–20,000 centipoises

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, plastic containers.
- **LABELING:** Label Gel in terms of the content of sodium fluoride (NaF) and in terms of the content of fluoride ion.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sodium Fluoride RS

Sodium Gluconate



$C_6H_{11}NaO_7$ 218.14

D-Gluconic acid, monosodium salt;
Monosodium D-gluconate [527-07-1].

DEFINITION

Sodium Gluconate contains NLT 98.0% and NMT 102.0% of $C_6H_{11}NaO_7$.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩
  Sample solution: 1 in 20
  Acceptance criteria: Meets the requirements
- **B. THIN-LAYER CHROMATOGRAPHY**
  Standard solution: 10 mg/mL of USP Potassium Gluconate RS in water. [NOTE—Heat in a water bath at 60° if necessary to dissolve.]
  Sample solution: 10 mg/mL of Sodium Gluconate in water. [NOTE—Heat in a water bath at 60° if necessary to dissolve.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel
  Application volume: 5 µL
  Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
  Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Develop in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool and spray with the *Spray reagent*. Heat the plate at 110° for 10 min.
  Acceptance criteria: The principal spot from the *Sample solution* corresponds in color, size, and R_F value to that from the *Standard solution*.

ASSAY

- **PROCEDURE**
  Sample: 150 mg
  Analysis: Transfer the *Sample* to a suitable conical flask and dissolve in 75 mL of glacial acetic acid, warming if necessary to dissolve. Cool, add quinaldine red TS, and titrate with 0.1 N perchloric acid VS to a colorless endpoint. Each mL of 0.1 N perchloric acid is equivalent to 21.81 mg of $C_6H_{11}NaO_7$.
  Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Sample: 1.0 g
  Acceptance criteria: The *Sample* shows no more chloride than corresponds to 1 mL of 0.020 N hydrochloric acid (NMT 0.07%).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Sample: 2.0 g
  Acceptance criteria: The *Sample*, dissolved in boiling water, shows no more sulfate than corresponds to 1 mL of 0.020 N sulfuric acid (NMT 0.05%).
- **LEAD** ⟨251⟩
  Test preparation: 1.0 g in 25 mL of water
  Acceptance criteria: NMT 10 ppm
- **REDUCING SUBSTANCES**
  Sample: 1.0 g
  Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of 0.1 N iodine VS, and 10 mL of 3 N hydrochloric acid, and titrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, omitting the specimen, and note the difference in volumes required (see *Titrimetry* ⟨541⟩). Each mL of the difference in volume of 0.1 N sodium thiosulfate consumed is equivalent to 2.7 mg of reducing substances (as dextrose).
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Potassium Gluconate RS

Sodium Hypochlorite Solution

NaClO 74.44

Hypochlorous acid, sodium salt;
Sodium hypochlorite [7681-52-9].

DEFINITION

Sodium Hypochlorite Solution contains NLT 4.0% and NMT 6.0%, by weight, of sodium hypochlorite (NaClO).
[CAUTION—This solution is not suitable for application to wounds.]

IDENTIFICATION

- **A.**
  Sample: Sodium Hypochlorite Solution
  Acceptance criteria: The *Sample* at first colors red litmus blue and then bleaches it.
- **B.**
  Sample: Sodium Hypochlorite Solution
  Acceptance criteria: The *Sample* imparts an intense yellow color to a nonluminous flame.

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ASSAY

• **PROCEDURE**

Sample solution: 3 g of Sodium Hypochlorite Solution

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Dilute the *Sample solution* with 50 mL of water, and add 2 g of potassium iodide and 10 mL of 6 N acetic acid. Titrate the liberated iodine with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Calculate the percentage of sodium hypochlorite (NaClO) in the portion of Sodium Hypochlorite Solution taken:

$$\text{Result} = [(V - B) \times N \times (F/W)] \times 100$$

V = sample *Titrant* volume (mL)
B = blank *Titrant* volume (mL)
N = normality of the *Titrant* (mEq/mL)
F = 37.22 mg/mEq
W = sample weight (mg)

Acceptance criteria: 4.0%–6.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, at a temperature not exceeding 25°.

Sodium Hypochlorite Compounded Topical Solution

DEFINITION

Sodium Hypochlorite Compounded Topical Solution contains NLT 0.20 g and NMT 0.32 g of sodium hypochlorite (NaClO) in 1000 mL of Topical Solution.

Prepare Sodium Hypochlorite Compounded Topical Solution as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Sodium Hypochlorite Solution	5.0 mL
Monobasic Sodium Phosphate, monohydrate	1.02 g
Dibasic Sodium Phosphate, anhydrous	17.61 g
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Dibasic Sodium Phosphate, anhydrous* and the *Monobasic Sodium Phosphate, monohydrate* in about 500 mL of *Purified Water*. Add the *Sodium Hypochlorite Solution* and sufficient *Purified Water* to bring the preparation to final volume, and mix.

[NOTE—The source of the *Sodium Hypochlorite Solution* may be commercial unscented laundry bleach, nominally 5.25% (w/v) provided that the commercial laundry bleach was acquired recently.]

ASSAY

• **PROCEDURE**

Sample: 50.0 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: To the *Sample* in a glass-stoppered flask add 0.5 g of potassium iodide and 10 mL of 6 N acetic acid. Titrate the liberated iodine with *Titrant*, adding 2 mL of starch TS as the endpoint is approached. Perform a blank determination and make any necessary correction. Each mL of *Titrant* is equivalent to 3.722 mg of sodium hypochlorite (NaClO).

Acceptance criteria: 0.20–0.32 g in 1000 mL

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 7.8–8.2

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant, 1-L plastic containers, and store at controlled room temperature.

• **BEYOND-USE DATE:** NMT 7 days after the date on which it was compounded when stored at controlled room temperature

• **LABELING:** Label it to indicate that its strength is 0.025%, and to state the *Beyond-Use Date*. [NOTE—For external use only; it may be applied to wounds and burns.]

Sodium Iodide

NaI 149.89

Sodium iodide [7681-82-5].

DEFINITION

Sodium Iodide contains NLT 99.0% and NMT 101.5% of NaI, calculated on the anhydrous basis.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Iodide* ⟨191⟩

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample: 500 mg

Analysis: Dissolve the *Sample* in 10 mL of water. Add 35 mL of hydrochloric acid, and titrate with 0.05 M potassium iodate VS until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. Each mL of 0.05 M potassium iodate is equivalent to 14.99 mg of NaI.

Acceptance criteria: 99.0%–101.5% on the anhydrous basis

IMPURITIES

• **IODATE**

Iodate solution: Dilute 1 mL of potassium iodate solution (1 in 2500) with water to 100 mL.

Standard solution: Dissolve 100 mg of Sodium Iodide in ammonia- and carbon dioxide-free water and add 1 mL of *Iodate solution* to obtain 10 mL of solution. Transfer to a color-comparison tube.

Sample solution: Dissolve 1.1 g in sufficient ammonia- and carbon dioxide-free water to obtain 10 mL of solution. Transfer to a color-comparison tube.

Analysis: To each color comparison tube add 1 mL of starch TS and 0.25 mL of 1.0 N sulfuric acid, and mix.

Acceptance criteria: Any color produced in the *Sample solution* does not exceed that in the *Standard solution* (NMT 4 ppm).

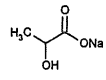
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• **THIOSULFATE AND BARIUM**
Sample solution: Dissolve 0.5 g in 10 mL of ammonia- and carbon dioxide-free water.
Analysis: Add 2 drops of 2 N sulfuric acid to the *Sample solution.*
Acceptance criteria: No turbidity develops within 1 min.
• **POTASSIUM**
Sample solution: Dissolve 1.0 g in 2 mL of water.
Acceptance criteria: The *Sample solution* yields no precipitate with 1.0 mL of sodium bitartrate TS.
• **LIMIT OF NITRATE, NITRITE, AND AMMONIA**
Sample solution: Dissolve 1.0 g in 5 mL of water.
Analysis: To the *Sample solution* contained in a test tube of 40-mL capacity add 5 mL of 1 N sodium hydroxide and about 200 mg of aluminum wire. Insert a pledget of purified cotton in the upper portion of the test tube, and place a piece of moistened red litmus paper over the mouth of the tube. Heat the test tube and its contents on a steam bath for 15 min.
Acceptance criteria: No blue coloration of the paper is discernible.

SPECIFIC TESTS
• **ALKALINITY**
Sample solution: Dissolve 1.0 g in 10 mL of water.
Analysis: Add 0.15 mL of 0.10 N sulfuric acid and 1 drop of phenolphthalein TS to the *Sample solution.*
Acceptance criteria: No red color is produced.
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

Sodium Lactate Injection



$C_3H_5NaO_3$ 112.06
Propanoic acid, 2-hydroxy-, monosodium salt.
Sodium lactate [72-17-3].

» Sodium Lactate Injection is sterile Sodium Lactate Solution in Water for Injection, or a sterile solution of Lactic Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_3H_5NaO_3$.

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL. The label includes also the warning: "Not for use in the treatment of lactic acidosis."
Identification—Overlay 2 mL of Injection on 5 mL of a 1 in 100 solution of catechol in sulfuric acid: a deep red color is produced at the zone of contact.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 2.0 USP Endotoxin Units per mEq.
pH ⟨791⟩: between 6.0 and 7.3, the Injection being diluted with water, if necessary, to approximately 0.16 M (20 mg per mL).
Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Pipet into a small beaker a volume of Injection, equivalent to about 300 mg of sodium lactate, and evaporate to dryness. Add to the residue 60 mL of a 1 in 5 mixture of acetic anhydride in glacial acetic acid, and stir until the residue is completely dissolved. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 11.21 mg of $C_3H_5NaO_3$.

Sodium Lactate Solution

» Sodium Lactate Solution is an aqueous solution containing not less than 50.0 percent, by weight, of monosodium lactate. It contains not less than 98.0 percent and not more than 102.0 percent of the labeled amount of $C_3H_5NaO_3$.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate its content of sodium lactate.
Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Lactate* ⟨191⟩.
pH ⟨791⟩: between 5.0 and 9.0.
Chloride ⟨221⟩—A portion, equivalent to 1 g of sodium lactate, shows no more chloride than corresponds to 0.7 mL of 0.020 N hydrochloric acid (0.05%).
Sulfate—To 10 mL of a solution (1 in 100) add 2 drops of hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced.
Sugars—To 10 mL of hot alkaline cupric tartrate TS add 5 drops of Solution: no red precipitate is formed.
Limit of citrate, oxalate, phosphate, or tartrate—Dilute 5 mL with recently boiled and cooled water to 50 mL. To 4 mL of this solution add 6 N ammonium hydroxide or 3 N hydrochloric acid, if necessary, to bring the pH to between 7.3 and 7.7. Add 1 mL of calcium chloride TS, and heat in a boiling water bath for 5 minutes: the solution remains clear.

Limit of methanol and methyl esters—
Potassium permanganate and *phosphoric acid solution*—Dissolve 3 g of potassium permanganate in a mixture of 15 mL of phosphoric acid and 70 mL of water. Dilute with water to 100 mL.
Oxalic acid and *sulfuric acid solution*—Cautiously add 50 mL of sulfuric acid to 50 mL of water, mix, cool, add 5 g of oxalic acid, and mix to dissolve.
Standard preparation—Prepare a solution containing 10.0 mg of methanol in 100 mL of dilute alcohol (1 in 10).
Test preparation—Place 40.0 g in a glass-stoppered, round-bottom flask, add 10 mL of water, and add cautiously 30 mL of 5 N potassium hydroxide. Connect a condenser to the flask, and steam-distill, collecting the distillate in a suitable 100-mL graduated vessel containing 10 mL of alcohol. Continue the distillation until the volume in the receiver reaches approximately 95 mL, and dilute the distillate with water to 100.0 mL.
Procedure—Transfer 10.0 mL each of the *Standard preparation* and the *Test preparation* to 25-mL volumetric

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flasks, to each add 5.0 mL of *Potassium permanganate and phosphoric acid solution*, and mix. After 15 minutes, to each add 2.0 mL of *Oxalic acid and sulfuric acid solution*, stir with a glass rod until the solution is colorless, add 5.0 mL of fuchsin-sulfurous acid TS, and dilute with water to volume. After 2 hours, concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 575 nm, with a suitable spectrophotometer, using water as the blank: the absorbance of the solution from the *Test preparation* is not greater than that from the *Standard preparation* (0.025%).

Assay—Weigh accurately into a suitable flask a volume of Solution, equivalent to about 300 mg of sodium lactate, add 60 mL of a 1 in 5 mixture of acetic anhydride in glacial acetic acid, mix, and allow to stand for 20 minutes. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 11.21 mg of $C_3H_5NaO_3$.

Sodium Monofluorophosphate



Na_2PFO_3 143.95

Phosphorofluoridic acid, disodium salt.
Disodium phosphorofluoridate [10163-15-2].

» Sodium Monofluorophosphate contains not less than 91.7 percent and not more than 100.5 percent of Na_2PFO_3, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Sodium Fluoride RS

Identification—

A: Place about 1 g in a platinum crucible in a well-ventilated hood, and add 15 mL of sulfuric acid. Cover the crucible with a piece of clear, polished glass, and heat on a steam bath for 1 hour. Remove the glass cover, rinse it in water, and dry: the glass surface exposed to vapors from the crucible is etched.

B: A solution (1 in 10) with silver nitrate TS yields a white precipitate, which is soluble in diluted nitric acid and in dilute ammonium hydroxide (1 in 2).

C: A solution responds to the tests for *Sodium* ⟨191⟩.

pH ⟨791⟩: between 6.5 and 8.0, in a solution (1 in 50).

Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 0.2% of its weight.

Arsenic, *Method I* ⟨211⟩: 3 ppm.

Limit of fluoride ion— [NOTE—Use plasticware throughout this test.]

Buffer solution—To 55 g of sodium chloride in a 1000-mL volumetric flask add 500 mg of sodium citrate, 255 g of sodium acetate, and 300 mL of water. Shake to dissolve, and cautiously add 115 mL of glacial acetic acid with mixing. Cool to room temperature, add 300 mL of isopropyl alcohol, dilute with water to volume, and mix: the pH of this solution is between 5.0 and 5.5.

Standard stock solution—Dissolve an accurately weighed quantity of USP Sodium Fluoride RS quantitatively in water to obtain a solution containing 1105 µg per mL. Each mL of this solution contains 500 µg of fluoride ion. Store in a tightly closed, plastic container.

Standard preparations—To four 100-mL volumetric flasks transfer, respectively, 2.0-, 4.0-, 10.0-, and 20.0-mL portions of the *Standard stock solution*, dilute each with *Buffer solution* to volume, and mix to obtain solutions having fluoride ion concentrations of 10, 20, 50, and 100 µg per mL, respectively.

Test preparation—Transfer about 1.8 g, accurately weighed, to a 100-mL volumetric flask, add water to dissolve, dilute with water to volume, and mix. Transfer 20.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix.

Procedure—Concomitantly measure the potential (see *pH* ⟨791⟩), in mV, of the *Standard preparations* and of the *Test preparation* with a pH meter capable of a minimum reproducibility of ±0.2 mV, equipped with a glass-sleeved calomel-fluoride specific-ion electrode system. [NOTE—When taking measurements, immerse the electrodes in the solution, which has been transferred to a 150-mL plastic beaker containing a plastic-coated stirring bar. Allow to stir on a magnetic stirrer having an insulated top until equilibrium is attained (1 to 2 minutes), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.] Plot the logarithm of the fluoride-ion concentrations, in µg per mL, of each *Standard preparation* versus potential, in mV. From the measured potential of the *Test preparation* and the standard curve, determine the concentration, in µg per mL, of fluoride ion in the *Test preparation:* not more than 1.2% is found.

Assay—

Monochloroacetate buffer—Dissolve 189 g of monochloroacetic acid and 55 g of sodium hydroxide in about 1500 mL of water. Cool, dilute with water to 2000 mL, and mix.

0.025 M Thorium nitrate—Dissolve 13.8 g of thorium nitrate in about 800 mL of water, and filter the solution into a 1000-mL volumetric flask. Dilute with water to volume, and mix. Standardize this solution as follows. Transfer 20.0 mL of *Standard stock solution,* prepared as directed in the test for *Limit of fluoride ion,* to a 150-mL beaker containing 50 mL of water. Add 3 drops of sodium alizarinsulfonate TS, mix, and adjust the acidity by the careful addition, successively, of sodium hydroxide solution (1 in 50) and dilute hydrochloric acid (1 in 160), until the pink color has just been discharged. Add 1 mL of *Monochloroacetate buffer,* and titrate with *0.025 M Thorium nitrate* to a permanent pink color. Calculate the molarity of the thorium nitrate titrant taken by the equation:

$$M = (S)/[(4)(41.99V)]$$

in which S is the weight, in mg, of USP Sodium Fluoride RS in the portion of *Standard stock solution* taken, 41.99 is the molecular weight of sodium fluoride, and V is the volume, in mL, of titrant consumed.

Procedure—Transfer about 1.8 g of Sodium Monofluorophosphate, accurately weighed, to a 100-mL volumetric flask. Add about 50 mL of water, mix to effect solution, dilute with water to volume, and mix. Transfer 20.0 mL of this solution to the reaction flask of a suitable fluoride steam distilling apparatus containing 10 lime-glass beads measuring 5 mm in diameter and 70 mL of dilute sulfuric acid (1 in 2). Distill, by passing steam through the solution in the reaction flask and applying heat to the flask, collecting the distillate in a 250-mL volumetric flask. Control the temperature of the solution in the reaction flask so that it does not exceed 140°. Change receivers when the flask is filled to volume with distillate, and continue distilling into a 400-mL beaker until an additional 150 mL to 200 mL of distillate tailing

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has been collected. Mix the solution in the 250-mL volumetric flask, and transfer 50.0 mL to a 150-mL beaker. To the 150-mL beaker add 50 mL of water and 3 drops of sodium alizarinsulfonate TS, and mix. Adjust the acidity of this solution by the careful addition, successively, of sodium hydroxide solution (1 in 50) and dilute hydrochloric acid (1 in 160), until the pink color has just been discharged. Add 1 mL of *Monochloroacetate buffer*, titrate with *0.025 M Thorium nitrate* to a permanent pink color, and record the volume, in mL, of titrant consumed as V_A. Add 3 drops of sodium alizarinsulfonate TS to the distillation tail in the 400-mL beaker, proceed as directed previously with the adjustment of the acidity, the addition of *Monochloroacetate buffer*, and the titration with *0.025 M Thorium nitrate*, and record the volume, in mL, of titrant consumed as V_B. Calculate the percentage of Na_2PFO_3 in the Sodium Monofluorophosphate taken by the formula:

$$[(2)(143.95)(5V_A + V_B)(M)/(W)] - (143.95/18.9984)(F)$$

in which M is the molarity of *0.025 M Thorium nitrate*; W is the weight, in g, of the Sodium Monofluorophosphate taken; F is the percentage of fluoride ions determined as directed in the test for *Limit of fluoride ion*; 143.95 is the molecular weight of sodium monofluorophosphate; 18.9984 is the atomic weight of fluorine; and the other terms are as defined therein.

Sodium Nitrite

$NaNO_2$ 69.00
Nitrous acid, sodium salt;
Sodium nitrite [7632-00-0].

DEFINITION
Sodium Nitrite contains NLT 98.0% and NMT 102.0% of sodium nitrite ($NaNO_2$), calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Chemical Identification Tests, Sodium* ⟨191⟩**:** A solution meets the requirements of the tests.
- **B. IDENTIFICATION TESTS—GENERAL,** *Chemical Identification Tests, Nitrite* ⟨191⟩**:** A solution meets the requirements of the tests.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: 2.7 mM sodium carbonate and 0.3 mM sodium bicarbonate in water
Standard solution: 0.12 mg/mL of USP Sodium Nitrite RS in water
Sample solution: 0.12 mg/mL of Sodium Nitrite in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with suppression
Columns
Guard: 4-mm × 50-mm, packing L105
Analytical: 4-mm × 200-mm; 9-µm packing L105
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: NLT 4 times the retention time of nitrite
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for sodium and nitrite ions are 1.0 and 1.85, respectively.]
Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 3000 theoretical plates
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sodium nitrite ($NaNO_2$) in the portion of Sodium Nitrite taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sodium Nitrite RS in the *Standard solution* (mg/mL)
C_U = concentration of Sodium Nitrite in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **LIMIT OF ALUMINUM, IRON, AND SELENIUM**

Internal standard stock solution: To 2 mL of each of commercially available 1000 ppm scandium (for aluminum) and yttrium (for iron) and 10^4 ppm germanium (for selenium) standards in a 100-mL volumetric flask, add 2 mL of nitric acid and dilute with water to volume.
Internal standard solution: To 0.5 mL of *Internal standard stock solution* in a 10-mL volumetric flask, add 0.5 mL of nitric acid and dilute with water to volume.
Blank solution: Dilute with water to volume, 0.125 mL of *Internal standard solution*, 0.5 mL of nitric acid, and 0.5 mL of 80 ppm gold solution in a 50-mL volumetric flask.
Standard solutions: 2, 5, 50, and 100 ppb solutions for aluminum and selenium; 0, 50, 100, 500, and 1000 ppb for iron as follows. To suitable amounts of commercially available aluminum, selenium, and iron standard solutions in a 50-mL volumetric flask, add 0.125 mL of *Internal standard solution*, 0.5 mL of nitric acid, and 0.5 mL of 80 ppm gold solution, and dilute with water to volume.
Sample solution: To 1 g of sodium nitrite in a 50-mL volumetric flask, add 0.125 mL of *Internal standard solution*, 2.5 mL of nitric acid, and 0.5 mL of 80 ppm gold solution, and dilute with water to volume.
Instrumental conditions
(See *Plasma Spectrochemistry* ⟨730⟩.)
Mode: Inductively coupled plasma–mass spectrometer (ICP–MS)
Spectrometer: Quadrupole mass spectrometer
Detector: Ion detector
System suitability
Sample: *Standard solutions*
Suitability requirements: Before analyzing samples, the instrument must pass a suitable performance check. Generate the calibration curve using the corresponding *Standard solutions* for each element under test. The linear regression coefficient is NLT 0.999.
Analysis
Sample: *Sample solution*
Determine the concentration of each element in the *Sample solution* using the calibration curve.
Acceptance criteria: See *Table 1*.

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Table 1

Element	Isotope (amu)	Acceptance Criteria, NMT (ppm)
Aluminum	27	2
Iron	57	10
Selenium	82	30

• **LIMIT OF SODIUM NITRATE**

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability: Proceed as directed in the *Assay*.

[NOTE—The relative retention times for nitrite and nitrate are 1.0 and about 1.85, respectively.]

Analysis

Sample: *Sample solution*

Calculate the percentage of sodium nitrate ($NaNO_3$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of nitrate from the *Sample solution*
r_S = peak response of nitrite from the *Sample solution*
F = relative response factor for nitrate, 0.7

Acceptance criteria: NMT 0.4%

• **LIMIT OF CALCIUM AND POTASSIUM**

Internal standard solution: 40 ppm scandium solution prepared from a suitable commercially available 1000 ppm high purity scandium standard solution in water

Blank solution: To 0.5 mL of *Internal standard solution* in a 50-mL volumetric flask, add 0.5 mL of nitric acid and dilute with water to volume.

Standard solutions: 2.5, 5.0, 7.5, and 10.0 ppm of calcium and potassium prepared as follows. To suitable amounts of the respective commercially available calcium and potassium standard solutions in a suitable volumetric flask, add 0.5 mL of *Internal standard solution* and 0.5 mL of nitric acid, and dilute with water to 50 mL.

Sample solution: To 1 g of sodium nitrite in a 50-mL volumetric flask, add 0.5 mL of *Internal standard solution* and 2.5 mL of nitric acid, and dilute with water to volume.

Instrumental conditions

(See *Plasma Spectrochemistry* ⟨730⟩.)

Mode: Inductively coupled plasma–mass spectrometer (ICP–MS)

Spectrometer: Quadrupole mass spectrometer

Detector: Ion detector

System suitability

Sample: *Standard solutions*

Suitability requirements: Before analyzing samples, the instrument must pass a suitable performance check. Generate the calibration curve using the *Standard solutions* of the corresponding elements. The linear regression coefficient is NLT 0.999.

Analysis

Sample: *Sample solution*

Determine the concentration of each element in the *Sample solution* using the calibration curve.

Acceptance criteria: See *Table 2*.

Table 2

Element	Isotope (amu)	Acceptance Criteria, NMT (ppm)
Calcium	44	100
Potassium	39	50

• **LIMIT OF CARBONATE**

All carbonate solutions must be prepared fresh and stored in tightly sealed vials and stored in a cool area away from excessive heat.

Standard stock solution: 1 mg/mL of carbonate, prepared as follows. Dissolve 0.177 g of sodium carbonate in a 100-mL volumetric flask, and dilute with water to volume.

Standard solutions: Prepare solutions of 0.50, 1.0, 2.0, 4.0, and 5.0 µg/mL carbonate in water from *Standard stock solution*.

Sample solution: Transfer 1.0 g of Sodium Nitrite into a 100-mL volumetric flask, and dilute with water to volume.

System suitability: Before analyzing samples, the total organic carbon (TOC) analyzer must pass a suitable performance check. Generate the calibration curve using the *Standard solutions*. The linear regression coefficient is NLT 0.995; %RSD for each Standard is NMT 10%.

Analysis

Sample: *Sample solution*

Calculate the percentage of carbonate present in the sample taken:

$$\text{Result} = [(C_t \times D)/W] \times 100$$

C_t = concentration of carbonate (g/mL)
D = sample dilution, 100 mL
W = weight of sample taken (g)

Acceptance criteria: NMT 0.02%

• **TOTAL NON-PURGEABLE ORGANIC CONTENT**

Standard stock solution: Equivalent to 250 µg/mL of total organic carbon prepared as follows. Weigh 9 mg of USP Sucrose RS in a suitable flask, and add 15 mL of water.

Standard solutions: 0.5, 2.0, and 10 µg/mL of total organic carbon in water from *Standard stock solution*

Sample solution: Dissolve and dilute with water to volume, 5.0 g of Sodium Nitrite in a 100-mL volumetric flask.

System suitability: Before analyzing samples, the total organic carbon analyzer instrument must pass a suitable performance check. Generate the calibration curve using the *Standard solutions*. The linear regression coefficient is NLT 0.99; %RSD for the 2 µg/mL and 10 µg/mL calibration standards is NMT 15%.

Analysis

Sample: *Sample solution*

Calculate the amount, in ppm, of non-purgeable organic content present in the sample taken:

$$\text{Result} = [(C_t \times D)/W]$$

C_t = amount of non-purgeable organic content as determined by the analyzer (µg/g)
D = sample dilution, 100 mL
W = weight of sample taken (g)

Acceptance criteria: NMT 10 ppm

• **LIMIT OF SULFATE**

Standard solution: 0.0148 mg/mL of anhydrous sodium sulfate in water

Sample solution: 100 mg/mL of Sodium Nitrite in water

Analysis

Sample: *Sample solution*

Evaporate 5 mL of the *Sample solution* to dryness. Add 1 mL of dilute hydrochloric acid (prepared by adding 1 mL of hydrochloric acid to 19 mL water). Pass through a prewashed small filter paper, and wash with two 2-mL portions of water. Dilute to 10 mL, and add 1 mL of barium chloride TS. Compare turbidity in the *Standard solution* and *Sample solution*, 10 min after adding the barium chloride TS.

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Acceptance criteria: Any turbidity in the *Sample solution* should not exceed the turbidity in the *Standard solution* (NMT 0.01%).

• **LIMIT OF CHLORIDE**

Standard solution: 0.0165 mg/mL of sodium chloride in water

Sample solution: 100 mg/mL of Sodium Nitrite in water

Analysis

Samples: *Standard solution* and *Sample solution*

To 2.0 mL of the *Sample solution* in a suitable test tube add 10 mL of water and 1 mL of glacial acetic acid and boil for 5 min. Pass through a chlorine-free filter paper, and dilute with water to 20 mL. Dilute 1 mL of the *Standard solution* with water to 20 mL in a separate test tube. Add 1 mL of nitric acid and 1 mL of 0.1 N silver nitrate. Allow to stand for 5 min protected from light and compare using a black background.

Acceptance criteria: Any turbidity in the *Sample solution* should not exceed the turbidity in the *Standard solution* (NMT 50 ppm).

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.1 USP Endotoxin Units/mg of sodium nitrite

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩: NMT 100 cfu/g, total aerobic microbial count and NMT 20 cfu/g, total yeasts and molds

• **pH** ⟨791⟩: 7.0–9.0

• **INSOLUBLE MATTER**

Sample solution: 100 mg/mL of Sodium Nitrite in water

Analysis: Heat the *Sample solution* to boiling in a covered beaker at about 100° for 1 h. Pass the hot solution through a suitable crucible filter of 10–15 µm pore size. Wash the beaker and filter with hot water, dry at 105°, cool in a desiccator, and weigh the residue.

Acceptance criteria: NMT 50 ppm

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over silica gel for 4 h.

Acceptance criteria: NMT 0.25%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers and store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Endotoxin RS

USP Sodium Nitrite RS

USP Sucrose RS

Sodium Nitrite Injection

DEFINITION

Sodium Nitrite Injection is a sterile solution of Sodium Nitrite in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of sodium nitrite ($NaNO_2$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Chemical Identification Tests, Sodium* ⟨191⟩: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Chemical Identification Tests, Nitrite* ⟨191⟩: Meets the requirements

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: 2.7 mM sodium carbonate and 0.3 mM sodium bicarbonate in water

Standard solution: 0.12 mg/mL of USP Sodium Nitrite RS in water

Sample solution: Nominally equivalent to 0.12 mg/mL of sodium nitrite in water from a suitable volume of Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: Ion chromatography

Detector: Conductivity with suppression

Columns

Guard: 4-mm × 50-mm; packing L105

Analytical: 4-mm × 200-mm; 9-µm packing L105

Flow rate: 1.5 mL/min

Injection volume: 25 µL

Run time: NLT 4 times the retention time of nitrite

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for sodium and nitrite ions are 1.0 and 1.85, respectively.]

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sodium nitrite ($NaNO_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sodium Nitrite RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of sodium nitrite in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **LIMIT OF SODIUM NITRATE**

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability: Proceed as directed in the *Assay*.

[NOTE—The relative retention times for nitrite and nitrate are 1.0 and about 1.85, respectively.]

Analysis

Sample: *Sample solution*

Calculate the percentage of sodium nitrate ($NaNO_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of nitrate from the *Sample solution*

r_S = peak response of nitrite from the *Sample solution*

F = relative response factor of sodium nitrate, 0.7

Acceptance criteria: NMT 0.5%

• **LIMIT OF NITROGEN OXIDE (NO_x) RELATED IMPURITIES**

Blank solution 1: 0.1 N potassium chloride prepared as follows. Dissolve 7.46 g of potassium chloride in 1 L of water.

Blank solution 2: Acidified 0.1 N potassium chloride prepared as follows. In a 500-mL volumetric flask pipet 15-mL of 1 N hydrochloric acid and dilute with 0.1 N potassium chloride to volume.

Standard solution: 4.920 mg/mL of sodium nitrate in water equivalent to 1000 µg/mL of nitrogen

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Sample solution: Dilute 10.0 mL of Injection with water to 50.0 mL. Store between 2° and 8°. Use 40 mL for each measurement.

System suitability

[NOTE—Under acidic conditions of pH less than 1.8, the nitrite is converted to equivalent amounts of nitrogen oxides with different oxidation states.]

Samples: *Blank solution 2* and *Standard solution*

Suitability requirements: Generate a calibration curve of the mV readings for *Blank solution 2*, and a sequential addition of 100 µL, 100 µL, 800 µL, and 9 mL of the *Standard solution* to *Blank solution 2* versus the log of the concentrations of the nitrogen oxide (NO_x) in the solution. Use a pH meter equipped with a suitable nitrogen oxide (NO_x) electrode to obtain the mV readings. Perform a linear regression without the zero concentration point and determine the slope and correlation coefficient (R). The slope must be between 54 and 60 mV and the linear regression coefficient is NLT 0.995.

Analysis

Samples: *Blank solution 1*, *Blank solution 2*, *Standard solution*, and *Sample solution*

Analyze the samples in the following order and obtain the pH meter readings in mV for 40-mL portions each of *Blank solution 1* in duplicate, *Sample solution* in duplicate, and *Blank solution 1*; and 100 mL portions each of *Blank solution 2*, and *Blank solution 2* plus 100 µL of *Standard solution*. [NOTE—This solution contains 1 µg/mL of nitrogen oxide (NO_x).] Repeat the measurements.

Acceptance criteria: The mV reading obtained for the *Sample solution* is NMT the mV reading obtained for *Blank solution 2* with 100 µL of the *Standard solution* (NMT 1 ppm).

SPECIFIC TESTS

- **pH** ⟨791⟩: 7.0–9.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 4.0 USP Endotoxin Units/mL of sodium nitrite
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers of Type I glass. Protect from direct light. Do not freeze. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sodium Nitrite RS

Sodium Nitroprusside

$Na_2[Fe(CN)_5NO] \cdot 2H_2O$ 297.95

Ferrate(2-), pentakis(cyano-C)nitrosyl-, disodium, dihydrate, (*OC*-6-22)-.

Disodium pentacyanonitrosylferrate(2-) dihydrate [13755-38-9].

Sodium nitroferricyanide dihydrate.

Anhydrous 261.92

[14402-89-2].

» Sodium Nitroprusside contains not less than 99.0 percent of $Na_2[Fe(CN)_5NO] \cdot 2H_2O$.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Sodium Nitroprusside RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
[NOTE—Use low-actinic glassware.]
Solution: 1 in 135.
Medium: water.
Wavelength range: 350 nm to 700 nm.

B: Dissolve 5 mg in 2 mL of water, and add 2 drops of acetone and 0.5 mL of 2 N sodium hydroxide: an orange color is produced. Add 2 mL of acetic acid: the color changes to purple.

C: A solution (1 in 4) imparts an intense yellow color to a nonluminous flame.

Water Determination, *Method I* ⟨921⟩ **:** between 9.0% and 15.0%.

Insoluble substances—Dissolve 10.0 g in 50 mL of water, heat the solution on a steam bath for 30 minutes, filter, wash the residue with water, and dry at 105° to constant weight: the weight of the residue is not greater than 1 mg (0.01%).

Chloride—

Standard chloride solution—Dissolve 42.4 mg of potassium chloride in water to make 100.0 mL of solution. Each mL of this solution contains 0.2 mg of chloride.

Procedure—Transfer 1.0 g of Sodium Nitroprusside to a 250-mL conical flask, transfer 1.0 mL of *Standard chloride solution* to a similar flask, and add to each flask 85 mL of water. To the flask containing the substance under test, add 15 mL of cupric sulfate solution (83 in 1000), mix, and allow any undissolved particles to settle. Carefully add cupric sulfate solution (83 in 1000) to the flask containing the diluted *Standard chloride solution*, with mixing, so that its color matches that of the test solution in the first flask. Filter the contents of each flask, and discard the first 25 mL of the filtrate. To 10 mL of the subsequent filtrate from each flask add 2 mL of nitric acid, and mix. Add 1 mL of 1 N silver nitrate to each, and again mix: the test solution so treated becomes no more turbid than the treated *Standard chloride solution* (0.02%).

Limit of ferricyanide—Dissolve 500 mg in 20 mL of ammonium acetate TS, previously adjusted with 1 N acetic acid to a pH of 4.62. Divide this solution into halves, and transfer each half to a separate 50-mL volumetric flask, identified as *A* and *B*, respectively. To flask *B* add 1.0 mL of a freshly prepared solution of potassium ferricyanide containing 78 µg per mL. To both flasks add 5 mL of ferrous ammonium sulfate solution (1 in 1000), dilute with water to volume, and mix. Allow the flasks to stand for 1 hour, and concomitantly determine the absorbance of the solutions at the wavelength of maximum absorbance at about 720 nm, using as a blank a solution prepared by dissolving 250 mg of the specimen in 10 mL of the pH 4.62 ammonium acetate TS and diluting with water to 50 mL. The absorbance of the solution in flask *A* is not greater than the absorbance of the solution in flask *B* minus the absorbance of the solution in flask *A* (0.02% of ferricyanide).

Limit of ferrocyanide—Dissolve 2.0 g in 40 mL of water, divide the solution into halves, and transfer each half to a separate 50-mL volumetric flask, identified as *A* and *B*, respectively. To flask *B* add 2 mL of a freshly prepared solution of potassium ferrocyanide containing 200 µg per mL. To both

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flasks add 0.2 mL of ferric chloride TS, dilute with water to volume, and mix. Allow to stand for 20 minutes, accurately timed, and concomitantly measure the absorbance of the solutions at the wavelength of maximum absorbance at about 695 nm, using as a blank a solution prepared by dissolving 1.0 g of the specimen in water to make 50 mL: the absorbance of the solution in flask *A* is not greater than the absorbance of the solution in flask *B* minus the absorbance of the solution in flask *A* (0.02% of ferrocyanide).

Sulfate—

Standard sulfate solution—Dissolve 15 mg of anhydrous sodium sulfate in water to make 100.0 mL of solution. Each mL of this solution contains 0.1 mg of sulfate.

Procedure—Dissolve 5.0 g of Sodium Nitroprusside in water to make 250.0 mL of solution, and filter the solution into a flat-bottom, 250-mL graduated flask. Transfer 5.0 mL of *Standard sulfate solution* to a similar flask, and dilute to the same volume as the test solution. To each flask add 10 drops of glacial acetic acid and 5 mL of 1 N barium chloride, and allow to stand for 10 minutes. Place both flasks over a fluorescent light source, and observe: the turbidity in the treated test solution is not more intense than that of the treated *Standard sulfate solution* (0.01%).

Other requirements—Where the label states that Sodium Nitroprusside is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and for *Bacterial endotoxins* under *Sodium Nitroprusside for Injection*. Where the label states that *Sodium Nitroprusside* must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Sodium Nitroprusside for Injection*.

Assay—Dissolve about 500 mg of Sodium Nitroprusside, accurately weighed, in 130 mL of chloride-free water. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride electrode system. Each mL of 0.1 N silver nitrate is equivalent to 14.90 mg of $Na_2[Fe(CN)_5NO] \cdot 2H_2O$.

Sodium Nitroprusside for Injection

» Sodium Nitroprusside for Injection is Sodium Nitroprusside suitable for parenteral use. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of sodium nitroprusside ($Na_2[Fe(CN)_5NO] \cdot 2H_2O$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

USP Reference standards ⟨11⟩—
USP Sodium Nitroprusside RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

Identification—To 50 mg contained in a small test tube add 10 mL of ascorbic acid solution (1 in 50), and mix. Add 1 mL of dilute hydrochloric acid (1 in 10), mix, and add dropwise, while mixing, 1 to 2 mL of 1 N sodium hydroxide: a transient blue color is produced.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.05 USP Endotoxin Unit per µg of sodium nitroprusside.

Water Determination, *Method I* ⟨921⟩: not more than 15.0%.

Other requirements—It responds to the *Identification* test *A* under *Sodium Nitroprusside*. It meets also the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩, and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

pH 7.1 buffer—Dissolve 1.36 g of monobasic potassium phosphate and 5.2 mL of a 1 in 4 solution of tetrabutylammonium hydroxide in methanol in water to make 1000 mL, and adjust with phosphoric acid or with the tetrabutylammonium hydroxide solution to a pH of 7.1.

Mobile phase—Prepare a suitable filtered mixture of *pH 7.1 buffer* and acetonitrile (about 70:30).

[NOTE—Use low-actinic glassware throughout the following sections.]

Standard preparation—Dissolve an accurately weighed quantity of USP Sodium Nitroprusside RS, in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

Assay preparation 1 (where the label states only the total contents of the container)—Transfer the contents of 1 container of Sodium Nitroprusside for Injection to a 100-mL volumetric flask with the aid of *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Dilute an accurately measured volume of this solution quantitatively with *Mobile phase* to obtain a solution containing about 0.05 mg of $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ per mL.

Assay preparation 2 (where the label states the quantity of $Na_2[Fe(CN_5)NO] \cdot 2H_2O$ in a given volume of constituted solution)—Constitute Sodium Nitroprusside for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution thus obtained quantitatively and stepwise with *Mobile phase* to obtain a solution containing about 0.05 mg of $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 30-cm column that contains 10-µm packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

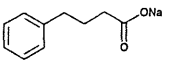
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ in the container or in the portion of constituted solution taken by the formula:

$$L(C/D)(r_U/r_S)$$

in which L is the labeled quantity, in mg of $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ in the container, or in the volume of constituted solution taken; C is the concentration, in mg per mL, of USP Sodium Nitroprusside RS in the *Standard preparation; D* is the concentration, in mg of $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ per mL, of *Assay preparation 1* or of *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the volume of constituted solution taken, respectively, and the extent of dilution; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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Sodium Phenylbutyrate



$C_{10}H_{11}NaO_2$ 186.18
Benzenebutanoic acid, sodium salt;
Sodium 4-phenylbutyrate;
Sodium 4-phenylbutanoate. [1716-12-7].

DEFINITION
Sodium Phenylbutyrate contains NLT 99.0% and NMT 101.0% of sodium phenylbutyrate ($C_{10}H_{11}NaO_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL ⟨191⟩, *Sodium***
  Sample solution: Dissolve 150 mg in 2 mL of water.
  Acceptance criteria: Meets the requirements of test *A*

ASSAY
- **PROCEDURE**
  Sample: 150 mg
  Analysis: Disperse the *Sample* in 50 mL of anhydrous acetic acid. [NOTE—The opalescence of the solution disappears during the titration.] Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 18.62 mg of sodium phenylbutyrate ($C_{10}H_{11}NaO_2$).
  Acceptance criteria: 99.0%–101.0% on the anhydrous basis

IMPURITIES
- **LIMIT OF PHENYLBUTYRATE RELATED COMPOUND C**
  Silylation solution: To 2 mL of bis(trimethylsilyl) trifluoroacetamide add 0.04 mL of chlorotrimethylsilane, and mix.
  Sample solution: Dissolve 50.0 mg of Sodium Phenylbutyrate in 3 mL of water, and add 0.5 mL of hydrochloric acid. Extract with 2 quantities, each of 5 mL, of methylene chloride. Evaporate the combined methylene chloride extracts to dryness in a vial with a screw cap, and add 0.5 mL of the *Silylation solution*. Seal the vial, and heat at 70 ± 5° for 20 min.
  Standard stock solution: 0.5 mg/mL of USP Phenylbutyrate Related Compound C RS in methylene chloride
  Diluted standard stock solution: 0.05 mg/mL of USP Phenylbutyrate Related Compound C RS in methylene chloride from *Standard stock solution*
  Standard solution: Place an amount of the *Diluted standard stock solution*, equivalent to 0.05 mg, in a vial with a screw cap, evaporate to dryness, and add 0.5 mL of the *Silylation solution*. Seal the vial, and heat at 70 ± 5° for 20 min.
  System suitability solution: Prepare a solution containing 0.4 mg/mL of USP Sodium Phenylbutyrate RS in water. To 3 mL of this solution add 0.1 mL of hydrochloric acid. Extract with 2 quantities, each of 5 mL, of methylene chloride. Combine the methylene chloride extracts in a vial with a screw cap, and add 2 mL of *Standard stock solution*. Evaporate to dryness, and add 0.5 mL of the *Silylation solution*. Seal the vial, and heat at 70 ± 5° for 20 min.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: GC
  Detector: Flame ionization
  Column: 25-m × 0.25-mm fused silica capillary; coated with a 1.0-µm film of phase G27
  Temperatures
  Injector: 270°
  Detector: 270°
  Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	5
50	10	270	5

  Carrier gas: Helium
  Flow rate: 0.9 mL/min
  Injection volume: 1 µL
  Split ratio: 1:100
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for phenylbutyrate related compound C and phenylbutyrate are about 0.98 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 3.0 between the phenylbutyrate related compound C and phenylbutyrate peaks
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of phenylbutyrate related compound C in the portion of Sodium Phenylbutyrate taken:

$$\text{Result} = (r_U/r_S) \times (W_S/W_U) \times 100$$

r_U = peak response of phenylbutyrate related compound C from the *Sample solution*
r_S = peak response of phenylbutyrate related compound C from the *Standard solution*
W_S = amount of USP Phenylbutyrate Related Compound C RS taken to prepare the *Standard solution* (mg)
W_U = amount of Sodium Phenylbutyrate taken to prepare the *Sample solution* (mg)

  Acceptance criteria: NMT 0.1%
- **ORGANIC IMPURITIES**
  Mobile phase: Methanol, glacial acetic acid, and water (49:1:50)
  Impurity stock solution A: 0.1 mg/mL of USP Phenylbutyrate Related Compound A RS in methanol
  Impurity standard solution A: Dilute 1.0 mL of *Impurity stock solution A* with water to 50.0 mL. This solution contains 2 µg/mL of USP Phenylbutyrate Related Compound A RS.
  Impurity stock solution B: 0.02 mg/mL of USP Phenylbutyrate Related Compound B RS in methanol
  Impurity standard solution B: Dilute 1.0 mL of *Impurity stock solution B* with water to 50.0 mL. This solution contains 0.4 µg/mL of USP Phenylbutyrate Related Compound B RS.
  System suitability solution: Prepare a solution containing 20 mg/mL of USP Sodium Phenylbutyrate RS in methanol. Transfer 10 mL of this solution to a 50-mL volumetric flask, add 1.0 mL of *Impurity stock solution B*, and dilute with water to volume.
  Sensitivity solution: Dilute 6.0 mL of *Impurity standard solution A* with water to 10.0 mL. This solution contains 1.2 µg/mL of USP Phenylbutyrate Related Compound A RS.

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Sample solution: 4.0 mg/mL of Sodium Phenylbutyrate, prepared as follows. Dissolve it first in methanol, using 20% of the final volume, and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated end-capped packing L1
Flow rate: 1.3 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 6 between phenylbutyrate and phenylbutyrate related compound B, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Impurity standard solution A*, *Impurity standard solution B*, and *Sample solution*
Calculate the percentage of phenylbutyrate related compound A or phenylbutyrate related compound B in the portion of Sodium Phenylbutyrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of respective impurity in the *Sample solution*
r_S = peak response of phenylbutyrate related compound A or phenylbutyrate related compound B in the respective *Impurity standard solution*
C_S = concentration of USP Phenylbutyrate Related Compound A RS or USP Phenylbutyrate Related Compound B RS in the respective *Impurity standard solution* (mg/mL)
C_U = concentration of Sodium Phenylbutyrate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Sodium Phenylbutyrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity in the *Sample solution*
r_S = peak response of phenylbutyrate related compound A in *Impurity standard solution A*
C_S = concentration of USP Phenylbutyrate Related Compound A RS in *Impurity standard solution A* (mg/mL)
C_U = concentration of Sodium Phenylbutyrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenylbutyrate related compound A	0.3	0.1
Phenylbutyrate related compound B	0.7	0.01
Sodium phenylbutyrate	1.0	—
Any individual unspecified impurity	—	0.05
Total impurities	—	0.1

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 0.5%
• **pH** ⟨791⟩
Sample solution: 20 mg/mL in carbon dioxide-free water
Acceptance criteria: 6.5–7.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve under argon in tight containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sodium Phenylbutyrate RS
USP Phenylbutyrate Related Compound A RS
3-Benzoylpropionic acid;
4-Oxo-4-phenylbutanoic acid.
$C_{10}H_{10}O_3$ 178.18
USP Phenylbutyrate Related Compound B RS
α-Tetralone;
3,4-Dihydronaphthalen-1(2*H*)-one.
$C_{10}H_{10}O$ 146.19
USP Phenylbutyrate Related Compound C RS
4-Cyclohexylbutanoic acid.
$C_{10}H_{18}O_2$ 170.25

Sodium Phenylbutyrate Compounded Oral Suspension

DEFINITION
Sodium Phenylbutyrate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sodium phenylbutyrate ($C_{10}H_{11}O_2Na$).
Prepare Sodium Phenylbutyrate Compounded Oral Suspension 200 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Sodium Phenylbutyrate powder[a]	20 g
Vehicle: a 1:1 mixture of Ora-Sweet[b] (regular or sugar-free) and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Ucyclyd Pharma, Inc., Scottsdale, AZ.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the *Sodium Phenylbutyrate powder* in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a sodium phenylbutyrate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and 5 mM phosphoric acid (40:60). Filter and degas.

USP Monographs

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Standard solution: 0.1 mg/mL of sodium phenylbutyrate in *Mobile phase*. [NOTE—The *Standard solution* should be prepared from the appropriate reference material.]
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.1 mg/mL of sodium phenylbutyrate from Oral Suspension and *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 218 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 60°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for sodium phenylbutyrate is about 3.0 min.]
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium phenylbutyrate ($C_{10}H_{11}O_2Na$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of sodium phenylbutyrate in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium phenylbutyrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 7.0–8.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

Dibasic Sodium Phosphate

$Na_2HPO_4 \cdot 12H_2O$ 358.14
$Na_2HPO_4 \cdot 7H_2O$ 268.07
$Na_2HPO_4 \cdot 2H_2O$ 177.99
$Na_2HPO_4 \cdot H_2O$ 159.94
Na_2HPO_4 141.96
Phosphoric acid, disodium salt, dodecahydrate; Disodium hydrogen phosphate, dodecahydrate [10039-32-4].
Phosphoric acid, disodium salt, heptahydrate; Disodium hydrogen phosphate heptahydrate [7782-85-6].
Phosphoric acid, disodium salt, dihydrate; Disodium hydrogen phosphate, dihydrate [10028-24-7].
Phosphoric acid, sodium salt, monohydrate; Disodium hydrogen phosphate, monohydrate [118830-14-1].
Phosphoric acid, disodium salt, hydrate; Disodium hydrogen phosphate hydrate [10140-65-5].
Anhydrous [7558-79-4].

DEFINITION
Dibasic Sodium Phosphate is dried or contains one, two, seven, or twelve molecules of water of hydration. It contains NLT 98.0% and NMT 100.5% of Na_2HPO_4, calculated on the dried basis.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩
Sample solution: Equivalent to 1 part of Na_2HPO_4 in 30
Acceptance criteria: The solution meets the requirements.
• **B. IDENTIFICATION TESTS—GENERAL,** *Phosphate* ⟨191⟩
Sample solution: Equivalent to 1 part of Na_2HPO_4 in 30
Acceptance criteria: The solution meets the requirements.

ASSAY
• **PROCEDURE**
Sample: Equivalent to 2.5 g of Na_2HPO_4
Sample solution: Transfer the *Sample* to a 250-mL beaker. Add 50 mL of water and 40.0 mL of 1 N hydrochloric acid, and stir until dissolved.
Blank: Transfer 40.0 mL of 1 N hydrochloric acid to a 250-mL beaker. Add 50 mL of water.
Analysis: Titrate the *Blank* with 1 N sodium hydroxide VS, and record the volume of 1 N sodium hydroxide VS consumed. Titrate the excess acid in the *Sample solution* potentiometrically with 1 N sodium hydroxide VS to the inflection point at about pH 4, and record the buret reading. Subtract this buret reading from that of the *Blank*, and designate the volume of 1 N sodium hydroxide VS resulting from this subtraction as *A*. Continue the titration with 1 N sodium hydroxide VS to the inflection point at about pH 8.8, record the buret reading, and calculate the volume (*B*) of 1 N sodium hydroxide required in the titration between the two inflection points (pH 4–8.8). Where *A* is equal to or less than *B*, each mL of the volume *A* of 1 N sodium hydroxide is equivalent to 142.0 mg of Na_2HPO_4. Where *A* is greater than *B*, each mL of the volume $(2B - A)$ of 1 N sodium hydroxide is equivalent to 142.0 mg of Na_2HPO_4.
Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES
• **INSOLUBLE SUBSTANCES**
Sample solution: Equivalent to 5.0 g of Na_2HPO_4 in 100 mL of hot water
Analysis: Filter through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° for 2 h.
Acceptance criteria: NMT 20 mg (NMT 0.4%)
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample: Equivalent to 0.5 g of Na_2HPO_4
Acceptance criteria: Shows no more chloride than corresponds to 0.42 mL of 0.020 N hydrochloric acid (NMT 0.06%)
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample: Equivalent to 0.1 g of Na_2HPO_4
Acceptance criteria: Shows no more sulfate than corresponds to 0.2 mL of 0.020 N sulfuric acid (NMT 0.2%)
• **ARSENIC,** *Method I* ⟨211⟩
Test preparation: Equivalent to 187.5 mg of Na_2HPO_4 in 35 mL of water
Acceptance criteria: NMT 16 ppm

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SPECIFIC TESTS
- **Loss on Drying** ⟨731⟩: Dry a sample at 130° to constant weight.
  Acceptance criteria: See *Table 1*.

Table 1

Hydrate Form	Acceptance Criteria
Dried	NMT 5.0%
Monohydrate	10.3%–12.0%
Dihydrate	18.5%–21.5%
Heptahydrate	43.0%–50.0%
Dodecahydrate	55.0%–64.0%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate whether it is dried or is the monohydrate, the dihydrate, the heptahydrate, or the dodecahydrate.

Monobasic Sodium Phosphate

NaH_2PO_4 119.98

$NaH_2PO_4 \cdot H_2O$ 137.99

$NaH_2PO_4 \cdot 2H_2O$ 156.01

Phosphoric acid, monosodium salt, monohydrate; Monosodium phosphate monohydrate [10049-21-5].
Phosphoric acid, monosodium salt, dihydrate; Monosodium phosphate dihydrate [13472-35-0].
Anhydrous [7558-80-7].

DEFINITION
Monobasic Sodium Phosphate contains one or two molecules of water of hydration, or is anhydrous. It contains NLT 98.0% and NMT 103.0% of monobasic sodium phosphate (NaH_2PO_4), calculated on the anhydrous basis.

IDENTIFICATION
- **A. Identification Tests—General,** *Sodium* ⟨191⟩
  Sample solution: 50 mg/mL
  Acceptance criteria: Meets the requirements
- **B. Identification Tests—General,** *Phosphate* ⟨191⟩
  Sample solution: 50 mg/mL
  Acceptance criteria: Meets the requirements

ASSAY
- **Procedure**
  Sample: 2.5 g
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 1 N sodium hydroxide VS
  Endpoint detection: Visual
  Analysis: Dissolve the *Sample* in 10 mL of cold water. Add 20 mL of a cold, saturated solution of sodium chloride, add phenolphthalein TS, and then titrate with *Titrant*, keeping the temperature of the solution between 10° and 15° during the entire titration. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 120.0 mg of monobasic sodium phosphate (NaH_2PO_4).
  Acceptance criteria: 98.0%–103.0% on the anhydrous basis

IMPURITIES
- **Insoluble Substances**
  Sample solution: Equivalent to 10.0 g of $NaH_2PO_4 \cdot H_2O$ in 100 mL of hot water
  Analysis: Pass through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° for 2 h.
  Acceptance criteria: NMT 20 mg (0.2%)
- **Chloride and Sulfate,** *Chloride* ⟨221⟩
  Sample: Equivalent to 1.0 g of $NaH_2PO_4 \cdot H_2O$
  Control: 0.20 mL of 0.020 N hydrochloric acid
  Acceptance criteria: NMT 0.014%; the *Sample* shows no more chloride than the *Control*.
- **Chloride and Sulfate,** *Sulfate* ⟨221⟩
  Sample: Equivalent to 0.20 g of $NaH_2PO_4 \cdot H_2O$
  Control: 0.30 mL of 0.020 N sulfuric acid
  Acceptance criteria: NMT 0.15%; the *Sample* shows no more sulfate than the *Control*.
- **Aluminum, Calcium, and Related Elements**
  Sample solution: Equivalent of 1.0 g of $NaH_2PO_4 \cdot H_2O$ in 10 mL of water
  Acceptance criteria: The *Sample solution* does not become turbid when rendered slightly alkaline to litmus paper with 6 N ammonium hydroxide.
- **Arsenic,** *Method I* ⟨211⟩
  Test preparation: Equivalent of 0.375 g of $NaH_2PO_4 \cdot H_2O$ in 35 mL of water
  Acceptance criteria: NMT 8 ppm

SPECIFIC TESTS
- **pH** ⟨791⟩
  Sample solution: Equivalent to 1.0 g of $NaH_2PO_4 \cdot H_2O$ in 20 mL of water
  Acceptance criteria: 4.1–4.5
- **Water Determination,** *Method I* ⟨921⟩
  Test preparation: For the monohydrate, before performing the test, the sample may be ground to a fine powder in an atmosphere of temperature and relative humidity known not to influence the results.
  Acceptance criteria: See *Table 1*.

Table 1

Hydrate Form	Acceptance Criteria (%)
Anhydrous	<2.0
Monohydrate	10.0–15.0
Dihydrate	18.0–26.5

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.
- **Labeling:** Label it to indicate whether it is anhydrous, monohydrate, or dihydrate.

Sodium Phosphates Injection

» Sodium Phosphates Injection is a sterile solution of Monobasic Sodium Phosphate and Dibasic Sodium Phosphate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$)

USP Monographs

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and dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$). It contains no bacteriostat or other preservative.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
Labeling—The label states the sodium content in terms of milliequivalents in a given volume, and states also the phosphorus content in terms of millimoles in a given volume. Label the Injection to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration and that once opened any unused portion is to be discarded. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Phosphate* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.10 USP Endotoxin Units per mg of sodium phosphates.
Particulate Matter in Injections ⟨788⟩: meets the requirements under small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay for monobasic sodium phosphate—Transfer an accurately measured volume of Injection, equivalent to about 300 mg of anhydrous monobasic sodium phosphate, to a 100-mL beaker, and dilute with water to about 50 mL. Place the electrodes of a suitable pH meter in the solution, and titrate with 0.1 N sodium hydroxide VS to the inflection point to a pH of about 8.8. Each mL of 0.1 N sodium hydroxide is equivalent to 13.80 mg of $NaH_2PO_4 \cdot H_2O$.
Assay for dibasic sodium phosphate—Transfer an accurately measured volume of Injection, equivalent to about 300 mg of anhydrous dibasic sodium phosphate, to a 100-mL beaker, and dilute with water to about 50 mL. Place the electrodes of a suitable pH meter in the solution, and titrate with 0.1 N hydrochloric acid VS to the inflection point to a pH of about 4.0. Each mL of 0.1 N hydrochloric acid is equivalent to 26.81 mg of $Na_2HPO_4 \cdot 7H_2O$.

Sodium Phosphates Compounded Injection

DEFINITION

Sodium Phosphates Compounded Injection contains NLT 95.0% and NMT 105.0% of the labeled amount of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$) and dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$). It contains no bacteriostat or other preservative.

Prepare Sodium Phosphates Compounded Injection containing 3 mM/mL of phosphorus and 4.0 mEq/mL of sodium as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Monobasic Sodium Phosphate (anhydrous)[a]	24 g
Dibasic Sodium Phosphate (anhydrous)	14.2 g
Sterile Water for Injection, a sufficient quantity to make	100 mL

[a] If anhydrous is not used, waters of hydration should be included in calculating the appropriate amount of sodium phosphates.

Dissolve the *Dibasic Sodium Phosphate (anhydrous)* in 50 mL of *Sterile Water for Injection*. Dissolve the *Monobasic Sodium Phosphate (anhydrous)* in the solution, and bring to final volume with *Sterile Water for Injection*. [NOTE—May need to heat to 77° to fully dissolve. If heating is required, allow to reach room temperature prior to passing through a filter.] Pass through a filter of 1.2-µm pore size to remove particulate matter, and sterilize by autoclave.

ASSAY

• **PROCEDURE FOR SODIUM**
Mobile phase: 8 mM methanesulfonic acid
Standard solution: 0.025 mg/mL of sodium prepared with USP Sodium Chloride RS and water
Sample solution: Transfer 1 mL of Injection into a 100-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4.6-mm × 25-cm; packing L97
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for sodium is about 3.6 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sodium from the *Sample solution*
r_S = peak response of sodium from the *Standard solution*
C_S = concentration of sodium in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

• **PROCEDURE FOR PHOSPHATE**
Mobile phase: 40 mM sodium hydroxide
Standard solution: 0.230 mg/mL of phosphate prepared from USP Dibasic Potassium Phosphate RS in water
Sample solution: Transfer 0.2 mL of Injection into a 250-mL volumetric flask and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4.6-mm × 25-cm; packing L103
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for phosphorus is about 5.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phosphate (PO_4) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phosphate from the *Sample solution*
r_S = peak response of phosphate from the *Standard solution*
C_S = concentration of phosphate in the *Standard solution* (mg/mL)
C_U = nominal concentration of phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.3–6.3
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 1.10 USP Endotoxin Units/mg of sodium phosphates
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in single-dose glass containers. Store at controlled room temperature.

Change to read:

- **Beyond-Use Date:** ▲In the absence of passing a sterility test and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility and endotoxins test is passed, NMT 120 days after the date on which it was compounded when stored at controlled room temperature.▲ (CN 1-May-2020)
- **Labeling:** Label it to state the *Beyond-Use Date*. The label states the sodium content in terms of milliequivalents in a given volume, and states also the phosphorus content in terms of millimoles in a given volume.
- **USP Reference Standards** ⟨11⟩
  USP Dibasic Potassium Phosphate RS
  USP Sodium Chloride RS

Sodium Phosphates Oral Solution

» Sodium Phosphates Oral Solution is a solution of Dibasic Sodium Phosphate and Monobasic Sodium Phosphate, or Dibasic Sodium Phosphate and Phosphoric Acid, in Purified Water. It contains, in each 100 mL, not less than 16.2 g and not more than 19.8 g of dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$), and not less than 43.2 g and not more than 52.8 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$).

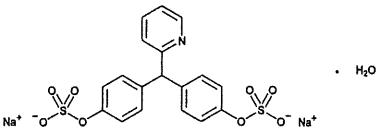
Packaging and storage—Preserve in tight containers.
Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Phosphate* ⟨191⟩.
Specific gravity ⟨841⟩: between 1.333 and 1.366.
pH ⟨791⟩: between 4.4 and 5.2.
Assay—Pipet 25.0 mL of Oral Solution into a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 25.0 mL of this stock solution to a 250-mL beaker, and add 15.0 mL of 0.5 N sodium hydroxide and 75 mL of water. Proceed as directed in the *Assay* under *Sodium Phosphates Rectal Solution*, beginning with "Titrate the excess base."

Sodium Phosphates Rectal Solution

» Sodium Phosphates Rectal Solution is a solution of Dibasic Sodium Phosphate and Monobasic Sodium Phosphate, or Dibasic Sodium Phosphate and Phosphoric Acid, in Purified Water. It contains, in each 100 mL, not less than 5.4 g and not more than 6.6 g of dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$), and not less than 14.4 g and not more than 17.6 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$).

Packaging and storage—Preserve in tight, single-unit containers, at controlled room temperature.
Identification—It meets the requirements of the tests for *Sodium* ⟨191⟩ and for *Phosphate* ⟨191⟩.
Specific gravity ⟨841⟩: between 1.112 and 1.136.
pH ⟨791⟩: between 5.0 and 5.8.
Assay—Pipet 5.0 mL of Rectal Solution into a 250-mL beaker, and add 15.0 mL of 0.5 N sodium hydroxide VS and 95 mL of water. Titrate the excess base potentiometrically with 0.5 N hydrochloric acid VS to the first inflection point, at a pH of about 9.2. Record the volume, *A*, in mL, of 0.5 N hydrochloric acid consumed. Continue the titration to the second inflection point, at a pH of about 4.4, and record the total volume, *B*, in mL, of 0.5 N hydrochloric acid required in the titration. For a blank determination, transfer 15.0 mL of 0.5 N sodium hydroxide into a 250-mL beaker, add 100 mL of water, and immediately titrate potentiometrically with 0.5 N hydrochloric acid VS. Record the volume, *C*, in mL, of 0.5 N hydrochloric acid consumed. Each mL of the volume ($C - A$) of 0.5 N hydrochloric acid is equivalent to 69.0 mg of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$). Each mL of the volume ($B - C$) of 0.5 N hydrochloric acid is equivalent to 134.0 mg of dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$).

Sodium Picosulfate



$C_{18}H_{13}NNa_2O_8S_2 \cdot H_2O$ 499.42
$C_{18}H_{13}NNa_2O_8S_2$ 481.41
4,4′-(2-Pyridylmethylene)diphenyl bis(hydrogen sulfate) disodium salt, monohydrate;
Disodium 4,4′-(pyridin-2-ylmethanediyl)dibenzenesulfate 4,4′-(Pyridin-2-ylmethylene)bisphenyl bis(sodium sulfate), monohydrate [10040-45-6].

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DEFINITION

Sodium Picosulfate contains NLT 98.5% and NMT 100.5% of sodium picosulfate ($C_{18}H_{13}NNa_2O_8S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*: Meets the requirements for test *A*

ASSAY

- **PROCEDURE**

Sample solution: Dissolve 400 mg of Sodium Picosulfate in 80 mL of methanol.

Analysis: Titrate with 0.1 N perchloric acid VS (see *Titrimetry* ⟨541⟩), determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 48.14 mg of sodium picosulfate ($C_{18}H_{13}NNa_2O_8S_2$).

Acceptance criteria: 98.5%–100.5% on the anhydrous basis

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩: A 1.0-g portion shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid: NMT 0.02%.
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩: A 500-mg portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid: NMT 0.04%.
- **ORGANIC IMPURITIES**

Buffer: 2.3 g/L of dibasic sodium phosphate dihydrate in water. For each liter of solution, add 200 mg of cetylrimethylammonium bromide, and adjust with phosphoric acid to a pH of 7.5.

Mobile phase: Acetonitrile and *Buffer* (45:55). [NOTE—If necessary, vary the buffer/acetonitrile proportion in 10-mL increments per L of *Mobile phase* in order to fulfill the resolution requirement.]

Impurity solution: 0.025 mg/mL of USP Sodium Picosulfate Related Compound A RS in *Mobile phase*

System suitability solution: 0.5 mg/mL of sodium picosulfate and 0.5 µg/mL of USP Sodium Picosulfate Related Compound A RS (from *Impurity solution*), in *Mobile phase*

Sample solution: 0.5 mg/mL of Sodium Picosulfate in *Mobile phase*

Diluted sample solution: 0.5 µg/mL of Sodium Picosulfate in *Mobile phase*, from *Sample solution*

Sensitivity solution: 0.25 µg/mL of Sodium Picosulfate in *Mobile phase*, from *Diluted sample solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 263 nm

Column: 4.6-mm or 4.0-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 4 between sodium picosulfate related compound A and sodium picosulfate, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample solution* and *Diluted sample solution*

Calculate the percentage of each impurity in the portion of Sodium Picosulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for sodium picosulfate from the *Diluted sample solution*

C_S = concentration of Sodium Picosulfate in the *Diluted sample solution* (mg/mL)

C_U = concentration of Sodium Picosulfate in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak below 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria (%)
4,4′-[(Pyridin-2-yl) methylene]bisphenol	0.5	2.0	0.2
4-[(Pyridin-2-yl)(4-hydroxyphenyl)methyl]-phenyl sodium sulfate (sodium picosulfate related compound A)	0.7	1.4	0.2
Sodium picosulfate	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.5

SPECIFIC TESTS

- **COLOR OF SOLUTION**

Standard solution: Mix 0.75 mL of *Matching Fluid O* (see *Color and Achromicity* ⟨631⟩) with 99.25 mL of dilute hydrochloric acid (10 g/L).

Sample solution: Dissolve 2.5 g of Sodium Picosulfate in 50 mL of carbon dioxide-free water. [NOTE—Retain the remaining portion of the *Sample solution* for the test for *Acidity and Alkalinity*.]

Analysis: Proceed as directed in *Color and Achromicity* ⟨631⟩.

Acceptance criteria: The *Sample solution* is not more intensely colored than the *Standard solution*.

- **ACIDITY AND ALKALINITY**

Analysis: To 10 mL of the portion of *Sample solution* retained from the test for *Color of Solution* add a drop of phenolphthalein TS.

Acceptance criteria: The solution is colorless. NMT 0.25 mL of 0.01 N sodium hydroxide is required to change the color of the indicator to pink.

- **WATER DETERMINATION,** *Method Ia* ⟨921⟩: 3.0%–5.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Picosulfate RS

USP Sodium Picosulfate Related Compound A RS

4-[(Pyridin-2-yl)(4-hydroxyphenyl)methyl]phenyl sodium sulfate.

$C_{18}H_{14}NNaO_5S$ 379.36

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Sodium Polystyrene Sulfonate

Benzene, diethenyl-, polymer with ethenylbenzene, sulfonated, sodium salt.
Divinylbenzene copolymer with styrene, sulfonated, sodium salt.

» Sodium Polystyrene Sulfonate is a cation-exchange resin prepared in the sodium form. Each g exchanges not less than 110 mg and not more than 135 mg of potassium, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
Labeling—Sodium Polystyrene Sulfonate that is intended for preparing suspensions for oral or rectal administration may be labeled Sodium Polystyrene Sulfonate for Suspension.
Water Determination, *Method I* ⟨921⟩: not more than 10.0%.
Limit of ammonium salts—Place 1 g in a 50-mL beaker, add 5 mL of 1 N sodium hydroxide, cover the beaker with a watch glass having a moistened strip of red litmus paper on the underside, and allow to stand for 15 minutes: the litmus paper shows no blue color.

Sodium content—
Sodium solution—Dissolve in water an accurately weighed quantity of sodium chloride to make a solution containing 5.00 mg of sodium per mL.
Standard graph—Into four 1-liter flasks pipet, respectively, 0, 1, 2, and 3 mL of *Sodium solution*. To each flask add 0.1 mL of nitric acid, 0.1 mL of sulfuric acid, and 10 mL of low-sodium, low-potassium, nonionic surfactant solution (1 in 50), dilute with water to volume, and mix. Adjust the scale of a suitable flame spectrophotometer to a reading of 100 at a wavelength of 588 nm with the solution containing 15 mg of sodium per L. Determine the instrument readings on the other three solutions, and plot the observed readings, on ruled coordinate paper, as the ordinate, and the concentration of sodium, in mg per liter, as the abscissa. The line intersects the ordinate at, or below, a scale reading of 25 ("blank reading").
Procedure—Ash the equivalent of 1 g of Sodium Polystyrene Sulfonate, accurately weighed, with a slight excess of sulfuric acid. Add 1 mL of nitric acid and a few mL of water to the residue. Warm to dissolve, and transfer with water to a 1-liter volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, add 1 mL of low-sodium, low-potassium, non-ionic surfactant solution (1 in 50), dilute with water to volume, and mix. Determine the instrument reading concomitantly with the readings obtained for plotting the *Standard graph*, and determine the sodium concentration, in mg per liter, by interpolation from the *Standard graph*. Calculate the percentage of sodium taken by the formula:

$$A/W$$

in which A is the weight, in mg, of sodium found per L and W is the weight, in g, of Sodium Polystyrene Sulfonate taken. The sodium content is not less than 9.4% and not more than 11.5%, calculated on the anhydrous basis.

Potassium exchange capacity—
Potassium solution—Dissolve an accurately weighed quantity of potassium chloride in water to make a solution containing 5.00 mg of potassium per mL.
Sodium solution—Dissolve an accurately weighed quantity of sodium chloride in water to make a solution containing 4.00 mg of sodium per mL.
Standard graph—Identify five 1-liter volumetric flasks by the numbers *1*, *2*, *3*, *4*, and *5*. In that order pipet into the flasks 4, 3, 2, 1, and 0 mL, respectively, of *Sodium solution*, and in the same order 0, 1, 2, 3, and 4 mL, respectively, of *Potassium solution*. To each flask add 10 mL of low-sodium, low-potassium, nonionic surfactant solution (1 in 50), dilute with water to volume, and mix. Adjust the scale of a suitable flame spectrophotometer to 100 with solution from flask *5* at 766 nm. Determine the instrument readings with solutions from flasks *4*, *3*, *2*, and *1*. On ruled coordinate paper, plot the observed instrument readings as the ordinate, and the concentrations, in mg per liter, of potassium as the abscissa.
Procedure—Pipet 100 mL of *Potassium solution* into a glass-stoppered flask containing about 1.6 g of Sodium Polystyrene Sulfonate, accurately weighed, shake by mechanical means for 15 minutes, filter, and discard the first 20 mL of the filtrate. Pipet 5 mL of the filtrate into a 1-liter volumetric flask, add 10 mL of low-sodium, low-potassium, nonionic surfactant solution (1 in 50), dilute with water to volume, and mix. Observe the flame spectrophotometer readings of the exchanged solution concomitantly with those obtained for plotting the *Standard graph*, and determine the potassium concentration, in mg per liter, by interpolation from the *Standard graph*. Calculate the quantity, in mg per g, of potassium adsorbed on the resin taken by the formula:

$$(X - 20Y)/W$$

in which X is the weight, in mg, of potassium in 100 mL of *Potassium solution* before exchange; Y is the weight, in mg, of potassium per L as interpolated from the *Standard graph;* and W is the weight, in g, of Sodium Polystyrene Sulfonate taken, expressed on the anhydrous basis.

Sodium Polystyrene Sulfonate Suspension

» Sodium Polystyrene Sulfonate Suspension is a suspension of Sodium Polystyrene Sulfonate in an aqueous vehicle that may contain suitable suspending or stabilizing agents. It exchanges not less than 110 mg and not more than 135 mg of potassium for each g of the labeled amount of sodium polystyrene sulfonate.

Packaging and storage—Preserve in well-closed containers, protected from freezing and from excessive heat.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—Its total aerobic microbial count does not exceed 100 cfu per mL, its total combined molds and yeasts count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Pseudomonas aeruginosa*.

Sodium content—
Sodium solution and *Standard graph*—Prepare as directed in the test for *Sodium content* under *Sodium Polystyrene Sulfonate*.
Procedure—Transfer an accurately measured quantity of Suspension, freshly mixed and free from air bubbles, equivalent to about 1 g of sodium polystyrene sulfonate, to a suitable crucible, heat at 80° until dry, and ash the residue with a slight excess of sulfuric acid. Proceed as directed for

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Procedure in the test for *Sodium content* under *Sodium Polystyrene Sulfonate*, beginning with "Add 1 mL of nitric acid." Calculate the percentage of sodium taken by the formula:

$$A/L$$

in which A is the quantity, in mg, of sodium found per liter, and L is the quantity, in g, of sodium polystyrene sulfonate in the portion of Suspension taken, based on the labeled amount: the sodium content is not less than 9.4% and not more than 11.5%.

Potassium exchange capacity—

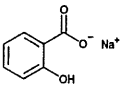
Potassium solution, *Sodium solution*, and *Standard graph*—Prepare as directed in the test for *Potassium exchange capacity* under *Sodium Polystyrene Sulfonate*.

Procedure—Transfer an accurately measured quantity of Suspension, freshly mixed and free from air bubbles, equivalent to about 1.6 g of sodium polystyrene sulfonate, to a suitable glass-stoppered flask, add 100.0 mL of *Potassium solution*, shake by mechanical means for 15 minutes, filter, and discard the first 20 mL of the filtrate. Proceed as directed for *Procedure* in the test for *Potassium exchange capacity* under *Sodium Polystyrene Sulfonate*, beginning with "Pipet 5 mL of the filtrate." Calculate the quantity, in mg, of potassium adsorbed on each g of the sodium polystyrene sulfonate taken by the formula:

$$(X - 20Y)/L$$

in which X is the quantity, in mg, of potassium in 100 mL of *Potassium solution* before exchange; Y is the quantity, in mg, of potassium per L as interpolated from the *Standard graph;* and L is the labeled quantity, in g, of sodium polystyrene sulfonate in the portion of Suspension taken.

Sodium Salicylate



$C_7H_5NaO_3$ 160.10

Benzoic acid, 2-hydroxy-, monosodium salt; Monosodium salicylate [54-21-7].

DEFINITION

Sodium Salicylate contains NLT 98.0% and NMT 102.0% of sodium salicylate ($C_7H_5NaO_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩
  Sample solution: 50 mg/mL of Sodium Salicylate in water
  Analysis: Proceed as directed for *Sodium* in the chapter.
  Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, trifluoroacetic acid, and water (40: 0.1: 60)
Standard solution: 0.04 mg/mL of USP Sodium Salicylate RS in *Mobile phase*
Sample solution: 0.04 mg/mL of Sodium Salicylate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Column temperature: 30°
Flow rate: 0.2 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.8
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sodium salicylate ($C_7H_5NaO_3$) in the portion of Sodium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sodium Salicylate RS in the *Standard solution* (mg/mL)
C_U = concentration of Sodium Salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **SULFITE** or **THIOSULFATE**

Sample solution: 1.0 g of Sodium Salicylate in 20 mL of water
Analysis: Add 1 mL of hydrochloric acid to the *Sample solution*, and filter the liquid.
Acceptance criteria: NMT 0.15 mL of 0.10 N iodine is required to produce a yellow color in the filtrate.

- **ORGANIC IMPURITIES**

Mobile phase: Methanol, trifluoroacetic acid, and water (40: 0.1: 60)
Standard stock solution: 0.125 mg/mL of USP Sodium Salicylate RS, 0.25 mg/mL of USP Salicylic Acid Related Compound A RS, 0.125 mg/mL of USP Salicylic Acid Related Compound B RS, and 0.05 mg/mL of USP Phenol RS in *Mobile phase*
Standard solution: 1.25 µg/mL of USP Sodium Salicylate RS, 2.5 µg/mL of USP Salicylic Acid Related Compound A RS, 1.25 µg/mL of USP Salicylic Acid Related Compound B RS, and 0.5 µg/mL of USP Phenol RS in *Mobile phase* from *Standard stock solution*
Sample solution: 2.5 mg/mL of Sodium Salicylate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Column temperature: 30°
Flow rate: 0.2 mL/min
Injection volume: 2 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between salicylic acid related compound A and phenol; NLT 3.0 between phenol and salicylic acid related compound B
Relative standard deviation: NMT 2% for each peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid related compound A, salicylic acid related compound B, or phenol in the portion of Sodium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid related compound A, salicylic acid related compound B, or phenol from the *Sample solution*
r_S = peak response of salicylic acid related compound A, salicylic acid related compound B, or phenol from the *Standard solution*
C_S = concentration of USP Salicylic Acid Related Compound A RS, USP Salicylic Acid Related Compound B RS, or USP Phenol RS in the *Standard solution* (mg/mL)
C_U = concentration of Sodium Salicylate in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Sodium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of salicylic acid related compound B from the *Standard solution*
C_S = concentration of USP Salicylic Acid Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Sodium Salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A	0.3	0.1
Phenol	0.4	0.02
Salicylic acid related compound B	0.6	0.05
Salicylic acid	1.0	—
Any other individual impurity	—	0.05
Total impurities	—	0.2

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Salicylic Acid Related Compound A RS
4-Hydroxybenzoic acid.
$C_7H_6O_3$ 138.12
USP Salicylic Acid Related Compound B RS
4-Hydroxyisophthalic acid.
$C_8H_6O_5$ 182.13
USP Sodium Salicylate RS

Sodium Salicylate Tablets

DEFINITION
Sodium Salicylate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of sodium salicylate ($C_7H_5NaO_3$).

IDENTIFICATION
• **A.**
Sample: 50 mg/mL of sodium salicylate in water from powdered Tablets. Filter and use the filtrate.
Acceptance criteria: The sample imparts an intense yellow color to a nonluminous flame.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, trifluoroacetic acid, and water (40: 0.1: 60). Prepare by adding 1 mL of trifluoroacetic acid to a solution containing 400 mL of methanol and 600 mL of water.
Standard solution: 0.04 mg/mL of USP Sodium Salicylate RS in *Mobile phase*
Sample stock solution: Nominally 0.4 mg/mL of sodium salicylate from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume of *Mobile phase*, and sonicate for 10 min. Allow the solution to cool to room temperature and then dilute with *Mobile phase* to volume.
Sample solution: Nominally 0.04 mg/mL of sodium salicylate in *Mobile phase* from *Sample stock solution*. Pass through a suitable filter of 0.20-µm pore size, discarding the first 2–3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Column temperature: 30°
Flow rate: 0.2 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.8
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium salicylate ($C_7H_5NaO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

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C_S = concentration of USP Sodium Salicylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: A known concentration of USP Sodium Salicylate RS in *Medium*
Sample solution: Filter portions of the solution under test, and dilute with water, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 230 nm
Analysis: Determine the amount of sodium salicylate ($C_7H_5NaO_3$) dissolved from UV absorbance of the *Sample solution*, in comparison with that of the *Standard solution*.
Tolerances: NLT 75% (*Q*) of the labeled amount of sodium salicylate ($C_7H_5NaO_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase: Methanol, trifluoroacetic acid, and water (40: 0.1: 60). Prepare by adding 1 mL of trifluoroacetic acid to a solution containing 400 mL of methanol and 600 mL of water.
System suitability solution: 0.5 mg/mL of USP Sodium Salicylate RS, 0.5 µg/mL of USP Salicylic Acid Related Compound A RS, 0.5 µg/mL of USP Salicylic Acid Related Compound B RS, and 0.5 µg/mL of USP Phenol RS in *Mobile phase*
Standard solution: 2.5 µg/mL of USP Sodium Salicylate RS in *Mobile phase*
Sample solution: Nominally 2.5 mg/mL of sodium salicylate from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume of *Mobile phase*, and sonicate for 15 min. Allow the solution to cool to room temperature and then dilute with *Mobile phase* to volume. Centrifuge the solution, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Column temperature: 30°
Flow rate: 0.2 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between salicylic acid related compound A and phenol; NLT 3.0 between phenol and salicylic acid related compound B, *System suitability solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of sodium salicylate from the *Standard solution*
C_S = concentration of USP Sodium Salicylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A[a]	0.3	—
Phenol[a]	0.4	—
Salicylic acid related compound B[a]	0.6	—
Salicylic acid	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] These are process impurities, which are included in the table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩
USP Phenol RS
USP Salicylic Acid Related Compound A RS
4-Hydroxybenzoic acid.
$C_7H_6O_3$ 138.12
USP Salicylic Acid Related Compound B RS
4-Hydroxyisophthalic acid.
$C_8H_6O_5$ 182.13
USP Sodium Salicylate RS

Sodium Sulfate

$Na_2SO_4 \cdot 10H_2O$ 322.19
Sulfuric acid disodium salt, decahydrate.
Disodium sulfate decahydrate [7727-73-3].
Anhydrous 142.04
[7757-82-6].

» Sodium Sulfate contains ten molecules of water of hydration, or is anhydrous. It contains not less than 99.0 percent of Na_2SO_4, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 30°.
Labeling—Label it to indicate whether it is the decahydrate or is anhydrous.
Identification—A solution (1 in 20) responds to the tests for *Sodium* ⟨191⟩ and for *Sulfate* ⟨191⟩.

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Acidity or alkalinity—To 10 mL of a solution, containing the equivalent of 1.0 g of $Na_2SO_4 \cdot 10H_2O$ in 20 mL of water, add 1 drop of bromothymol blue TS: not more than 0.50 mL of either 0.010 N hydrochloric acid or 0.010 N sodium hydroxide is required to change the color of the solution.
Loss on drying ⟨731⟩—Dry at 105° for 4 hours: the decahydrate loses between 51.0% and 57.0% of its weight, and the anhydrous form loses not more than 0.5% of its weight.
Chloride ⟨221⟩—A portion equivalent to 1.0 g of $Na_2SO_4 \cdot 10H_2O$ shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (0.02%).
Assay—Weigh accurately a portion of Sodium Sulfate, equivalent to about 400 mg of anhydrous sodium sulfate, dissolve in 200 mL of water, and add 1 mL of hydrochloric acid. Heat to boiling, and gradually add, in small portions and while constantly stirring, an excess of hot barium chloride TS (about 8 mL). Heat the mixture on a steam bath for 1 hour, collect the precipitate of barium sulfate on a retentive, ashless filter paper, wash until free from chloride when tested with silver nitrate TS, and place the filter into a suitable tared crucible. Carefully burn away the paper, and ignite at 800 ± 25° to constant weight. The weight of the barium sulfate so obtained, multiplied by 0.6086, represents its equivalent of Na_2SO_4.

Sodium Sulfate Injection

» Sodium Sulfate Injection is a sterile, concentrated solution of Sodium Sulfate in Water for Injection, which upon dilution is suitable for parenteral use. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $Na_2SO_4 \cdot 10H_2O$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
Labeling—Label it to indicate that it is to be diluted before injection to render it isotonic (3.89% of $Na_2SO_4 \cdot 10H_2O$).
Identification—It responds to the tests for *Sodium* ⟨191⟩ and for *Sulfate* ⟨191⟩.
Pyrogen—When diluted with water for injection to contain 3.89% of $Na_2SO_4 \cdot 10H_2O$, it meets the requirements of the *Pyrogen Test* ⟨151⟩.
pH ⟨791⟩: between 5.0 and 6.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 400 mg of sodium sulfate ($Na_2SO_4 \cdot 10H_2O$), to a suitable vessel. Dilute if necessary, to 200 mL, and proceed as directed in the *Assay* under *Sodium Sulfate*, beginning with "add 1 mL of hydrochloric acid." The weight of the barium sulfate so obtained, multiplied by 1.3804, represents its equivalent of $Na_2SO_4 \cdot 10H_2O$.

Sodium Sulfide

$Na_2S \cdot 9H_2O$ 240.18
Sodium sulfide nonahydrate;
Disodium sulfide nonahydrate [1313-84-4].

DEFINITION
Sodium Sulfide contains NLT 98.0% and NMT 103.0% of sodium sulfide ($Na_2S \cdot 9H_2O$).

IDENTIFICATION
• **A.**
Conduct this test in a fume hood.
Sample: 100 mg
Analysis: To the *Sample* add 10 mL of 3 N hydrochloric acid, and cover the top of the container with filter paper saturated with a 0.2 M lead acetate solution.
Acceptance criteria: A brownish or silvery-black color forms on the paper.

ASSAY
• **PROCEDURE**
Sample solution: Dissolve 275 mg of Sodium Sulfide in 30 mL of filtered and degassed water. Add 50.0 mL of 0.1 N iodine while mixing, add 2 mL of hydrochloric acid, and allow to stand for 15 min in a dark place.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Titrate the *Sample solution* with *Titrant* to a pale yellow color. Add 5 mL of starch TS, and titrate with *Titrant* to a clear endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 12.01 mg of sodium sulfide ($Na_2S \cdot 9H_2O$).
Acceptance criteria: 98.0%–103.0%

IMPURITIES
• **LIMIT OF IRON**
Sample solution: 50 mg/mL in water
Acceptance criteria: The solution is clear and colorless.
• **LIMIT OF SULFITE AND THIOSULFATE**
Sample solution: Dissolve 3.0 g of Sodium Sulfide in 200.0 mL of filtered and degassed water. Add 100.0 mL of 0.2 N zinc sulfate, and allow to stand for 30 min. Filter, and add 1 mL of starch TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.01 N iodine VS
Endpoint detection: Visual
Analysis: Titrate 100.0 mL of the filtrate from the *Sample solution* with *Titrant*.
Acceptance criteria: NMT 3.0 mL of *Titrant* is required.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cool place.

Sodium Sulfide Topical Gel

DEFINITION
Sodium Sulfide Topical Gel contains NLT 90.0% and NMT 120.0% of the labeled amount of sodium sulfide ($Na_2S \cdot 9H_2O$) in a suitable gel base.

IDENTIFICATION
• **A.**
Conduct this test in a fume hood.
Sample: 100 mg
Analysis: Add 10 mL of 3 N hydrochloric acid to the *Sample*, and cover the top of the container with filter paper saturated with 0.2 M lead acetate solution.

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Acceptance criteria: A brownish or silvery-black color forms on the paper.

ASSAY

• **PROCEDURE**

Solution A: 67.0 g/L of edetate disodium and 36.0 g/L of ascorbic acid in 2.0 M sodium hydroxide solution

Sample solution: Transfer a portion of the Topical Gel, equivalent to 30 mg of sodium sulfide, to a 100-mL volumetric flask. Add 50 mL of *Solution A*, and shake by mechanical means until the Topical Gel is dispersed. Dilute with *Solution A* to volume.

Titrimetric system

Mode: Direct titration

Titrant: 0.01 M lead perchlorate VS

Endpoint detection: Potentiometric

Analysis: Transfer 30.0 mL of the *Sample solution* to a suitable beaker, and titrate with *Titrant*, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.01 M lead perchlorate is equivalent to 2.4018 mg of sodium sulfide ($Na_2S \cdot 9H_2O$).

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 11.5–13.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature or in a cool place.

Sodium Thiosulfate

$Na_2S_2O_3 \cdot 5H_2O$ 248.18

$Na_2S_2O_3$ 158.11

Thiosulfuric acid, disodium salt, pentahydrate; Disodium thiosulfate pentahydrate [10102-17-7]. Anhydrous [7772-98-7].

DEFINITION

Sodium Thiosulfate contains NLT 99.0% and NMT 100.5% of sodium thiosulfate ($Na_2S_2O_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A▲** (CN 1-May-2020)

• **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

Sample solution: 0.1 g/mL in water

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample: 800 mg

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N iodine VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 30 mL of water. Adjust with a suitable solution of dilute hydrochloric acid or dilute sodium hydroxide to a pH of 6.2–6.7, if necessary. Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of *Titrant* is equivalent to 15.81 mg of sodium thiosulfate ($Na_2S_2O_3$).

Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES

• **CALCIUM**

Sample solution: Dissolve 1 g in 20 mL of water.

Analysis: To the *Sample solution* add a few mL of ammonium oxalate TS.

Acceptance criteria: No turbidity is produced.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method III* ⟨921⟩

Sample: 1.0 g

Analysis: Dry the *Sample* under vacuum at 40°–45° for 16 h.

Acceptance criteria: 32.0%–37.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Thiosulfate RS

Sodium Thiosulfate Injection

DEFINITION

Sodium Thiosulfate Injection is a sterile solution of Sodium Thiosulfate in freshly boiled Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$).

IDENTIFICATION

• **A. PROCEDURE**

Sample solution: Equivalent to 0.1 g/mL of sodium thiosulfate in water

Analysis: Add a few drops of iodine TS.

Acceptance criteria: The color is discharged.

• **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

Sample solution: Equivalent to 0.1 g/mL of sodium thiosulfate in water

Acceptance criteria: Meets the requirements

• **C. IDENTIFICATION TESTS—GENERAL,** *Thiosulfate* ⟨191⟩

Sample solution: Equivalent to 0.1 g/mL of sodium thiosulfate in water

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample solution: Equivalent to 1 g of sodium thiosulfate from Injection. Adjust with a suitable solution of dilute hydrochloric acid or dilute sodium hydroxide to a pH of 6.2–6.7, and dilute with water to about 20 mL.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N iodine VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of *Titrant* is equivalent to 24.82 mg of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$).

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.03 USP Endotoxin Units/mg of sodium thiosulfate

• **pH** ⟨791⟩: 6.0–9.5

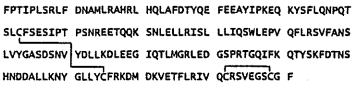
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers of Type I glass.

Somatropin



$C_{990}H_{1528}N_{262}O_{300}S_7$ 22,124.77
[12629-01-5].

DEFINITION

Somatropin is a protein hormone consisting of 191 amino acid residues, and its structure corresponds to the major component of the growth hormone extracted from human pituitary glands. It is produced as a lyophilized powder or bulk solution by methods based on recombinant DNA technology. The presence of host-cell DNA and host-cell protein impurities in Somatropin is process specific—the limits of these impurities are determined by validated methods. Manufacturers must demonstrate a correlation between the *Assay* and a validated and approved growth-promotion-based bioassay. It may contain excipients.

[NOTE—One mg of anhydrous Somatropin is equivalent to 3.0 USP Somatropin Units.]

IDENTIFICATION

- **A.** The retention time of the somatropin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Chromatographic Purity*, except that a *Standard solution* is also chromatographed and prepared by reconstituting a vial of USP Somatropin RS with *Diluent* to obtain a known concentration of about 2 mg/mL.
- **B. PEPTIDE MAPPING**

Solution A: Trifluoroacetic acid and water (1:999). Filter, and degas.

Solution B: Water, trifluoroacetic acid, and acetonitrile (100:1:899)

Solution C: 0.05 M solution of tris(hydroxymethyl) aminomethane (Tris). Adjust with hydrochloric acid to a pH of 7.5.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	80	20
40	75	25
65	50	50
70	20	80
71	100	0
86	100	0

Trypsin solution: 1 mg/mL of trypsin in *Solution C*. Store in a freezer, if necessary.

Standard stock solution: 2.0 mg/mL of USP Somatropin RS in *Solution C*

Standard solution: To 1 mL of the *Standard stock solution* add 30 µL of *Trypsin solution*. Cap the tube, and place it in a water bath at 37° for 4 h. [NOTE—If this solution is not injected immediately, store it in a freezer.]

Sample stock solution: 2.0 mg/mL of Somatropin in *Solution C*

Sample solution: To 1 mL of the *Sample stock solution* add 30 µL of *Trypsin solution*. Cap the tube, and place it in a water bath at 37° for 4 h. [NOTE—If this solution is not injected immediately, store it in a freezer.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 100 µL

Analysis
Samples: *Standard solution* and *Sample solution*, separately injected
[NOTE—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

Acceptance criteria: The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*.

- **C. SOMATROPIN BIOIDENTITY TESTS** ⟨126⟩**:** Meets the requirements

[NOTE—The bioidentity test may be performed either on the Somatropin bulk drug substance or on the finished pharmaceutical product.]

ASSAY

- **SOMATROPIN CONTENT**

Buffer solution: Dissolve 5.18 g of dibasic sodium phosphate and 3.65 g of monobasic sodium phosphate in 950 mL of water. Adjust with phosphoric acid or sodium hydroxide solution to a pH of 7.0. Dilute with water to 1000 mL.

Mobile phase: Isopropyl alcohol and *Buffer solution* (3:97). Filter, and degas.

Diluent: *Buffer solution* and water (1:1.5)

System suitability solution: Place 1 vial of USP Somatropin RS in an oven at 50° for 12–24 h. Remove from the oven, and dissolve the contents of the vial in *Diluent* to obtain a solution with a known concentration of about 1 mg/mL and a dimer content of 1%–2%.

Standard solution: Known concentration of about 1 mg/mL of USP Somatropin RS in *Diluent*

Sample solution: About 1 mg/mL of accurately weighed Somatropin in *Diluent*, or by diluting a bulk solution of Somatropin with *Diluent*. [NOTE—If necessary, the amount of protein in the solution can be determined with the test for *Total Protein Content*.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 7.8-mm × 30-cm; packing L33
Column temperature: Ambient
Flow rate: 0.6 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NMT 0.4 for the ratio of the valley height, between the dimer and the monomer, and the dimer peak height
Tailing factor: NMT 1.7 for the monomer (major) peak

USP Monographs

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Analysis
Samples: *Standard solution* and *Sample solution*, separately injected
Record the chromatograms for NLT twice the retention time of the somatropin monomer (major) peak, and measure the peak responses for the monomer.
Calculate the concentration, in mg/mL, of somatropin in the *Sample solution* taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of the monomer from the *Sample solution*
r_S = peak response of the monomer from the *Standard solution*
C_S = concentration of USP Somatropin RS in the *Standard solution* (mg/mL)

Acceptance criteria: NLT 910 µg of somatropin/mg on the anhydrous basis. When prepared as a bulk solution, it contains NLT 910 µg of somatropin/mg of total protein.

IMPURITIES

• **CHROMATOGRAPHIC PURITY**
Diluent: 0.05 M Tris in water. Adjust with hydrochloric acid to a pH of 7.5.
Mobile phase: *n*-Propyl alcohol and degassed *Diluent* (29:71). Filter.
System suitability solution: 2.0 mg/mL of Somatropin in *Diluent*. Pass through a filter to sterilize or add sodium azide to a final concentration of 0.01%, and allow to stand at room temperature for 24 h. [NOTE—Use within 48 h of preparation, or store the solution in a refrigerator until ready to use.]
Sample solution: 2.0 mg/mL of Somatropin in *Diluent*. [NOTE—Maintain the solutions between 2° and 8°, and use within 24 h. If an automatic injector is used, maintain the temperature between 2° and 8°.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L26
Column temperature: 45°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.0 between somatropin and its adjacent peak
Tailing factor: 0.9–1.8 for the somatropin (major) peak
Analysis
Sample: *Sample solution*
Calculate the percentage of impurities in the portion of Somatropin taken:

$$\text{Result} = [A_U/(A_U + A_S)] \times 100$$

A_U = sum of all the peak responses other than the somatropin (major) peak and disregarding any peak due to the solvent from the *Sample solution*
A_S = peak response of somatropin from the *Sample solution*

Acceptance criteria: NMT 6.0% of total impurities

• **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**
Buffer solution, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis
Sample: *Sample solution*
Measure the areas of the main peak and of the peaks eluting before the main peak, excluding the solvent peaks.
Calculate the percentage of high molecular weight proteins in the portion of Somatropin taken:

$$\text{Result} = [A_H/(A_H + A_M)] \times 100$$

A_H = sum of the areas of the high molecular weight peaks
A_M = peak area of the monomer from the *Sample solution*

Acceptance criteria: NMT 4% of high molecular weight proteins

SPECIFIC TESTS

• **TOTAL PROTEIN CONTENT**
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
The method is used in the calculation of total protein in the assay of bulk solution.
Buffer solution: 0.025 M solution of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 7.0.
Sample solution: Mix a weighed quantity of Somatropin bulk solution with a weighed quantity of *Buffer solution* to obtain a solution with an absorbance value between 0.5 and 1.0 at the wavelength of maximum absorbance at 280 nm.
Analysis: Determine the absorbance of the *Sample solution* using a spectrophotometric cell of path length 1 cm, at the wavelength of maximum absorbance around 280 nm and at 320 nm, using *Buffer solution* as the blank.
Calculate the protein content, in mg, in the portion of Somatropin taken:

$$\text{Result} = (A_{max} - A_{320}) \times (V/0.82)$$

A_{max} = absorbance value of the *Sample solution* at the wavelength of maximum absorbance
A_{320} = absorbance value of the *Sample solution* at 320 nm
V = volume of the *Sample solution*

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 3×10^2 cfu/g, the test being performed on 0.2–0.3 g of powder, accurately weighed.
• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*: NMT 10%, when prepared as a lyophilized powder
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 10 USP Endotoxin Units/mg of Somatropin

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store between –10° and –25°.
• **LABELING:** The labeling states that the material is of recombinant DNA origin.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Somatropin RS

Somatropin for Injection

DEFINITION

Somatropin for Injection is a sterile, lyophilized mixture of Somatropin with one or more suitable buffering and stabilizing agents. Manufacturers must demonstrate a

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correlation between the *Assay* and a validated and approved growth-promotion-based bioassay.
[NOTE—One mg of anhydrous Somatropin is equivalent to 3.0 USP Somatropin Units.]

IDENTIFICATION

- **A.** The retention time of the somatropin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Chromatographic Purity*, except that a *Standard solution* is also chromatographed and prepared by reconstituting a vial of USP Somatropin RS with *Diluent* to obtain a known concentration of about 2 mg/mL.
- **B. SOMATROPIN BIOIDENTITY TESTS** ⟨126⟩**:** Meets the requirements
[NOTE—The bioidentity test may be performed either on the Somatropin bulk drug substance or on the finished pharmaceutical product.]

ASSAY

- **SOMATROPIN CONTENT**

Buffer solution: 5.18 g of dibasic sodium phosphate and 3.65 g of monobasic sodium phosphate in 950 mL of water. Adjust with phosphoric acid or sodium hydroxide solution to a pH of 7.0. Dilute with water to 1000 mL.
Mobile phase: Isopropyl alcohol and *Buffer solution* (3:97). Filter and degas.
Diluent: *Buffer solution* and water (1:1.5)
System suitability solution: Place 1 vial of USP Somatropin RS in an oven at 50° for 12–24 h. Remove from the oven, and dissolve the contents of the vial in *Diluent* to obtain a solution with a known concentration of about 1 mg/mL and a dimer content of 1%–2%.
Standard solution: Known concentration of about 1 mg/mL of USP Somatropin RS in *Diluent*
Sample solution: Dissolve the contents of a suitable number of containers in *Diluent* to obtain a concentration of 1 mg/mL of somatropin.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 7.8-mm × 30-cm; packing L33
Column temperature: Ambient
Flow rate: 0.6 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NMT 0.4 for the ratio of the valley height, between the dimer and the monomer, and the dimer peak height
Tailing factor: NMT 1.7 for the monomer (major) peak
Analysis
Samples: *Standard solution* and *Sample solution*, separately injected
Record the chromatograms for NLT twice the retention time of the somatropin monomer (major) peak, and measure the peak responses for the monomer.
Calculate the percentage of somatropin per container:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/N) \times 100$$

r_U = peak response of the monomer from the *Sample solution*
r_S = peak response of the monomer from the *Standard solution*
C_S = concentration of USP Somatropin RS in the *Standard solution* (mg/mL)
V = total volume of the *Sample solution* (mL)
N = number of containers used to obtain the *Sample solution*

Acceptance criteria: 89.0%–110.0%

IMPURITIES

- **CHROMATOGRAPHIC PURITY**

Diluent: 0.05 M tris(hydroxymethyl)aminomethane in water. Adjust with hydrochloric acid to a pH of 7.5.
Mobile phase: *n*-Propyl alcohol and degassed *Diluent* (29:71). Filter.
System suitability solution: 2.0 mg/mL of somatropin in *Diluent*. Pass through a filter to sterilize or add sodium azide to a final concentration of 0.01%, and allow to stand at room temperature for 24 h. [NOTE—Use within 48 h of preparation, or store the solution in a refrigerator until ready to use.]
Sample solution: 2.0 mg/mL of somatropin in *Diluent*. [NOTE—Maintain the solutions between 2° and 8°, and use within 24 h. If an automatic injector is used, maintain the temperature between 2° and 8°.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L26
Column temperature: 45°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.0 between somatropin and its adjacent peak
Tailing factor: 0.9–1.8 for the somatropin (major) peak
Analysis
Sample: *Sample solution*
Calculate the percentage of impurities in the portion of Injection taken:

$$\text{Result} = [A_U/(A_U + A_S)] \times 100$$

A_U = sum of all the peak responses other than the somatropin (major) peak, disregarding any peak due to the solvent
A_S = peak response of somatropin

Acceptance criteria: NMT 12% of total impurities

- **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

Buffer solution, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *System suitability solution* and *Sample solution*
Measure the areas of the main peak and of the peaks eluting before the main peak, excluding the solvent peaks.
Calculate the percentage of high molecular weight proteins in the portion of Injection taken:

$$\text{Result} = [A_H/(A_H + A_M)] \times 100$$

A_H = sum of the areas of the high molecular weight peaks
A_M = peak area of the monomer from the *Sample solution*

Acceptance criteria: NMT 6% of high molecular weight proteins

USP Monographs

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SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 20 USP Endotoxin Units/mg of Somatropin
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 2° and 8°.
- **LABELING:** The labeling states that the material is of recombinant DNA origin.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Somatropin RS

Sorbitol Solution

DEFINITION
Sorbitol Solution is an aqueous solution containing NLT 64.0% of D-sorbitol ($C_6H_{14}O_6$). The amounts of total sugars, other polyhydric alcohols, and any hexitol anhydrides, if detected, are not included in the requirements or in the calculated amount under *General Notices, 5.60.10. Other Impurities in USP and NF Articles.*

IDENTIFICATION
- **A. PROCEDURE**
  Sample solution: Dissolve 1.4 g of Sorbitol Solution in 75 mL of water.
  Analysis: Transfer 3 mL of the *Sample solution* to a 15-cm test tube. Add 3 mL of freshly prepared catechol solution (1 in 10), and mix. Add 6 mL of sulfuric acid, mix again, and gently heat the tube in a flame for 30 s.
  Acceptance criteria: A deep pink or wine-red color appears.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. LIMIT OF DIETHYLENE GLYCOL AND ETHYLENE GLYCOL**
  Diluent: Acetone and water (96:4)
  Standard solution: 0.08 mg/mL of USP Diethylene Glycol RS and 0.08 mg/mL of USP Ethylene Glycol RS in *Diluent*
  Sample solution: Transfer 2.0 g of Sorbitol Solution to a 25-mL volumetric flask. Add 1.0 mL of *Diluent* to the flask, and mix on a vortex mixer for 3 min. Add the remaining *Diluent* to the flask to volume in three equal portions. Mix on a vortex mixer for about 3 min after each addition of *Diluent*. Pass a portion of the supernatant layer obtained through a 0.45-µm nylon filter. Discard the first 2 mL of the filtrate, and collect the rest of the filtrate for analysis. [NOTE—Acetone is used to precipitate sorbitol.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: GC
  Detector: Flame ionization
  Column: 0.32-mm × 15-m fused-silica capillary column; 0.25-µm layer of phase G46
  Temperature
  Detector: 300°
  Injector port: 240°
  Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	—	70	2
70	50	300	5

  Carrier gas: Helium
  Flow rate: 3.0 mL/min
  Injection size: 1.0 µL
  Injection type: Split injection. The split ratio is about 10:1. [NOTE—A split liner, deactivated with glass wool, is used.]
  System suitability
  Sample: *Standard solution*
  [NOTE—Diethylene glycol elutes after ethylene glycol in the chromatogram.]
  Suitability requirements
  Resolution: NLT 30 between ethylene glycol and diethylene glycol
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Based on the *Standard solution*, identify the peaks of ethylene glycol and diethylene glycol. Compare peak areas of ethylene glycol and diethylene glycol in the *Standard solution* and the *Sample solution*.
  Acceptance criteria
  Diethylene glycol: The peak area of diethylene glycol in the *Sample solution* is NMT the peak area of diethylene glycol in the *Standard solution*, corresponding to NMT 0.10% of diethylene glycol in Sorbitol Solution.
  Ethylene glycol: The peak area of ethylene glycol in the *Sample solution* is NMT the peak area of ethylene glycol in the *Standard solution*, corresponding to NMT 0.10% of ethylene glycol in Sorbitol Solution.

ASSAY
- **PROCEDURE**
  Mobile phase: Water
  System suitability solution: 4.8 mg/g of mannitol and 4.8 mg/g of USP Sorbitol RS in water
  Standard solution: 4.8 mg/g of USP Sorbitol RS in water
  Sample solution: 6.0 mg/g of Sorbitol Solution in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Refractive index
  Column: 7.8-mm × 10-cm; packing L34
  Temperature
  Detector: 35°
  Column: 50 ± 2°
  Flow rate: 0.7 mL/min
  Injection size: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for mannitol and sorbitol are 0.6 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between sorbitol and mannitol, *System suitability solution*
  Relative standard deviation: NMT 2.0% for sorbitol, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_6H_{14}O_6$ in the Sorbitol Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

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C_S = concentration of USP Sorbitol RS in the *Standard solution* (mg/g)
C_U = concentration of Sorbitol Solution in the *Sample solution* (mg/g)

Acceptance criteria: NLT 64.0%

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%, calculated on the anhydrous basis, determined on a 2-g portion
- **Limit of Nickel**

Solution A: A saturated ammonium pyrrolidine dithiocarbamate solution (containing 10 mg/mL of ammonium pyrrolidine dithiocarbamate)

Sample solution: Dissolve and dilute 20.0 g of Sorbitol Solution to 100 mL with diluted acetic acid. Add 2.0 mL of *Solution A* and 10.0 mL of methyl isobutyl ketone, and shake for 30 s. Protect from bright light. Allow the two layers to separate, and use the methyl isobutyl ketone layer.

Standard solutions: Prepared as directed for *Sample solution*, except to prepare three solutions by adding 0.5, 1.0, and 1.5 mL of nickel standard solution TS.

Blank solution: Prepare as directed for *Sample solution*, except to omit the use of the Sorbitol Solution.

Spectrometric conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 232.0 nm (maximum absorbance)

Lamp: Nickel hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions, Sample solution,* and *Blank solution*

Set the instrument to zero using the *Blank solution.* Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at least three times each. Record the average of the steady readings for each of these solutions. Between each measurement, aspirate the *Blank solution*, and ascertain that the reading returns to zero. Plot the absorbances of the *Standard solutions* and the *Sample solution* versus the added quantity of nickel. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of nickel in the *Sample solution.*

Acceptance criteria: NMT 1 ppm, calculated on the anhydrous basis

ORGANIC IMPURITIES

- **Procedure: Reducing Sugars**

Sample: Equivalent to 3.3 g of sorbitol on the anhydrous basis from Sorbitol Solution

Analysis: To the *Sample*, add 3 mL of water, 20.0 mL of cupric citrate TS, and a few glass beads. Heat so that boiling begins after 4 min, and maintain boiling for 3 min. Cool rapidly, and add 40 mL of diluted acetic acid, 60 mL of water, and 20.0 mL of 0.05 N iodine VS. With continuous shaking, add 25 mL of a mixture of 6 mL of hydrochloric acid and 94 mL of water. When the precipitate has dissolved, titrate the excess of iodine with 0.05 N sodium thiosulfate VS using 2 mL of starch TS, added toward the end of the titration, as an indicator.

Acceptance criteria: NLT 12.8 mL of 0.05 N sodium thiosulfate VS is required, corresponding to NMT 0.3% of reducing sugars, on the anhydrous basis, as glucose.

[NOTE—The amount determined in this test is not included in the calculated amount under *General Notices, 5.60.10. Other Impurities in USP and NF Articles.*]

SPECIFIC TESTS

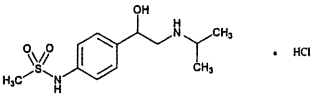
- **pH** ⟨791⟩: 5.0–7.5, in a 14% (w/w) solution of Sorbitol Solution in carbon dioxide-free water
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 28.5%–31.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Diethylene Glycol RS
USP Ethylene Glycol RS
USP Sorbitol RS

Sotalol Hydrochloride



$C_{12}H_{20}N_2O_3S \cdot HCl$ 308.83

Methanesulfonamide, *N*-[4-[1-hydroxy-2-[(1-methylethyl) amino]ethyl]phenyl]-, monohydrochloride; 4′-[1-Hydroxy-2-(isopropylamino)ethyl]methanesulfonanilide monohydrochloride [959-24-0].

DEFINITION

Sotalol Hydrochloride contains NLT 98.5% and NMT 101.5% of sotalol hydrochloride ($C_{12}H_{20}N_2O_3S \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B.**

▲The retention time of the sotalol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Transfer about 1.08 g of sodium 1-octanesulfonate to a 1000-mL volumetric flask. Dissolve in 10 mL of glacial acetic acid and about 70 mL of water. Add 720 mL of water and dilute with acetonitrile to volume.

Diluent: Acetonitrile and water (1:4)

Internal standard solution: 4.5 mg/mL of caffeine in *Diluent*

Standard stock solution: 2 mg/mL of USP Sotalol Hydrochloride RS in *Diluent*

Standard solution: 0.2 mg/mL of USP Sotalol Hydrochloride RS prepared as follows. Transfer 10.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask. Dilute with *Diluent* to volume.

Sample stock solution: 2 mg/mL of Sotalol Hydrochloride in *Diluent*

Sample solution: 0.2 mg/mL of Sotalol Hydrochloride prepared as follows. Transfer 10 mL of *Sample stock solution* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask. Dilute with *Diluent* to volume.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 3.9-mm × 30-cm; ▲10-µm▲ 2S (USP41) packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for caffeine and sotalol are 0.39 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 8.5 between caffeine and sotalol
Relative standard deviation: ▲NMT 0.55%▲ 2S (USP41)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sotalol hydrochloride ($C_{12}H_{20}N_2O_3S \cdot HCl$) in the portion of Sotalol Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of sotalol to caffeine from the *Sample solution*
R_S = peak area ratio of sotalol to caffeine from the *Standard solution*
C_S = concentration of USP Sotalol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Sotalol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5%

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.5%

Delete the following:

▲• **HEAVY METALS,** *Method II* ⟨231⟩**:** 0.002%▲ (Official 1-Jan-2018)

Delete the following:

▲• **LIMIT OF METHANOL, ISOPROPYL ALCOHOL, AND ACETONE**
Standard stock solution: Transfer 10 µL each of methanol, isopropyl alcohol, and acetone to a 100-mL volumetric flask. Dilute with N,N-dimethylacetamide to volume, and mix.
Standard solution: 0.04 mg/mL of each methanol, isopropyl alcohol, and acetone in N,N-dimethylacetamide prepared by diluting 10.0 mL of *Standard stock solution* with 10.0 mL of N,N-dimethylacetamide
Sample solution: 10 mg/mL of Sotalol Hydrochloride prepared by transferring 100 mg of Sotalol Hydrochloride to a 25-mL flask, dissolve in 10 ml of N,N-dimethylacetamide. [NOTE—Do not dilute to volume.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2.0-mm × 1.8-m; glass with 5% phase G16 on 60- to 80-mesh support S12
Temperatures
Injector: 200°
Detector: 300°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70°	—	70°	5
70°	30°	180°	3

Carrier gas: Helium
Flow rate: 30 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between methanol and acetone and between acetone and isopropyl alcohol
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of methanol, isopropyl alcohol, and acetone in the portion of Sotalol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the corresponding analyte from the *Sample solution*
r_S = peak area of the corresponding analyte from the *Standard solution*
C_S = concentration of the appropriate analyte in the *Standard solution* (mg/mL)
C_U = concentration of Sotalol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methanol	1.0	0.3
Acetone	1.4	0.3
Isopropyl alcohol	2.7	0.3
Total	—	0.5▲ 2S (USP41)

Change to read:

• **ORGANIC IMPURITIES**
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 6 µg/mL each of USP Sotalol Hydrochloride RS, USP Sotalol Related Compound A RS, USP Sotalol Related Compound B RS, and USP Sotalol Related Compound C RS in *Mobile phase*
Sample solution: 2 mg/mL of Sotalol Hydrochloride in *Mobile phase*
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between sotalol ▲▲ 2S (USP41) related compound A and sotalol ▲▲ 2S (USP41)
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of each sotalol related compound in the portion of Sotalol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak ▲response▲ 2S (USP41) of the corresponding sotalol related compound from the *Sample solution*

r_S = peak ▲response▲ 2S (USP41) of the corresponding sotalol related compound from the *Standard solution*

C_S = concentration of the corresponding Reference Standard in the *Standard solution* (µg/mL)

C_U = concentration of Sotalol Hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of ▲any unspecified impurities▲ 2S (USP41) in the portion of Sotalol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = ▲sum of all the peak responses of all unspecified impurities▲ 2S (USP41) from the *Sample solution*

r_S = peak ▲response▲ 2S (USP41) of sotalol from the *Standard solution*

C_S = concentration of USP Sotalol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Sotalol Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sotalol ▲▲ 2S (USP41) related compound B	0.65	0.3
Sotalol ▲▲ 2S (USP41)	1.0	—
Sotalol ▲▲ 2S (USP41) related compound A	1.2	0.3
Sotalol ▲▲ 2S (USP41) related compound C	1.4	0.4
▲Any unspecified impurities▲ 2S (USP41)	—	▲0.10▲ 2S (USP41)
Total impurities	—	0.5

SPECIFIC TESTS

- **CONTENT OF CHLORIDE**

Sample: 310 mg

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis: Transfer the *Sample* to a glass beaker, and dissolve in 100 mL of water and 10 mL of glacial acetic acid. Titrate with *Titrant*, and determine the endpoint potentiometrically. Each milliliter of *Titrant* is equivalent to 3.545 mg of chloride (Cl).

Acceptance criteria: 11.1%–11.9%

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 125 mg/mL in methanol

Acceptance criteria: –0.7° to +0.7°

- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sotalol Hydrochloride RS

USP Sotalol Related Compound A RS

▲*N*-[4-(Isopropylglycyl)phenyl]methanesulfonamide hydrochloride.▲ 2S (USP41)

$C_{12}H_{18}N_2O_3S \cdot HCl$ 306.81

USP Sotalol Related Compound B RS

N-(4-Formylphenyl)methanesulfonamide.

$C_8H_9NO_3S$ 199.22

USP Sotalol Related Compound C RS

▲*N*-{4-[2-(Isopropylamino)ethyl]phenyl} methanesulfonamide.▲ 2S (USP41)

$C_{12}H_{20}N_2O_2S$ ▲256.36▲ 2S (USP41)

Sotalol Hydrochloride Compounded Oral Suspension

DEFINITION

Sotalol Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sotalol hydrochloride ($C_{12}H_{20}N_2O_3S \cdot HCl$).

Prepare Sotalol Hydrochloride Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Sotalol Hydrochloride tablets[a] equivalent to	600 mg of sotalol hydrochloride
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus,[b] a sufficient quantity to make	120 mL

[a] Betapace 120-mg tablets, Berlex Laboratories, Wayne, NJ.

[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Sotalol Hydrochloride tablets* in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a sotalol hydrochloride liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 5 mM octanesulfonic acid (25:75), adjusted to a pH of 3.2. Pass through a nylon 66 filter of 0.45-µm pore size, and degas.

Standard solution: 20 µg/mL of USP Sotalol Hydrochloride RS in *Mobile phase*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 20 µg/mL of sotalol hydrochloride from Oral Suspension and *Mobile phase*. Centrifuge.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.0-mm × 15-cm; 5-µm packing L1

Flow rate: 0.4 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

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[NOTE—The retention time for sotalol hydrochloride is about 5.1 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sotalol hydrochloride ($C_{12}H_{20}N_2O_3S \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sotalol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of sotalol hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 3.8–4.8

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sotalol Hydrochloride RS

Sotalol Hydrochloride Tablets

» Sotalol Hydrochloride Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of sotalol hydrochloride ($C_{12}H_{20}N_2O_3S \cdot HCl$).

Packaging and storage—Preserve in well-closed containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Sotalol Hydrochloride RS

Identification—Weigh and powder a quantity of the Tablets, equivalent to about 250 mg of sotalol hydrochloride, and transfer to a 50-mL volumetric flask. Add 25 mL of methanol, and shake for 10 minutes. Dilute with methanol to volume, mix, and filter: the filtrate so obtained meets the requirements for *Identification* test *B* under *Sotalol Hydrochloride.*

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{12}H_{20}N_2O_3S \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 230 nm on filtered portions of the solution under test, in comparison with a Standard solution having a known concentration of USP Sotalol Hydrochloride RS in the same *Medium.*

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{12}H_{20}N_2O_3S \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Assay—

Phosphate buffer—Dissolve 6.8 g of monobasic potassium phosphate in about 800 mL of water. Dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (9:1).

Internal standard solution—Transfer about 1.8 g of caffeine, accurately weighed, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume, and mix.

Standard preparation—Transfer about 80 mg of USP Sotalol Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask. Add 2.5 mL of 1 N hydrochloric acid, dissolve in and dilute with water to volume, mix, and filter. Transfer 10.0 mL of the filtrate and 25.0 mL of the *Internal standard solution* to a 200-mL volumetric flask. Dilute with water to volume, and mix.

Assay preparation—Transfer an appropriate number of Tablets, equivalent to about 800 mg of sotalol hydrochloride, to a 250-mL volumetric flask. Add 25 mL of 1 N hydrochloric acid, and swirl. Dilute with water to about three-fourths of the volume of the flask, and shake for 15 minutes. Dilute with water to volume, mix, and filter. Transfer 10.0 mL of this solution and 25.0 mL of the *Internal standard solution* to a 200-mL volumetric flask. Dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the relative retention times for sotalol and caffeine are about 1.0 and 2.0, respectively; the resolution, *R,* between caffeine and sotalol is not less than 6.0; and the relative standard deviation for replicate injections, determined from peak area ratios, is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of sotalol hydrochloride ($C_{12}H_{20}N_2O_3S \cdot HCl$) in the portion of Tablets taken by the formula:

$$5000C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Sotalol Hydrochloride RS in the *Standard preparation;* and R_U and R_S are the peak area ratios of sotalol to caffeine obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Soybean Oil

[8001-22-7].

DEFINITION

Soybean Oil is the refined fixed oil obtained from the seeds of the soya plant *Glycine max* Merr. (Fam. Fabaceae). It may contain suitable antioxidants.

IDENTIFICATION

- **A. IDENTITY BY FATTY ACID COMPOSITION**
  Analysis: Proceed as directed in the test for *Fats and Fixed Oils, Fatty Acid Composition.*
  Acceptance criteria: Meets the composition profile of fatty acids in *Table 1*

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• **B. Identity by Triglyceride Profile**
Analysis: Proceed as directed in *Identification of Fixed Oils by Thin-Layer Chromatography* ⟨202⟩.
Acceptance criteria: Meets the requirements in the chapter

IMPURITIES

• **Alkaline Impurities**
Sample: 10 mL
Analysis: Mix 10 mL of acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. If necessary, neutralize the solution to a green color with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide. Add the *Sample*, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid VS to change the color of the upper layer to yellow.
Acceptance criteria: NMT 0.1 mL of 0.01 N hydrochloric acid is required.

SPECIFIC TESTS

• **Fats and Fixed Oils,** *Acid Value* ⟨401⟩**:** NMT 0.3
• **Fats and Fixed Oils,** *Peroxide Value* ⟨401⟩
Solution A: Glacial acetic acid and chloroform (60:40)
Solution B: Prepare a saturated solution of potassium iodide in freshly boiled and cooled water, and store it protected from light. Discard it if it gives a color on addition of *Solution A* and starch TS.
Sample: 10 g
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.01 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a conical flask. Add 30 mL of *Solution A*, and swirl to dissolve. Add 0.5 mL of *Solution B*, swirl the flask for 1 min, accurately timed, and add 30 mL of water. Titrate with *Titrant*, with vigorous agitation, to a light yellow color. Add 0.5 mL of starch TS, and continue the titration until the blue color has disappeared. Perform a blank test, and make any necessary correction.
Calculate the peroxide value:

$$\text{Result} = [(V \times N)/W] \times F$$

V = volume of sodium thiosulfate (mL)
N = normality of the sodium thiosulfate solution (mEq/mL)
W = weight of Soybean Oil taken (g)
F = conversion factor, 1000 g/kg

Acceptance criteria: NMT 10.0
• **Fats and Fixed Oils,** *Fatty Acid Composition* ⟨401⟩**:**
Soybean Oil exhibits the composition profile of fatty acids shown in *Table 1*, as determined in the chapter.

Table 1

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
<14	0	≤0.1
14	0	≤0.2
16	0	9.0–13.0
16	1	≤0.3
18	0	2.5–5.0
18	1	17.0–30.0
18	2	48.0–58.0
18	3	5.0–11.0
20	0	≤1.0
20	1	≤1.0
22	0	≤1.0
22	1	≤0.3
24	0	≤0.5

• **Fats and Fixed Oils,** *Unsaponifiable Matter* ⟨401⟩**:** NMT 1.5%
• **Sterol Composition**
Sample A: Transfer 5 g of Soybean Oil to a 250-mL conical flask, add 50 mL of an alcoholic potassium hydroxide solution prepared by dissolving 12 g of potassium hydroxide in 10 mL of water and diluting with alcohol to 100 mL, and heat the flask on a steam bath under a suitable condenser to maintain reflux for 1 h, swirling frequently. Cool to a temperature below 25°, and transfer the contents of the flask to a separator with a polytetrafluoroethylene stopcock, rinsing the flask with two 50-mL portions of water that are added to the separator (do not use grease on the stopcock). Extract with three 100-mL portions of ether, combining the ether extracts in another separator containing 40 mL of water. Gently rotate or shake the separator for a few minutes. [Note—Violent agitation may result in the formation of a difficult-to-separate emulsion.] Allow the mixture to separate, and discard the lower aqueous phase. Wash the ether extract with two additional 40-mL portions of water, and discard the lower aqueous phase. Wash the ether extract successively with a 40-mL portion of potassium hydroxide solution (3 in 100) and a 40-mL portion of water. Repeat this potassium hydroxide solution–water wash sequence three times. Wash the ether extract with 40-mL portions of water until the last washing is not reddened by the addition of 2 drops of phenolphthalein TS. Transfer the ether extract to a tared flask, and rinse the separator with 10 mL of ether, adding the rinsings to the flask. Evaporate the ether on a steam bath, and add 6 mL of acetone to the residue. Remove the acetone in a current of air, and dry the residue at 105°.
Reference A: Transfer 5 g of sunflower oil to a 250-mL conical flask, and proceed as directed in *Sample A*, beginning with "add 50 mL of an alcoholic potassium hydroxide solution prepared by dissolving 12 g of potassium hydroxide in 10 mL of water and diluting with alcohol to 100 mL".
Separation of the sterol fraction by LC
Mobile phase: Isopropyl alcohol and *n*-hexane (1:99)
Sample solution A: Transfer *Sample A* with three 4-mL quantities of ether to a 15-mL test tube. Evaporate to dryness under a stream of nitrogen. Dissolve *Sample A* in *Mobile phase* to obtain a solution with an approximate concentration of 40 mg/mL. Add a few drops of isopropyl alcohol to improve the solubility. [Note—3 drops are normally sufficient to ensure complete solubilization.] Pass through a membrane filter (nominal 0.45-µm pore size).
Reference solution A: Prepare as directed for *Sample solution A* except use *Reference A* instead of *Sample A*.
Chromatographic system
(See *Chromatography* ⟨621⟩.)
Mode: LC
Detector: UV 210 nm
Columns
Guard: 4.6-mm × 0.5-cm (or 4.6-mm × 1.0-cm); 5-µm packing L3 with a 6-nm pore size
Analytical: 4.6-mm × 25-cm; 5-µm packing L3 with a 6-nm pore size

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Flow rate: 1.0 mL/min
Injection volume: 50 µL

Identification of peaks due to sterols
Samples: *Sample solution A* and *Reference solution A*
Sterol identification: The sterol fraction elutes at the end of the chromatogram. Locate the fraction to be collected by using the chromatogram from *Reference solution A*. The chromatogram from *Reference solution A* shows two or three principal peaks, which elute at approximately 21–35 min depending on the column used.
Sterol collection: Collect the fraction at the detector outlet in a 15-mL tube with a screw cap. Evaporate the solvent under a stream of nitrogen. [NOTE—If necessary, to increase the sample amount for later analysis, make the second injection of 50 µL on the HPLC column and collect the fraction at the detector outlet in the same 15-mL test tube with a screw cap. Evaporate the solvent under a stream of nitrogen.]

Determination of sterols by GC
Sample solution B: Dissolve the residue of the sterol fraction obtained from *Sample solution A* in the previous LC step in 0.2 mL of anhydrous pyridine and 0.2 mL of a mixture of 1 volume of chlorotrimethylsilane and 99 volumes of bis(trimethylsilyl)trifluoroacetamide. Insert the stopper into the test tube tightly and heat at 80° for 20 min. Allow to cool and use the liquid phase.
Reference solution B: Dissolve 9 parts of the residue of the sterol fraction obtained from *Reference solution A* in the previous LC step, 1 part of cholesterol in 0.2 mL of anhydrous pyridine, and 0.2 mL of a mixture of 1 volume of chlorotrimethylsilane and 99 volumes of bis(trimethylsilyl)trifluoroacetamide. Insert the stopper into the test tube tightly, and heat at 80° for 20 min. Allow to cool and use the liquid phase.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 30-m fused-silica capillary; 0.25-µm layer of phase G27
Temperatures
Injection port: 290°
Detector: 290°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
260	—	260	38
260	5	290	5

Carrier gas: Helium
Flow rate: 2.6 mL/min
Injection volume: 1–3 µL (depending on the expected amount of sterols in the test sample).
Injection type: Split injection; split ratio is 25:1

System suitability
Sample: *Reference solution B*
The chromatogram from *Reference solution B* shows five principal peaks corresponding to cholesterol, campesterol, stigmasterol, β-sitosterol, and Δ7-stigmastenol.
[NOTE—The retention times of the sterols with reference to β-sitosterol are given in *Table 3*.]

Table 3

Identification	Relative Retention Time
Cholesterol	0.65
Brassicasterol	0.71
24-Methylene-cholesterol	0.81
Campesterol	0.82
Campestanol	0.84
Stigmasterol	0.88
Δ7-Campesterol	0.93
Δ5,23-Stigmastadienol	0.95
Clerosterol	0.96
β-Sitosterol	1.00
Sitostanol	1.02
Δ5-Avenasterol	1.03
Δ5,24-Stigmastadienol	1.09
Δ7-Stigmastenol	1.13
Δ7-Avenasterol	1.17

Suitability requirements
Resolution: NLT 3.0 between the campesterol and stigmasterol peaks

Analysis
Samples: *Sample solution B* and *Reference solution B*
Use the chromatogram from *Reference solution B* to identify the peaks due to cholesterol, campesterol, stigmasterol, β-sitosterol and Δ7-stigmastenol. Identify the peaks due to the sterols in the chromatogram from *Sample solution B* using the chromatograms from *Reference solution B* and the relative retention times with reference to β-sitosterol (main peak) given in *Table 3*.
Calculate the percentage of the content of each sterol in the sterol fraction of Soybean Oil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

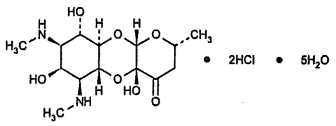
r_U = area of the peak due to the sterol component to be determined
r_T = sum of the areas of the peaks due to the components indicated in *Table 3*

Acceptance criteria: NMT 0.3% of brassicasterol
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 0.1%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **LABELING:** Label it to indicate the name and quantity of any added antioxidant. Where Soybean Oil is intended for use in the manufacture of injectable dosage forms, it is so labeled.
- **OTHER REQUIREMENTS:** For Soybean Oil intended for use in injectable dosage forms, which is specified in the labeling, the requirements for *Unsaponifiable Matter, Acid Value, Peroxide Value,* and *Water, Method Ic* in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Vehicles and added substances, Nonaqueous vehicles* must be met.

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Spectinomycin Hydrochloride



$C_{14}H_{24}N_2O_7 \cdot 2HCl \cdot 5H_2O$ 495.35
4*H*-Pyrano[2,3-*b*][1,4]benzodioxin-4-one, decahydro-4a,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)-, dihydrochloride, pentahydrate, [2*R*-(2α,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ)]-.
(2*R*,4a*R*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-Decahydro-4a,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)-4*H*-pyrano[2,3-*b*]1,4]benzodioxin-4-one dihydrochloride pentahydrate [22189-32-8].
Anhydrous 405.28
[21736-83-4].

» Spectinomycin Hydrochloride has a potency equivalent to not less than 603 µg of spectinomycin ($C_{14}H_{24}N_2O_7$) per mg.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Spectinomycin Hydrochloride RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy* **: *197M*** ▲ (CN 1-May-2020)—Do not dry specimen.
Crystallinity ⟨695⟩: meets the requirements.
Bacterial Endotoxins Test ⟨85⟩ —Where the label states that Spectinomycin Hydrochloride is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.09 USP Endotoxin Unit per mg of spectinomycin.
Sterility Tests ⟨71⟩ —Where the label states that Spectinomycin Hydrochloride is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 3.8 and 5.6, in a solution containing 10 mg per mL.
Water Determination, *Method I* ⟨921⟩: between 16.0% and 20.0%.
Residue on ignition ⟨281⟩: not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Assay—
Internal standard solution—Dissolve triphenylantimony in dimethylformamide to obtain a solution containing about 2 mg per mL.
Standard preparation—Transfer about 30 mg of USP Spectinomycin Hydrochloride RS, accurately weighed, to a glass-stoppered, 25-mL conical flask. Add 10.0 mL of *Internal standard solution* and 1.0 mL of hexamethyldisilazane, and shake intermittently for 1 hour.
Assay preparation—Proceed as directed under *Standard preparation* using Spectinomycin Hydrochloride.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and contains a 3-mm × 60-cm glass column packed with 5 percent phase G27 on 80- to 100-mesh support S1AB. The column and detector are maintained at about 190° and 220°, respectively, and the injection port at about 215°, and dry helium is used as the carrier gas at a flow rate of about 45 mL per minute. Chromatograph the *Standard preparation,* and record the chromatogram as directed for *Procedure:* the resolution, *R,* between the major peaks is not less than 2.0; and the relative standard deviation of the peak response ratios, R_S, from replicate injections of the *Standard preparation* is not more than 3.5%.
Procedure—Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the ratio, R_U, of the response of the spectinomycin peak to the response of the internal standard peak in the chromatogram from the *Assay preparation,* and similarly calculate the ratio, R_S, in the chromatogram from the *Standard preparation.* Calculate the quantity, in µg, of $C_{14}H_{24}N_2O_7$ in the portion of Spectinomycin Hydrochloride taken to prepare the *Assay preparation* by the formula:

$$P(W_S)(R_U/R_S)$$

in which *P* is the potency of USP Spectinomycin Hydrochloride RS, in µg of spectinomycin per mg; and W_S is the weight, in mg, of USP Spectinomycin Hydrochloride RS taken from the *Standard preparation;* and the other terms are as defined above.

Spectinomycin for Injectable Suspension

» Spectinomycin for Injectable Suspension contains an amount of Spectinomycin Hydrochloride equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of spectinomycin ($C_{14}H_{24}N_2O_7$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Spectinomycin Hydrochloride RS

Change to read:

Identification—▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020). Do not dry specimen.
pH ⟨791⟩: between 4.0 and 7.0, in the suspension constituted as directed in the labeling.
Other requirements—It conforms to the Definition, and meets the requirements for *Crystallinity, Bacterial endotoxins, Sterility, Water,* and *Residue on ignition* under *Spectinomycin Hydrochloride.* It meets also the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Internal standard solution , *Standard preparation,* and *Chromatographic system*—Prepare as directed in the *Assay* under *Spectinomycin Hydrochloride.*

USP Monographs

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Assay preparation 1—Suspend the contents of 1 container of Spectinomycin for Injectable Suspension in water, and dilute quantitatively with water to obtain a stock solution containing about 20 mg of spectinomycin per mL. Transfer 1.0 mL of this solution to a glass-stoppered, 25-mL conical flask, and freeze-dry. Add 10.0 mL of *Internal standard solution* and 1.0 mL of hexamethyldisilazane, and shake intermittently for 1 hour.

Assay preparation 2 (where the label states the quantity of spectinomycin in a given volume of constituted suspension)—Constitute 1 container of Spectinomycin for Injectable Suspension in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Dilute an accurately measured portion of the constituted suspension quantitatively with water to obtain a stock solution containing about 20 mg of spectinomycin per mL. Transfer 1.0 mL of this solution to a glass-stoppered, 25-mL conical flask, and freeze-dry. Add 10.0 mL of *Internal standard solution* and 1.0 mL of hexamethyldisilazane, and shake intermittently for 1 hour.

Procedure—Proceed as directed in the *Assay* under *Spectinomycin Hydrochloride*. Calculate the quantity, in g, of $C_{14}H_{24}N_2O_7$ in the container of Spectinomycin for Injectable Suspension taken to prepare *Assay preparation 1* taken by the formula:

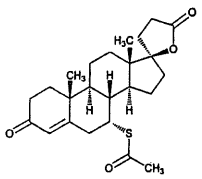
$$(L_1/D_1)(P/1000)(W_S)(R_U/R_S)$$

in which L_1 is the labeled quantity, in g, of $C_{14}H_{24}N_2O_7$ in the container, and D_1 is the concentration, in mg per mL, of spectinomycin in the stock solution used to prepare *Assay preparation 1*, on the basis of the labeled quantity in the container and the extent of dilution, and the other terms are as defined therein. Calculate the quantity, in mg, of $C_{14}H_{24}N_2O_7$ in each mL of constituted Injectable Suspension taken to prepare *Assay preparation 2* taken by the formula:

$$(L_2/D_2)(P/1000)(W_S)(R_U/R_S)$$

in which L_2 is the labeled quantity, in mg, of $C_{14}H_{24}N_2O_7$ in each mL of constituted suspension of Spectinomycin for Injectable Suspension, and D_2 is the concentration, in mg per mL, of spectinomycin in the stock solution used to prepare *Assay preparation 2*, on the basis of the labeled quantity in each mL of constituted suspension and the extent of dilution.

Spironolactone



$C_{24}H_{32}O_4S$ 416.57

Pregn-4-ene-21-carboxylic acid, 7-(acetylthio)-17-hydroxy-3-oxo-, γ-lactone, (7α,17α)-;
17-Hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate;
(2′*R*)-7α-(Acetylthio)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione [52-01-7].

DEFINITION

Spironolactone contains NLT 97.0% and NMT 103.0% of spironolactone ($C_{24}H_{32}O_4S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and water (60:40)
Standard solution: 0.5 mg/mL of USP Spironolactone RS in a mixture of acetonitrile and water (1:1)
Sample solution: 0.5 mg/mL of Spironolactone in a mixture of acetonitrile and water (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of spironolactone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.10%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of spironolactone ($C_{24}H_{32}O_4S$) in the portion of Spironolactone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of spironolactone from the *Sample solution*
r_S = peak response of spironolactone from the *Standard solution*
C_S = concentration of USP Spironolactone RS in the *Standard solution* (mg/mL)
C_U = concentration of Spironolactone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **LIMIT OF MERCAPTO COMPOUNDS**

Sample solution: Shake 2.0 g with 30 mL of water, and filter.
Analysis: To 15 mL of the *Sample solution* add 3 mL of starch TS, and titrate with 0.010 N iodine. Perform a blank determination, and make any necessary correction.
Acceptance criteria: NMT 0.10 mL of 0.010 N iodine is consumed.

- **ORGANIC IMPURITIES**

Diluent: Acetonitrile and water (50:50)
Mobile phase: Acetonitrile, tetrahydrofuran, methanol, and water (15:20:425:540)
Standard stock solutions: 2 mg/mL of USP Spironolactone RS and 0.2 mg/mL each of USP Spironolactone Related Compound A RS, USP Spironolactone Related Compound B RS, USP Spironolactone Related Compound C RS, USP Spironolactone Related Compound D RS, USP Spironolactone Epimer Mixture RS, and USP Spironolactone Related Compound I RS prepared

USP Monographs

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separately as follows. Dissolve each suitable amount of the corresponding USP Reference Standard in a suitable volumetric flask in about 10% of the total volume of tetrahydrofuran. Dilute with *Diluent* to volume.

Standard solution: 0.002 mg/mL of USP Spironolactone RS and 0.006 mg/mL of USP Spironolactone Related Compound A RS in *Diluent* from the corresponding *Standard stock solutions*

System suitability solution: 0.02 mg/mL of USP Spironolactone RS and 0.006 mg/mL each of USP Spironolactone Related Compound A RS, USP Spironolactone Related Compound B RS, USP Spironolactone Related Compound C RS, USP Spironolactone Related Compound D RS, USP Spironolactone Epimer Mixture RS, and USP Spironolactone Related Compound I RS in *Diluent* from the corresponding *Standard stock solutions*

Sensitivity solution: 0.001 mg/mL of USP Spironolactone RS in *Diluent* from the corresponding *Standard stock solution*

Sample solution: 2 mg/mL of Spironolactone prepared as follows. Dissolve a suitable amount of Spironolactone in about 10% of the total volume of tetrahydrofuran and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2.8 times the retention time of the spironolactone peak

System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.2 between spironolactone and spironolactone related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0% for the spironolactone peak, *Standard solution*
Signal-to-noise ratio: NLT 20, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of spironolactone related compound A in the portion of Spironolactone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of spironolactone related compound A from the *Sample solution*
r_S = peak response of spironolactone related compound A from the *Standard solution*
C_S = concentration of USP Spironolactone Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Spironolactone in the *Sample solution* (mg/mL)

Calculate the percentage of each impurity, other than spironolactone related compound A, in the portion of Spironolactone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity, other than spironolactone related compound A, from the *Sample solution*
r_S = peak response of spironolactone from the *Standard solution*
C_S = concentration of USP Spironolactone RS in the *Standard solution* (mg/mL)
C_U = concentration of Spironolactone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Spironolactone related compound B	0.95	0.2
Spironolactone	1.0	—
Spironolactone related compound A	1.2	0.2
Spironolactone related compound C	1.5	0.2
Spironolactone related compound D	1.6	0.3
Spironolactone epimer	1.7	0.3
Spironolactone related compound I	1.9	0.1
Any unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

- **Optical Rotation** ⟨781S⟩, *Procedures*, *Specific Rotation*
  Sample solution: 10 mg/mL in alcohol
  Acceptance criteria: –41° to –45°
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩
  USP Spironolactone RS
  USP Spironolactone Related Compound A RS
  (2′*R*)-3′,4′-Dihydro-5′*H*-spiro[androst-4,6-diene-17,2′-furan]-3,5′-dione.
  $C_{22}H_{28}O_3$ 340.46
  USP Spironolactone Related Compound B RS
  (2′*R*)-7α-(Acetylthio)-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione.
  $C_{24}H_{30}O_4S$ 414.56
  USP Spironolactone Related Compound C RS
  (2′*R*)-3′,4′-Dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione.
  $C_{22}H_{30}O_3$ 342.47
  USP Spironolactone Related Compound D RS
  (2′*R*)-7α-(Acetyldisulfanyl)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione.
  $C_{24}H_{32}O_4S_2$ 448.64
  USP Spironolactone Epimer Mixture RS
  It contains a mixture of approximately 39% of spironolactone and 56% of 7-epispironolactone:
  (2′*R*)-7β-(Acetylthio)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione.
  $C_{24}H_{32}O_4S$ 416.57
  USP Spironolactone Related Compound I RS
  S-[17α-(Ethoxymethyl)-17-hydroxy-3-oxoandrost-4-en-7α-yl]ethanethioate.
  $C_{24}H_{36}O_4S$ 420.61

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Spironolactone Compounded Oral Suspension

DEFINITION

Spironolactone Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$). Prepare Spironolactone Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Spironolactone tablet(s)[a] equivalent to	500 mg
Ora-Blend[b], a sufficient quantity to make	100 mL

[a] Spironolactone 25-mg tablet, Qualitest Pharmaceuticals, Huntsville, AL.
[b] Perrigo Pharmaceuticals, Allegan, MI.

Crush the *Spironolactone tablet(s)* to a fine powder, and pass through a 40-mesh sieve. Wet the powder with a small amount of *Ora-Blend*, and triturate to make a smooth paste. Add the *Ora-Blend* to make the mortar contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of the *Ora-Blend*. Add sufficient *Ora-Blend* to bring to final volume. Shake to mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Mix 435 mL of water with 2.7 mL of phosphoric acid and 50 mL of methanol. Combine the solution with 515 mL of acetonitrile and mix well. Filter and degas.

Standard solution: 0.2 mg/mL of USP Spironolactone RS in *Mobile phase*

Sample solution: Shake each bottle of Oral Suspension thoroughly. Transfer 1.0 mL of Oral Suspension into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well to dissolve.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for spironolactone is about 10.7 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of spironolactone from the *Sample solution*
r_S = peak response of spironolactone from the *Standard solution*
C_S = concentration of spironolactone in the *Standard solution* (mg/mL)
C_U = nominal concentration of spironolactone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.6–4.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Spironolactone RS

Spironolactone Compounded Oral Suspension, Veterinary

DEFINITION

Spironolactone Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$).

Prepare Spironolactone Compounded Oral Suspension, Veterinary 25 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Spironolactone tablets[a] equivalent to	2.5 g
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus[b], a sufficient quantity to make	100 mL

[a] Spironolactone 25-mg tablets, Mylan, Morgantown, WV.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder with a pestle. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a spironolactone liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 20 mM dibasic ammonium phosphate (55:45). Filter and degas.

Diluent: Acetonitrile and water (50:50)

Standard stock solution: 1.0 mg/mL of USP Spironolactone RS in *Diluent*

Standard solution: Pipet 2.5 mL of the *Standard stock solution* to a 10-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution with a nominal concentration of 0.25 mg/mL.

Sample solution: Shake each bottle of Oral Suspension, Veterinary thoroughly by hand. Pipet 1.0 mL of the Oral Suspension, Veterinary into a 100-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution with a nominal concentration of 0.25 mg/mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for spironolactone is about 10.4 min.]
Suitability requirements
Column efficiency: NLT 9000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of spironolactone in the *Standard solution* (mg/mL)
C_U = nominal concentration of spironolactone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **PH** ⟨791⟩**:** 3.7–4.9

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label to indicate that it is for veterinary use only.
• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded, when stored at 2°–8° or controlled room temperature
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Spironolactone RS

Spironolactone Tablets

DEFINITION
Spironolactone Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$).

IDENTIFICATION
• **A.**
Standard solution: 4 mg/mL of USP Spironolactone RS in methanol
Sample solution: Equivalent to 4 mg/mL of spironolactone from finely powdered Tablets in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Developing solvent system: Chloroform, ethyl acetate, and methanol (20:20:10)
Application volume: 10 µL
Analysis: Develop the plate until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol and water (600:400)
Diluent: Acetonitrile and water (50:50)
Standard solution: 0.5 mg/mL of USP Spironolactone RS in *Diluent*
Sample stock solution: Weigh NLT 10 Tablets, and transfer to a suitable volumetric flask. [NOTE—The target concentration is about 1 mg/mL.] Add a sufficient quantity of *Diluent*, shake for about 30 min, and sonicate for 30 min or until the Tablets are disintegrated. Cool the solution to room temperature, dilute with *Diluent* to volume, and centrifuge a suitable portion of the mixture.
Sample solution: Nominally 0.5 mg/mL in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Spironolactone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of spironolactone in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid containing 0.1% of sodium lauryl sulfate; 1000 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: A known concentration of USP Spironolactone RS in *Medium*. [NOTE—A volume of alcohol not exceeding 1% of the final volume of the solution may be used to prepare the *Standard solution*.]
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, as necessary in comparison with the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 242 nm
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Determine the amount of spironolactone ($C_{24}H_{32}O_4S$) dissolved using UV absorption of the samples used.

Tolerances: NLT 75% (Q) of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Spironolactone RS

Spironolactone and Hydrochlorothiazide Compounded Oral Suspension

DEFINITION

Spironolactone and Hydrochlorothiazide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

Prepare an oral suspension containing 5 mg/mL of spironolactone and 5 mg/mL of hydrochlorothiazide as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Spironolactone and Hydrochlorothiazide tablets[a] equivalent to	500 mg of spironolactone and 500 mg of hydrochlorothiazide
Vehicle: a 1:1 mixture of Ora-Sweet[b] (regular or sugar-free) and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Spironolactone and hydrochlorothiazide 25-mg/25-mg tablets, Mylan Pharmaceutical Inc., Morgantown, WV.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Spironolactone and Hydrochlorothiazide tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a spironolactone and hydrochlorothiazide liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and water (70:30). Filter and degas.

Standard solution: 0.1 mg/mL of USP Spironolactone RS and 0.1 mg/mL of USP Hydrochlorothiazide RS in methanol

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.1 mg/mL of spironolactone and 0.1 mg/mL of hydrochlorothiazide from Oral Suspension and methanol. Centrifuge.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention times for hydrochlorothiazide and spironolactone are about 3.5 and 7.4 min, respectively.]
Suitability requirements
Relative standard deviation: NMT 2.0% for spironolactone and NMT 2.0% for hydrochlorothiazide for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Spironolactone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of spironolactone in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Oral Suspension taken by the same formula, changing the terms to refer to hydrochlorothiazide.

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded, when stored in a refrigerator or controlled at room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydrochlorothiazide RS
  USP Spironolactone RS

Spironolactone and Hydrochlorothiazide Tablets

DEFINITION

Spironolactone and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of spironolactone ($C_{24}H_{32}O_4S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and water (700:300)

Standard solution: 50 µg/mL each of USP Spironolactone RS and USP Hydrochlorothiazide RS in methanol

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Sample stock solution: Nominally equivalent to about 0.25 mg/mL of spironolactone prepared as follows. Transfer a portion of NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add about 70 mL of methanol, shake by mechanical means for 30 min, dilute with methanol to volume, mix, and centrifuge.
Sample solution: Transfer 20.0 mL of the supernatant clear liquid of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for hydrochlorothiazide and spironolactone are 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between hydrochlorothiazide and spironolactone
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of spironolactone ($C_{24}H_{32}O_4S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of spironolactone or hydrochlorothiazide from the *Sample solution*
r_S = peak response of spironolactone or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Spironolactone RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of spironolactone or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid containing 0.1% sodium lauryl sulfate; 900 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: 12.5 µg/mL each of USP Spironolactone RS and USP Hydrochlorothiazide RS in a mixture of methanol and *Medium* (1:1)
Sample solution: Transfer a 5.0-mL portion of the solution under test to a 10-mL volumetric flask, and dilute with methanol to volume.
Solution A: Acetonitrile
Solution B: 4.5 g/L of monobasic potassium phosphate in water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	25	75
10	75	25
18	75	25
25	25	75

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for hydrochlorothiazide and spironolactone are 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between hydrochlorothiazide and spironolactone
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 75% (*Q*) of each of the labeled amounts of spironolactone ($C_{24}H_{32}O_4S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Content Uniformity* for both spironolactone ($C_{24}H_{32}O_4S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydrochlorothiazide RS
USP Spironolactone RS

Stannous Fluoride

SnF_2 156.71
Tin fluoride (SnF_2).
Tin fluoride (SnF_2) [7783-47-3].

» Stannous Fluoride contains not less than 71.2 percent of stannous tin (Sn^{++}), and not less than 22.3 percent and not more than 25.5 percent of fluoride (F), calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Sodium Fluoride RS

Identification—
A: To 5 mL of a solution (1 in 100) in a test tube add 2 mL of calcium chloride TS: a fine, white precipitate of calcium fluoride is formed.
B: Mix on a spot plate 2 drops of a solution (1 in 100) with 2 drops of silver nitrate TS: a brown-black precipitate is formed.
C: Add 1 drop of a solution (1 in 100) to 2 drops of mercuric chloride TS: a white, silky precipitate is formed. On further

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addition of the solution (1 in 100), a brown-black precipitate is formed.

pH ⟨791⟩: between 2.8 and 3.5, in a freshly prepared 0.4% solution.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Water-insoluble substances—Transfer about 10 g, accurately weighed, to a 400-mL plastic beaker, add 200 mL of water, and stir with a plastic rod for 3 minutes, or until no more solid dissolves. Filter through a tared filtering crucible, and wash thoroughly, first with ammonium fluoride solution (1 in 100), then with water. [NOTE—Prepare and use the filtering crucible in a well-ventilated hood.] Dry the residue at 105° for 4 hours, cool, and weigh: the weight of the residue does not exceed 0.2%.

Antimony—

Rhodamine B solution—Dissolve 20 mg of rhodamine B in 200 mL of 0.5 N hydrochloric acid.

Standard preparation—Transfer 55.0 mg of antimony potassium tartrate, accurately weighed, to a 200-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, add 6 N hydrochloric acid to volume, and mix.

Test preparation—Transfer 1.0 g of Stannous Fluoride, accurately weighed, to a 50-mL volumetric flask, add 6 N hydrochloric acid to volume, and mix.

Procedure—Pipet 5 mL each of the *Standard preparation* and the *Test preparation* into separate 125-mL separators, add 15 mL of hydrochloric acid and 1 g of ceric sulfate, and allow to stand for 5 minutes, with occasional shaking. Add 500 mg of hydroxylamine hydrochloride, and shake for 1 minute. Pipet 15 mL of isopropyl ether into the mixture, shake for 30 seconds, add 7 mL of water, and mix. Cool in a water bath at room temperature for 10 minutes, shake for 30 seconds, allow the layers to separate, and discard the aqueous phase. Add 20 mL of *Rhodamine B solution*, shake for 30 seconds, and discard the aqueous layer. Decant the ether layer from the top of the separator, and centrifuge, if necessary, to obtain a clear solution. Concomitantly determine the absorbances of the ether solutions from the *Test preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 550 nm, with a suitable spectrophotometer, using water as the blank: the absorbance of the *Test preparation* does not exceed that of the *Standard preparation* (0.005%).

Assay for stannous ion—

0.1 N Potassium iodide-iodate—In a 1000-mL volumetric flask, dissolve 3.567 g of potassium iodate, previously dried at 110° to constant weight, in 200 mL of oxygen-free water containing 1 g of sodium hydroxide and 10 g of potassium iodide, dilute with oxygen-free water to volume, and mix. Standardize this solution by titrating a solution prepared from an accurately weighed quantity of reagent tin (Sn) and hydrochloric acid. Each mL of *0.1 N Potassium iodide-iodate* is equivalent to 5.935 mg of Sn.

Procedure—Transfer about 250 mg of Stannous Fluoride, accurately weighed, to a 500-mL conical flask, and add 300 mL of hot, recently boiled 3 N hydrochloric acid. While passing a stream of an oxygen-free inert gas over the surface of the liquid, swirl the flask to dissolve the Stannous Fluoride, and cool to room temperature. Add 5 mL of potassium iodide TS, and titrate in an inert atmosphere with *0.1 N Potassium iodide-iodate*, adding 3 mL of starch TS as the endpoint is approached. Each mL of *0.1 N Potassium iodide-iodate* is equivalent to 5.935 mg of Sn^{++}.

Assay for fluoride— [NOTE—Store all solutions, except *Buffer solution*, in plastic containers.]

Buffer solution—Dissolve 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of (1,2-cyclohexylenedinitrilo) tetraacetic acid in 500 mL of water. Adjust with 5 N sodium hydroxide to a pH of 5.25 ± 0.25, dilute with water to 1000 mL, and mix.

Standard preparations—Dissolve an accurately weighed quantity of USP Sodium Fluoride RS quantitatively in water to obtain a solution containing 420 µg per mL. Each mL of this solution *(Standard preparation A)* contains 190 µg of fluoride ion (10^{-2} M). Transfer 25.0 mL of *Standard preparation A* to a 250-mL volumetric flask, dilute with water to volume, and mix. This solution *(Standard preparation B)* contains 19 µg of fluoride ion per mL (10^{-3} M). Transfer 25.0 mL of *Standard preparation B* to a 250-mL volumetric flask, dilute with water to volume, and mix. This solution *(Standard preparation C)* contains 1.9 µg of fluoride ion per mL (10^{-4} M).

Assay preparation—Transfer to a 250-mL volumetric flask about 100 mg of Stannous Fluoride, accurately weighed. Add 50 mL of water, mix vigorously for 5 minutes, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Pipet 20 mL of each *Standard preparation* and of the *Assay preparation* into separate plastic beakers each containing a plastic-coated stirring bar. Pipet 20 mL of *Buffer solution* into each beaker. Concomitantly measure the potentials (see *pH* ⟨791⟩), in mV, of the solutions from the *Standard preparations* and of the solution from the *Assay preparation*, with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride-specific ion-indicating electrode and a calomel reference electrode. [NOTE—When taking measurements, immerse the electrodes in the solution, stir on a magnetic stirrer having an insulated top until equilibrium is attained (1 to 2 minutes), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.] Plot the logarithms of the fluoride-ion concentrations, in µg per mL, of the *Standard preparations* versus potential, in mV. From the measured potential of the *Assay preparation* and the standard reponse line, determine the concentration, *C*, in µg per mL, of fluoride ion in the *Assay preparation*. Calculate the percentage of fluoride (F) in the portion of Stannous Fluoride taken by the formula:

$$125C/W$$

in which *C* is the determined concentration of fluoride, in µg per mL, in the *Assay preparation*, and *W* is the weight, in mg, of Stannous Fluoride taken.

Stannous Fluoride Gel

DEFINITION

Stannous Fluoride Gel contains NLT 95.0% and NMT 115.0% of the labeled amount of stannous fluoride (SnF_2) in a suitable medium containing a suitable viscosity-inducing agent.

[NOTE—If Glycerin is used as the medium in the preparation of this Gel, use Glycerin that has a low water content; that is, Glycerin having a specific gravity of NLT 1.2607, corresponding to a concentration of 99.5%.]

IDENTIFICATION

• **A.**

Sample solution: Nominally 1 mg/mL of stannous fluoride from Gel in water

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Analysis: Add 2 mL of calcium chloride TS to 5 mL of the *Sample solution*.
Acceptance criteria: A fine, white precipitate of calcium fluoride is formed.

• **B.**
Sample solution: Nominally 1 mg/mL of stannous fluoride from Gel in water
Analysis: On a spot plate, mix 2 drops of *Sample solution* with 2 drops of silver nitrate TS.
Acceptance criteria: A brown-black precipitate is formed.

• **C.**
Sample solution: Nominally 1 mg/mL of stannous fluoride from Gel in water
Analysis: Add 1 drop of *Sample solution* to 2 drops of mercuric chloride TS.
Acceptance criteria: A white, silky precipitate is formed. On further addition of the *Sample solution*, a brown-black precipitate is formed.

ASSAY

• **PROCEDURE**

[NOTE—Store all solutions except the *Buffer solution* in plastic containers.]

Buffer solution: Dissolve 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of (1,2-cyclohexylenedinitrilo) tetraacetic acid in 500 mL of water. Adjust with 5 N sodium hydroxide to a pH of 5.25 ± 0.25, and dilute with water to 1000 mL.
Standard solution A: 420 µg/mL of USP Sodium Fluoride RS in water, equivalent to 190 µg/mL (10^{-2} M) of fluoride ion
Standard solution B: 19 µg/mL (10^{-3} M) of fluoride ion in water from *Standard solution A*
Standard solution C: 1.9 µg/mL (10^{-4} M) of fluoride ion in water from *Standard solution B*
Sample solution: Nominally 0.08 mg/mL of stannous fluoride from Gel in water
Analysis
Samples: *Standard solutions A–C* and *Sample solution*
Transfer accurately 20 mL each of *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution* into separate plastic beakers, each containing a plastic-coated stirring bar. Transfer accurately 20 mL of *Buffer solution* into each beaker. Concomitantly measure the potentials (see *pH* ⟨791⟩), in millivolts, of each solution with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride-specific ion-indicating electrode and a suitable reference electrode.
[NOTE—When taking measurements, immerse the electrodes in the solution, stir on a magnetic stirrer having an insulated top until equilibrium is attained (1–2 min), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.]
Plot the logarithms of the fluoride-ion concentrations, in µg/mL, of *Standard solution A*, *Standard solution B*, and *Standard solution C* versus potential, in millivolts. From the measured potential of the *Sample solution* and the standard response line, determine the concentration (C), in µg/mL, of fluoride ion in the *Sample solution*.
Calculate the concentration of the labeled amount of stannous fluoride (SnF_2), in mg/g, in the portion of Gel taken:

$$\text{Result} = (C/C_U) \times (M_{r1}/M_{r2})$$

C = concentration of fluoride ion in the *Sample solution* (µg/mL)
C_U = nominal concentration of stannous fluoride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of stannous fluoride, 156.71
M_{r2} = twice the atomic weight of fluorine, 38.0

Acceptance criteria: 95.0%–115.0%

SPECIFIC TESTS

• **CONTENT OF TOTAL TIN**
Potassium chloride solution: 19.1 mg/mL potassium chloride in water
Standard stock solution: 1 mg/mL of tin prepared as follows. Transfer an appropriate amount of tin (Sn) to a suitable volumetric flask. Add hydrochloric acid to about 20% of the final volume of the flask, and swirl to dissolve. Add 20% of the flask volume of water into the flask, and allow to cool. Dilute with water to volume, and mix.
Standard solution A: 50.0 µg/mL of tin and 191 µg/mL of potassium chloride from the *Standard stock solution* and the *Potassium chloride solution* in water
Standard solution B: 100.0 µg/mL of tin and 191 µg/mL of potassium chloride from the *Standard stock solution* and the *Potassium chloride solution* in water
Standard solution C: 150.0 µg/mL of tin and 191 µg/mL of potassium chloride from the *Standard stock solution* and the *Potassium chloride solution* in water
Sample solution: Nominally 0.132 mg/mL of stannous fluoride from a portion of Gel prepared as follows. Transfer an accurately weighed quantity of Gel, equivalent to about 132 mg of stannous fluoride, to a plastic beaker. Add 80 mL of water and 20 mL of hydrochloric acid, and mix. Transfer this mixture to a 1000-mL volumetric flask, add 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.
Blank: *Potassium chloride solution*, hydrochloric acid, and water (1:2:97)
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometer
Analytical wavelength: 235.5 nm
Lamp: Tin hollow-cathode
Flame: Nitrous oxide-acetylene oxidizing flame
Analysis
Samples: *Blank*, *Standard solutions A–C*, and *Sample solution*
Use water to adjust the instrument to zero. Concomitantly determine the absorbance of *Blank*, each *Standard solution*, and the *Sample solution*. Aspirate water before and after each determination. Correct the absorbances of each *Standard solution* and the *Sample solution* by subtracting the absorbance of the *Blank*. Plot the corrected absorbances of each *Standard solution* versus concentration, in µg/mL, of tin, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C), in µg/mL, of tin in the *Sample solution*.
Calculate the concentration of tin, in mg/g, in the portion of Gel taken:

$$\text{Result} = C/C_U$$

C = concentration of tin in the *Sample solution* (µg/mL)
C_U = nominal concentration of stannous fluoride in the *Sample solution* (mg/mL)

[NOTE—Use this value to calculate the percentage of stannous ion for the test for the *Content of Stannous Ion*.]

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• **CONTENT OF STANNOUS ION**

Solution A: 5 mg/mL of sodium hydroxide and 50 mg/mL of potassium iodide in oxygen-free water

Potassium iodide-iodate titrant: 0.1 N potassium iodide-iodate solution prepared as follows. In a 1000-mL volumetric flask, dissolve 3.567 g of potassium iodate, previously dried at 110° to constant weight, in 200 mL of *Solution A*. Dilute with oxygen-free water to volume, and mix. Standardize this solution by titrating a solution prepared from an accurately weighed quantity of reagent tin (Sn) and hydrochloric acid. Each milliliter of 0.1 N *Potassium iodide-iodate titrant* is equivalent to 5.935 mg of tin (Sn).

Sample: A suitable amount of Gel, equivalent to 80 mg of stannous fluoride

Titrimetric system

Mode: Direct titration

Titrant: *Potassium iodide-iodate titrant*

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a capped plastic vessel equipped for titration in an inert atmosphere. Add a plastic coated stirring bar, 20 mL of recently boiled 3 N hydrochloric acid, and 5 mL of potassium iodide TS. Close the vessel, purge the system with an oxygen-free inert gas, and titrate immediately with *Potassium iodide-iodate titrant*, adding 2 mL of starch TS as the endpoint is approached.

Calculate the concentration of stannous ion (Sn^{++}), in mg/g, in the portion of Gel taken:

$$\text{Result} = (V \times F)/W$$

V = volume of the *Potassium iodide-iodate titrant* consumed (mL)

F = equivalency factor, 5.935 mg/mL of 0.1 N potassium iodide-iodate

W = weight of the portion of Gel taken (g)

Acceptance criteria: NLT 68.2% of the concentration of stannous fluoride as determined in the *Assay* and NLT 90.0% of the concentration of total tin as determined in the test for *Content of Total Tin*

• **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩

Sample: A portion of Gel

Analysis: Transfer the *Sample* to a suitable plastic container, insert the stopper securely, and allow to stand until the *Sample* is free from air bubbles. Place it in a water bath maintained at a temperature of 25 ± 0.5° until it adjusts to the temperature of the water bath (4 h or longer). Do not stir the *Sample* while it is in the bath. Remove the *Sample* from the bath, stir the *Sample* gently for 2 min, and without delay, using a rotational viscometer, determine the viscosity by using a spindle having a cylinder 1.27 cm in diameter and 0.16 cm high that is attached to a shaft 0.32 cm in diameter, with the distance from the top of the cylinder to the lower tip of the shaft being 2.54 cm and the immersion depth being 5.00 cm (No. 3 spindle). Operate the viscometer at 12 rpm, and record the scale reading at 1-min intervals for 4 min.

Calculate the viscosity, in centipoises, by multiplying the scale reading by 100.

Acceptance criteria: 600–170,000 centipoises

• **pH** ⟨791⟩

Sample solution: A freshly prepared mixture with water (50:50)

Acceptance criteria: 2.8–4.0

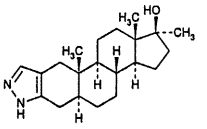
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Fluoride RS

Stanozolol



$C_{21}H_{32}N_2O$ 328.49

2′*H*-Androst-2-eno[3,2-*c*]pyrazol-17-ol, 17-methyl-, (5α, 17β)-.

17-Methyl-2′*H*-5α-androst-2-eno[3,2-*c*]pyrazol-17β-ol [10418-03-8].

» Stanozolol contains not less than 98.0 percent and not more than 100.5 percent of $C_{21}H_{32}N_2O$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Stanozolol RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution: 50 µg per mL.

Medium: alcohol.

Absorptivities at 224 nm, calculated on the dried basis, do not differ by more than 3.0%.

Specific rotation ⟨781S⟩: between +34° and +40°.

Test solution: 10 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry it at a pressure not exceeding 5 mm of mercury at 100° to constant weight: it loses not more than 1.0% of its weight.

Chromatographic purity—

Standard dilutions—Dissolve an accurately weighed quantity of USP Stanozolol RS in a mixture of chloroform and methanol (9:1) to obtain a solution having a known concentration of about 20 mg per mL. Dilute this solution with the same medium to obtain *Standard dilutions* having known concentrations of about 50, 100, 200, and 400 µg per mL, respectively.

Procedure—Score a 20- × 20-cm thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (binder-free) into channels 10 mm wide. Apply 10-µL portions, in two 5-µL increments, of a test solution prepared by dissolving Stanozolol in a mixture of chloroform and methanol (9:1) to obtain a solution containing about 20 mg per mL, and of each of the four *Standard dilutions* in the center of the channels at points about 2.5 cm from one edge of the plate. Develop the plate in a suitable chamber, lined with filter paper and previously equilibrated with 200 mL of a mixture of chloroform and

USP Monographs

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methanol (188:12), for 15 minutes, taking care to ensure that the filter paper has been wetted completely with the solvent mixture. Allow the plate to develop until the solvent front has moved about 15 cm above the line of application. Remove the plate, and allow the solvent to evaporate completely. Spray it with 20% sulfuric acid, and heat in an oven at 100° for 15 minutes. Examine the plate under long-wavelength UV light: the channel for the test solution exhibits its principal spot at the same R_F value as the spots for the *Standard dilutions*. Estimate the concentration of any spots in the channel for the test solution, other than the principal spot, by comparison with the spots from the *Standard dilutions*. The spots from the 50-, 100-, 200-, and 400-µg-per-mL dilutions correspond to 0.25%, 0.5%, 1.0%, and 2.0% of chromatographic impurities, respectively, and the sum of the chromatographic impurities in the test solution is not greater than 2.0%.

Assay—Dissolve about 700 mg of Stanozolol, accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.85 mg of $C_{21}H_{32}N_2O$.

Stanozolol Tablets

» Stanozolol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of stanozolol ($C_{21}H_{32}N_2O$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Stanozolol RS

Identification—Boil an amount of powdered Tablets, equivalent to about 2 mg of stanozolol, with 5 mL of benzene, filter, and evaporate on a steam bath to dryness. Add 3 mL of *p*-dimethylaminobenzaldehyde TS to the residue: a yellow color develops, which exhibits a green fluorescence under long-wavelength UV light.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 500 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Determine the amount of $C_{21}H_{32}N_2O$ dissolved by employing the following method.

Bromocresol purple solution—Mix 1.0 g of bromocresol purple with 1000 mL of dilute glacial acetic acid (1 in 50), and filter if necessary to obtain a clear solution.

Standard preparations— [NOTE—Prepare *Standard preparations* on the day of use.] Transfer about 50 mg of USP Stanozolol RS, accurately weighed, to a 50-mL volumetric flask, add 15.0 mL of methanol, and mix to dissolve. Add 5.0 mL of 1.0 N hydrochloric acid, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 200-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix. Separately pipet 2-mL, 4-mL, and 6-mL portions of the solution into three 60-mL separators, add accurately measured volumes of *Dissolution Medium* to adjust the volumes in each to 25.0 mL, and pipet 25 mL of *Dissolution Medium* into a fourth 60-mL separator.

Procedure—Pipet 25 mL of a filtered portion of the solution under test into a 60-mL separator. To this separator and to each of the four separators containing *Standard preparations* add 1.0 mL of *Bromocresol purple solution* and 10.0 mL of chloroform. Insert the stopper in each, shake gently for 1 minute, allow the phases to separate, and swirl if necessary to break up emulsions. Transfer the lower chloroform layers to separate 50-mL centrifuge tubes, insert the glass stoppers, and centrifuge for 5 minutes to clarify the solutions. Concomitantly determine the absorbances of the solutions obtained from the solution under test and from the *Standard preparation* in 1-cm cells, at the wavelength of maximum absorbance at about 420 nm, with a suitable spectrophotometer, using chloroform as the blank. Construct a standard plot of absorbances versus the concentrations of the solutions from the *Standard preparations*. From the plot so obtained, determine the amount of $C_{21}H_{32}N_2O$ dissolved in the solution from the solution under test.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{21}H_{32}N_2O$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩— [NOTE—Maintain the acid concentration at a uniform level in the solutions being compared spectrophotometrically; the same acidic alcohol solution is to be used throughout this procedure. Also, take precautions throughout this procedure to minimize evaporation.] Transfer 1 Tablet to a 25-mL volumetric flask, add 0.5 mL of water, and shake to disintegrate. Add about 20 mL of alcohol, heat on a steam bath, with occasional swirling, for 10 to 15 minutes, then cool, dilute with alcohol to volume, and mix. Filter through medium-porosity filter paper, taking precautions to minimize evaporation, discard the first 5 mL of the filtrate, and proceed as directed for *Assay preparations* in the *Assay*, beginning with "Transfer 5.0 mL of the filtrate."

Assay— [NOTE—Maintain the acid concentration at a uniform level in the solutions being compared spectrophotometrically; the same acidic alcohol solution is to be used throughout this procedure.]

Standard preparations—Dissolve a suitable quantity of USP Stanozolol RS, accurately weighed, in alcohol, and dilute quantitatively and stepwise with alcohol, if necessary, to obtain a stock solution having a known concentration of about 80 µg per mL. Transfer 5.0 mL of this stock solution to a 10-mL volumetric flask, dilute with alcohol to volume, and mix to prepare the *Neutral standard preparation*. Transfer another 5.0-mL portion of the stock solution to a second 10-mL volumetric flask, dilute with acidic alcohol (1.5 mL of hydrochloric acid in 100 mL of alcohol) to volume, and mix to prepare the *Acidic standard preparation*. The concentration of USP Stanozolol RS in the *Standard preparations* is about 40 µg per mL.

Assay preparations—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 4 mg of stanozolol, to a 50-mL volumetric flask, add about 25 mL of alcohol, and heat on a steam bath, with frequent swirling, for 15 minutes. Cool, dilute with alcohol to volume, mix, filter through medium-porosity filter paper, taking precautions to minimize evaporation, and discard the first 10 mL of the filtrate. Transfer 5.0 mL of the filtrate to a 10-mL volumetric flask, dilute with alcohol to volume, and mix to prepare the *Neutral assay preparation*. Transfer another 5.0-mL portion of the filtrate to a second 10-mL volumetric flask, dilute with acidic alcohol (1.5 mL of hydrochloric acid in 100 mL of alcohol) to volume, and mix to prepare the *Acidic assay preparation*.

Procedure—Concomitantly determine the absorbances of the acidic alcohol solution, the *Acidic standard preparation*, and the *Acidic assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 235 nm, with a suitable spectrophotometer, using alcohol, the *Neutral standard preparation*, and the *Neutral assay preparation*, respectively, as the blanks. Calculate the quantity, in mg, of $C_{21}H_{32}N_2O$ in the portion of Tablets taken by the formula:

USP Monographs

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$$0.1C(A_U - A_O)/(A_S - A_O)$$

in which C is the concentration, in µg per mL, of USP Stanozolol RS in the *Standard preparations;* and A_U, A_S, and A_O are the absorbances of the *Acidic assay preparation*, the *Acidic standard preparation*, and the acidic alcohol solution, respectively.

Topical Starch

DEFINITION

Topical Starch consists of the granules separated from the mature grain of corn [*Zea mays* L. (Fam. Gramineae)].

IDENTIFICATION

- **A.**
  Sample: 1 g
  Analysis: Prepare a smooth mixture of the *Sample* with 2 mL of cold water, stir it into 15 mL of boiling water, boil gently for 2 min, and cool.
  Acceptance criteria: A translucent, whitish jelly is produced.
- **B.** A water slurry of it is colored reddish violet to deep blue by iodine TS.

IMPURITIES

- **Residue on Ignition** ⟨281⟩
  Sample: 2.0 g
  Analysis: Ignite the *Sample* at a temperature of 575 ± 25°.
  Acceptance criteria: NMT 0.5%
- **Iron** ⟨241⟩
  Sample: The residue obtained in the test for *Residue on Ignition*
  Analysis: Dissolve the *Sample* in 4 mL of hydrochloric acid with the aid of gentle heating, dilute with water to 50 mL, and mix. Dilute 25 mL of the resulting solution with water to 47 mL.
  Acceptance criteria: NMT 10 µg/g
- **Sulfur Dioxide**
  Sample suspension: Mix 20 g with 200 mL water to obtain a smooth suspension, and filter.
  Analysis: To 100 mL of the clear filtrate add 3 mL of starch TS, and titrate with 0.01 N iodine VS to the first permanent blue color.
  Acceptance criteria: NMT 2.7 mL of 0.01 N iodine VS is consumed (0.008%).
- **Oxidizing Substances**
  Sample: 4.0 g
  Analysis: Add 50.0 mL of water to the *Sample* in a glass-stoppered, 125-mL conical flask. Insert the stopper, and swirl for 5 min. Decant into a glass-stoppered, 50-mL centrifuge tube, and spin to clarify. Transfer 30.0 mL of clear supernatant to a glass-stoppered, 125-mL conical flask. Add 1 mL of glacial acetic acid and 0.5–1.0 g of potassium iodide. Insert the stopper, swirl, and allow to stand for 25–30 min in the dark. Add 1 mL of starch TS, and titrate with 0.002 N sodium thiosulfate VS to the disappearance of the starch–iodine color. [Note—Each mL of 0.002 N sodium thiosulfate is equivalent to 34 µg of oxidant, calculated as hydrogen peroxide.]
  Acceptance criteria: NMT 12.6 mL of 0.002 N sodium thiosulfate VS is required (0.018%).

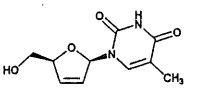
SPECIFIC TESTS

- **Botanic Characteristics:** Polygonal, rounded, or spheroidal granules up to about 35 µm in diameter, and usually having a circular or several-rayed central cleft.
- **Loss on Drying** ⟨731⟩
  Analysis: Dry a sample at 120° for 4 h.
  Acceptance criteria: NMT 14.0%
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count does not exceed 5×10^2 cfu/g, and the total combined molds and yeasts count does not exceed 5×10^1 cfu/g.
- **pH** ⟨791⟩
  Sample: 20.0 g ± 100 mg
  Analysis: Prepare a slurry of the *Sample* in 100 mL of water in a suitable nonmetallic container. Agitate continuously at a moderate rate for 5 min, then stop agitation, and immediately determine the pH potentiometrically to the nearest 0.1 unit.
  Acceptance criteria: 4.5–7.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Stavudine



$C_{10}H_{12}N_2O_4$ 224.21

Thymidine, 2′,3′-didehydro-3′-deoxy-;
1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine [3056-17-5].

DEFINITION

Stavudine contains NLT 98.0% and NMT 102.0% of stavudine ($C_{10}H_{12}N_2O_4$), calculated on an anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**
  All solutions containing stavudine must be prepared immediately before use and remain refrigerated until use.
  Solution A: 0.77 g/L of ammonium acetate
  Mobile phase: Acetonitrile and *Solution A* (5:95)
  Standard solution: 0.02 mg/mL of USP Stavudine RS in water
  Sample solution: 0.02 mg/mL of Stavudine in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 3.3-cm; 3-µm packing L1
  Flow rate: 0.7 mL/min
  Injection volume: 25 µL
  System suitability
  Sample: *Standard solution*
  [Note—The retention time of the stavudine peak is between 2.8 and 5.0 min.]

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Suitability requirements
Column efficiency: NLT 800 theoretical plates
Tailing factor: NMT 1.6
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of stavudine ($C_{10}H_{12}N_2O_4$) in the portion of Stavudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Stavudine RS in the *Standard solution* (mg/mL)
C_U = concentration of Stavudine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **ORGANIC IMPURITIES**

All solutions containing stavudine must be prepared immediately before use and remain refrigerated until use.
Solution A: 0.77 g/L of ammonium acetate in water
Solution B: Acetonitrile and *Solution A* (3.5: 96.5)
Solution C: Acetonitrile and *Solution A* (25:75)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
20	0	100
30	0	100
35	100	0
40	100	0

System suitability solution: 0.50 mg/mL of USP Stavudine System Suitability Mixture RS in water
Sample solution: 0.5 mg/mL of Stavudine in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2.1 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of the stavudine peak, or at least until the last impurity has eluted
System suitability
Sample: *System suitability solution*
[NOTE—The retention time of the main stavudine peak is 10.5 ± 2 min. The relative retention times for stavudine and thymine are listed in *Table 2*.]
Suitability requirements
Resolution: NLT 1.15 between the thymidine epimer and thymidine; NLT 1.0 between stavudine and α-stavudine
Column efficiency: NLT 9500 theoretical plates
Capacity factor, *k'*: NLT 4
Analysis
Samples: *System suitability solution* and *Sample solution*

Calculate the percentage of thymine in the portion of Stavudine taken:

$$\text{Result} = (r_U/r_T) \times F \times 100$$

r_U = peak response of thymine from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
F = relative response factor (see *Table 2*)

Calculate the percentage of all other impurities in the portion of Stavudine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard threshold is 0.03%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Thymine	0.28	0.69	0.5
Stavudine	1.0	—	—
Any other individual impurity	—	1.0	0.1
Total impurities[a]	—	—	1.0

[a] Including thymine.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL
Acceptance criteria: –40° to –45°, calculated on the anhydrous basis
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and humidity. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Stavudine RS
USP Stavudine System Suitability Mixture RS
It is a mixture of stavudine and the following related compounds: thymidine, thymine, alpha-stavudine, and *xylo*-thymidine.

Stavudine Capsules

DEFINITION

Stavudine Capsules contain NLT 90.0% and NMT 105.0% of the labeled amount of stavudine ($C_{10}H_{12}N_2O_4$).

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IDENTIFICATION

Change to read:

- **A.** ▲The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

All solutions containing stavudine must be prepared immediately before use and remain refrigerated until use.
Solution A: 0.77 g/L of ammonium acetate in water
Mobile phase: Acetonitrile and *Solution A* (5:95)
System suitability solution: 0.1 µg/mL each of ▲USP Zidovudine Related Compound C RS▲ 2S (USP41) and ▲USP Zidovudine Related Compound D RS▲ 2S (USP41) in water
Standard solution: 0.1 mg/mL of USP Stavudine RS in water. Use sonication to dissolve before dilution.
Sample solution: Nominally 0.1 mg/mL of stavudine prepared as follows. Open NLT 3 Capsules, and dissolve the contents in water. Dilute with water to obtain the final concentration.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 268 nm. ▲For *Identification A*, use a diode array detector in the range of 190–400 nm.▲ 2S (USP41)
Column: 4.6-mm × 3.3-cm; ▲3-µm▲ 2S (USP41) packing L1
Flow rate: 0.7 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The retention time of the stavudine peak in the *Standard solution* is between 2.8 and 5.0 min.]
Suitability requirements
Resolution: NLT 2.0 between ▲zidovudine related compound C▲ 2S (USP41) and ▲zidovudine related compound D; zidovudine related compound C▲ 2S (USP41) is resolved from the void volume, *System suitability solution*
▲▲ 2S (USP41)
Tailing factor: NMT 1.8, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of stavudine ($C_{10}H_{12}N_2O_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Stavudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of stavudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Solution A and **Mobile phase:** Prepare as directed in the *Assay*.
Sample solution: ▲Dilute with *Medium* to a concentration that is similar to the *Standard solution*.▲ 2S (USP41)
Standard solution: USP Stavudine RS in *Medium* with a concentration corresponding to that of the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 3.3-cm; ▲3-µm▲ 2S (USP41) packing L1
Flow rate: 0.7 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
▲▲ 2S (USP41)
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Determine the percentage of the labeled amount of stavudine ($C_{10}H_{12}N_2O_4$) dissolved, using the procedure set forth in the *Assay*, making any necessary modifications.
Tolerances: NLT 80% (*Q*) of the labeled amount of stavudine ($C_{10}H_{12}N_2O_4$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Solution A, Mobile phase, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Standard solution: 0.001 mg/mL of ▲USP Zidovudine Related Compound C RS.▲ 2S (USP41) Use sonication to dissolve before dilution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 268 nm
Column: 4.6-mm × 3.3-cm; ▲3-µm▲ 2S (USP41) packing L1
Flow rate: 0.7 mL/min
Injection volume: 20 µL
Run time: ▲NLT▲ 2S (USP41) 2.5 times the retention time of the stavudine peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The retention time of the stavudine peak in the *Standard solution* is between 2.8 and 5.0 min.]
Suitability requirements
Resolution: NLT 2.0 between ▲zidovudine related compound C▲ 2S (USP41) and ▲zidovudine related compound D; zidovudine related compound C▲ 2S (USP41) is resolved from the void volume, *System suitability solution*
▲▲ 2S (USP41)
Tailing factor: NMT 1.8, *Standard solution*

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Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of ▲zidovudine related compound C▲ 2S (USP41) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ▲zidovudine related compound C▲ 2S (USP41) from the *Sample solution*

r_S = peak response of ▲zidovudine related compound C▲ 2S (USP41) from the *Standard solution*

C_S = concentration of ▲USP Zidovudine Related Compound C RS▲ 2S (USP41) in the *Standard solution* (mg/mL)

C_U = nominal concentration of ▲stavudine▲ 2S (USP41) in the *Sample solution* (mg/mL)

Calculate the percentage of unknown impurities, not including ▲zidovudine related compound C,▲ 2S (USP41) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. The disregard threshold is 0.05% of the total sample related peak response.

Table 1

Name	Acceptance Criteria, NMT (%)
▲Zidovudine related compound C[a]▲ 2S (USP41)	1.0
Any other individual impurity	0.2
Total impurities[b]	2.0

▲[a] 5-Methylpyrimidine-2,4-(1*H*,3*H*)-dione.▲ 2S (USP41)

[b] Includes ▲zidovudine related compound C.▲ 2S (USP41)

SPECIFIC TESTS

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: Nominally 10 mg/mL of stavudine in water. Disperse a sufficient quantity of Capsule contents, equivalent to 200 mg of stavudine, in 50 mL of acetone. Bring to a boil, and pass through a fine-porosity filter. Precipitate the stavudine with 150 mL of heptane, filter the crystals, wash with heptane, and dry in air.

Acceptance criteria: −40° to −45°

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 3.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tightly closed containers, and store at controlled room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Stavudine RS

▲USP Zidovudine Related Compound C RS

Thymine;

5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.

$C_5H_6N_2O_2$ 126.11

USP Zidovudine Related Compound D RS

Thymidine;

[1-(2-Deoxy-β-D-ribofuranosyl)]thymine.

$C_{10}H_{14}N_2O_5$ 242.23▲ 2S (USP41)

Stavudine for Oral Solution

DEFINITION

Stavudine for Oral Solution, when reconstituted as directed in the labeling, yields a 1 mg/mL solution that contains NLT 90.0% and NMT 110.0% of the labeled amount of stavudine ($C_{10}H_{12}N_2O_4$). It may contain suitable flavors, preservatives, sweeteners, and stabilizers.

IDENTIFICATION

Change to read:

• **▲A.▲** (USP 1-Aug-2019) The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**

[NOTE—All testing solutions must be prepared immediately before use and remain refrigerated until use.]

Buffer: 1.93 mg/mL of ammonium acetate in water

Solution A: Methanol and *Buffer* (6:94)

Solution B: Methanol and *Buffer* (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	100	0
12.1	0	100
17	0	100
17.1	100	0
35	100	0

System suitability solution: 2.5 µg/mL each of ▲USP Zidovudine Related Compound C RS▲ (USP 1-Aug-2019) and ▲USP Zidovudine Related Compound D RS▲ (USP 1-Aug-2019) in water

Standard solution: 0.1 mg/mL of USP Stavudine RS in water

Sample solution: Nominally 0.1 mg/mL of stavudine in water, prepared as follows. Constitute Stavudine for Oral Solution as directed in the labeling. Dilute an accurately measured volume of Stavudine for Oral Solution quantitatively, and stepwise if necessary, with water.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 268 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)

Columns

Guard: 4-mm × 20-mm; ▲5-µm▲ (USP 1-Aug-2019) packing L1

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Analytical: 4.6-mm × 3.3-cm; ▲3-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 8.4 between ▲zidovudine related compound C▲ (USP 1-Aug-2019) and ▲zidovudine related compound D,▲ (USP 1-Aug-2019) *System suitability solution* ▲▲ (USP 1-Aug-2019)
Tailing factor: NMT 2 for the stavudine peak, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of stavudine ($C_{10}H_{12}N_2O_4$) in each milliliter of Stavudine for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Stavudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of stavudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
[NOTE—All testing solutions must be prepared immediately prior to use and remain refrigerated until use.]
▲Buffer,▲ (USP 1-Aug-2019) Solution A, Solution B, ▲Mobile phase,▲ (USP 1-Aug-2019) System suitability solution, Standard solution, Sample solution, Chromatographic system, and **▲System suitability:▲ (USP 1-Aug-2019)** *Assay*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Stavudine for Oral Solution taken:

$$\text{Result} = (r_U/r_T) \times 1/F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses including that of the main stavudine peak from the *Sample solution*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
▲Zidovudine related compound C[a]▲ (USP 1-Aug-2019)	0.24	0.69	1.0
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.5

[a] 5-Methylpyrimidine-2,4-(1*H*,3*H*)-dione.

SPECIFIC TESTS
• **PH** ⟨791⟩
Sample: Constitute as directed in the labeling.
Acceptance criteria: 5–7
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tightly closed containers, protected from excessive moisture. Store at controlled room temperature. After constitution, store the Stavudine for Oral Solution in tightly closed containers under refrigeration. Discard unused portion after 30 days.
• **LABELING:** The label contains directions for constitution of the powder and states the equivalent amount of stavudine ($C_{10}H_{12}N_2O_4$) in a given volume of the Stavudine for Oral Solution obtained after constitution.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Stavudine RS
▲USP Zidovudine Related Compound C RS
Thymine;
5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.
$C_5H_6N_2O_2$ 126.11
USP Zidovudine Related Compound D RS
Thymidine;
[1-(2-Deoxy-β-D-ribofuranosyl)]thymine.
$C_{10}H_{14}N_2O_5$ 242.23▲ (USP 1-Aug-2019)

Storax

DEFINITION
Storax is a balsam obtained from the trunk of *Liquidambar orientalis* Miller, known in commerce as Levant Storax, or of *Liquidambar styraciflua* L., known in commerce as American Storax (Fam. Hamamelidaceae).

CONTAMINANTS
• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Sample: 2 g
Analysis: Dry the *Sample* at 105° for 2 h.
Acceptance criteria: It loses NMT 20.0% of its weight.
• **ALCOHOL-INSOLUBLE SUBSTANCES**
Sample: 10 g of mixed Storax
Analysis: Heat the *Sample* in a beaker at 105° for 30 min, take up the residue in 100 mL of hot alcohol, pass through counter-balanced filters or a tared filter crucible, and wash the residue with small portions of hot alcohol until the last washing is colorless or practically so.
Acceptance criteria: The weight of the residue, after drying at 105° for 1 h, does not exceed 5.0% of the weight of Storax taken.

USP Monographs

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- **ALCOHOL-SOLUBLE SUBSTANCES**

Analysis: Evaporate the combined alcohol filtrate and washings obtained in the test for *Alcohol-Insoluble Substances* at a temperature not exceeding 60°, and dry the residue at 105° for 1 h.

Acceptance criteria: The weight of the yellow-to-brown residue of purified Storax is NLT 70.0% of the weight of Storax taken. [NOTE—The purified Storax obtained in the test for *Alcohol-Soluble Substances* meets the requirements of the following tests: *Fats and Fixed Oils, Acid Value* and *Saponification Value*, and *Cinnamic Acid*.]

- **FATS AND FIXED OILS** ⟨401⟩, *Acid Value*

Sample: 1 g of purified Storax

Analysis: Dissolve the *Sample* in 50 mL of neutralized alcohol, add 0.5 mL of phenolphthalein TS, and titrate with 0.5 N sodium hydroxide VS.

Acceptance criteria: 50–85 for Levant Storax; 36–85 for American Storax

- **FATS AND FIXED OILS** ⟨401⟩, *Saponification Value*

Sample: 2 g of purified Storax

Analysis: Mix the *Sample* with 50 mL of solvent hexane, add 25.0 mL of 0.5 N alcoholic potassium hydroxide VS, and allow the mixture to stand for 24 h with frequent agitation. Then add 0.5 mL of phenolphthalein TS, and titrate the excess alkali with 0.5 N hydrochloric acid VS.

Acceptance criteria: 160–200

- **CINNAMIC ACID**

Sample solution: 2 g of purified Storax in 25 mL of 0.5 N alcoholic potassium hydroxide

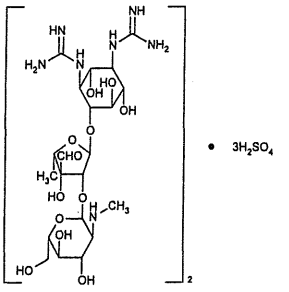
Analysis: Boil the *Sample solution* for 1 h under a reflux condenser. Add 0.5 mL of phenolphthalein TS, neutralize with 0.5 N sulfuric acid, and evaporate the alcohol on a steam bath. Dissolve the residue in 50 mL of water, and shake the solution with 20 mL of ether. Shake the separated ether with 5 mL of water, adding the washing to the water solution, and discard the ether extract. Add to the water solution 10 mL of diluted sulfuric acid, and shake with four 20-mL portions of ether. Wash the combined ether extracts with 5 mL of water, rejecting the water washing. Transfer to a flask, and distill off the ether. Add to the residue 100 mL of water, and boil the mixture vigorously for 15 min under a reflux condenser. Filter while hot, and allow the filtrate to cool to about 25°. White crystals of cinnamic acid separate. Collect and dry the cinnamic acid by vacuum filtration. Repeat the extraction of the residue twice by boiling each time under a reflux condenser, as previously described, with the filtrate from the preceding crystallization, and collect the additional cinnamic acid in the same crucible. Finally, wash the cinnamic acid with two 10-mL portions of ice-cold water, dry at 80°, and weigh. To 50 mg of the cinnamic acid obtained as directed above, add 5 mL of 2 N sulfuric acid, heat, and add potassium permanganate TS.

Acceptance criteria: The weight of the cinnamic acid is NLT 25.0% of the weight of purified Storax taken. A portion of the acid recrystallized from hot water melts at 134°–135°. After adding the 2 N sulfuric acid, and heating and adding potassium permanganate TS, the odor of benzaldehyde is perceptible.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Streptomycin Sulfate



$(C_{21}H_{39}N_7O_{12})_2 \cdot 3H_2SO_4$ 1457.38

D-Streptamine, *O*-2-deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-*O*-5-deoxy-3-C-formyl-α-L-lyxofuranosyl-(1→4)-*N*,*N*′-bis(aminoiminomethyl)-, sulfate (2:3) (salt).

Streptomycin sulfate (2:3) (salt) [3810-74-0].

» Streptomycin Sulfate has a potency equivalent to not less than 650 µg and not more than 850 µg of streptomycin ($C_{21}H_{39}N_7O_{12}$) per mg.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Streptomycin Sulfate RS

Identification—

A: Dissolve 5 g of ferric chloride in 50 mL of 0.1 N hydrochloric acid. Transfer 2.5 mL of this stock solution to a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Prepare *Iron reagent* at the time of use. Dissolve the specimen in water, and dilute with water to obtain a solution containing about 1 mg of streptomycin per mL. To 5 mL of this solution add 2.0 mL of 1 N sodium hydroxide, and heat in a water bath for 10 minutes. Cool in ice water for 3 minutes, then add 2.0 mL of 1.2 N hydrochloric acid, and mix. Add 5 mL of *Iron reagent*, and mix: a violet color is produced.

B: It responds to the tests for *Sulfate* ⟨191⟩.

pH ⟨791⟩: between 4.5 and 7.0, in a solution containing 200 mg of streptomycin per mL.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

Other requirements—Where the label states that Streptomycin Sulfate is sterile, it meets the requirements for *Sterility Tests* and *Bacterial endotoxins* under *Streptomycin for Injection*. Where the label states that Streptomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Streptomycin for Injection*.

Assay—

Mobile phase—Use 70 mM sodium hydroxide. During use, store in a plastic bottle flushed with a blanket of helium above the liquid surface. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

USP Monographs

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Standard preparation—Dissolve an accurately weighed quantity of USP Streptomycin Sulfate RS in water, and quantitatively dilute with water to obtain a solution having a known concentration of about 0.03 mg per mL. Sonicate for 1 minute, and mix.

Assay preparation—Transfer about 30 mg of Streptomycin Sulfate, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, sonicate for 1 minute, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with water to volume, and mix.

System suitability solution—Heat about 10 mL of the *Standard preparation* at 75° for 1 hour. Allow to cool.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with an electrochemical detector, a gold working electrode, a pH silver–silver chloride reference electrode, a 4-mm × 5-cm guard column that contains packing L46, and a 4-mm × 25-cm analytical column that contains packing L46. The electrochemical detector is used in the integrated amperometric mode with a range of 300 nC, an output of 1 V full scale, and a rise time of 0.5 second, positive polarity. The potential is programmed as follows.

Step	Time (seconds)	Potential (V)	Integration
1	0.00	+0.1	
2	0.20	+0.1	begins
3	0.40	+0.1	ends
4	0.41	−2.0	
5	0.42	−2.0	
6	0.43	+0.6	
7	0.44	−0.1	
8	0.50	−0.1	

The flow rate is about 0.5 mL per minute. Chromatograph the *System suitability solution,* and measure the peak areas as directed for *Procedure:* the relative retention times are about 0.5 for the main degradation product and 1.0 for streptomycin; and the resolution, *R,* between the two peaks is not less than 3. Chromatograph the *Standard preparation,* and measure the peak areas as directed for *Procedure:* the tailing factor is not more than 2; the column efficiency is not less than 1000 theoretical plates; and the relative standard deviation for replicate injections is not more than 5%.

[NOTE—If variation of retention time or increase of tailing occurs, clean the columns with 0.2 M sodium hydroxide. Carefully maintain the working and reference electrodes.]

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of streptomycin ($C_{21}H_{39}N_7O_{12}$) in each mg of Streptomycin Sulfate taken by the formula:

$$1000(CP/W_U)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Streptomycin Sulfate RS in the *Standard preparation; P* is the designated streptomycin content, in µg per mg, of streptomycin ($C_{21}H_{39}N_7O_{12}$) in USP Streptomycin Sulfate RS; W_U is the weight, in mg, of Streptomycin Sulfate taken to prepare the *Assay preparation;* and r_U and r_S are the streptomycin peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Streptomycin Injection

» Streptomycin Injection contains an amount of Streptomycin Sulfate equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of streptomycin ($C_{21}H_{39}N_7O_{12}$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—
USP Streptomycin Sulfate RS

Identification—

Iron reagent—Dissolve 5 g of ferric chloride in 50 mL of 0.1 N hydrochloric acid. Transfer 2.5 mL of this stock solution to a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Prepare the *Iron reagent* at the time of use.

Procedure—Dissolve the specimen in water, and dilute with water to obtain a solution containing about 1 mg of streptomycin per mL. To 5 mL of this solution add 2.0 mL of 1 N sodium hydroxide, and heat in a water bath for 10 minutes. Cool in ice water for 3 minutes, then add 2.0 mL of 1.2 N hydrochloric acid, and mix. Add 5 mL of *Iron reagent,* and mix: a violet color is produced.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.25 USP Endotoxin Unit per mg of streptomycin.

pH ⟨791⟩: between 5.0 and 8.0.

Other requirements—It meets the requirements for *Sterility* under *Streptomycin for Injection.* It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, Standard preparation, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Streptomycin Sulfate.*

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of streptomycin, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Streptomycin Sulfate.* Calculate the quantity, in mg, of streptomycin ($C_{21}H_{39}N_7O_{12}$) in each mL of the Injection taken by the formula:

$$20(CP/V)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken to prepare the *Assay preparation;* and the other terms are as defined therein.

Streptomycin for Injection

» Streptomycin for Injection contains an amount of Streptomycin Sulfate equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of streptomycin ($C_{21}H_{39}N_7O_{12}$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

USP Reference standards ⟨11⟩—
USP Streptomycin Sulfate RS

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Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Identification—

A: Dissolve 5 g of ferric chloride in 50 mL of 0.1 N hydrochloric acid. Transfer 2.5 mL of this stock solution to a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Prepare *Iron reagent* at the time of use. Dissolve the specimen in water, and dilute with water to obtain a solution containing about 1 mg of streptomycin per mL. To 5 mL of this solution add 2.0 mL of 1 N sodium hydroxide, and heat in a water bath for 10 minutes. Cool in ice water for 3 minutes, then add 2.0 mL of 1.2 N hydrochloric acid, and mix. Add 5 mL of *Iron reagent*, and mix: a violet color is produced.

B: It responds to the tests for *Sulfate* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.25 USP Endotoxin Unit per mg of streptomycin.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 4.5 and 7.0, in a solution containing 200 mg of streptomycin per mL.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—

Mobile phase, Standard preparation, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Streptomycin Sulfate.*

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Streptomycin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution containing about 0.025 mg of streptomycin per mL.

Assay preparation 2 (where the label states the quantity of streptomycin in a given volume of constituted solution)—Constitute Streptomycin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively, and stepwise if necessary, with water to obtain a solution containing about 0.025 mg of streptomycin per mL.

Procedure—Proceed as directed in the *Assay* under *Streptomycin Sulfate.* Calculate the quantity, in mg, of streptomycin ($C_{21}H_{39}N_7O_{12}$) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(CP/1000)(L/D)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of streptomycin ($C_{21}H_{39}N_7O_{12}$) in the container, or in the volume of constituted solution taken; D is the concentration, in mg of streptomycin per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container, or in the volume of constituted solution taken, respectively; and the other terms are as defined therein.

Strontium Chloride Sr 89 Injection

$^{89}SrCl_2$ 159.9

Strontium chloride ($^{89}SrCl_2$) [38270-90-5].

» Strontium Chloride Sr 89 Injection is a sterile solution of radioactive strontium (^{89}Sr) processed in the form of strontium chloride in Water for Injection.

Strontium Chloride Sr 89 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{89} Sr as strontium chloride expressed in megabecquerels per mL (or in millicuries per mL) at the time indicated in the labeling. The strontium chloride content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount.

Specific activity: not less than 2.96 MBq (80 µCi) per mg of strontium at the time indicated in the labeling.

Packaging and storage—Preserve in single-dose containers that are adequately shielded.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of strontium chloride expressed as mg of strontium per mL; the amount of ^{89} Sr as labeled strontium chloride expressed as total megabecquerels (or millicuries) and concentration as megabecquerels per mL (or millicuries per mL) on the date and time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{89}Sr is 50.5 days.

Radionuclide identification (see *Radioactivity* ⟨821⟩)—Strontium 89 decays by beta emission to stable Yttrium 89 with 0.01% of disintegrations going via the metastable daughter ^{89m} Y with a half-life of 16 seconds, with which it rapidly establishes radioactive equilibrium. Its gamma-ray spectrum is identical to that of a specimen of strontium 89 in equilibrium with ^{89m} Y exhibiting bremsstrahlung and a gamma photopeak of 0.909 MeV. [NOTE—Use a plastic container to perform measurements.]

Bacterial Endotoxins Test ⟨85⟩—It meets the requirements, the limit of endotoxin content being not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 4.0 and 7.5.

Radionuclidic purity (see *Radioactivity* ⟨821⟩)—[NOTE—Use a plastic container to perform the following test.] Using a suitable counting assembly, determine the radioactivity of each gamma-emitting radionuclidic impurity, in kBq per MBq (or µCi per mCi) of ^{89}Sr, in the Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩. The total activity of all gamma-emitting impurities is not greater than 370 kBq per 37 MBq (or 10 µCi per mCi) of ^{89} Sr at the expiration date stated in the labeling.

Chemical purity (Limit of aluminum)— [NOTE—The *Standard preparations* and the *Test preparation* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Nitric acid diluent—Dilute 40 mL of nitric acid with water to 1000 mL.

USP Monographs

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Standard preparations—Transfer 2000 mg of aluminum metal to a 1000-mL volumetric flask, add 50 mL of 6 N hydrochloric acid, swirl to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Nitric acid diluent* to volume, and mix. Transfer 1.0-, 2.0-, and 4.0-mL portions of this solution to separate 100-mL volumetric flasks, dilute with *Nitric acid diluent* to volume, and mix. These solutions contain 0.01, 0.02, and 0.04 µg of Al per mL, respectively.

Test preparation—Transfer 1.0 mL of Injection to a 100-mL volumetric flask, and carefully add 4 mL of nitric acid. Dilute with water to volume, and mix.

Procedure—Determine the absorbances of the *Standard preparations* and the *Test preparation* at the aluminum emission line at 309.3 nm with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with an aluminum hollow-cathode lamp and a flameless electrically heated furnace, using *Nitric acid diluent* as the blank. Plot the absorbances of the *Standard preparations* versus the contents of Al, in µg per mL, drawing the straight line best fitting the three points. From the graph so obtained, determine the quantity, in µg, of Al in each mL of the *Test preparation*. Calculate the quantity, in µg per g, of Al in the specimen taken by multiplying this value by 100: the limit is 2 µg per g.

Assay for radioactivity—Using a suitable counting assembly, determine the radioactivity, in MBq (or mCi) per mL, of the Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it is not subject to the recommendation on *Container Content*.

Assay—

Potassium chloride solution—Dissolve 1.9 g of potassium chloride in water, dilute with water to 1000 mL, and mix. This solution contains 1000 µg of potassium per mL.

Strontium stock solution—Transfer 1.685 g of strontium carbonate, accurately weighed, to a 100-mL volumetric flask, and add water to dissolve. Add 10 mL of hydrochloric acid, dilute with water to volume, and mix to obtain a solution containing 10,000 µg of strontium per mL.

Standard preparations—Pipet 10 mL of *Strontium stock solution* into a 100-mL volumetric flask. Add 10 mL of hydrochloric acid, dilute with *Potassium chloride solution* to volume, and mix. Transfer 10 mL and 20 mL, respectively, of this solution into separate 100-mL volumetric flasks, dilute with *Potassium chloride solution* to volume, and mix. These *Standard preparations* contain 100 and 200 µg of strontium per mL.

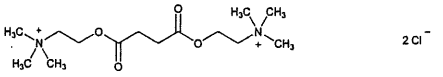
Assay preparation—Pipet 0.1 mL of Injection into a small beaker. Add 5 mL of *Potassium chloride solution*, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the strontium emission line of 407.8 nm with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a strontium hollow-cathode lamp and a nitrous oxide–acetylene flame, using *Potassium chloride solution* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of strontium, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, in µg per mL, of strontium in the *Assay preparation*. Calculate the quantity, in µg, of strontium in each mL of the Injection taken by the formula:

$$51C$$

in which C is the concentration, in µg per mL, of strontium in the *Assay preparation*.

Succinylcholine Chloride



$C_{14}H_{30}Cl_2N_2O_4$ (anhydrous) 361.31

$C_{14}H_{30}Cl_2N_2O_4 \cdot 2H_2O$ 397.34

Ethanaminium, 2,2′-[(1,4-dioxo-1,4-butanediyl)bis(oxy)] bis[*N*,*N*,*N*-trimethyl]-, dichloride;
Choline chloride succinate (2:1) [71-27-2].
Dihydrate [6101-15-1].

DEFINITION

Succinylcholine Chloride contains NLT 96.0% and NMT 102.0% of succinylcholine chloride ($C_{14}H_{30}Cl_2N_2O_4$), calculated on the anhydrous basis.

[**CAUTION**—Succinylcholine chloride is a neuromuscular blocking agent. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Prepare a 1 in 10 solution of 1 N aqueous tetramethylammonium chloride in methanol. Adjust with hydrochloric acid to a pH of about 3.0.

Standard solution: 8.8 mg/mL of USP Succinylcholine Chloride RS prepared as follows. Transfer a suitable amount of USP Succinylcholine Chloride RS to a suitable volumetric flask and dissolve in 40% of the total volume of water. Dilute with *Mobile phase* to volume while mixing.

Sample solution: 8.8 mg/mL of Succinylcholine Chloride prepared as follows. Transfer a suitable amount of Succinylcholine Chloride to a suitable volumetric flask and dissolve in 40% of the total volume of water. Dilute with *Mobile phase* to volume while mixing.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4-mm × 25-cm; 10-µm packing L3
Flow rate: 0.75 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of succinylcholine chloride ($C_{14}H_{30}Cl_2N_2O_4$) in the portion of Succinylcholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Succinylcholine Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Succinylcholine Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Buffer: 3.85 g/L of anhydrous sodium 1-pentanesulfonate, 2.9 g/L of sodium chloride, and 1% (v/v) 1 N sulfuric acid in water
Mobile phase: Acetonitrile and *Buffer* (5:95)
System suitability solution: 0.5 mg/mL each of USP Citric Acid RS and USP Succinic Acid RS in *Mobile phase*
Standard solution: 0.05 mg/mL of USP Succinylmonocholine Chloride RS in *Mobile phase*
Sample solution: 10 mg/mL of Succinylcholine Chloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Autosampler temperature: 4°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.9 between citric acid and succinic acid, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Succinylcholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of succinylmonocholine chloride from the *Standard solution*
C_S = concentration of USP Succinylmonocholine Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Succinylcholine Chloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Edetate disodium[a]	0.18	—	—
Succinic acid	0.22	1.6	0.1
Unidentified impurity 1[b]	0.32	1.0	0.4
Unidentified impurity 2[b]	0.32	1.0	
Succinylmonocholine	0.49	1.0	0.4
Succinylcholine	1.0	—	—
Any unspecified impurity	—	1.0	0.2
Total impurities[b, c]	—	—	1.5

[a] Included for identification purposes only. Begin integration after this peak, if present.
[b] May occur as a doublet. Acceptance criteria is for the sum of both peaks.
[c] Total impurities include the sum of the results in the tests for *Organic Impurities* and *Limit of Choline*.

Change to read:

- **LIMIT OF CHOLINE**

▲Solution A: 0.62 g/L of methanesulfonic acid
Solution B: 4.8 g/L of methanesulfonic acid
Mobile phase: See *Table 2*. [NOTE—Alternatively, the *Mobile phase* can be generated electrolytically using an automatic eluant generator.]

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
17	100	0
18	0	100
36	0	100
37	100	0
43	100	0▲ (USP 1-Aug-2019)

System suitability solution: 10 µg/mL of USP Choline Chloride RS and 5 µg/mL of USP Potassium Chloride RS
Standard solution: 8 µg/mL of USP Choline Chloride RS
Sample solution: 2 mg/mL of Succinylcholine Chloride. Store at 4° immediately following preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with suppression
Cell temperature: 35°
Columns
Guard: 2-mm × 5-cm; packing L98
Analytical: 2-mm × 25-cm; packing L97
Temperatures
Autosampler: 4°
▲Column: 35° ▲ (USP 1-Aug-2019)
Flow rate: 0.25 mL/min
Injection volume: 5 µL
▲ ▲ (USP 1-Aug-2019)
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for potassium and choline are 0.6 and 1.0, respectively.]

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Suitability requirements
Resolution: NLT 5.0 between choline and potassium
Relative standard deviation: NMT 3.0% for choline
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of choline in the portion of Succinylcholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of choline from the *Sample solution*
r_S = peak response of choline from the *Standard solution*
C_S = concentration of USP Choline Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Succinylcholine Chloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of choline, 104.17
M_{r2} = molecular weight of choline chloride, 139.62

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 10.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Choline Chloride RS
USP Citric Acid RS
USP Potassium Chloride RS
USP Succinic Acid RS
USP Succinylcholine Chloride RS
USP Succinylmonocholine Chloride RS
Ethanaminium, 2-(carboxy-1-oxopropoxy)-*N,N,N*-trimethyl-, chloride.
$C_9H_{18}ClNO_4$ 239.70

Succinylcholine Chloride Injection

DEFINITION
Succinylcholine Chloride Injection is a sterile solution of Succinylcholine Chloride in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous succinylcholine chloride ($C_{14}H_{30}Cl_2N_2O_4$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 1 mg/mL of USP Succinylcholine Chloride RS in water
Sample solution: Nominally 1 mg/mL of succinylcholine chloride from Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 1 µL
Developing solvent system: Acetone and 1 N hydrochloric acid (1:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter. To locate the spots, heat the plate at 105° for 5 min, cool, and spray with potassium bismuth iodide TS, then heat again at 105° for 5 min.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
[NOTE—Because the *Mobile phase* used in this procedure has a fairly high concentration of chloride ion and a low pH, it is advisable to rinse the entire system with water following use of this *Mobile phase*.]
Mobile phase: Prepare a solution (1 in 10) of 1 N tetramethylammonium chloride in methanol. Pass this solution through a 0.45-µm membrane filter, and adjust with hydrochloric acid to a pH of 3.0.
Standard solution: 8.8 mg/mL of USP Succinylcholine Chloride RS prepared as follows. Transfer 88 mg of USP Succinylcholine Chloride RS to a 10-mL volumetric flask and add a volume of water corresponding to the solvent composition of the *Sample solution*. Dilute with *Mobile phase* to volume. Prepare the *Standard solution* concurrently with the *Sample solution*.
Sample solution: Nominally 8.0 mg/mL of succinylcholine chloride prepared as follows. Transfer a volume of Injection equivalent to 80 mg of anhydrous succinylcholine chloride to a 10-mL volumetric flask and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4-mm × 25-cm; packing L3
Flow rate: 0.75 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous succinylcholine chloride ($C_{14}H_{30}Cl_2N_2O_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Succinylcholine Chloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of succinylcholine chloride in the *Sample solution* (mg/mL)

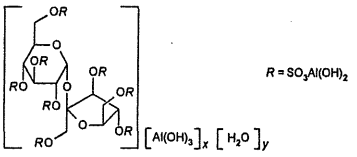
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 2.0 USP Endotoxin Units/mg of succinylcholine chloride
• **PH** ⟨791⟩: 3.0–4.5
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass, in a refrigerator.
• **LABELING:** Label it to indicate, as its expiration date, the month and year NMT 2 years from the month during which the Injection was last assayed and released by the manufacturer.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Succinylcholine Chloride RS

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Sucralfate



$Al_8(OH)_{16}(C_{12}H_{14}O_{35}S_8)[Al(OH)_3]_x[H_2O]_y$ in which x = 8 to 10, and y = 22 to 31.

α-D-Glucopyranoside, β-D-fructofuranosyl-, octakis(hydrogen sulfate), aluminum complex.

Sucrose octakis(hydrogen sulfate) aluminum complex [54182-58-0].

» Sucralfate is the hydrous basic aluminum salt of sucrose octasulfate. It contains the equivalent of not less than 30.0 percent and not more than 38.0 percent of sucrose octasulfate ($C_{12}H_{14}O_{35}S_8$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Potassium Sucrose Octasulfate RS

[NOTE—Sucrosofate Potassium is USAN].

α-D-Glucopyranoside, 1,3,4,6-tetra-*O*-sulfo-β-D-fructofuranosyl, tetrakis (hydrogen sulfate), octapotassium salt, heptahydrate.

$C_{12}H_{14}K_8O_{35}S_8 \cdot 7H_2O$ 1413.64

[CAS-76578-81-9].

Anhydrous.

$C_{12}H_{14}K_8O_{35}S_8$ 1287.53

[CAS-73264-44-5].

Clarity and color of solution—Dissolve 1.0 g in 10 mL of 2 N sulfuric acid: the solution is clear and practically colorless.

Identification—

A: The retention time of the sucrose octasulfate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Add 100 mL of 0.1 N hydrochloric acid to about 500 mg of Sucralfate, and boil the mixture gently, with stirring, for 20 minutes until the sample is completely dissolved. Neutralize with 0.1 N sodium hydroxide, and allow to cool. Add 4 mL of alkaline cupric tartrate TS. Boil a small amount of this solution: a red precipitate of cuprous oxide is produced.

C: A solution in 3 N hydrochloric acid meets the requirements of the tests for *Aluminum* ⟨191⟩.

Acid-neutralizing capacity—Transfer about 250 mg, accurately weighed, to a 250-mL screw-capped bottle, add 100.0 mL of 0.1 N hydrochloric acid, previously heated to 37°, cap the bottle, place it in a 37° water bath, and stir the contents continuously for 1 hour. Cool to room temperature, and transfer 20.0 mL to a 100-mL beaker. Add 30 mL of water, and titrate with 0.1 N sodium hydroxide VS to a pH of 3.5. Perform a blank titration on a mixture of water and 0.1 N hydrochloric acid (30: 20.0). Calculate the mEq of acid consumed per g of Sucralfate taken by the formula:

$$5N(V_B - V_T)/W$$

where N is the exact normality of the sodium hydroxide VS; V_B and V_T are the volumes, in mL, of sodium hydroxide VS consumed by the blank and the test solution, respectively; and W is the weight, in g, of Sucralfate taken: not less than 12 mEq of acid is consumed.

Chloride ⟨221⟩—Transfer 500 mg to a 100-mL volumetric flask, add 30 mL of 2 N nitric acid, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL color comparison tube, add 3 mL of 2 N nitric acid and 2 mL of silver nitrate TS, dilute with water to 50 mL, and mix. Allow to stand, protected from direct sunlight, for 5 minutes. The sample shows no more turbidity than that produced in a solution containing 0.35 mL of 0.020 N hydrochloric acid: not more than 0.50% of chloride is found.

Arsenic, *Method II* ⟨211⟩: 4 ppm.

Limit of pyridine and 2-methylpyridine—

Internal standard solution—Transfer 1.0 mL of 3-methyl pyridine to a 50-mL volumetric flask, dilute with chloroform to volume, and mix. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

Standard stock solution—Transfer about 0.5 g each of 2-methylpyridine and pyridine to a 50-mL volumetric flask, dissolve in chloroform, dilute with chloroform to volume, and mix. Quantitatively dilute 5.0 mL of this solution with chloroform to 50.0 mL. Transfer 5.0 mL of this solution to a 50.0 mL volumetric flask, dilute with chloroform to volume, and mix.

Standard solution—Transfer 5.0 mL of *Standard stock solution* to a 20-mL volumetric flask, add 1.0 mL of the *Internal standard solution*, dilute with chloroform to volume, and mix.

Test solution—Sonicate about 1 g of Sucralfate, accurately weighed, in 10.0 mL of 1 M sodium hydroxide until a uniformly cloudy mixture is obtained. Extract this solution with three 5-mL portions of chloroform, and collect the chloroform extracts in a 20-mL volumetric flask. Add 1.0 mL of the *Internal standard solution*, dilute with chloroform to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a split injection system, and a 0.53-mm × 10-m capillary column coated with a 2.65-µm layer of phase G27. Helium is used as the carrier gas, at a pressure of 36-mm of mercury. The column temperature is maintained at 50°. The injection port temperature and the detector temperature are maintained at 150° and 200°, respectively. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative retention times of pyridine, 2-methylpyridine, and 3-methylpyridine are about 0.42, 0.72, and 1.0, respectively; the resolution, R, between pyridine and 2-methylpyridine is not less than 3.5; the resolution, R, between 2-methylpyridine and 3-methylpyridine is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Separately calculate the quantities, in µg, of pyridine and 2-methylpyridine, if present, in the portion of Sucralfate taken by the formula:

$$20C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of pyridine or 2-methylpyridine in the *Standard solution;* and R_U and R_S are the peak response ratios of the analyte to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.05% each of pyridine and 2-methylpyridine is found.

Limit of sucrose heptasulfate—

Mobile phase—Dissolve 99.1 g of ammonium sulfate in 900 mL of water, dilute with water to 1000 mL, and mix. Adjust with phosphoric acid to a pH of 3.5 ± 0.1, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

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Standard solution—Prepare as directed for the *Standard preparation* in the *Assay*.

Chromatographic system—Prepare as directed in the *Assay*.

Test solution—Prepare as directed for *Assay preparation* in the *Assay*.

Procedure—Inject about 50 µL of the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for sucrose heptasulfate and 1.0 for sucrose octasulfate. The ratio of the peak response of the sucrose heptasulfate peak to that of the sucrose octasulfate peak is not more than 0.1.

Aluminum content—Transfer about 1.0 g, accurately weighed, to a 250-mL volumetric flask, add 10 mL of 6.0 N hydrochloric acid, mix, and heat with continuous stirring in a water bath at 70° for 5 minutes. Cool to room temperature, dilute with water to volume, and mix. Filter the solution, discarding the first portion of the filtrate. Transfer 25.0 mL of the filtrate to a 250-mL beaker, add 25.0 mL of 0.05 M edetate disodium VS, add 20 mL of acetic acid-ammonium acetate buffer TS, and mix. Heat in a water bath at 70° for 5 minutes. Cool to room temperature, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 1.349 mg of aluminum: between 15.5% and 18.5% of aluminum is found, calculated on an "as is" basis.

Assay—

Mobile phase—Dissolve 132 g of ammonium sulfate in 900 mL of water, dilute with water to 1000 mL, and mix. Adjust with phosphoric acid to a pH of 3.5 ± 0.1, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Potassium Sucrose Octasulfate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 10 mg of anhydrous potassium sucrose octasulfate (as determined from the concentration of USP Potassium Sucrose Octasulfate RS corrected for water content by a titrimetric water determination) per mL.

Assay preparation—Transfer about 450 mg of Sucralfate, accurately weighed, to a 35-mL centrifuge tube, and shake at a moderate rate on a vortex mixer. While shaking add 10.0 mL of a mixture of 4.0 N sulfuric acid and 2.2 N sodium hydroxide (1:1). Sonicate with swirling for 5 minutes, keeping the temperature of the mixture below 30°. Without delay transfer the tube to a vortex mixer and while shaking at moderate rate, add an accurately measured volume, *V*, in mL, of 0.1 N sodium hydroxide to bring the pH of the solution to approximately 2, and dilute the solution with (15.0 − *V*) mL of water. Shake for 1 minute, and centrifuge for 5 minutes. Separate the clear supernatant layer, and allow it to stand at room temperature until the pH stabilizes. If the pH is not between 2.3 and 3.5, repeat the test using a different volume of 0.1 N sodium hydroxide. Use the clear supernatant layer.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector and a 3.9-mm × 30-cm column that contains packing L8. The detector and column temperatures are maintained at 30°. The flow rate is about 1 mL per minute.

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the sucrose octasulfate peak is not less than 400 theoretical plates; the tailing factor for the sucrose octasulfate peak is not more than 4.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sucrose octasulfate ($C_{12}H_{14}O_{35}S_8$) in the portion of Sucralfate taken by the formula:

$$(974.75/1287.53)(25C)(r_U/r_S)$$

in which 974.75 and 1287.53 are the molecular weights of sucrose octasulfate and anhydrous potassium sucrose octasulfate, respectively; *C* is the concentration, in mg per mL, of anhydrous potassium sucrose octasulfate in the *Standard preparation;* and r_U and r_S are the peak responses of sucrose octasulfate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Sucralfate Tablets

» Sucralfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of sucralfate ($Al_8(OH)_{16}(C_{12}H_{14}O_{35}S_8)[Al(OH)_3]_x[H_2O]_y$) corresponding to not less than 30.6 percent and not more than 37.4 percent of sucrose octasulfate ($C_{12}H_{14}O_{35}S_8$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Potassium Sucrose Octasulfate RS

[NOTE—Sucrosofate Potassium is USAN].

α-D-Glucopyranoside, 1,3,4,6-tetra-*O*-sulfo-β-D-fructofuranosyl, tetrakis (hydrogen sulfate), octapotassium salt, heptahydrate.

$C_{12}H_{14}K_8O_{35}S_8 \cdot 7H_2O$ 1413.64

[CAS-76578-81-9].

Anhydrous.

$C_{12}H_{14}K_8O_{35}S_8$ 1287.53

[CAS-73264-44-5].

Identification—

A: The retention time of the sucrose octasulfate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Shake a portion of finely powdered Tablets, equivalent to about 1 g of sucralfate, with 3 N hydrochloric acid, and filter: the solution so obtained meets the requirements of *Identification* test *C* under *Sucralfate*.

Disintegration ⟨701⟩: 15 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Acid-neutralizing capacity—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of sucralfate, to a 250-mL screw-capped bottle, and proceed as directed in the test for *Acid-neutralizing capacity* under *Sucralfate*, beginning with "add 100.0 mL of 0.1 N hydrochloric acid": not less than 12 mEq of acid is consumed.

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Sucralfate*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 450 mg of sucralfate, to a 35-mL

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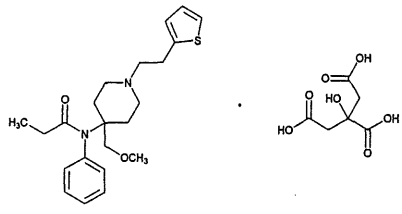
centrifuge tube, and shake at a moderate rate on a vortex mixer. Proceed as directed for *Assay preparation* in the *Assay* under *Sucralfate* beginning with "While shaking, add 10.0 mL."

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Sucralfate*. Calculate the quantity, in mg, of sucrose octasulfate ($C_{12}H_{14}O_{35}S_8$) in the portion of Tablets taken by the formula:

$$(974.75/1287.53)(25C)(r_U/r_S)$$

in which the terms are as defined therein.

Sufentanil Citrate



$C_{22}H_{30}N_2O_2S \cdot C_6H_8O_7$ 578.67

Propanamide, *N*-[4-(methoxymethyl)-1-[2-(thienyl)ethyl]-4-piperidinyl]-*N*-phenyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1);
N-[4-(Methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidyl] propionanilide citrate (1:1) [60561-17-3].

DEFINITION

Sufentanil Citrate contains NLT 98.0% and NMT 102.0% of sufentanil citrate ($C_{22}H_{30}N_2O_2S \cdot C_6H_8O_7$), calculated on the dried basis.

[**CAUTION**—Handle Sufentanil Citrate with great care because it is a potent opioid analgesic. Great care should be taken to prevent inhaling particles of Sufentanil Citrate and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 50 µg/mL
Medium: Use *Mobile phase*, prepared as directed in the *Assay*.
Acceptance criteria: Meets the requirements

- **C. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩

Sample solution: Dissolve 500 mg in 5 mL of water.
Analysis: Render the *Sample solution* alkaline with 1 N sodium hydroxide, and extract with three 5-mL portions of methylene chloride.
Acceptance criteria: The aqueous layer meets the requirements.

- **D.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, acetonitrile, and 0.13 M ammonium acetate (45:24:31). Adjust with glacial acetic acid or ammonium hydroxide to a pH of 7.2.
Standard solution: 0.075 mg/mL of USP Sufentanil Citrate RS in *Mobile phase*
Sample solution: 0.075 mg/mL of Sufentanil Citrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sufentanil citrate ($C_{22}H_{30}N_2O_2S \cdot C_6H_8O_7$) in the portion of Sufentanil Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sufentanil Citrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Sufentanil Citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase: Proceed as directed in the *Assay*.
Blank: 0.664 mg/mL of citric acid in *Mobile phase*
Sample solution: 0.75 mg/mL of Sufentanil Citrate in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*.
Analysis
Samples: *Blank* and *Sample solution*
Calculate the percentage of each impurity, disregarding any peaks corresponding to those found in the *Blank*, in the portion of Sufentanil Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses of all of the peaks

Acceptance criteria
Individual impurity: NMT 0.5%
Total impurities: NMT 1.0%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample under vacuum at 60° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Monographs

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sufentanil Citrate RS

Sufentanil Citrate Injection

DEFINITION
Sufentanil Citrate Injection is a sterile solution of Sufentanil Citrate in Water for Injection. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of sufentanil citrate ($C_{22}H_{30}N_2O_2S \cdot C_6H_8O_7$).
[**CAUTION**—Handle Sufentanil Citrate Injection with great care, because it is a potent opioid analgesic.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 50 µg/mL
Medium: Use *Mobile phase* prepared as directed in the *Assay*. [NOTE—For samples that do not require dilution to achieve 50 µg/mL, use Water for Injection as the medium for the *Standard solution*.]
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, acetonitrile, and 0.13 M ammonium acetate (45:24:31). Adjust with glacial acetic acid or ammonium hydroxide to a pH of 7.2.
Standard solution: 0.075 mg/mL of USP Sufentanil Citrate RS in water
Sample solution: Use Sufentanil Citrate Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sufentanil citrate ($C_{22}H_{30}N_2O_2S \cdot C_6H_8O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sufentanil Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sufentanil citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 6.25 USP Endotoxin Units/mL
• **pH** ⟨791⟩: 3.5–6.0
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sufentanil Citrate RS

Invert Sugar Injection

» Invert Sugar Injection is a sterile solution of a mixture of equal amounts of Dextrose and Fructose in Water for Injection, or an equivalent sterile solution produced by the hydrolysis of Sucrose, in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_6H_{12}O_6$. It contains no antimicrobial agents.
[NOTE—Invert Sugar Injection that is produced by mixing Dextrose and Fructose is exempt from the requirement of the test for *Completeness of inversion*.]

Packaging and storage—Preserve in single-dose containers, preferably of Type I or Type II glass, or of a suitable plastic material.
Labeling—The label states the total osmolar concentration in mOsmol per L.
Identification—Add a few drops of Injection to 5 mL of hot alkaline cupric tartrate TS: a copious red precipitate of cupric oxide is formed.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 USP Endotoxin Unit per mL.
pH ⟨791⟩: between 3.0 and 6.5.
Chloride ⟨221⟩—A 2.0-mL portion shows no more chloride than corresponds to 0.34 mL of 0.020 N hydrochloric acid (0.012%).
Limit of 5-hydroxymethylfurfural and related substances—Dilute an accurately measured volume of Injection, equivalent to 1.0 g of invert sugar, with water to 500.0 mL. Determine the absorbance of this solution in a 1-cm cell at 284 nm, with a suitable spectrophotometer, using water as the blank: the absorbance is not more than 0.25.

Completeness of inversion—
Mobile phase—Use filtered, degassed water.
Standard preparation—Prepare a solution in water containing known concentrations of about 0.25 mg of sucrose and about 12.5 mg of dextrose per mL.
Test preparation—Transfer a volume of Injection, equivalent to about 2.5 g of invert sugar, to a 100-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector and a 7.8-mm × 30-cm column that contains 9-µm packing L19, maintained at a constant temperature of about 40°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the sucrose elutes first, and the peak is baseline separated from the dextrose peak. The relative standard deviation for replicate injections is not more than 2.0%.

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Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the sucrose peaks. Calculate the quantity, in mg, of sucrose, in the volume of the Injection taken by the formula:

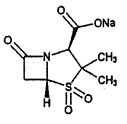
$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of sucrose in the *Standard preparation*, and r_U and r_S are the peak responses for sucrose obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 1.5% of the quantity of invert sugar in the volume of Injection taken, based on the value stated on the label, is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Pipet 50 mL of alkaline cupric tartrate TS into a 400-mL beaker, add 48 mL of water, mix, and pipet into the mixture 2 mL of Injection that has been diluted quantitatively with water, if necessary, to a 5.0% concentration. Cover the beaker with a watch glass, heat the solution, regulating the heat so that boiling begins in 4 minutes, and continue boiling for 2.0 minutes. Filter the hot solution at once through a tared porcelain filtering crucible, wash the precipitate with water maintained at 60°, then with 10 mL of alcohol. Dry at 105° to constant weight. Perform a blank determination, and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 204.0 mg and not more than 224.4 mg, corresponding to between 95.0 and 105.0 mg of $C_6H_{12}O_6$.

Sulbactam Sodium



$C_8H_{10}NNaO_5S$ 255.22

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-, 4,4-dioxide, sodium salt, (2*S*-*cis*)-;
Sodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide [69388-84-7].

DEFINITION

Sulbactam Sodium contains NLT 886 µg/mg and NMT 941 µg/mg of sulbactam ($C_8H_{11}NO_5S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer A: 5.4 g/L of monobasic potassium phosphate adjusted with dilute phosphoric acid to a pH of 4.0

Buffer B: 2.7 g/L of monobasic potassium phosphate adjusted with dilute phosphoric acid to a pH of 4.0

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer A (%)	Acetonitrile (%)
0	98	2
7.5	50	50
8.5	50	50
9.0	98	2
12.5	98	2

Standard solution: 0.7 mg/mL of USP Sulbactam RS prepared as follows. Dissolve a suitable amount of USP Sulbactam RS in acetonitrile, using 2% of the final volume, and dilute with *Buffer B* to volume.

Sample solution: 0.77 mg/mL of Sulbactam Sodium prepared as follows. Dissolve a suitable amount of Sulbactam Sodium in acetonitrile, using 2% of the final volume, and dilute with *Buffer B* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg, of sulbactam ($C_8H_{11}NO_5S$) in each mg of Sulbactam Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulbactam RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulbactam Sodium in the *Sample solution* (mg/mL)
P = potency of sulbactam in USP Sulbactam RS (mg/mg)
F = conversion factor, 1000 µg/mg

Acceptance criteria: 886–941 µg/mg on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer A, Buffer B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Diluent: 2 mL of acetonitrile diluted with *Buffer B* to 100 mL

System suitability solution: 0.7 µg/mL each of USP Sulbactam Related Compound A RS, USP Sulbactam Related Compound D RS, USP Sulbactam Related Compound E RS, and USP Sulbactam Related Compound F RS prepared as follows. Dissolve suitable amounts of USP Sulbactam Related Compound A RS, USP Sulbactam Related Compound D RS, USP Sulbactam Related Compound E RS, and USP Sulbactam Related Compound F RS in acetonitrile using 10% of the final volume, and sonicate as needed. Dilute with *Diluent* to volume. Protect this solution from light.

USP Monographs

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Standard stock solution: 0.7 mg/mL of USP Sulbactam RS prepared as follows. Dissolve a suitable amount of USP Sulbactam RS in acetonitrile, using 2% of the final volume, and dilute with *Buffer B* to volume.
Standard solution: 0.7 µg/mL of USP Sulbactam RS from *Standard stock solution* in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between sulbactam related compound D and sulbactam related compound E, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Sulbactam Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulbactam RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulbactam Sodium in the *Sample solution* (mg/mL)
P = potency of sulbactam in USP Sulbactam RS (mg/mg)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

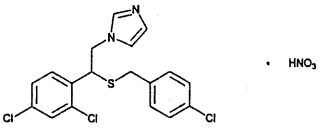
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulbactam related compound A[a]	0.4	1.7	0.5
Amoxicillin related compound A[b]	0.6	2.0	0.1
Sulbactam	1.0	—	—
6-Bromopenicillanic acid sulfone[c]	1.6	1.0	0.2
Sulbactam related compound D[d]	2.0	2.0	0.1
Sulbactam related compound E[e]	2.1	1.0	0.2
Sulbactam related compound F[f]	2.5	1.7	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 3-Sulfino-D-valine; (2*S*)-2-Amino-3-methyl-3-sulfinobutanoic acid.
[b] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[c] (2*S*,5*R*,6*R*)-6-Bromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide.
[d] 6-Bromopenicillanic acid; (2*S*,5*R*,6*R*)-6-Bromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[e] 6,6-Dibromopenicillanic acid sulfone; (2*S*,5*R*)-6,6-Dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide.
[f] 6,6-Dibromopenicillanic acid; (2*S*,5*R*)-6,6-Dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Sulbactam Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Unit/mg of sulbactam.
- **STERILITY TESTS** ⟨71⟩: Where the label states that Sulbactam Sodium is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulbactam RS
  USP Sulbactam Related Compound A RS
  (2*S*)-2-Amino-3-methyl-3-sulfinobutanoic acid.
  $C_5H_{11}NO_4S$ 181.21
  USP Sulbactam Related Compound D RS
  (2*S*,5*R*,6*R*)-6-Bromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, potassium salt.
  $C_8H_9BrKNO_3S$ 318.23
  USP Sulbactam Related Compound E RS
  (2*S*,5*R*)-6,6-Dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide.
  $C_8H_9Br_2NO_5S$ 391.03
  USP Sulbactam Related Compound F RS
  (2*S*,5*R*)-6,6-Dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
  $C_8H_9Br_2NO_3S$ 359.03

Sulconazole Nitrate



$C_{18}H_{15}Cl_3N_2S \cdot HNO_3$ 460.76
1*H*-Imidazole, 1-[2-[[(4-chlorophenyl)methyl]thio]-2-(2,4-dichlorophenyl)ethyl]-, mononitrate, (±)-;
(±)-1-[2,4-Dichloro-β-[(*p*-chlorobenzyl)thio] phenethyl] imidazole mononitrate [61318-91-0].

DEFINITION
Sulconazole Nitrate contains NLT 98.0% and NMT 102.0% of sulconazole nitrate ($C_{18}H_{15}Cl_3N_2S \cdot HNO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Nitrate*: Meets the requirements of test *A*

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• **C.** The retention time of the major peak for sulconazole of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 7.7 g of ammonium acetate in 992 mL of water and add 3.3 mL of glacial acetic acid. Pass through a suitable filter of 0.45-µm pore size under vacuum and degas with helium purging.

Solution A: Methanol, water, and *Buffer* (200:640:160)

Solution B: Methanol and acetonitrile (40:60)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	60	40
8.0	10	90
8.1	60	40
10.0	60	40

Diluent: Methanol and water (60:40)

Standard solution: 0.15 mg/mL of USP Sulconazole Nitrate RS in *Diluent*, prepared as follows. Transfer an appropriate quantity of USP Sulconazole Nitrate RS to a suitable volumetric flask, dissolve in 80% of the flask volume of *Diluent*, sonicate until dissolved, and dilute with *Diluent* to volume.

Sample solution: 0.15 mg/mL of Sulconazole Nitrate in *Diluent*, prepared as follows. Transfer an appropriate quantity of Sulconazole Nitrate to a suitable volumetric flask, dissolve in 80% of the flask volume of *Diluent*, sonicate until dissolved, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.1-mm × 10-cm; 1.7-µm packing L1

Flow rate: 0.5 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulconazole nitrate ($C_{18}H_{15}Cl_3N_2S \cdot HNO_3$) in the portion of Sulconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulconazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 1.5 µg/mL each of USP Sulconazole Nitrate RS and USP Sulconazole Related Compound A RS in *Diluent*, prepared as follows. Transfer appropriate quantities of USP Sulconazole Nitrate RS and USP Sulconazole Related Compound A RS to a suitable volumetric flask, dissolve in 80% of the flask volume of *Diluent*, sonicate until dissolved, and dilute with *Diluent* to volume.

Sample solution: 1.5 mg/mL of Sulconazole Nitrate in *Diluent*, prepared as follows. Transfer an appropriate quantity of Sulconazole Nitrate to a suitable volumetric flask, dissolve in 80% of the flask volume of *Diluent*, sonicate until dissolved, and dilute with *Diluent* to volume.

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Relative standard deviation: NMT 2.0% for sulconazole nitrate and sulconazole related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulconazole related compound A in the portion of Sulconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulconazole related compound A from the *Sample solution*

r_S = peak response of sulconazole related compound A from the *Standard solution*

C_S = concentration of USP Sulconazole Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulconazole Nitrate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Sulconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of sulconazole nitrate from the *Standard solution*

C_S = concentration of USP Sulconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Sulconazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulconazole related compound A	0.61	0.1
Sulconazole nitrate	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

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SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum at 80° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers, protected from light.
• **USP Reference Standards** ⟨11⟩
USP Sulconazole Nitrate RS
USP Sulconazole Related Compound A RS
1-[2-(4-Chlorobenzyl)sulfinyl-2-(2,4-dichlorophenyl) ethyl]-1*H*-imidazole.
$C_{18}H_{15}Cl_3N_2OS$ 413.74

Triple Sulfa Vaginal Cream

DEFINITION
Triple Sulfa Vaginal Cream contains NLT 90.0% and NMT 110.0% of the labeled amounts of sulfathiazole ($C_9H_9N_3O_2S_2$), sulfacetamide ($C_8H_{10}N_2O_3S$), and sulfabenzamide ($C_{13}H_{12}N_2O_3S$).

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Acetonitrile, 1 M tetrabutylammonium hydroxide, and water (22:10:78). Adjust with dilute phosphoric acid (1 in 10) to a pH of 7.7 ± 0.2, and mix for 5 min. If necessary, adjust to a pH of 7.7 ± 0.2, using dilute phosphoric acid (1 in 50) or 1 M tetrabutylammonium hydroxide.
Internal standard solution: 10 mg/mL of sulfapyridine in acetone
Standard solution: 0.068 mg/mL of USP Sulfathiazole RS, 0.058 mg/mL of USP Sulfacetamide RS, and 0.074 mg/mL of USP Sulfabenzamide RS prepared as follows. Transfer 34 mg of USP Sulfathiazole RS, 29 mg of USP Sulfacetamide RS, and 37 mg of USP Sulfabenzamide RS to a 50-mL volumetric flask. Add 2.0 mL of *Internal standard solution* and 30 mL of acetone, and shake for 10 min. If necessary, sonicate to effect solution. Dilute with acetone to volume. Pipet 5 mL of this solution into a 50-mL volumetric flask, and evaporate on a steam bath with the aid of a gentle stream of nitrogen to dryness. Dissolve the residue in *Mobile phase*, and dilute with *Mobile phase* to volume.
Sample solution: Nominally 0.069 mg/mL of sulfathiazole, 0.058 mg/mL of sulfacetamide, and 0.074 mg/mL of sulfabenzamide prepared as follows. Using a plastic syringe equipped with a suitable cannula, transfer a quantity of Vaginal Cream, nominally equivalent to 173 mg of sulfathiazole, 144 mg of sulfacetamide, and 184 mg of sulfabenzamide, to a 250-mL volumetric flask. Add 10.0 mL of *Internal standard solution* and 100 mL of acetone, and warm the flask on a steam bath while swirling the contents to dissolve the cream. Cool to room temperature, and dilute with acetone to volume. Pass the solution through filter paper, discarding the first 10 mL of the filtrate. Pipet 5 mL of the filtrate so obtained into a 50-mL volumetric flask, and evaporate on a steam bath with the aid of a gentle stream of nitrogen to dryness. Dissolve the residue in *Mobile phase*, and dilute with *Mobile phase* to volume. Cool the solution in an ice bath for 10 min, pass the cold solution through filter paper, discarding the first 10–15 mL of the filtrate, and collect 5 mL for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for sulfacetamide, sulfapyridine, sulfathiazole, and sulfabenzamide are about 0.8, 1.0, 1.8, and 2.5, respectively.]
Suitability requirements
Resolution: NLT 2.0 between sulfacetamide and sulfapyridine
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of sulfathiazole ($C_9H_9N_3O_2S_2$), sulfacetamide ($C_8H_{10}N_2O_3S$), and sulfabenzamide ($C_{13}H_{12}N_2O_3S$) in the portion of Vaginal Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of sulfathiazole, sulfacetamide, or sulfabenzamide to the internal standard from the *Sample solution*
R_S = peak response ratio of sulfathiazole, sulfacetamide, or sulfabenzamide to the internal standard from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulfathiazole, sulfacetamide, or sulfabenzamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 3.0–4.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed, light-resistant containers, or in collapsible tubes.
• **USP Reference Standards** ⟨11⟩
USP Sulfabenzamide RS
USP Sulfacetamide RS
USP Sulfathiazole RS

Triple Sulfa Vaginal Inserts

DEFINITION
Triple Sulfa Vaginal Inserts contain NLT 90.0% and NMT 110.0% of the labeled amounts of sulfathiazole ($C_9H_9N_3O_2S_2$), sulfacetamide ($C_8H_{10}N_2O_3S$), and sulfabenzamide ($C_{13}H_{12}N_2O_3S$).

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, 1 M tetrabutylammonium hydroxide, and water (22:10:78). Adjust with dilute phosphoric acid (1 in 10) to a pH of 7.7 ± 0.2, and mix for 5 min. If necessary, adjust to a pH of 7.7 ± 0.2, using dilute phosphoric acid (1 in 50) or 1 M tetrabutylammonium hydroxide.

Internal standard solution: 10 mg/mL of sulfapyridine in acetone

Standard solution: 0.068 mg/mL of USP Sulfathiazole RS, 0.058 mg/mL of USP Sulfacetamide RS, and 0.074 mg/mL of USP Sulfabenzamide RS prepared as follows. Transfer 34 mg of USP Sulfathiazole RS, 29 mg of USP Sulfacetamide RS, and 37 mg of USP Sulfabenzamide RS to a 50-mL volumetric flask. Add 2.0 mL of *Internal standard solution* and 30 mL of acetone, and shake for 10 min. If necessary, sonicate to effect solution. Dilute with acetone to volume. Pipet 5 mL of this solution into a 50-mL volumetric flask, and evaporate on a steam bath with the aid of a gentle stream of nitrogen to dryness. Dissolve the residue in *Mobile phase*, and dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.069 mg/mL of sulfathiazole, 0.058 mg/mL of sulfacetamide, and 0.074 mg/mL of sulfabenzamide prepared as follows. Transfer a weighed portion of the powder (NLT 10 Vaginal Inserts), equivalent to about 173 mg of sulfathiazole, 144 mg of sulfacetamide, and 184 mg of sulfabenzamide, to a 250-mL volumetric flask. Add 10.0 mL of water, and shake for 10 min. Add 10.0 mL of *Internal standard solution* and 100 mL of acetone, and shake for 30 min at low speed on a mechanical shaker. Dilute with acetone to volume, and allow to stand for 30 min. Pipet 5 mL of the clear supernatant into a 50-mL volumetric flask, and evaporate on a steam bath with the aid of a gentle stream of nitrogen to dryness. Dissolve the residue in *Mobile phase*, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for sulfacetamide, sulfapyridine, sulfathiazole, and sulfabenzamide are 0.8, 1.0, 1.8, and 2.5, respectively.]

Suitability requirements

Resolution: NLT 2.0 between sulfacetamide and sulfapyridine

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of sulfathiazole ($C_9H_9N_3O_2S_2$), sulfacetamide ($C_8H_{10}N_2O_3S$), and sulfabenzamide ($C_{13}H_{12}N_2O_3S$) in the portion of Vaginal Inserts taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of sulfathiazole, sulfacetamide, or sulfabenzamide to the internal standard from the *Sample solution*

R_S = peak response ratio of sulfathiazole, sulfacetamide, or sulfabenzamide to the internal standard from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of sulfathiazole, sulfacetamide, or sulfabenzamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Time: 30 min

Acceptance criteria: Meet the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation* for sulfathiazole, sulfacetamide, and sulfabenzamide

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

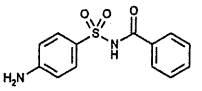
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfabenzamide RS

USP Sulfacetamide RS

USP Sulfathiazole RS

Sulfabenzamide



$C_{13}H_{12}N_2O_3S$ 276.31

Benzamide, *N*-[(4-aminophenyl)sulfonyl]-;
N-Sulfanilylbenzamide [127-71-9].

DEFINITION

Sulfabenzamide contains NLT 99.0% and NMT 100.5% of sulfabenzamide ($C_{13}H_{12}N_2O_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.**

Sample: 100 mg

Analysis: Suspend the *Sample* in 2 mL of water, and add 100 mg of sodium bicarbonate.

Acceptance criteria: It dissolves with effervescence (distinction from sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, and sulfaguanidine).

ASSAY

• **PROCEDURE**

Sample: 800 mg of Sulfabenzamide

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium methoxide VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 125-mL conical flask, and dissolve in 25 mL of dimethylformamide. Add 3 drops of thymol blue TS (prepared with methanol), and titrate with *Titrant* to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 27.63 mg of sulfabenzamide ($C_{13}H_{12}N_2O_3S$).

Acceptance criteria: 99.0%–100.5% on the dried basis

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IMPURITIES

• **SELENIUM** ⟨291⟩
Sample solution: 300 mg of Sulfabenzamide
Standard solution: Use 3 mL of *Stock Solution*.
Acceptance criteria: NMT 10 ppm
• **ORDINARY IMPURITIES** ⟨466⟩
Visualization: 1
Eluant: Chloroform, methanol, and glacial acetic acid (90:5:5)
Standard solution and **Sample solution:** Use methanol as solvent.

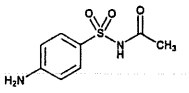
SPECIFIC TESTS

• **CLARITY AND COLOR OF SOLUTION**
Sample: 2.0 g
Analysis: Dissolve the *Sample* in 15 mL of 1 N sodium hydroxide, with warming.
Acceptance criteria: A colorless to pale yellow solution having NMT a slight turbidity is produced.
• **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩**:** Between 180° and 184°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfabenzamide RS

Sulfacetamide



$C_8H_{10}N_2O_3S$ 214.24
Acetamide, *N*-[(4-aminophenyl)sulfonyl]-;
N-Sulfanilylacetamide [144-80-9].

DEFINITION

Sulfacetamide contains NLT 99.0% and NMT 100.5% of sulfacetamide ($C_8H_{10}N_2O_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Solution A: 1% glacial acetic acid
Solution B: Methanol
Diluent: Methanol and *Solution A* (10:90)
Mobile phase See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
9.5	10	90
9.6	90	10
14	90	10

Standard solution: 0.2 mg/mL of USP Sulfacetamide RS in *Diluent*. Sonicate for 5 min to dissolve.
Sample solution: 0.2 mg/mL of Sulfacetamide in *Diluent*. Sonicate for 5 min to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 12.5-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.9–1.8, *Standard solution*
Relative standard deviation: NMT 0.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfacetamide ($C_8H_{10}N_2O_3S$) in the portion of Sulfacetamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulfacetamide from the *Sample solution*
r_S = peak response of sulfacetamide from the *Standard solution*
C_S = concentration of USP Sulfacetamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfacetamide in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample: 1 g
Analysis: Digest the *Sample* with 50 mL of water at about 70° for 5 min. Cool immediately to room temperature, and filter.
Acceptance criteria: 0.04%; a 25-mL portion of the filtrate shows no more sulfate than corresponds to 0.2 mL of 0.02 N sulfuric acid.
• **SELENIUM** ⟨291⟩
Sample: 200 mg
Acceptance criteria: 30 ppm
• **ORGANIC IMPURITIES**
Mobile phase: Methanol, water, and glacial acetic acid (10:89:1)
Diluent: Methanol and *Solution A* (10:90)
System suitability solution: 0.2 mg/mL of USP Sulfacetamide RS and 0.05 mg/mL of USP Sulfanilamide RS in *Diluent*. Sonicate for 5 min to dissolve.
Standard solution: 2 µg/mL of USP Sulfacetamide RS and 4 µg/mL of USP Sulfanilamide RS in water
Sample solution: 2 mg/mL of Sulfacetamide in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between sulfacetamide and sulfanilamide, *System suitability solution*
Tailing factor: NMT 1.5 for sulfacetamide, *Standard solution*
Relative standard deviation: NMT 2.0% for sulfacetamide, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfanilamide in the portion of Sulfacetamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulfanilamide from the *Sample solution*
r_S = peak response of sulfanilamide from the *Standard solution*
C_S = concentration of USP Sulfanilamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfacetamide in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Sulfacetamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of sulfacetamide from the *Standard solution*
C_S = concentration of USP Sulfacetamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfacetamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

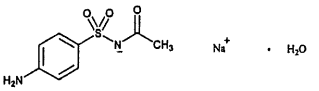
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulfanilamide	0.6	0.2
Sulfacetamide	1.0	—
Any single unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 0.5%
• **REACTION**
Sample solution: 6.67 mg/mL
Acceptance criteria: Acid to litmus
• **CLARITY AND COLOR OF SOLUTION**
Sample: 200 mg
Analysis: Dissolve the *Sample* in 5 mL of 1 N sodium hydroxide.
Acceptance criteria: A yellow to faintly yellow solution having NMT a trace of turbidity is produced.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfacetamide RS
USP Sulfanilamide RS
p-Aminobenzenesulfonamide.
$C_6H_8N_2O_2S$ 172.20

Sulfacetamide Sodium



$C_8H_9N_2NaO_3S \cdot H_2O$ 254.24
$C_8H_9N_2NaO_3S$ 236.23
Acetamide, *N*-[(4-aminophenyl)sulfonyl]-, monosodium salt, monohydrate;
N-Sulfanilylacetamide monosodium salt monohydrate [6209-17-2].
Anhydrous [127-56-0].

DEFINITION
Sulfacetamide Sodium contains NLT 99.0% and NMT 100.5% of sulfacetamide sodium ($C_8H_9N_2NaO_3S$), calculated on the anhydrous basis.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.**
Sample solution: 1 g in 25 mL of water
Analysis: Adjust the *Sample solution* with 6 N acetic acid to a pH of 4–5, and filter. Wash the precipitate with water, and use the filtrate.
Acceptance criteria: The filtrate responds to the test for *Identification Tests—General, Sodium* ⟨191⟩.
• **C.**
Sample solution: 100 mg in 5 mL of water
Analysis: Add 5 drops of cupric sulfate TS to the *Sample solution*.
Acceptance criteria: A light bluish green precipitate is formed, and it remains unchanged on standing.
• **D.**
Sample solution: 500 mg in 10 mL of dilute hydrochloric acid (1 in 10)
Analysis 1: To about one-half of the *Sample solution* add 2 mL of trinitrophenol TS.
Acceptance criteria 1: A very heavy flocculent or almost gelatinous precipitate is formed.
Analysis 2: To the remainder of the *Sample solution* add 3 drops of formaldehyde TS.
Acceptance criteria 2: A white precipitate is formed, and it changes to orange on standing (distinction from sulfamethoxypyridazine).

ASSAY
• **PROCEDURE**
Solution A: 1% glacial acetic acid
Solution B: Methanol
Mobile phase: See *Table 1*.

USP Monographs

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
9.5	10	90
9.6	90	10
14	90	10

Standard solution: 0.2 mg/mL of USP Sulfacetamide Sodium RS in water
Sample solution: 0.2 mg/mL of Sulfacetamide Sodium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.9–1.8, *Standard solution*
Relative standard deviation: NMT 0.5% for sulfacetamide, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfacetamide sodium ($C_8H_9N_2NaO_3S$) in the portion of Sulfacetamide Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulfacetamide from the *Sample solution*
r_S = peak response of sulfacetamide from the *Standard solution*
C_S = concentration of USP Sulfacetamide Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfacetamide Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES

• **SELENIUM** ⟨291⟩
Sample: 200 mg
Acceptance criteria: 30 ppm
• **ORGANIC IMPURITIES**
Mobile phase: Methanol, water, and glacial acetic acid (10:89:1)
System suitability solution: 0.2 mg/mL of USP Sulfacetamide Sodium RS and 0.05 mg/mL of USP Sulfanilamide RS in *Mobile phase*
Standard solution: 2 µg/mL of USP Sulfacetamide Sodium RS and 4 µg/mL of USP Sulfanilamide RS in *Mobile phase*
Sample solution: 2 mg/mL of Sulfacetamide Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between sulfacetamide and sulfanilamide, *System suitability solution*
Tailing factor: NMT 1.5 for sulfacetamide, *Standard solution*
Relative standard deviation: NMT 2.0% for sulfacetamide, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfanilamide in the portion of Sulfacetamide Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulfanilamide from the *Sample solution*
r_S = peak response of sulfanilamide from the *Standard solution*
C_S = concentration of USP Sulfanilamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfacetamide Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Sulfacetamide Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of sulfacetamide from the *Standard solution*
C_S = concentration of USP Sulfacetamide Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfacetamide Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulfanilamide	0.6	0.2
Sulfacetamide	1.0	—
Any single unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS

• **PH** ⟨791⟩
Sample solution: 50 mg/mL
Acceptance criteria: 8.0–9.5
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 8.1%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfacetamide Sodium RS
USP Sulfanilamide RS
p-Aminobenzenesulfonamide.
$C_6H_8N_2O_2S$ 172.20

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Sulfacetamide Sodium Ophthalmic Ointment

DEFINITION

Sulfacetamide Sodium Ophthalmic Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of sulfacetamide sodium ($C_8H_9N_2NaO_3S \cdot H_2O$). It is sterile.

IDENTIFICATION

• **A.**

Sample: Nominally 1 g of sulfacetamide sodium from a quantity of Ophthalmic Ointment

Analysis: Dissolve the *Sample* in 100 mL of ether in a separator, and extract the mixture with 25 mL of water. Wash the extract with 25 mL of ether, and warm the water extract on a steam bath to remove the last traces of ether. Adjust with 6 N acetic acid to a pH of 4–5, and filter. Wash the precipitate with water, and dry at 105° for 2 h. Use the precipitate in *Identification B*, *C*, and *D*.

Acceptance criteria: The sulfacetamide melts at 180°–184°.

• **B.**

Sample: 500 mg of the sulfacetamide from *IdentificationA*

Analysis: Place the *Sample* in a test tube, and heat gently until it boils.

Acceptance criteria: An oily liquid, which has the characteristic odor of acetamide, condenses on the walls of the test tube (distinction from the sublimates of sulfadiazine, sulfamerazine, and sulfamethazine, which are solids at room temperature).

• **C.**

Sample solution: 100 mg of the sulfacetamide from *IdentificationA* in 5 mL of water

Analysis: Add 5 drops of cupric sulfate TS to the *Sample solution*.

Acceptance criteria: A light bluish-green precipitate is formed, and it remains unchanged on standing.

• **D.**

Sample solution: 500 mg of the sulfacetamide from *IdentificationA* in 10 mL of dilute hydrochloric acid (1 in 10)

Analysis 1: To about one-half of the *Sample solution* add 2 mL of trinitrophenol TS.

Acceptance criteria 1: A very heavy flocculent or almost gelatinous precipitate is formed.

Analysis 2: To the remainder of the *Sample solution* add 3 drops of formaldehyde TS.

Acceptance criteria 2: A white precipitate is formed, and it changes to orange on standing (distinction from sulfamethoxypyridazine).

ASSAY

• **PROCEDURE**

Diluent: 20% methanol

Mobile phase: Methanol, glacial acetic acid, and water (10:1:89)

Standard stock solution: 5 mg/mL of USP Sulfacetamide Sodium RS prepared as follows. Transfer 50 mg of USP Sulfacetamide Sodium RS to a 40-mL centrifuge tube. Add 10.0 mL of *Diluent*, insert the stopper, and mix using a vortex mixer for 3 min to dissolve the Reference Standard. Add 7.5 mL of heptane, insert the stopper, and mix using a vortex mixer for another 3 min. Centrifuge to effect separation of the phases. Withdraw, and discard the upper heptane layer.

Standard solution: 0.03 mg/mL of USP Sulfacetamide Sodium RS in *Diluent* from the *Standard stock solution*

System suitability solution: 0.03 mg/mL of sulfanilamide in the *Standard solution*

Sample stock solution: Nominally 5 mg/mL of sulfacetamide sodium prepared as follows. Transfer 100 mg of sulfacetamide sodium from a quantity of Ophthalmic Ointment to a 40-mL centrifuge tube. Add 15.0 mL of heptane, insert the stopper, and mix using a vortex mixer for 3 min to dissolve the Ophthalmic Ointment. Add 20.0 mL of *Diluent*, insert the stopper, and mix using a vortex mixer for 3 min. Centrifuge to effect separation of the phases. Withdraw, and discard the upper heptane layer.

Sample solution: Nominally 0.03 mg/mL of sulfacetamide sodium in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 90 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3 between the sulfacetamide and sulfanilamide peaks, *System suitability solution*

Column efficiency: NLT 1500 theoretical plates, determined from the analyte peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sulfacetamide sodium ($C_8H_9N_2NaO_3S \cdot H_2O$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulfacetamide Sodium RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)

C_U = nominal concentration of sulfacetamide sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of sulfacetamide sodium monohydrate, 254.24

M_{r2} = molecular weight of anhydrous sulfacetamide sodium, 236.23

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfacetamide Sodium RS

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Sulfacetamide Sodium Ophthalmic Solution

DEFINITION

Sulfacetamide Sodium Ophthalmic Solution is a sterile solution containing NLT 90.0% and NMT 110.0% of the labeled amount of sulfacetamide sodium ($C_8H_9N_2NaO_3S \cdot H_2O$). It may contain suitable buffers, stabilizers, and antimicrobial agents.

IDENTIFICATION

• **A.**

Sample: Nominally 1 g of sulfacetamide sodium from a volume of Ophthalmic Solution

Analysis: Dilute the *Sample* with water to 25 mL, adjust with 6 N acetic acid to a pH of 4–5, and filter. Wash the precipitate with water, and dry at 105° for 2 h. Use the precipitate in *Identification* tests *B* and *C*.

Acceptance criteria: The sulfacetamide melts at 180°–184°.

• **B.**

Sample: 500 mg of the sulfacetamide from *Identification* test *A*

Analysis: Place the *Sample* in a test tube, and heat gently until it boils.

Acceptance criteria: An oily liquid, which has the characteristic odor of acetamide, condenses on the walls of the test tube (distinction from the sublimates of sulfadiazine, sulfamerazine, and sulfamethazine, which are solids at room temperature).

• **C.**

Sample solution: 500 mg of *Identification* test *A* in 10 mL of dilute hydrochloric acid (1 in 10)

Analysis 1: To about one-half of the *Sample solution* add 2 mL of trinitrophenol TS.

Acceptance criteria 1: A very heavy flocculent or almost gelatinous precipitate is formed.

Analysis 2: To the remainder of the *Sample solution* add 3 drops of formaldehyde TS.

Acceptance criteria 2: A white precipitate is formed, and it changes to orange on standing (distinction from sulfamethoxypyridazine).

ASSAY

• **PROCEDURE**

Diluent: 20% methanol

Mobile phase: Methanol, glacial acetic acid, and water (10:1:89)

Standard stock solution: 5 mg/mL of USP Sulfacetamide Sodium RS prepared as follows. Transfer 50 mg of USP Sulfacetamide Sodium RS to a 40-mL centrifuge tube. Add 10.0 mL of *Diluent*, insert the stopper, and mix using a vortex mixer for 3 min to dissolve the Reference Standard. Add 7.5 mL of heptane, insert the stopper, and mix using a vortex mixer for another 3 min. Centrifuge to effect separation of the phases. Withdraw, and discard the upper heptane layer.

Standard solution: 0.03 mg/mL of USP Sulfacetamide Sodium RS in *Diluent* from the *Standard stock solution*

System suitability solution: 0.03 mg/mL of sulfanilamide in the *Standard solution*

Sample stock solution: Nominally 1 mg/mL of sulfacetamide sodium prepared as follows. Transfer 100 mg of sulfacetamide from a volume of Ophthalmic Solution, freshly mixed and free of air bubbles, to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: 0.03 mg/mL of sulfacetamide sodium from Ophthalmic Solution in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 90 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3 between the sulfacetamide and sulfanilamide peaks, *System suitability solution*

Column efficiency: NLT 1500 theoretical plates, determined from the analyte peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sulfacetamide sodium ($C_8H_9N_2NaO_3S \cdot H_2O$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulfacetamide Sodium RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)

C_U = nominal concentration of sulfacetamide sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of sulfacetamide sodium monohydrate, 254.24

M_{r2} = molecular weight of anhydrous sulfacetamide sodium, 236.23

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a cool place.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfacetamide Sodium RS

Sulfacetamide Sodium Topical Suspension

DEFINITION

Sulfacetamide Sodium Topical Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sulfacetamide sodium ($C_8H_9N_2NaO_3S$).

IDENTIFICATION

• **A.** The retention time of the sulfacetamide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (125:3:875)

Internal standard solution: 5 mg/mL of sulfathiazole sodium

USP Monographs

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Standard stock solution: 0.25 mg/mL of USP Sulfacetamide Sodium RS and 0.1 mg/mL of *p*-hydroxybenzoic acid prepared as follows. Transfer 25 mg of USP Sulfacetamide Sodium RS and 10 mg of *p*-hydroxybenzoic acid to a 100-mL volumetric flask. Dissolve in water, add 5 mL of the *Internal standard solution*, and dilute with water to volume.
Standard solution: 0.02 mg/mL of USP Sulfacetamide Sodium RS and 8 µg/mL of *p*-hydroxybenzoic acid in water from the *Standard stock solution*
Sample stock solution: Transfer 250 mg of Topical Suspension to a 125-mL conical flask, add 5 mL of the *Internal standard solution*, and dilute with 95 mL of water.
Sample solution: 2 mL of the *Sample stock solution* diluted with water to 25 mL. Centrifuge, and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for sulfanilamide, sulfacetamide, sulfathiazole, and *p*-hydroxybenzoic acid are about 0.2, 0.5, 1.0, and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between sulfathiazole and *p*-hydroxybenzoic acid
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous sulfacetamide sodium ($C_8H_9N_2NaO_3S$) in the portion of Topical Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of sulfacetamide to sulfathiazole from the *Sample solution*
R_S = peak area ratio of sulfacetamide to sulfathiazole from the *Standard solution*
C_S = concentration of USP Sulfacetamide Sodium RS in the *Standard* solution (mg/mL)
C_U = nominal concentration of sulfacetamide sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/mL, and the total combined molds and yeasts count does not exceed 50 cfu/mL. It meets the requirements of the test for *Pseudomonas aeruginosa*.
• **pH** ⟨791⟩: 6.5–7.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfacetamide Sodium RS

Sulfacetamide Sodium and Prednisolone Acetate Ophthalmic Ointment

DEFINITION
Sulfacetamide Sodium and Prednisolone Acetate Ophthalmic Ointment is a sterile ointment containing NLT 90.0% and NMT 110.0% of the labeled amounts of sulfacetamide sodium ($C_8H_9N_2NaO_3S \cdot H_2O$) and prednisolone acetate ($C_{23}H_{30}O_6$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the tests for *Sulfacetamide Sodium* and *Prednisolone Acetate* in the *Assay*.
• **B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the tests for *Sulfacetamide Sodium* and *Prednisolone Acetate* in the *Assay*.

ASSAY
• **SULFACETAMIDE SODIUM**
Diluent: Dilute methanol (1 in 5)
Mobile phase: Methanol, glacial acetic acid, and water (100:10:890), filtered and degassed
Standard solution: Transfer about 50 mg of USP Sulfacetamide Sodium RS to a 40-mL centrifuge tube. Add 10.0 mL of *Diluent*, insert the stopper in the tube, and mix using a vortex mixer for about 3 min to dissolve. Add 7.5 mL of heptane, insert the stopper in the tube, and mix using a vortex mixer for another 3 min. Centrifuge to effect separation of the phases. Withdraw and discard the upper heptane layer. Transfer 3.0 mL of the bottom layer to a 500-mL volumetric flask, add *Diluent* to volume, and mix.
System suitability solution: Dissolve 3 mg of sulfanilamide in 100 mL of the *Standard solution*, and mix.
Sample solution: Transfer a quantity of Ophthalmic Ointment nominally equivalent to about 100 mg of sulfacetamide sodium to a 40-mL centrifuge tube. Add 15.0 mL of heptane, insert the stopper in the tube, and mix using a vortex mixer for about 3 min to dissolve the Ophthalmic Ointment. Add 20.0 mL of *Diluent*, insert the stopper in the tube, and mix using a vortex mixer for 3 min. Centrifuge to effect separation of the phases. Withdraw and discard the upper heptane layer. Transfer 3.0 mL of the bottom layer to a 500-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 254-nm diode array
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 90 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3 between the sulfacetamide and sulfanilamide peaks, *System suitability solution*
Column efficiency: NLT 1500 theoretical plates, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of the labeled amount of sulfacetamide sodium ($C_8H_9N_2NaO_3S \cdot H_2O$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sulfacetamide sodium from the *Sample solution*
r_S = peak response of sulfacetamide sodium from the *Standard solution*
C_S = concentration of USP Sulfacetamide Sodium RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulfacetamide sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sulfacetamide sodium monohydrate, 254.24
M_{r2} = molecular weight of anhydrous sulfacetamide sodium, 236.23

Acceptance criteria: 90.0%–110.0%

• **PREDNISOLONE ACETATE**

Diluent: Dilute methanol (9 in 10)

Mobile phase: Acetonitrile and water (400:600), filtered and degassed

Internal standard solution: 0.7 mg/mL of norethindrone in *Diluent*

Standard stock solution: 0.8 mg/mL of USP Prednisolone Acetate RS in *Diluent*

Standard solution: 0.04 mg/mL of USP Prednisolone Acetate RS prepared as follows. Transfer 5.0 mL of *Standard stock solution* to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Diluent* to volume, and mix.

Sample solution: Transfer a quantity of Ophthalmic Ointment nominally equivalent to about 4 mg of prednisolone acetate to a 50-mL centrifuge tube. Add 10.0 mL of heptane, and mix using a vortex mixer for about 2 min to dissolve the Ophthalmic Ointment. Add 5.0 mL of *Internal standard solution* and 20.0 mL of *Diluent*, and mix using a vortex mixer for 2 min. Centrifuge to effect separation of the phases. Withdraw and discard the upper heptane layer. Transfer the lower layer to a 100-mL volumetric flask. Add *Diluent* to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: 254-nm diode array

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 40 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for prednisolone acetate and norethindrone are about 1.0 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 4.5 between the prednisolone and norethindrone peaks

Column efficiency: NLT 3000 theoretical plates for the prednisolone peak

Tailing factor: NMT 2.5 for the prednisolone peak

Relative standard deviation: NMT 1.5% for the peak response ratio of prednisolone acetate to norethindrone

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of prednisolone acetate to the internal standard peak from the *Sample solution*
R_S = peak response ratio of prednisolone acetate to the internal standard peak from the *Standard solution*
C_S = concentration of USP Prednisolone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of prednisolone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

• **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes that are tamper-proof so that sterility is assured at time of first use.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Prednisolone Acetate RS
USP Sulfacetamide Sodium RS

Sulfacetamide Sodium and Prednisolone Acetate Ophthalmic Suspension

» Sulfacetamide Sodium and Prednisolone Acetate Ophthalmic Suspension is a sterile, aqueous suspension containing not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sulfacetamide sodium ($C_8H_9N_2NaO_3S.H_2O$) and prednisolone acetate ($C_{23}H_{30}O_6$). It may contain suitable preservatives, buffers, stabilizers, and suspending agents.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩**—**

USP Prednisolone Acetate RS
USP Sulfacetamide Sodium RS

Identification—

A: Pass about 25 mL of the well-mixed Ophthalmic Suspension through a fine, sintered-glass filter, saving the filtrate. Wash the crystals in the funnel with a small amount of water. Dry the crystals at 105° for 3 hours: the IR absorption spectrum of a potassium bromide dispersion of the crystals exhibits maxima only at the same wavelengths as that of a similar preparation of USP Prednisolone Acetate RS.

B: To the filtrate saved from *Identification* test *A*, add 6 N acetic acid dropwise until the pH is between 4 and 5. Allow crystals of sulfacetamide to develop. Filter the crystals, wash with a small amount of water, and dry at 105° for 2 hours: the IR absorption spectrum of a potassium bromide dispersion of the crystals so obtained exhibits maxima only at the same wavelengths as a preparation of USP Sulfacetamide Sodium RS, similarly treated.

Sterility Tests ⟨71⟩: meets the requirements.

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pH ⟨791⟩: between 6.0 and 7.4.

Assay for sulfacetamide sodium—

Mobile phase—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (890:100:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Sulfacetamide Sodium RS in a mixture of water and methanol (4:1), and dilute quantitatively, and stepwise if necessary, with the same solvent mixture to obtain a solution having a known concentration of about 30 µg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 100 mg of sulfacetamide sodium, to a 100-mL volumetric flask, dilute with a mixture of water and methanol (4:1) to volume, and mix. Dilute 3.0 mL of this solution with the same solvent mixture to 100.0 mL, and mix.

System suitability preparation—Dissolve about 3 mg of sulfanilamide in 100 mL of the *Standard preparation*, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined for the analyte peak is not less than 1500 theoretical plates; the resolution, *R*, between the sulfacetamide and sulfanilamide peaks is not less than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 90 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sulfacetamide sodium ($C_8H_9N_2NaO_3S.H_2O$) in each mL of the Ophthalmic Suspension taken by the formula:

$$3.33(254.24/236.23)C(r_U/r_S)$$

in which 254.24 and 236.23 are the molecular weights of sulfacetamide sodium monohydrate and anhydrous sulfacetamide sodium, respectively; *C* is the concentration, in µg per mL, calculated on the anhydrous basis, of USP Sulfacetamide Sodium RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for prednisolone acetate—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Prednisolone Acetate RS in methanol to obtain a solution containing about 2 mg per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, and dilute with a solvent mixture prepared by dissolving 2.72 g of monobasic potassium phosphate in 300 mL of water and 700 mL of methanol. The *Standard preparation* has a known concentration of about 0.04 mg per mL.

Assay preparation—Using a "To contain" pipet, transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 10 mg of prednisolone acetate, to a 250-mL volumetric flask. Rinse the pipet with the solvent mixture described under *Standard preparation*, collecting the rinsings in the flask, dilute with the same solvent mixture to volume, and mix.

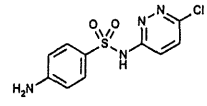
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of prednisolone acetate ($C_{23}H_{30}O_6$) in each mL of the Ophthalmic Suspension taken by the formula:

$$250(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Prednisolone Acetate RS in the *Standard preparation; V* is the volume, in mL, of Ophthalmic Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Sulfachlorpyridazine



$C_{10}H_9ClN_4O_2S$ 284.72

N^1-(6-Chloro-3-pyridazinyl)sulfanilamide [80-32-0].

» Sulfachlorpyridazine contains not less than 97.0 percent and not more than 103.0 percent of $C_{10}H_9ClN_4O_2S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Sulfachlorpyridazine RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the main peak in the chromatogram of the *Assay preparation*, obtained as directed in the *Assay*, corresponds to that of the main peak observed in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

Clarity and color of solution—Dissolve 1.0 g of it in 50 mL of 0.1 N methanolic hydrochloric acid prepared by diluting 8.6 mL of hydrochloric acid with methanol to obtain 1000 mL of solvent: a clear solution is produced that is not deeper in color than pale yellow.

Acidity—Prepare a suspension of 3.0 g of it in 150.0 mL of carbon dioxide-free water, and heat at 70° for 5 minutes, maintaining the suspension. Cool rapidly in an ice bath to 20 ± 0.5°, stirring by mechanical means. Filter the suspension using vacuum, and collect the filtrate. Titrate 25.0 mL of the clear filtrate with 0.1 N sodium hydroxide VS, using 2 drops of thymolphthalein TS as the indicator. Transfer a second

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25.0-mL portion of the clear filtrate to a 250-mL conical flask, add 10 mL of hydrochloric acid, and cool in an ice bath to 15°. Add about 25 g of crushed ice, prepared from frozen purified water, and titrate with 0.1 M sodium nitrite VS, stirring vigorously, until the titrated solution produces an immediate, stable, blue color on starch-iodide paper. The volume of 0.1 N sodium hydroxide consumed in the titration of the first 25.0-mL portion of the filtrate does not exceed the volume of 0.1 M sodium nitrite consumed in the titration of the second 25.0-mL portion of the filtrate by more than 0.5 mL.

Loss on drying ⟨731⟩: Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Assay—

pH 2.5 phosphate buffer—Dissolve 14 g of monobasic potassium phosphate in 1600 mL of water, adjust with phosphoric acid to a pH of 2.5 ± 0.1, dilute with water to 2000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *pH 2.5 phosphate buffer* and methanol (700:300). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Prepare a stock solution of USP Sulfachlorpyridazine RS in methanol having a known concentration of about 0.5 mg per mL. Transfer 3.0 mL of this stock solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter this solution through a nylon filter having a porosity of 0.5 µm or finer, and use the filtrate as the *Standard preparation*. The *Standard preparation* contains about 15 µg of USP Sulfachlorpyridazine RS per mL.

Assay preparation—Transfer about 50 mg of Sulfachlor pyridazine, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume, and mix. Transfer 3.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter this solution through a filter having a porosity of 0.5 µm or finer, and use the filtrate as the *Assay preparation*.

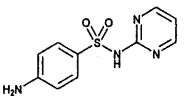
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 265-nm detector, a 4.6-mm × 25-cm analytical column containing 5-µm packing L1, and a guard column containing 5-µm packing L1, and is maintained at about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{10}H_9ClN_4O_2S$ in the portion of Sulfachlorpyridazine taken by the formula:

$$(10/3)(C)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Sulfachlorpyridazine RS in the *Standard preparation;* and r_U and r_S are the sulfachlorpyridazine peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Sulfadiazine



$C_{10}H_{10}N_4O_2S$ 250.28

Benzenesulfonamide, 4-amino-*N*-2-pyrimidinyl-; *N*¹-2-Pyrimidinylsulfanilamide [68-35-9].

DEFINITION

Sulfadiazine contains NLT 98.0% and NMT 102.0% of $C_{10}H_{10}N_4O_2S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**

Sample: 50 mg

Analysis: Carefully melt the *Sample* in a small test tube.

Acceptance criteria: A reddish brown color develops upon melting, and the fumes evolved during decomposition do not discolor moistened lead acetate test paper (distinction from sulfathiazole).

- **C.**

Sample: 1 g

Analysis 1: Gently heat the *Sample* in a small test tube until a sublimate is formed. Collect a few mg of the sublimate with a glass rod, and mix in a test tube with 1 mL of a solution (1 in 20) of resorcinol in alcohol. Add 1 mL of sulfuric acid, and mix by shaking.

Acceptance criteria 1: A deep red color appears at once.

Analysis 2: Cautiously dilute the mixture obtained in *Analysis 1* with 25 mL of ice-cold water, and add an excess of 6 N ammonium hydroxide.

Acceptance criteria 2: A blue or reddish blue color is produced.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, water, and glacial acetic acid (12:87:1)

Standard solution: 1 mg/mL of USP Sulfadiazine RS in 0.025 N sodium hydroxide

Sample solution: 1 mg/mL of Sulfadiazine in 0.025 N sodium hydroxide

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%, five injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulfadiazine ($C_{10}H_{10}N_4O_2S$) in the portion of Sulfadiazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of sulfadiazine from the *Sample solution*
r_S = peak response of sulfadiazine from the *Standard solution*
C_S = concentration of USP Sulfadiazine RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfadiazine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **SELENIUM** ⟨291⟩
  Sample: 200 mg
  Acceptance criteria: NMT 30 ppm
- **ORDINARY IMPURITIES** ⟨466⟩
  Diluent: Toluene and dimethylformamide (2:1)
  Standard solutions: 0.008, 0.041, 0.08, and 0.17 mg/mL in *Diluent*
  Sample solution: 8.3 mg/mL in *Diluent*
  Eluant: Chloroform, methanol, and ammonium hydroxide (30:12:1)
  Visualization: 11

SPECIFIC TESTS

- **CLARITY AND COLOR OF SOLUTION**
  Sample: 1 g
  Analysis: Dissolve the *Sample* in a mixture of 20 mL of water and 5 mL of 1 N sodium hydroxide.
  Acceptance criteria: The solution is clear and not more deeply colored than pale yellow.
- **ACIDITY**
  Sample: 2 g
  Analysis: Digest the *Sample* with 100 mL of water at about 70° for 5 min. Cool at once to room temperature, and filter. To 25.0 mL of the filtrate add 2 drops of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide.
  Acceptance criteria: NMT 0.20 mL is required to produce a pink color.
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample at 105° for 2 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulfadiazine RS

Sulfadiazine Tablets

DEFINITION

Sulfadiazine Tablets contain NLT 95.0% and NMT 105.0% of the labeled quantity of $C_{10}H_{10}N_4O_2S$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. MELTING RANGE OR TEMPERATURE,** *Class Ia* ⟨741⟩
  Sample solution: Equivalent to 100 mg/mL of sulfadiazine from finely powdered Tablets in chloroform
  Analysis: Transfer the *Sample solution* to a small filter. Wash with 5 mL of chloroform, and discard the filtrate. Triturate the residue with 10 mL of 6 N ammonium hydroxide for 5 min, add 10 mL of water, and filter. Warm the filtrate until most of the ammonia is expelled, cool, and add 6 N acetic acid until the reaction is distinctly acid: a precipitate of sulfadiazine is formed. Collect the precipitate on a filter, wash it with cold water, and dry at 105° for 1 h.
  Acceptance criteria: The sulfadiazine melts at 250°–254°.

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile, glacial acetic acid, and water (12:1:87)
  Standard solution: 1 mg/mL of USP Sulfadiazine RS in 0.025 N sodium hydroxide
  Sample solution: 1 mg/mL of sulfadiazine from powdered Tablets in 0.025 N sodium hydroxide. [NOTE—Weigh NLT 20 Tablets, shake the solution for 30 min, and centrifuge, if necessary.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 2 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5 for sulfadiazine
  Relative standard deviation: NMT 2.0% (five replicate injections)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{10}H_{10}N_4O_2S$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulfadiazine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulfadiazine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 2: 75 rpm
  Time: 90 min
  Detector: UV, 242 nm
  Analysis: Determine the quantity of $C_{10}H_{10}N_4O_2S$ dissolved by using UV absorption on filtered portions of the solution under test, in comparison with a Standard solution having a known concentration of USP Sulfadiazine RS in the same medium. Use *Medium* as the blank and cells with a path length of 0.1 cm.
  Tolerances: NLT 70% (Q) of the labeled amount of $C_{10}H_{10}N_4O_2S$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

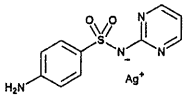
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulfadiazine RS

USP Monographs

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Silver Sulfadiazine



$C_{10}H_9AgN_4O_2S$ 357.14

Benzenesulfonamide, 4-amino-*N*-2-pyrimidinyl-, monosilver(1+) salt;
N[1]-2-Pyrimidinylsulfanilamide monosilver(1+) salt [22199-08-2].

DEFINITION

Silver Sulfadiazine contains NLT 98.0% and NMT 102.0% of silver sulfadiazine ($C_{10}H_9AgN_4O_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197D, or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Silver*

Sample: 1 g in 15 mL of ammonium hydroxide and 15 mL of water in a 50-mL volumetric flask. Dilute with water to volume, and mix.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: 0.77 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.2 ± 0.1.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
4	98	2
16	40	60
20	40	60
22	20	80
32	20	80
34	98	2
40	98	2

Diluent: Acetonitrile, ammonium hydroxide, and water (5:6:90)
Standard solution: 0.05 mg/mL of USP Silver Sulfadiazine RS in *Diluent*. Sonicate, if necessary, to dissolve.
Sample solution: 0.05 mg/mL of Silver Sulfadiazine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of silver sulfadiazine ($C_{10}H_9AgN_4O_2S$) in the portion of Silver Sulfadiazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Silver Sulfadiazine RS in the *Standard solution* (mg/mL)
C_U = concentration of Silver Sulfadiazine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS

- **CONTENT OF SILVER**

Sample solution: Add 500 mg to 150 mL of water and 50 mL of nitric acid, and stir for 15 min.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N potassium thiocyanate VS or 0.1 N ammonium thiocyanate VS
Endpoint detection: Potentiometric
Analysis: Titrate the *Sample solution* with *Titrant* using a silver-based indicator electrode and a double-junction reference electrode. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N potassium thiocyanate VS or 0.1 N ammonium thiocyanate VS is equivalent to 10.79 mg of silver.
Acceptance criteria: 29.3%–30.5% of silver is found.

IMPURITIES

- **LIMIT OF NITRATE**

Standard solution: 200 µg/mL of nitrate from potassium nitrate
Sample solution: Add 30.0 mL of water to 2 g of Silver Sulfadiazine, stir for 20 min, and pass through a suitable, nitrate-free filter.
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 408 nm
Blank: Deionized water
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Pipet 3 mL of the *Sample solution* and *Blank* into separate test tubes. Pipet 1 mL of the *Standard solution* and 2 mL of water into a third test tube. Cool the three test tubes in an ice bath. Slowly add 7.0 mL of cold chromotropic acid solution, prepared by dissolving 50 mg of chromotropic acid in 100 mL of cold sulfuric acid, to each test tube, while swirling, and allow the test tubes to remain in the ice bath for 3 min after the addition of the chromotropic acid solution. Remove the test tubes from the ice bath, and allow to stand for 30 min. Concomitantly determine the absorbances of the *Sample solution* and the *Standard solution* with a suitable spectrophotometer, against the *Blank*.
Calculate the percentage of nitrate content in the portion of Silver Sulfadiazine taken:

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$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of nitrate in the *Standard solution* (µg/mL)
C_U = concentration of Silver Sulfadiazine in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.1%

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 1.0 mg/mL of USP Silver Sulfadiazine RS and 0.001 mg/mL each of USP Sulfanilic Acid RS, USP Sulfanilamide RS, and sulfaguanidine in *Diluent*

Standard solution: 0.001 mg/mL of USP Silver Sulfadiazine RS in *Diluent*

Sample solution: 1.0 mg/mL of Silver Sulfadiazine in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for sulfaguanidine and sulfanilamide are about 0.65 and 0.71, respectively.]

Suitability requirements

Resolution: NLT 2.0 between sulfaguanidine and sulfanilamide, *System suitability solution*

Relative standard deviation: NMT 5.0% for the sulfadiazine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Silver Sulfadiazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of silver sulfadiazine from the *Standard solution*
C_S = concentration of USP Silver Sulfadiazine RS in the *Standard solution* (mg/mL)
C_U = concentration of Silver Sulfadiazine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfanilic acid	0.27	0.27	0.3
Sulfaguanidine[a, b]	0.65	0.85	—
Sulfanilamide[b]	0.71	1.0	—
2-Aminopyrimidine[c]	0.82	0.48	0.3
Sulfadiazine[d]	1.00	—	—
Acetylsulfadiazine[e]	1.14	1.0	0.2
Specified unidentified impurity[f]	1.28	1.0	0.15
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 4-Amino-*N*-carbamimidoylbenzenesulfonamide.
[b] These are controlled using the limit for individual unspecified impurities.
[c] Pyrimidin-2-amine.
[d] 4-Amino-*N*-2-pyrimidinyl-benzenesulfonamide.
[e] *N*-{4-[*N*-(Pyrimidin-2-yl)sulfamoyl]phenyl}acetamide.
[f] This compound is a specified unknown impurity. No information is available about the chemical structure or chemical name for this impurity.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 1 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Silver Sulfadiazine RS
USP Sulfanilamide RS
4-Aminobenzenesulfonamide.
$C_6H_8N_2O_2S$ 172.20
USP Sulfanilic Acid RS
4-Aminobenzenesulfonic acid.
$C_6H_7NO_3S$ 173.19

Silver Sulfadiazine Cream

DEFINITION

Silver Sulfadiazine Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of silver sulfadiazine ($C_{10}H_9AgN_4O_2S$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: Dissolve 35 mg of USP Sulfadiazine RS in 4 mL of ammonium hydroxide, and add 16 mL of methanol.

Sample solution: Transfer a quantity of Cream equivalent to 50 mg of silver sulfadiazine to a stoppered 50-mL conical flask. Add 4 mL of ammonium hydroxide, and mix until a smooth paste has been obtained. Add 16 mL of methanol, shake for 5 min, and filter.

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (7:4:1). Prepare the developing solvent by first mixing the chloroform and methanol, then adding the ammonium hydroxide.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, phosphoric acid, and water (99:1:900)

Diluent: Ammonium hydroxide in water (1 in 10)

Internal standard solution: 2 mg/mL of sulfamerazine in *Diluent*

Standard stock solution: 1 mg/mL of USP Silver Sulfadiazine RS in *Diluent*

Standard solution A: 0.29 mg/mL of USP Silver Sulfadiazine RS prepared by mixing 10.0 mL of *Standard stock solution*, 20 mL of alcohol, and 5.0 mL of *Internal standard solution*

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Standard solution B: 14 µg/mL of USP Silver Sulfadiazine RS prepared by mixing 2.0 mL of *Standard solution A* and 40 mL of *Mobile phase*

Sample stock solution: Nominally 0.29 mg/mL of silver sulfadiazine prepared as follows. Transfer a quantity of Cream equivalent to 10 mg of silver sulfadiazine to a 50-mL round-bottom centrifuge tube. Add about 30 mL of methanol, tightly seal the tube with a cap containing an inert liner, and mix, using a vortex mixer, for about 15 s. Centrifuge for 15 min. Aspirate, and discard the methanol. Care should be taken to avoid aspirating any of the residue. Transfer 5.0 mL of the *Internal standard solution* into the tube, and add 20 mL of alcohol. Replace the cap, and mix. Heat in a water bath at 60°, with periodic mixing, for 15 min to melt and disperse the Cream. While the mixture is still hot, transfer 10.0 mL of *Diluent* to the tube, and cool to room temperature.

Sample solution: Nominally 14 µg/mL of silver sulfadiazine prepared by mixing 2.0 mL of *Sample stock solution* and 40 mL of *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution B*
Suitability requirements
Resolution: NLT 2.0 between sulfadiazine and sulfamerazine
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution B* and *Sample solution*
Calculate the percentage of the labeled amount of silver sulfadiazine ($C_{10}H_9AgN_4O_2S$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio from the *Sample solution*
R_S = peak response ratio from *Standard solution B*
C_S = concentration of USP Silver Sulfadiazine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of silver sulfadiazine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, and *Pseudomonas aeruginosa*. The total aerobic microbial count does not exceed 23 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g.
• **pH** ⟨791⟩
Analysis: Determine from the supernatant of a 50-mg/mL mixture of Cream in water
Acceptance criteria: 4.0–7.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in collapsible tubes or in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Silver Sulfadiazine RS
USP Sulfadiazine RS

Sulfadiazine Sodium

$C_{10}H_9N_4NaO_2S$ 272.26
Benzenesulfonamide, 4-amino-*N*-2-pyrimidinyl-, monosodium salt;
*N*¹-2-Pyrimidinylsulfanilamide monosodium salt [547-32-0].

DEFINITION
Sulfadiazine Sodium contains NLT 99.0% and NMT 100.5% of sulfadiazine sodium ($C_{10}H_9N_4NaO_2S$), calculated on the dried basis.

IDENTIFICATION
• **A.**
Sample: 1.5 g
Analysis: Dissolve the *Sample* in 25 mL of water, and add 3 mL of 6 N acetic acid. A white precipitate of sulfadiazine is formed. Collect the precipitate on a filter, wash it well with cold water, and dry at 105° for 1 h.

Change to read:

Acceptance criteria 1: The infrared spectrum of the residue in ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020) matches the infrared spectrum of similarly prepared USP Sulfadiazine RS.
Acceptance criteria 2: The melting range of the residue as determined by the method for *Class 1a* in *Melting Range or Temperature* ⟨741⟩ is 250°–254°.
• **B. Identification Tests—General,** *Sodium* ⟨191⟩
Sample: 500 mg
Analysis: Ignite the *Sample*, and use the residue.
Acceptance criteria: Meets the requirements

ASSAY
• **Procedure**
Analysis: Proceed as directed in *Nitrite Titration* ⟨451⟩. Each mL of 0.1 M sodium nitrite is equivalent to 27.23 mg of sulfadiazine sodium ($C_{10}H_9N_4NaO_2S$).
Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES
• **Selenium** ⟨291⟩
Sample: 200 mg
Acceptance criteria: NMT 30 ppm

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP Reference Standards** ⟨11⟩
USP Sulfadiazine RS

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Sulfadiazine Sodium Injection

DEFINITION

Sulfadiazine Sodium Injection is a sterile solution of Sulfadiazine Sodium in Water for Injection. It contains, in each mL, NLT 237.5 mg and NMT 262.5 mg of sulfadiazine sodium ($C_{10}H_9N_4NaO_2S$).

IDENTIFICATION

• **A.**

Sample: Nominally 1.5 g of sulfadiazine sodium from 6 mL of Injection

Analysis: Dilute the *Sample* to 25 mL with water, and add 3 mL of 6 N acetic acid. A white precipitate of sulfadiazine is formed. Collect the precipitate on a filter, wash it well with cold water, and dry at 105° for 1 h.

Acceptance criteria 1: The melting range of the residue as determined by the method for *Class 1a* in *Melting Range or Temperature* ⟨741⟩ is 250°–254°.

Change to read:

Acceptance criteria 2: The infrared spectrum of the residue in ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020) matches the infrared spectrum of similarly prepared USP Sulfadiazine RS.

ASSAY

• **PROCEDURE**

Sample solution: Transfer a volume of Injection, equivalent to 500 mg of sulfadiazine sodium, into a beaker or a casserole.

Analysis: Proceed as directed in *Nitrite Titration* ⟨451⟩, beginning with "Add 20 mL of hydrochloric acid". Each mL of 0.1 M sodium nitrite is equivalent to 27.23 mg of sulfadiazine sodium ($C_{10}H_9N_4NaO_2S$).

Acceptance criteria: 237.5–262.5 mg/mL

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.1 USP Endotoxin Unit per mg of sulfadiazine sodium

• **pH** ⟨791⟩: 8.5–10.5

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

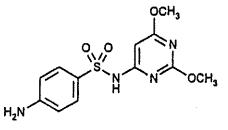
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose, light-resistant containers of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfadiazine RS

Sulfadimethoxine



$C_{12}H_{14}N_4O_4S$ 310.33

Benzenesulfonamide, 4-amino-*N*-(2,6-dimethoxy-4-pyrimidinyl)-.

N^1-(2,6-Dimethoxy-4-pyrimidinyl)sulfanilamide [122-11-2].

» Sulfadimethoxine contains not less than 98.0 percent and not more than 102.0 percent of $C_{12}H_{14}N_4O_4S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Sulfadimethoxine RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution: 1 in 100,000.

Medium: alcohol.

Absorptivities, calculated on the dried basis, at 272 nm do not differ by more than 3.0%.

C: To about 100 mg, add 3 mL of 2.5 N sodium hydroxide and 50 mL of water, mix until dissolved, and dilute with water to 100 mL. To about 5 mL of this solution, add 100 mg of phenol, and heat to boiling. Cool the solution, and add 0.5 mL of sodium hypochlorite TS and 3 drops of 2.5 N sodium hydroxide: a yellow color is produced.

D: To about 10 mg dissolved in 2 mL of diluted hydrochloric acid, add 3 drops of sodium nitrite solution (1 in 100), and dilute with water to 4 mL: the solution turns yellow. Add 1 mL of 2.5 N sodium hydroxide containing 10 mg of 2-naphthol: a red-orange precipitate is formed.

Melting range ⟨741⟩: between 197° and 202°.

Loss on drying ⟨731⟩: Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Assay—

Mobile phase—Dissolve 6 g of monobasic sodium phosphate in water to make 1000 mL. Adjust with 50% (w/v) sodium hydroxide solution to a pH of 7.0. Prepare a mixture of this solution and methanol (300:100). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Sulfadimethoxine RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL. Protect this solution from light.

Assay preparation—Transfer about 20 mg of Sulfadimethoxine, accurately weighed, to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and swirl to dissolve. Dilute this solution with *Mobile phase* to volume, and mix. Protect this solution from light.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg,

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of $C_{12}H_{14}N_4O_4S$ in the portion of Sulfadimethoxine taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Sulfadimethoxine RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Sulfadimethoxine Soluble Powder

» Sulfadimethoxine Soluble Powder contains Sulfadimethoxine Sodium equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of sulfadimethoxine ($C_{12}H_{14}N_4O_4S$).

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Sulfadimethoxine RS
Identification—Shake a quantity equivalent to about 1 g with 5 mL of diluted hydrochloric acid and 10 mL of water. Filter, and to the filtrate add 2.5 N sodium hydroxide dropwise until a precipitate forms and redissolves. Add diluted hydrochloric acid dropwise until a precipitate forms. Collect the precipitate on a very fine filter, and wash it with water and with ether: the sulfadimethoxine so obtained meets the requirements for the *Identification* tests under *Sulfadimethoxine.*
Minimum fill ⟨755⟩: meets the requirements.
pH ⟨791⟩: between 7.0 and 8.0, in a solution (1 in 20).

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Sulfadimethoxine.*
Assay preparation—Transfer an accurately weighed portion of Powder, equivalent to about 50 mg of sulfadimethoxine, to a 250-mL volumetric flask, add about 200 mL of *Mobile phase,* and swirl to dissolve. Dilute with *Mobile phase* to volume, and mix. Protect this solution from light.
Procedure—Proceed as directed in the *Assay* under *Sulfadimethoxine.* Calculate the quantity, in mg, of sulfadimethoxine ($C_{12}H_{14}N_4O_4S$) in the portion of Powder taken by the formula:

$$250C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Sulfadimethoxine RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Sulfadimethoxine Oral Suspension

» Sulfadimethoxine Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of sulfadimethoxine ($C_{12}H_{14}N_4O_4S$).

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Sulfadimethoxine RS
Identification—Shake a quantity equivalent to about 50 mg with 50 mL of water, and add 2.5 N sodium hydroxide dropwise until the solution becomes clear. Add diluted hydrochloric acid dropwise until a precipitate forms. Collect the precipitate on a very fine filter, and wash it with water and with ether: the sulfadimethoxine so obtained meets the requirements for the *Identification* tests under *Sulfadimeth oxine.*
pH ⟨791⟩: between 5.0 and 7.0.

Assay—
Mobile phase,Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Sulfadimethoxine.*
Assay preparation—Transfer an accurately measured volume of Suspension, previously mixed and free from air bubbles, equivalent to about 50 mg of sulfadimethoxine, to a 250-mL volumetric flask, add about 200 mL of *Mobile phase,* and swirl to dissolve. Dilute with *Mobile phase* to volume, and mix. Protect this solution from light.
Procedure—Proceed as directed in the *Assay* under *Sulfadimethoxine.* Calculate the quantity, in mg, of sulfadimethoxine ($C_{12}H_{14}N_4O_4S$) in each mL of the Oral Suspension taken by the formula:

$$250(C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation;* and the other terms are as defined therein.

Sulfadimethoxine Tablets

» Sulfadimethoxine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of sulfadimethoxine ($C_{12}H_{14}N_4O_4S$).

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.
Labeling—Label the Tablets to indicate that they are for veterinary use only.
USP Reference standards ⟨11⟩—
USP Sulfadimethoxine RS
Identification—Shake a quantity of finely powdered Tablets, equivalent to about 1 g of sulfadimethoxine, with 5 mL of diluted hydrochloric acid and 10 mL of water. Filter, and to the filtrate add 2.5 N sodium hydroxide dropwise until a precipitate forms and redissolves. Add diluted hydrochloric acid dropwise until a precipitate forms. Collect the filtrate on a very fine filter, and wash it with water and with ether: the sulfadimethoxine so obtained meets the requirements for the *Identification* tests under *Sulfadimethoxine.*
Disintegration ⟨701⟩: 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

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Assay—

Mobile phase, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Sulfadimethoxine*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of sulfadimethoxine, to a 250-mL volumetric flask, add about 200 mL of *Mobile phase*, and swirl to dissolve. Dilute with *Mobile phase* to volume, and mix. Filter if necessary to obtain a clear solution. Protect this solution from light.

Procedure—Proceed as directed in the *Assay* under *Sulfadimethoxine*. Calculate the quantity, in mg, of sulfadimethoxine ($C_{12}H_{14}N_4O_4S$) in the portion of Tablets taken by the formula:

$$250C(r_U/r_S)$$

in which the terms are as defined therein.

Sulfadimethoxine Sodium

$C_{12}H_{13}N_4NaO_4S$ 332.31

Benzenesulfonamide, 4-amino-*N*-(2,6-dimethoxy-4-pyrimidinyl)-, monosodium salt.

N^1-(2,6-Dimethoxy-4-pyrimidinyl)sulfanilamide monosodium salt [1037-50-9].

» Sulfadimethoxine Sodium contains not less than 98.0 percent and not more than 102.0 percent of $C_{12}H_{13}N_4NaO_4S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Sulfadimethoxine RS

Identification—

A: To about 100 mg, add 50 mL of water, mix until dissolved, and dilute with water to 100 mL. To about 5 mL of this solution add 100 mg of phenol, and heat to boiling. Cool the solution, and add 0.5 mL of sodium hypochlorite TS and 3 drops of 2.5 N sodium hydroxide: a yellow color is produced.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

C: Dissolve 10 mg in 2 mL of diluted hydrochloric acid, add 3 drops of sodium nitrite solution (1 in 100), and dilute with water to 4 mL: the solution turns yellow. Add 1 mL of 2.5 N sodium hydroxide containing 10 mg of 2-naphthol: a red-orange precipitate is formed.

pH ⟨791⟩: between 8.0 and 9.5, in a solution (1 in 20).

Loss on drying ⟨731⟩—Dry it at 125° for 3 hours: it loses not more than 5.0% of its weight.

Assay—

Mobile phase, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Sulfadimethoxine*.

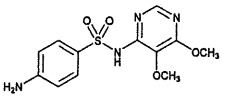
Assay preparation—Transfer about 60 mg of Sulfadi methoxine Sodium, accurately weighed, to a 250-mL volumetric flask, add about 200 mL of *Mobile phase*, and swirl to dissolve. Dilute with *Mobile phase* to volume, and mix. Protect this solution from light.

Procedure—Proceed as directed in the *Assay* under *Sulfadimethoxine*. Calculate the quantity, in mg, of $C_{12}H_{13}N_4NaO_4S$ in the portion of Sulfadimethoxine Sodium taken by the formula:

$$(332.31/310.34)(250C)(r_U/r_S)$$

in which 332.31 and 310.34 are the molecular weights of sulfadimethoxine sodium and sulfadimethoxine, respectively; and the other terms are as defined therein.

Sulfadoxine



$C_{12}H_{14}N_4O_4S$ 310.33

Benzenesulfonamide, 4-amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)-;

N^1-(5,6-Dimethoxy-4-pyrimidinyl)sulfanilamide [2447-57-6].

DEFINITION

Sulfadoxine contains NLT 99.0% and NMT 101.0% of sulfadoxine ($C_{12}H_{14}N_4O_4S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 6 µg/mL in 0.1 N sodium hydroxide

Acceptance criteria: Meets the requirements

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Add 1 mL of phosphoric acid to water, and dilute to 1000 mL.

Mobile phase: Acetonitrile and *Solution A* (17:83)

Standard solution: 0.4 mg/mL of USP Sulfadoxine RS prepared as follows. Dissolve an appropriate amount of USP Sulfadoxine RS in a suitable container with acetonitrile using 17% of the final volume, then dilute with *Solution A* to volume.

Sample solution: 0.4 mg/mL of sulfadoxine prepared as follows. Dissolve an appropriate amount of sulfadoxine in a suitable container with acetonitrile using 17% of the final volume, then dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.0-mm × 10-cm; 3-µm packing L11

Flow rate: 0.3 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

USP Monographs

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Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfadoxine ($C_{12}H_{14}N_4O_4S$) in the portion of Sulfadoxine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulfadoxine RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfadoxine in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
Diluent: Alcohol and ammonium hydroxide (9:1)
Standard solution: 0.10 mg/mL of USP Sulfadoxine RS in *Diluent*
Sample solution: 20 mg/mL of Sulfadoxine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 10 µL
Developing solvent system: Chloroform, methanol, and dimethylformamide (20:2:1)
Spray reagent A: 1-in-10 solution of sulfuric acid in alcohol
Spray reagent B: 1-in-200 solution of *N*-(1-naphthyl) ethylenediamine dihydrochloride in alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Separately apply the *Sample solution* and the *Standard solution* to the *Adsorbent*. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and air dry. Spray the dried plate with *Spray reagent A*, and expose to nitrous fumes generated by adding 7 M sulfuric acid dropwise to a solution containing 10% sodium nitrite and 3% potassium iodide. Dry the plate in a current of warm air for 15 min, and spray with *Spray reagent B*.
Acceptance criteria: No spot in the *Sample solution*, other than the principal spot, is greater in size and intensity than the spot from the *Standard solution*.

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 197°–200°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfadoxine RS

Sulfadoxine and Pyrimethamine Tablets

DEFINITION
Sulfadoxine and Pyrimethamine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of sulfadoxine ($C_{12}H_{14}N_4O_4S$) and NLT 90.0% and NMT 110.0% of the labeled amount of pyrimethamine ($C_{12}H_{13}ClN_4$).

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution* for pyrimethamine and sulfadoxine, as obtained in the *Assay*.
• **B.**
Diluent: Ammonium hydroxide and methanol (1 in 50)
Standard solution A: 10 mg/mL of USP Sulfadoxine RS in *Diluent*
Standard solution B: 0.5 mg/mL of USP Pyrimethamine RS in *Diluent*
Sample solution: Vigorously shake 700 mg of finely ground Tablet powder with 50 mL of *Diluent* for 3 min, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Heptane, chloroform, solution of methanol in alcohol (1 in 20), and glacial acetic acid (4:4:4:1)
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Allow the solvent front to move about two-thirds of the length of the plate, remove the plate, dry, and examine under short-wavelength UV light.
Acceptance criteria: The R_F values of the principal spots from the *Sample solution* correspond to the R_F values of the principal spots from the corresponding *Standard solution*.

ASSAY
• **PROCEDURE**
Solution A: 1 mL of phosphoric acid in 1000 mL of water
Mobile phase: Acetonitrile and *Solution A* (17:83)
Standard solution: 0.4 mg/mL of USP Sulfadoxine RS and 0.02 mg/mL of USP Pyrimethamine RS prepared as follows. Transfer suitable amounts of USP Sulfadoxine RS and USP Pyrimethamine RS to a suitable volumetric flask. Dissolve in acetonitrile using about 17% of the final flask volume, then dilute with *Solution A* to volume.
Sample stock solution: Transfer an equivalent of about 200 mg of sulfadoxine and 10 mg of pyrimethamine from NLT 10 finely powdered Tablets to a 100-mL volumetric flask. Add about 28 mL of acetonitrile and sonicate for about 30 min. Allow to cool and dilute with *Solution A* to volume.
Sample solution: Nominally 0.4 mg/mL of sulfadoxine and 0.02 mg/mL of pyrimethamine in *Mobile phase* from *Sample stock solution*. Pass through a PVDF filter of 0.45-µm pore size. Discard the first 5 mL and use the remaining filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 2.0-mm × 10-cm; 3-µm packing L11
Flow rate: 0.3 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*

USP Monographs

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Suitability requirements
Resolution: NLT 1.5 between pyrimethamine and sulfadoxine
Tailing factor: NMT 1.6 for sulfadoxine; NMT 2.0 for pyrimethamine
Relative standard deviation: NMT 2.0% for both sulfadoxine and pyrimethamine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of sulfadoxine ($C_{12}H_{14}N_4O_4S$) and pyrimethamine ($C_{12}H_{13}ClN_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

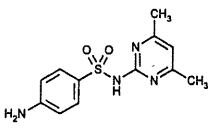
r_U = peak response of sulfadoxine or pyrimethamine from the *Sample solution*
r_S = peak response of sulfadoxine or pyrimethamine from the *Standard solution*
C_S = concentration of USP Sulfadoxine RS or USP Pyrimethamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulfadoxine or pyrimethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each for sulfadoxine and pyrimethamine

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: *pH 6.8 Phosphate Buffer*, prepared as directed in *Reagents, Indicators, and Solutions—Buffer Solutions*; 1000 mL
Apparatus 2: 75 rpm
Time: 30 min
Analysis: Determine the percentage of the labeled amounts of sulfadoxine ($C_{12}H_{14}N_4O_4S$) and pyrimethamine ($C_{12}H_{13}ClN_4$) dissolved, using the procedure set forth in the *Assay*, making any necessary modifications.
Tolerances: NLT 60% (*Q*) of the labeled amounts each of sulfadoxine ($C_{12}H_{14}N_4O_4S$) and pyrimethamine ($C_{12}H_{13}ClN_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements with respect to sulfadoxine and to pyrimethamine

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Pyrimethamine RS
USP Sulfadoxine RS

Sulfamethazine



$C_{12}H_{14}N_4O_2S$ 278.33
Benzenesulfonamide, 4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl)-.
N^1-(4,6-Dimethyl-2-pyrimidinyl)sulfanilamide [57-68-1].

» Sulfamethazine contains not less than 99.0 percent and not more than 100.5 percent of $C_{12}H_{14}N_4O_2S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Sulfamethazine RS
Clarity and color of solution—Dissolve 1.0 g in a mixture of 20 mL of water and 5 mL of 1 N sodium hydroxide: the solution is clear and not more deeply colored than pale yellow.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: To 0.10 g add 10 mL of water and just sufficient sodium hydroxide solution (1 in 250) to give a faint pink spot on phenolphthalein paper. Add 5 drops of cupric sulfate TS: a yellow-green precipitate is formed, and it becomes brown on standing.
Melting range ⟨741⟩: between 197° and 200°.
Acidity—Digest 3.0 g with 150 mL of carbon dioxide-free water at 70° for about 5 minutes, stirring occasionally to maintain suspension of the test specimen. Cool the mixture rapidly in an ice bath to 20 ± 0.5°, with mechanical stirring. Filter immediately, with vacuum, omitting washing of the solid but drying it thoroughly by suction. Add 2 drops of thymolphthalein TS to 25.0 mL of the filtrate, and titrate with 0.1 N sodium hydroxide VS. To a second 25.0-mL portion of the filtrate add 10 mL of hydrochloric acid. Cool the mixture to 15°, and titrate with 0.1 M sodium nitrite VS as directed under *Nitrite Titration* ⟨451⟩: the volume of 0.1 N sodium hydroxide consumed does not exceed the volume of 0.1 M sodium nitrite consumed by more than 0.5 mL.
Loss on drying ⟨731⟩—Dry about 1 g, accurately weighed, at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Selenium ⟨291⟩: 0.003%, a 200-mg test specimen being used.

Ordinary impurities ⟨466⟩—
Test solution: acetone.
Standard solution: acetone.
Eluant: a mixture of ethyl acetate, methanol, and ammonium hydroxide (17:6:5).
Visualization: 11.
Assay—Proceed with Sulfamethazine as directed under *Nitrite Titration* ⟨451⟩. Each mL of 0.1 M sodium nitrite is equivalent to 27.83 mg of $C_{12}H_{14}N_4O_2S$.

Sulfamethazine Granulated

» Sulfamethazine Granulated contains Sulfamethazine mixed with suitable diluents, carriers, and inactive ingredients. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of sulfamethazine ($C_{12}H_{14}N_4O_2S$).

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

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Labeling—Label it to indicate that it is for veterinary use only. Label it also to indicate that it is for manufacturing, processing, or repackaging.

USP Reference standards ⟨11⟩—
USP Sulfamethazine RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for sulfamethazine, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay.*

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 5 hours: it loses not more than 10% of its weight.

Powder fineness ⟨811⟩: not less than 95% passes a No. 20 sieve, and not more than 10% passes a No. 80 sieve.

Assay—

Mobile phase—Prepare a mixture of water, methanol, and glacial acetic acid (68:30:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Extractant—Prepare a mixture of 0.15 N hydrochloric acid and methanol (3:1).

Standard preparation—Prepare a solution of USP Sulfamethazine RS in *Extractant* having a known concentration of about 0.01 mg per mL.

Assay preparation—Transfer about 5 g of Sulfamethazine Granulated, accurately weighed, to a suitable container, add 250.0 mL of *Extractant,* and shake by mechanical means for 2 hours. Allow the mixture to settle, storing the mixture in a refrigerator if settling is allowed to continue overnight. Filter a portion of the supernatant, and transfer 10.0 mL of the clear filtrate to a 100-mL volumetric flask. Dilute with *Extractant* to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Extractant* to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, and use the filtrate as the *Assay preparation.* This solution contains about 0.01 mg of sulfamethazine per mL.

Derivatizing reagent—Dissolve 6.0 g of dimethylaminobenzaldehyde in 200 mL of glacial acetic acid, add 120 mL of methanol and 80 mL of water, mix, and degas. Prepare this reagent daily.

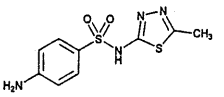
Chromatographic system—The liquid chromatograph is equipped with a guard column that contains packing L1, a 4.6-mm × 25-cm analytical column that contains 10-µm packing L1, a separate pump to deliver the *Derivatizing reagent* via a T-junction installed immediately postcolumn, a postcolumn derivatization coil consisting of 3-m × 0.5-mm inside-diameter polytef tubing, a flow cell, and a 450-nm detector. The *Mobile phase* flow rate is about 2 mL per minute, and the *Derivatizing reagent* flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, for the sulfamethazine peak is not less than 2.0; and the relative standard deviation for replicate injections is not more than 3.5%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the sulfamethazine peaks. Calculate the quantity, in mg, of sulfamethazine ($C_{12}H_{14}N_4O_2S$) in each g of the Sulfamethazine Granulated taken by the formula:

$$100{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Sulfamethazine RS in the *Standard preparation; W* is the quantity, in g, of Sulfamethazine Granulated taken to prepare the *Assay preparation;* and r_U and r_S are the sulfamethazine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Sulfamethizole



$C_9H_{10}N_4O_2S_2$ 270.33

Benzenesulfonamide, 4-amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-;
*N*¹-(5-Methyl-1,3,4-thiadiazol-2-yl) sulfanilamide [144-82-1].

DEFINITION

Sulfamethizole contains NLT 98.0% and NMT 101.0% of sulfamethizole ($C_9H_{10}N_4O_2S_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197M**▲ (CN 1-May-2020)
- **B.**
  Sample: 0.1 g
  Analysis: Add 5 mL of 3 N hydrochloric acid to the *Sample,* and boil gently for 5 min. Cool in an ice bath, then add 4 mL of 1% sodium nitrite solution, add water to make 10 mL, and place the mixture in an ice bath for 10 min. To 5 mL of the cooled mixture add a solution of 50 mg of 2-naphthol in 2 mL of 10% sodium hydroxide solution.
  Acceptance criteria: An orange-red precipitate is formed, and it darkens on standing.
- **C.**
  Sample: 20 mg
  Analysis: Suspend the *Sample* in 5 mL of water, add dropwise 1 N sodium hydroxide until dissolved, then add 2 or 3 drops of cupric sulfate TS.
  Acceptance criteria: A light green precipitate is formed, and it does not change on standing.
- **D.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**
  Mobile phase: Methanol, glacial acetic acid, and water (30:1:69)
  Standard stock solution: 0.4 mg/mL of USP Sulfamethizole RS in methanol
  Standard solution: 8 µg/mL of USP Sulfamethizole RS from the *Standard stock solution* in *Mobile phase*
  Sample stock solution: 0.4 mg/mL of Sulfamethizole in methanol
  Sample solution: 8 µg/mL of Sulfamethizole from the *Sample stock solution* in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; 10-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 2000 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%

USP Monographs

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfamethizole ($C_9H_{10}N_4O_2S_2$) in the portion of Sulfamethizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulfamethizole RS in the *Standard solution* (µg/mL)
C_U = concentration of Sulfamethizole in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Sample solution: A 25.0-mL portion of the filtrate prepared in the test for *Acidity*
  Acceptance criteria: 0.014%; shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid.
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Sample solution: A 25.0-mL portion of the filtrate prepared in the test for *Acidity*
  Acceptance criteria: 0.04%; shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid.
- **SELENIUM** ⟨291⟩: NMT 0.003%
- **ORDINARY IMPURITIES** ⟨466⟩
  Standard solution: Methanol
  Sample solution: Methanol
  Eluant: Acetone
  Visualization: 1
  Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 208°–212°
- **ACIDITY**
  Sample: 2.0 g
  Analysis: Digest the *Sample* with 100 mL of water at 70° for 5 min, cool immediately to about 20°, and filter. To 25.0 mL of the filtrate add 2 drops of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide. Save the remainder of the filtrate for the tests for *Chloride* and *Sulfate*.
  Acceptance criteria: NMT 0.50 mL is required for neutralization.
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 0.5%
- **CLARITY AND COLOR OF SOLUTION**
  Sample: 1.0 g
  Analysis: Dissolve the *Sample* in 20 mL of water and 5 mL of 1 N sodium hydroxide.
  Acceptance criteria: The solution is clear and not more than pale yellow.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulfamethizole RS

Sulfamethizole Oral Suspension

DEFINITION
Sulfamethizole Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sulfamethizole ($C_9H_{10}N_4O_2S_2$), in a buffered aqueous suspension.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
  Sample: Equivalent to 500 mg of sulfamethizole from Oral Suspension
  Analysis: Transfer the *Sample* to a 50-mL centrifuge tube, add 30 mL of water, mix, and centrifuge the mixture. Decant, and discard the supernatant. Resuspend the residue in 15 mL of water, mix, and centrifuge the mixture. Decant, and discard the clear supernatant. Repeat the washing procedure an additional two times. Dissolve the residue in a mixture of 10 mL of 6 N ammonium hydroxide and 10 mL of water, and filter. Warm the filtrate until most of the ammonia is expelled, cool, and add 6 N acetic acid until the mixture is distinctly acid. A precipitate of sulfamethizole is formed. Collect the precipitate on a filter, wash it well with cold water, and dry at 105° for 2 h.
  Acceptance criteria: The infrared spectrum of the residue matches the infrared spectrum of similarly prepared USP Sulfamethizole RS.

ASSAY
- **PROCEDURE**
  Sample: An equivalent to 500 mg of sulfamethizole from Oral Suspension
  Analysis: Transfer the *Sample* to a beaker. Add 50 mL of water and 20 mL of hydrochloric acid, stir until dissolved, cool to 15°, and slowly titrate with 0.1 M sodium nitrite VS. Determine the endpoint potentiometrically using suitable electrodes. Each mL of 0.1 M sodium nitrite is equivalent to 27.03 mg of sulfamethizole ($C_9H_{10}N_4O_2S_2$).
  Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements for Oral Suspension packaged in single-unit containers
- **DELIVERABLE VOLUME** ⟨698⟩: Meets the requirements for Oral Suspension packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulfamethizole RS

Sulfamethizole Tablets

DEFINITION
Sulfamethizole Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of sulfamethizole ($C_9H_{10}N_4O_2S_2$).

USP Monographs

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Sample: Equivalent to 500 mg of sulfamethizole from finely powdered Tablets

Analysis: Triturate the *Sample* with 5 mL of chloroform, and transfer to a small filter. Wash with another 5-mL portion of chloroform, and discard the filtrate. Triturate the residue with 10 mL of 6 N ammonium hydroxide for 5 min, add 10 mL of water, and filter. Warm the filtrate until most of the ammonia is expelled, cool, and add 6 N acetic acid until the mixture is distinctly acid. A precipitate of sulfamethizole is formed. Collect the precipitate on a filter, wash it well with cold water, and dry at 105° for 2 h.

Acceptance criteria: The infrared spectrum of the residue matches the infrared spectrum of similarly prepared USP Sulfamethizole RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (30:1:69)

Standard stock solution: 0.4 mg/mL of USP Sulfamethizole RS in methanol

Standard solution: 8 µg/mL of USP Sulfamethizole RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: Nominally 0.4 mg/mL of sulfamethizole prepared as follows. Transfer an equivalent to 40 mg of sulfamethizole from NLT 20 finely powdered Tablets to a 250-mL screw-capped bottle. Add 100.0 mL of methanol to the bottle, cap the bottle, shake by mechanical means for 30 min, and filter.

Sample solution: 8 µg/mL of sulfamethizole from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sulfamethizole ($C_9H_{10}N_4O_2S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulfamethizole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of sulfamethizole in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Prepare a solution having a known concentration of USP Sulfamethizole RS in *Medium*.

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: Absorption maximum at about 267 nm

Analysis: Calculate the percentage of the labeled amount of sulfamethizole ($C_9H_{10}N_4O_2S_2$) dissolved.

Tolerances: NLT 75% (*Q*) of the labeled amount of sulfamethizole ($C_9H_{10}N_4O_2S_2$) is dissolved.

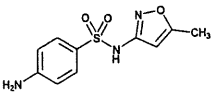
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfamethizole RS

Sulfamethoxazole



$C_{10}H_{11}N_3O_3S$ 253.28

Benzenesulfonamide, 4-amino-*N*-(5-methyl-3-isoxazolyl)-; N^1-(5-Methyl-3-isoxazolyl)sulfanilamide [723-46-6].

DEFINITION

Sulfamethoxazole contains NLT 98.0% and NMT 102.0% of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 13.6 g/L of potassium dihydrogen phosphate adjusted with a 20-g/L solution of potassium hydroxide to a pH of 5.3

Mobile phase: Methanol and *Buffer* (30:70)

Standard solution: 0.1 mg/mL each of USP Sulfamethoxazole RS and USP Sulfamethoxazole Related Compound A RS in *Mobile phase*. Sonicate at 45° with intermittent shaking to dissolve before final dilution.

Sample solution: 0.1 mg/mL of Sulfamethoxazole in *Mobile phase*. Sonicate at 45° with intermittent shaking to dissolve before final dilution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

USP Monographs

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Mode: LC
Detector: UV 210 nm
Column: 4-mm × 25-cm; 5-µm packing L7
Flow rate: 0.9 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for sulfamethoxazole and sulfamethoxazole related compound A are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 3.5 between the sulfamethoxazole and sulfamethoxazole related compound A peaks
Relative standard deviation: NMT 0.73% for the sulfamethoxazole peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) in the portion of Sulfamethoxazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulfamethoxazole from the *Sample solution*
r_S = peak response of sulfamethoxazole from the *Standard solution*
C_S = concentration of USP Sulfamethoxazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfamethoxazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: Use the *Standard solution* from the *Assay*.
Peak identification solution: 1 µg/mL each of USP Sulfamethoxazole Related Compound A RS, USP Sulfamethoxazole Related Compound B RS, USP Sulfamethoxazole Related Compound C RS, USP Sulfanilic Acid RS, and USP Sulfanilamide RS in *Mobile phase*. Sonicate, if necessary, to dissolve before final dilution.
Standard solution: 1 µg/mL each of USP Sulfamethoxazole RS and USP Sulfamethoxazole Related Compound F RS in *Mobile phase*. Sonicate, if necessary, to dissolve before final dilution.
Sample solution: 1 mg/mL of Sulfamethoxazole in *Mobile phase*. Sonicate at 45° with intermittent shaking to dissolve before final dilution.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between the sulfamethoxazole and sulfamethoxazole related compound A peaks, *System suitability solution*
Relative standard deviation: NMT 5.0% for the sulfamethoxazole peak, *Standard solution*
Analysis
Samples: *Peak identification solution, Standard solution,* and *Sample solution*
Calculate the percentage of sulfamethoxazole related compound F in the portion of Sulfamethoxazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulfamethoxazole related compound F from the *Sample solution*
r_S = peak response of sulfamethoxazole related compound F from the *Standard solution*
C_S = concentration of USP Sulfamethoxazole Related Compound F RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfamethoxazole in the *Sample solution* (mg/mL)

Calculate the percentage of any individual impurity in the portion of Sulfamethoxazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of sulfamethoxazole from the *Standard solution*
C_S = concentration of USP Sulfamethoxazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfamethoxazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.03%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulfanilic acid	0.26	0.10
Sulfanilamide	0.35	0.10
Sulfamethoxazole related compound F	0.45	0.10
Sulfamethoxazole related compound C	0.50	0.10
Sulfamethoxazole	1.0	—
Sulfamethoxazole related compound A	1.2	0.10
Sulfamethoxazole related compound B	2.1	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.30

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfamethoxazole RS
USP Sulfamethoxazole Related Compound A RS
N-{4-[*N*-(5-Methylisoxazol-3-yl)sulfamoyl]phenyl} acetamide.
$C_{12}H_{13}N_3O_4S$ 295.31
USP Sulfamethoxazole Related Compound B RS
4-Amino-*N*-{4-[*N*-(5-methylisoxazol-3-yl)sulfamoyl] phenyl}benzenesulfonamide.
$C_{16}H_{16}N_4O_5S_2$ 408.45

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USP Sulfamethoxazole Related Compound C RS
5-Methylisoxazol-3-amine.
$C_4H_6N_2O$ 98.10
USP Sulfamethoxazole Related Compound F RS
4-Amino-*N*-(3-methylisoxazol-5-yl)benzenesulfonamide.
$C_{10}H_{11}N_3O_3S$ 253.28
USP Sulfanilamide RS
4-Aminobenzenesulfonamide.
$C_6H_8N_2O_2S$ 172.20
USP Sulfanilic Acid RS
4-Aminobenzenesulfonic acid.
$C_6H_7NO_3S$ 173.19

Sulfamethoxazole Oral Suspension

DEFINITION
Sulfamethoxazole Oral Suspension contains NLT 95.0% and NMT 110.0% of the labeled quantity of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$).

IDENTIFICATION
• **A.**
Standard solution: Dissolve 100 mg of USP Sulfamethoxazole RS in 5 mL of ammonium hydroxide. Dilute with methanol to 50.0 mL.
Sample solution: Transfer a quantity of Oral Suspension, equivalent to 100 mg of sulfamethoxazole, into a 50-mL centrifuge tube. Add 5 mL of ammonium hydroxide, and shake gently. Add 25 mL of methanol, shake thoroughly for 3 min, centrifuge, decant the supernatant into a 50-mL volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 50 µL
Developing solvent system: Alcohol, *n*-heptane, chloroform, and glacial acetic acid (25:25:25:7)
Spray reagent: 0.10 g of *p*-dimethylaminobenzaldehyde in 1 mL of hydrochloric acid. Dilute with alcohol to 100 mL.
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the *Samples*, and allow the spots to dry. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent*.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.
• **B.**
Sample: Transfer a quantity of Oral Suspension, equivalent to 500 mg of sulfamethoxazole, into a 50-mL centrifuge tube. Add 25 mL of water, mix, and centrifuge. Decant and discard the supernatant, resuspend the residue in 25 mL of water, mix, and centrifuge again. Decant and discard the clear supernatant. Repeat the washing procedure an additional two times.
Analysis: Dissolve the residue from the *Sample* in 10 mL of hydrochloric acid, and add 15 mL of sodium nitrite solution (1 in 100) and 5 mL of sodium hydroxide solution (1 in 10) containing 10 mg of 2-naphthol.
Acceptance criteria: A red-orange precipitate is produced.

ASSAY
• **PROCEDURE**
Sample solution: Mix a volume of Oral Suspension, equivalent to 1 g of sulfamethoxazole, with 20 mL of glacial acetic acid and 40 mL of water.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 M sodium nitrite VS
Endpoint detection: Potentiometric
Analysis: Add 15 mL of hydrochloric acid to the *Sample solution*. Cool to 15°, and titrate immediately with *Titrant*, using a calomel–platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 25.33 mg of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$).
Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for oral suspension packaged in single-unit containers
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for oral suspension packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfamethoxazole RS

Sulfamethoxazole Tablets

DEFINITION
Sulfamethoxazole Tablets contain NLT 95.0% and NMT 105.0% of the labeled quantity of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$).

IDENTIFICATION
• **A.**
Standard solution: Dissolve 100 mg of USP Sulfamethoxazole RS in 5 mL of ammonium hydroxide. Dilute with methanol to 50.0 mL.
Sample solution: Transfer a quantity of finely powdered Tablets, equivalent to 100 mg of sulfamethoxazole, to a 50-mL centrifuge tube. Add 5 mL of ammonium hydroxide, and shake gently. Add 25 mL of methanol, shake thoroughly for 3 min, centrifuge, decant the supernatant into a 50-mL volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 50 µL
Developing solvent system: Alcohol, *n*-heptane, chloroform, and glacial acetic acid (25:25:25:7)
Spray reagent: 0.10 g of *p*-dimethylaminobenzaldehyde in 1 mL of hydrochloric acid. Dilute with alcohol to 100 mL.
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the *Samples*, and allow the spots to dry. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent*.

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Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

• **B.**

Sample: A portion of finely powdered Tablets equivalent to 100 mg of sulfamethoxazole

Analysis: To the *Sample* add 2 mL of hydrochloric acid, 3 mL of sodium nitrite solution (1 in 100), and 1 mL of sodium hydroxide solution (1 in 10) containing 10 mg of 2-naphthol.

Acceptance criteria: A red-orange precipitate is formed.

ASSAY

• **PROCEDURE**

Sample solution: Dissolve NLT 20 finely powdered Tablets, equivalent to 500 mg of sulfamethoxazole, with 20 mL of glacial acetic acid and 40 mL of water, and add 15 mL of hydrochloric acid.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 M sodium nitrite VS

Endpoint detection: Potentiometric

Analysis: Cool the *Sample solution* to 15°, and titrate immediately with *Titrant* to a potentiometric endpoint using a calomel–platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 25.33 mg of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$).

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Dilute hydrochloric acid (7 in 100); 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Sulfamethoxazole RS in *Medium* at a known concentration

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium* if necessary, in comparison to the *Standard solution.*

Instrumental conditions

Mode: UV

Analytical wavelength: 265 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) dissolved.

Tolerances: NLT 80% (*Q*) of the labeled amount of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfamethoxazole RS

Sulfamethoxazole and Trimethoprim Injection

DEFINITION

Sulfamethoxazole and Trimethoprim Injection is a sterile solution of Sulfamethoxazole and Trimethoprim in Water for Injection, which, when diluted with Dextrose Injection, is suitable for intravenous infusion. It contains NLT 90.0% and NMT 110.0% of the labeled quantities of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$).

IDENTIFICATION

• **A.** The R_F values of the principal spots of the *Sample solution* correspond to those of the *Standard solutions* of USP Trimethoprim RS (R_F about 0.5) and USP Sulfamethoxazole RS (R_F about 0.7), as obtained in the *Impurities* tests.

ASSAY

• **PROCEDURE**

Mobile phase: Mix 1400 mL of water, 400 mL of acetonitrile, and 2.0 mL of triethylamine in a 2000-mL volumetric flask. Allow to equilibrate to room temperature, and adjust with 0.2 N sodium hydroxide or dilute glacial acetic acid (1 in 100) to a pH of 5.9 ± 0.1. Dilute with water to volume, and pass through a filter of 0.45-µm pore size.

Standard stock solution: 0.32 mg/mL of USP Trimethoprim RS and 0.32*J* mg/mL of USP Sulfamethoxazole RS in methanol, where *J* is the ratio of the labeled amount, in mg, of sulfamethoxazole to the labeled amount, in mg, of trimethoprim in the dosage form

Standard solution: 0.032 mg/mL of USP Trimethoprim RS per mL and 0.032*J* mg/mL of USP Sulfamethoxazole RS per mL in *Mobile phase* from *Standard stock solution*

Sample stock solution: Transfer a volume of Injection, equivalent to 80 mg of sulfamethoxazole, to a 50-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Nominally 0.16 mg/mL of sulfamethoxazole in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for trimethoprim and sulfamethoxazole are 1.0 and 1.8, respectively.]

Suitability requirements

Resolution: NLT 5.0 between sulfamethoxazole and trimethoprim

Tailing factor: NMT 2.0 for trimethoprim and sulfamethoxazole

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of trimethoprim ($C_{14}H_{18}N_4O_3$) and sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trimethoprim or sulfamethoxazole from the *Sample solution*

r_S = peak response of trimethoprim or sulfamethoxazole from the *Standard solution*

C_S = concentration of the USP Trimethoprim RS or USP Sulfamethoxazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of trimethoprim or sulfamethoxazole in the *Sample solution* (mg/mL)

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Acceptance criteria: 90.0%–110.0% each of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$)

IMPURITIES

• **LIMIT OF TRIMETHOPRIM DEGRADATION PRODUCT**

Solution A: 0.06 N hydrochloric acid solution

Standard solution A: 48 mg/mL of USP Trimethoprim RS in chloroform and methanol (1:1)

Standard solution B: 240 µg/mL of USP Trimethoprim RS in a mixture of chloroform and methanol (1:1) from *Standard solution A*

Sample solution: Transfer a volume of Injection, equivalent to 48 mg of trimethoprim and 240 mg of sulfamethoxazole, to a glass-stoppered, 50-mL centrifuge tube. Add 15 mL of *Solution A*, and mix. Add 15 mL of chloroform, shake for 30 s, and centrifuge at high speed for 3 min. Transfer the supernatant layer to a 125-mL separator. Extract the chloroform layer in the centrifuge tube with 15 mL of *Solution A*, centrifuge at high speed, and add the extract to the separator. Add 2 mL of sodium hydroxide solution (100 mg/mL) to the solution in the separator, and extract with three 20-mL portions of chloroform, collecting the organic layer in a 125-mL conical flask. Evaporate the chloroform under a stream of nitrogen to dryness. Dissolve the residue in 1 mL of a mixture of chloroform and methanol (1:1).

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (97: 7.5: 1)

Spray reagent: Freshly prepared mixture of ferric chloride solution (100 mg/mL) and potassium ferricyanide solution (50 mg/mL) (1:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* to separate points on the plate. Develop the chromatogram using *Developing solvent system* until the solvent front has moved at least 12 cm. Remove the plate from the developing chamber, air-dry, and spray with *Spray reagent*. Locate the bands by viewing under short-wavelength UV light. Trimethoprim produces a spot at about R_F 0.5, and the trimethoprim degradation product produces a spot at about R_F 0.6–0.7. [NOTE—There may be spots due to concentrate excipients at about R_F 0.1.]

Acceptance criteria: Any spot from the *Sample solution* at about R_F 0.6–0.7 is not greater in size and intensity than the spot produced by *Standard solution B* at about R_F 0.5, corresponding to NMT 0.5%.

• **LIMIT OF SULFANILAMIDE AND SULFANILIC ACID**

Solution A: Methanol and dehydrated alcohol (5:95)

Solution B: Dilute 1 mL of ammonium hydroxide with *Solution A* to 100 mL

Standard solution A: 10 mg/mL of USP Sulfamethoxazole RS in *Solution B*

Standard solution B: 0.05 mg/mL of USP Sulfanilamide RS in *Solution B*

Standard solution C: 0.03 mg/mL of USP Sulfanilic Acid RS in *Solution B*

Sample solution: Transfer a volume of Injection, equivalent to 32 mg of trimethoprim and 160 mg of sulfamethoxazole, into a 25-mL graduated cylinder. Dilute with *Solution B* to 16 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: *Solution A*, heptane, chloroform, and glacial acetic acid (30:30:30:10)

Spray reagent (Modified Ehrlich's reagent): 100 mg of *p*-dimethylaminobenzaldehyde in 1 mL of hydrochloric acid. Dilute with alcohol to 100 mL.

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Apply the *Samples* to separate points on the plate. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved NLT 12 cm. Remove the plate from the developing chamber, air-dry, spray with *Spray reagent*, and allow the plate to stand for 15 min. Sulfamethoxazole produces a spot at about R_F 0.7.

Acceptance criteria: Any spots from the *Sample solution* at about R_F 0.5 or 0.1 are not greater in size or intensity than spots produced by *Standard solution B* and *Standard solution C*, respectively, corresponding to NMT 0.5% of sulfanilamide and 0.3% of sulfanilic acid.

SPECIFIC TESTS

• **pH** ⟨791⟩: 9.5–10.5

• **PYROGEN TEST** ⟨151⟩: Meets the requirements, the test dose being 0.5 mL/kg

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose, light-resistant containers, preferably of Type I glass. It may be packaged in 50-mL multiple-dose containers.

• **LABELING:** Label it to indicate that it is to be diluted with 5% Dextrose Injection before administration.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfamethoxazole RS

USP Sulfanilamide RS

p-Aminobenzenesulfonamide.

$C_6H_8N_2O_2S$ 172.20

USP Sulfanilic Acid RS

Benzenesulfonic acid, 4-amino-.

$C_6H_7NO_3S$ 173.19

USP Trimethoprim RS

Sulfamethoxazole and Trimethoprim Oral Suspension

DEFINITION

Sulfamethoxazole and Trimethoprim Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amounts of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

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ASSAY

• **PROCEDURE**

Mobile phase: Mix 1400 mL of water, 400 mL of acetonitrile, and 2.0 mL of triethylamine in a 2000-mL volumetric flask. Allow to equilibrate to room temperature, and adjust with 0.2 N sodium hydroxide or dilute glacial acetic acid (1 in 100) to a pH of 5.9 ± 0.1. Dilute with water to volume, and pass through a filter of 0.45-µm pore size.

Standard stock solution: 0.32 mg/mL of USP Trimethoprim RS and 0.32*f* mg/mL of USP Sulfamethoxazole RS in methanol, where *f* is the ratio of the labeled amount, in mg, of sulfamethoxazole to the labeled amount, in mg, of trimethoprim in the dosage form

Standard solution: 0.032 mg/mL of USP Trimethoprim RS per mL and 0.032*f* mg/mL of USP Sulfamethoxazole RS per mL in *Mobile phase* from *Standard stock solution*

Sample stock solution: Transfer a volume of Oral Suspension, equivalent to 80 mg of sulfamethoxazole, to a 50-mL volumetric flask with the aid of 30 mL of methanol. Sonicate the mixture for 10 min with occasional shaking. Allow to equilibrate to room temperature, dilute with methanol to volume, and centrifuge. Use the filtrate in the preparation of the *Sample solution*.

Sample solution: Nominally 0.16 mg/mL of sulfamethoxazole in *Mobile phase* from *Sample stock solution*. Filter the solution.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for trimethoprim and sulfamethoxazole are 1.0 and 1.8, respectively.]
Suitability requirements
Resolution: NLT 5.0 between sulfamethoxazole and trimethoprim
Tailing factor: NMT 2.0 for sulfamethoxazole and trimethoprim
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trimethoprim ($C_{14}H_{18}N_4O_3$) and sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trimethoprim or sulfamethoxazole from the *Sample solution*
r_S = peak response of trimethoprim or sulfamethoxazole from the *Standard solution*
C_S = concentration of the USP Trimethoprim RS or USP Sulfamethoxazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of trimethoprim or sulfamethoxazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$)

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements for oral suspension packaged in single-unit containers

• **DELIVERABLE VOLUME** ⟨698⟩: Meets the requirements for oral suspension packaged in multiple-unit containers

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Triethylamine and water (2.5: 2000). Adjust with glacial acetic acid to a pH of 5.9.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	80	20
25	60	40
30	20	80
31	90	10
35	90	10

Diluent: *Solution A* and *Solution B* (75:25)

System suitability solution: 1 mg/mL of USP Sulfamethoxazole RS; 0.2 mg/mL of USP Trimethoprim RS; 1 µg/mL each of USP Sulfamethoxazole N4-Glucoside RS, USP Sulfamethoxazole Related Compound C RS, and 0.3 µg/mL each of USP Trimethoprim Related Compound A RS, USP Trimethoprim Related Compound B RS, and USP Diaveridine RS in *Diluent*

Standard stock solution A: 0.3 mg/mL of USP Sulfamethoxazole N4-Glucoside RS, and 0.05 mg/mL each of USP Sulfamethoxazole Related Compound C RS and USP Sulfamethoxazole RS in *Diluent*. Sonicate for 5 min to dissolve.

Standard stock solution B: 0.03 mg/mL each of USP Trimethoprim RS, USP Trimethoprim Related Compound A RS, USP Trimethoprim Related Compound B RS, and USP Diaveridine RS in *Diluent*. Sonicate for 5 min to dissolve.

Standard solution: 0.03 mg/mL of USP Sulfamethoxazole N4-Glucoside RS; 5 µg/mL each of USP Sulfamethoxazole Related Compound C RS and USP Sulfamethoxazole RS from *Standard stock solution A*; and 1 µg/mL each of USP Trimethoprim RS, USP Trimethoprim Related Compound A RS, USP Trimethoprim Related Compound B RS, and USP Diaveridine RS from *Standard stock solution B* in *Diluent*

Sample solution: Nominally 1 mg/mL of sulfamethoxazole and 0.2 mg/mL of trimethoprim from a volume of Oral Suspension in *Diluent*. Sonicate for 15 min in *Diluent* before diluting to final volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between trimethoprim and trimethoprim related compound A; and NLT 1.5 between sulfamethoxazole and trimethoprim related compound B
Relative standard deviation: NMT 2.0% for sulfamethoxazole related compound C,

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sulfamethoxazole N_4-glucoside, diaveridine, trimethoprim, trimethoprim related compound A, sulfamethoxazole, and trimethoprim related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulfanilic acid and sulfanilamide in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of sulfanilic acid or sulfanilamide from the *Sample solution*

r_S = peak response of sulfamethoxazole from the *Standard solution*

C_S = concentration of USP Sulfamethoxazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of sulfamethoxazole in the *Sample solution* (mg/mL)

F = relative response factor

Calculate the percentage of sulfamethoxazole related compound C and sulfamethoxazole N_4-glucoside in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulfamethoxazole related compound C or sulfamethoxazole N_4-glucoside from the *Sample solution*

r_S = peak response of sulfamethoxazole related compound C or sulfamethoxazole N_4-glucoside from the *Standard solution*

C_S = concentration of USP Sulfamethoxazole Related Compound C RS or USP Sulfamethoxazole N4-Glucoside RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of sulfamethoxazole in the *Sample solution* (mg/mL)

Calculate the percentage of diaveridine, trimethoprim related compound A, trimethoprim related compound B, and any other unspecified degradation product in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diaveridine, trimethoprim related compound A, trimethoprim related compound B, or any other unspecified degradation product from the *Sample solution*

r_S = peak response of diaveridine, trimethoprim related compound A, trimethoprim related compound B, or trimethoprim (for any other unspecified degradation product) from the *Standard solution*

C_S = concentration of USP Diaveridine RS, USP Trimethoprim Related Compound A RS, USP Trimethoprim Related Compound B RS, or USP Trimethoprim RS (for any other unspecified degradation product) in the *Standard solution* (mg/mL)

C_U = nominal concentration of trimethoprim in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard limit: 0.01%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfanilic acid	0.18	2.4	0.3
Sulfanilamide	0.32	1.4	0.5
Sulfamethoxazole related compound C	0.36	—	0.2
Sulfamethoxazole related compound F[a]	0.50	—	—
Sulfamethoxazole N_4-glucoside	0.54	—	3.0
Diaveridine	0.62	—	0.5
Trimethoprim	0.71	—	—
Trimethoprim related compound A	0.74	—	0.5
Sulfamethoxazole related compound A[a]	0.81	—	—
Sulfamethoxazole	1.00	—	—
Trimethoprim related compound B	1.03	—	0.5
Any other unspecified degradation product	—	—	0.2

[a] Process related impurities monitored in the drug substance.

SPECIFIC TESTS

- **pH** ⟨791⟩: 5.0–6.5
- **Alcohol Determination,** *Method II* ⟨611⟩: NMT 0.5% of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature. Protect from light.
- **USP Reference Standards** ⟨11⟩

USP Alcohol Determination–Acetonitrile RS
USP Alcohol Determination–Alcohol RS
USP Diaveridine RS
5-(3,4-Dimethoxybenzyl)pyrimidine-2,4-diamine.
$C_{13}H_{16}N_4O_2$ 260.29
USP Sulfamethoxazole RS
USP Sulfamethoxazole N4-Glucoside RS
4-(Beta-D-glucopyranosylamino)-*N*-(5-methyl-3-isoxazolyl)-benzenesulfonamide.
$C_{16}H_{21}N_3O_8S$ 415.42
USP Sulfamethoxazole Related Compound C RS
5-Methyl-3-isoxamine.
$C_4H_6N_2O$ 98.10
USP Trimethoprim RS
USP Trimethoprim Related Compound A RS
4-Amino-5-(3,4,5-trimethoxybenzyl)pyrimidin-2-ol.
$C_{14}H_{17}N_3O_4$ 291.30
USP Trimethoprim Related Compound B RS
(2,4-Diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl) methanone.
$C_{14}H_{16}N_4O_4$ 304.30

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Sulfamethoxazole and Trimethoprim Tablets

DEFINITION

Sulfamethoxazole and Trimethoprim Tablets contain NLT 93.0% and NMT 107.0% of the labeled amounts of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$).

IDENTIFICATION

• **A.**

Standard solution A: 0.4 mg/mL of USP Trimethoprim RS in methanol

Standard solution B: 2 mg/mL of USP Sulfamethoxazole RS in methanol

Sample solution: Transfer an amount of finely ground Tablets, equivalent to 4 mg of trimethoprim, to a 10-mL volumetric flask, add 8 mL of methanol, and warm for several min on a steam bath with frequent shaking. Cool, dilute with methanol to volume, and centrifuge briefly.

Chromatographic system

Mode: TLC

Application volume: 5 µL

Developing solvent system: Chloroform, isopropyl alcohol, and diethylamine (6:5:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* to separate points about 3 cm from one end of the plate. Dry the spots in a current of warm air, and develop the plate with *Developing solvent system* in a chamber that is lined with filter paper. Remove the plate, dry, and examine under short-wavelength UV light.

Acceptance criteria: The trimethoprim and sulfamethoxazole spots from the solution under test have the same R_F values as the spots from the corresponding *Standard solutions*.

ASSAY

• **PROCEDURE**

Mobile phase: Mix 1400 mL of water, 400 mL of acetonitrile, and 2.0 mL of triethylamine in a 2000-mL volumetric flask. Allow to equilibrate to room temperature, and adjust with 0.2 N sodium hydroxide or dilute glacial acetic acid (1 in 100) to a pH of 5.9 ± 0.1. Dilute with water to volume, and pass through a filter of 0.45-µm pore size.

Standard stock solution: 0.32 mg/mL of USP Trimethoprim RS and 0.32*J* mg/mL of USP Sulfamethoxazole RS in methanol, where *J* is the ratio of the labeled amount, in mg, of sulfamethoxazole to the labeled amount, in mg, of trimethoprim in the dosage form

Standard solution: 0.032 mg/mL of USP Trimethoprim RS per mL and 0.032*J* mg/mL of USP Sulfamethoxazole RS per mL in *Mobile phase* from *Standard stock solution*

Sample stock solution: Transfer from finely powdered Tablets (NLT 20), an equivalent to 160 mg of sulfamethoxazole, to a 100-mL volumetric flask. Add 50 mL of methanol and sonicate, with intermittent shaking, for 5 min. Allow to equilibrate to room temperature, dilute with methanol to volume, and filter. Use the filtrate in the preparation of the *Sample solution*.

Sample solution: Nominally 0.16 mg/mL of sulfamethoxazole in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for trimethoprim and sulfamethoxazole are 1.0 and 1.8, respectively.]

Suitability requirements

Resolution: NLT 5.0 between sulfamethoxazole and trimethoprim

Tailing factor: NMT 2.0 for sulfamethoxazole and trimethoprim

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of trimethoprim ($C_{14}H_{18}N_4O_3$) and sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trimethoprim or sulfamethoxazole from the *Sample solution*

r_S = peak response of trimethoprim or sulfamethoxazole from the *Standard solution*

C_S = concentration of the USP Trimethoprim RS or USP Sulfamethoxazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of trimethoprim or sulfamethoxazole in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0% each of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Standard solution: USP Sulfamethoxazole RS and USP Trimethoprim RS at a known concentration

Sample solution: Pass a portion of the solution under test through a suitable filter.

Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$) dissolved by comparison of the peak responses obtained from a filtered aliquot of the solution under test with the peak responses from the corresponding component obtained from the *Standard solution*.

Calculate the percentage of each active component dissolved

Tolerances: NLT 70% (*Q*) of the labeled amount of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

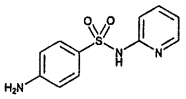
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfamethoxazole RS

USP Trimethoprim RS

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Sulfapyridine



$C_{11}H_{11}N_3O_2S$ 249.29
Benzenesulfonamide, 4-amino-*N*-2-pyridinyl-;
N^1-2-Pyridylsulfanilamide [144-83-2].

DEFINITION
Sulfapyridine contains NLT 99.0% and NMT 100.5% of sulfapyridine ($C_{11}H_{11}N_3O_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**
  Sample solution: Add 5 mL of 3 N hydrochloric acid to 0.1 g of Sulfapyridine.
  Analysis: Boil gently the *Sample solution* for 5 min, then cool in an ice bath. Add 4 mL of sodium nitrite solution (1 in 100), dilute with water to 10 mL, and place the mixture in the ice bath for 10 min. To 5 mL of the cooled mixture add a solution of 50 mg of 2-naphthol in 2 mL of sodium hydroxide solution (1 in 10).
  Acceptance criteria: An orange-red precipitate is formed, and it darkens on standing.

ASSAY
- **NITRITE TITRATION** ⟨451⟩**:** Each mL of 0.1 N sodium nitrite is equivalent to 24.93 mg of sulfapyridine ($C_{11}H_{11}N_3O_2S$).
  Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **SELENIUM** ⟨291⟩
  Sample: 200 mg
  Acceptance criteria: 30 ppm

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 190°–193°
- **ACIDITY**
  Sample: 2.0 g
  Analysis: Digest the *Sample* with 100 mL of water at 70° for 5 min, cool at once to 20°, and filter. To 25.0 mL of the filtrate add 2 drops of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide.
  Acceptance criteria: NMT 0.20 mL is required for neutralization.
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 0.5%
- **CLARITY AND COLOR OF SOLUTION**
  Sample solution: 1.0 g in a mixture of 20 mL of water and 5 mL of 1 N sodium hydroxide
  Acceptance criteria: The *Sample solution* is clear and not more deeply colored than pale yellow.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulfapyridine RS

Sulfapyridine Tablets

DEFINITION
Sulfapyridine Tablets contain NLT 95.0% and NMT 105.0% of the labeled quantity of sulfapyridine ($C_{11}H_{11}N_3O_2S$).

IDENTIFICATION
- **A. MELTING RANGE OR TEMPERATURE,** *Class Ia* ⟨741⟩
  Sample: Equivalent to 500 mg of sulfapyridine from finely powdered Tablets
  Analysis: Triturate the *Sample* with 5 mL of chloroform, and transfer to a small filter. Wash with another 5-mL portion of chloroform, and discard the filtrate. Triturate the residue with 10 mL of 6 N ammonium hydroxide for 5 min, add 10 mL of water, and filter. Warm the filtrate until most of the ammonia is expelled, cool, and add 6 N acetic acid until the reaction is distinctly acid. A precipitate of sulfapyridine is formed. Collect the precipitate on a filter, wash it well with cold water, and dry at 105° for 1 h. Also use the precipitate in *Identification* tests *B* and *C*.
  Acceptance criteria: The sulfapyridine so obtained melts between 189° and 192°.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)
  Sample: Use the precipitate obtained in *Identification* test *A*.
  Acceptance criteria: Meet the requirements
- **C.**
  Sample solution: Add 5 mL of 3 N hydrochloric acid to 0.1 g from the precipitate obtained in *Identification* test *A*.
  Analysis: Boil gently the *Sample solution* for 5 min, then cool in an ice bath. Add 4 mL of sodium nitrite solution (1 in 100), dilute with water to 10 mL, and place the mixture in the ice bath for 10 min. To 5 mL of the cooled mixture add a solution of 50 mg of 2-naphthol in 2 mL of sodium hydroxide solution (1 in 10).
  Acceptance criteria: An orange-red precipitate is formed, and it darkens on standing.

ASSAY
- **NITRITE TITRATION** ⟨451⟩
  Sample: Finely powdered Tablets (NLT 20 Tablets) equivalent to 500 mg of sulfapyridine
  Analysis: Proceed as directed in the chapter, beginning with "and transfer to a suitable open vessel". Each mL of 0.1 M sodium nitrite is equivalent to 24.93 mg of sulfapyridine ($C_{11}H_{11}N_3O_2S$).
  Acceptance criteria: 95.0%–105.0%

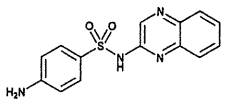
PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: 0.01 N hydrochloric acid; 900 mL
  Apparatus 2: 50 rpm
  Time: 60 min
  Standard solution: USP Sulfapyridine RS in *Medium*
  Sample solution: Filtered portion of solution under test, suitably diluted with 0.01 N sodium hydroxide in comparison to the *Standard solution*
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum absorbance at about 254 nm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of sulfapyridine ($C_{11}H_{11}N_3O_2S$) dissolved.
  Tolerances: NLT 70% (*Q*) of the labeled amount of sulfapyridine ($C_{11}H_{11}N_3O_2S$) is dissolved.

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- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulfapyridine RS

Sulfaquinoxaline



$C_{14}H_{12}N_4O_2S$ 300.34
N^1-2-Quinoxalinylsulfanilamide [59-40-5].

DEFINITION
Sulfaquinoxaline contains NLT 98.0% and NMT 101.0% of $C_{14}H_{12}N_4O_2S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: 10 µg/mL in 0.01 N sodium hydroxide
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Mobile phase: 2 g/L of monobasic ammonium phosphate in a mixture of acetonitrile, glacial acetic acid, tetrahydrofuran, ammonium hydroxide, and water (400:10:5:2:583). Pass through a filter of 0.5-µm or finer pore size.
  Standard solution: 0.06 mg/mL of USP Sulfaquinoxaline RS in 0.01 N sodium hydroxide
  Sample solution: 0.06 mg/mL of Sulfaquinoxaline in 0.01 N sodium hydroxide
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 25-cm; packing L1
  Flow rate: 1 mL/min
  Injection size: 15 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.2
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$) in the portion of Sulfaquinoxaline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulfaquinoxaline RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulfaquinoxaline in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Sample solution: 4 mg/mL, prepared as follows. Dissolve 400 mg of Sulfaquinoxaline in 4 mL of 1 N sodium hydroxide, dilute with methanol to 100 mL, and mix.
  Standard solution A: 0.12 mg/mL of USP Sulfaquinoxaline Related Compound A RS in methanol
  Standard solution B: 0.04 mg/mL of sulfanilamide in methanol
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Chloroform, methanol, and ammonium hydroxide (60:40:20)
  Analysis: Separately apply each solution to the TLC plate, and proceed as directed in the chapter. When the solvent front has moved about three-fourths the length of the plate, remove the plate from the chamber, mark the solvent front, allow it to air-dry, and examine the plate under short-wavelength UV light.
  Acceptance criteria: No spot corresponding to sulfaquinoxaline related compound A in the chromatogram of the *Sample solution* is more intense than the principal spot in the chromatogram of *Standard solution A* (NMT 3.0%); and no spot, other than the principal spot and the sulfaquinoxaline related compound A spot, in the chromatogram of the *Sample solution* is more intense than the principal spot in the chromatogram of *Standard solution B* (NMT 1.0%).

SPECIFIC TESTS
- **ACIDITY**
  Sample: 2 g
  Analysis: Digest the *Sample* with 100 mL of water at about 70° for 5 min, cool to about 20°, and filter. Titrate 50 mL of the filtrate with 0.1 N sodium hydroxide VS to a pH of 7.0.
  Acceptance criteria: NMT 0.2 mL is required
- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 4 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulfaquinoxaline Related Compound A RS
  N^1,N^2-Diquinoxalin-2-ylsulfanilamide.
  $C_{22}H_{16}N_6SO_2$ 428.50
  USP Sulfaquinoxaline RS

USP Monographs

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Sulfaquinoxaline Oral Solution

» Sulfaquinoxaline Oral Solution contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$).

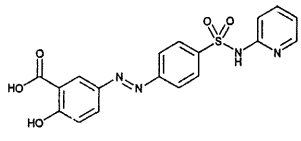
Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Sulfaquinoxaline RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: not less than 12.

Assay—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Sulfaquinoxaline*.
Standard preparation—Dissolve an accurately weighed quantity of USP Sulfaquinoxaline RS in 0.01 N sodium hydroxide to obtain a solution having a known concentration of about 0.7 mg per mL. Dilute an accurately measured volume of this solution quantitatively with water to obtain a solution having a known concentration of about 0.15 mg per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 300 mg of sulfaquinoxaline, to a 2000-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Separately inject equal volumes (about 8 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$) in each mL of the Oral Solution taken by the formula:

$$2000(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Sulfaquinoxaline RS in the *Standard preparation*, V is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*, and r_U and r_S are the sulfaquinoxaline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Sulfasalazine



$C_{18}H_{14}N_4O_5S$ 398.39
Benzoic acid, 2-hydroxy-5-[[4-[(2-pyridinylamino)sulfonyl] phenyl]azo]-;
5-[[*p*-(2-Pyridylsulfamoyl)phenyl]azo]salicylic acid [599-79-1].

DEFINITION
Sulfasalazine contains NLT 97.0% and NMT 101.5% of $C_{18}H_{14}N_4O_5S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. PROCEDURE**

Standard solution: Use the *Standard solution*, prepared as directed in the *Assay*.
Sample solution: Use the *Sample solution*, prepared as directed in the *Assay*.
Acceptance criteria: The visible absorption spectrum of the *Sample solution* corresponds to that of the *Standard solution*, as obtained from the *Assay*.

ASSAY
- **PROCEDURE**

Sample stock solution: 1.5 mg/mL of Sulfasalazine in 0.1 N sodium hydroxide
Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 1000-mL volumetric flask containing 750 mL of water, mix, add 20.0 mL of 0.1 N acetic acid, and dilute with water to volume.
Standard solution: 7.5 µg/mL of USP Sulfasalazine RS in the same medium as the *Sample solution*
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: Maximum at about 359 nm
Blank: Water
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Concomitantly determine the absorbances of the *Samples*. Calculate the percentage of $C_{18}H_{14}N_4O_5S$ in the portion of Sulfasalazine taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Sulfasalazine RS in the *Standard solution* (µg/mL)
C_U = concentration of Sulfasalazine in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–101.5% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.5%
- **Chloride and Sulfate,** *Chloride* ⟨221⟩

Analysis: Digest 2.0 g of Sulfasalazine with 100 mL of water at 70° for 5 min. Cool immediately to room temperature, and filter. Transfer a 25-mL portion of the filtrate to a 50-mL beaker (retain the remainder of this filtrate for the *Sulfate* test). Add 1 mL of nitric acid, mix, and allow to stand for 5 min. Pass through a fine texture, retentive filter paper (Whatman No. 42, or equivalent).
Acceptance criteria: The filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).
- **Chloride and Sulfate,** *Sulfate* ⟨221⟩

Analysis: Transfer a 25-mL portion of the filtrate from the *Chloride* test to a 50-mL beaker. Add 1 mL of 3 N hydrochloric acid, mix, and allow to stand for 5 min. Pass through a fine

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texture, retentive filter paper (Whatman No. 42, or equivalent).

Acceptance criteria: The filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).

ORGANIC IMPURITIES

• **Procedure**

Standard stock solution: 10 mg/mL of USP Sulfasalazine RS in a mixture of alcohol and 2 M ammonium hydroxide (4:1)

Standard solutions: Dilute aliquots of the *Standard stock solution* stepwise with the same medium to obtain solutions having concentrations of 200, 150, 100, and 20 µg/mL, corresponding to 2.0%, 1.5%, 1.0%, and 0.2%, respectively (*Standard solutions A, B, C,* and *D*).

Sample solution: 10 mg/mL of Sulfasalazine in a mixture of alcohol and 2 M ammonium hydroxide (4:1)

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform, acetone, and formic acid (12:6:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Allow the spots to dry, and develop the chromatogram in an unequilibrated chamber until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, dry with the aid of a current of hot air, and examine the plate under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. No spots, other than the principal spot, in the chromatogram of the *Sample solution* are larger or more intense than the principal spot of *Standard solution A* (2%), and the sum of the intensities of any secondary spots detected does not exceed 4%.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfasalazine RS

Sulfasalazine Tablets

DEFINITION

Sulfasalazine Tablets contain NLT 95.0% and NMT 105.0% of the labeled quantity of sulfasalazine ($C_{18}H_{14}N_4O_5S$).

IDENTIFICATION

• **PROCEDURE**

Standard solution: Use the *Standard solution*, prepared as directed in the *Assay*.

Sample solution: Use the *Sample solution*, prepared as directed in the *Assay*.

Acceptance criteria: The visible absorption spectrum of the *Sample solution* corresponds to that of the *Standard solution*, as prepared in the *Assay*.

ASSAY

• **PROCEDURE**

Sample stock solution: 1.5 mg/mL of sulfasalazine, from finely powdered Tablets (NLT 20 tablets), in 0.1 N sodium hydroxide

Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 1000-mL volumetric flask containing 750 mL of water, mix, add 20.0 mL of 0.1 N acetic acid, and dilute with water to volume.

Standard solution: 7.5 µg/mL of USP Sulfasalazine RS in the same medium as the *Sample solution*

Spectrometric conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: Maximum at about 359 nm

Blank: Water

Analysis

Samples: *Standard solution, Sample solution,* and *Blank*

Concomitantly determine the absorbances of the *Samples*. Calculate the percentage of $C_{18}H_{14}N_4O_5S$ in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Sulfasalazine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of sulfasalazine in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: *pH 7.5 Phosphate Buffer* (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Detector: UV 358 nm

Standard solution: USP Sulfasalazine RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

Tolerances: NLT 85% (Q) of the labeled amount of $C_{18}H_{14}N_4O_5S$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfasalazine RS

Sulfasalazine Delayed-Release Tablets

DEFINITION

Sulfasalazine Delayed-Release Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of sulfasalazine ($C_{18}H_{14}N_4O_5S$).

IDENTIFICATION

• **A.**

Standard solution and **Sample solution:** Proceed as directed in the *Assay*.

USP Monographs

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Acceptance criteria: The visible absorption spectrum of the *Sample solution* corresponds to that of the *Standard solution*, as prepared in the *Assay*.

ASSAY

• **PROCEDURE**

Standard solution: 7.5 µg/mL of USP Sulfasalazine RS in the same medium as the *Sample solution*

Sample stock solution: Nominally 1.5 mg/mL of sulfasalazine prepared as follows. Dissolve an appropriate amount of sulfasalazine from finely powdered Tablets (NLT 20) in 0.1 N sodium hydroxide in a suitable volumetric flask.

Sample solution: Nominally 7.5 µg/mL of sulfasalazine prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 1000-mL volumetric flask containing 750 mL of water. Mix, add 20.0 mL of 0.1 N acetic acid, and dilute with water to volume.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: Maximum at about 359 nm

Blank: Water

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Concomitantly determine the absorbances of the *Samples*.

Calculate the percentage of the labeled amount of sulfasalazine ($C_{18}H_{14}N_4O_5S$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Sulfasalazine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of sulfasalazine in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩**:** Proceed as directed in the *Procedure* for *Method B* in *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms*.

Acid stage

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 120 min

At the end of 120 min, determine the amount of sulfasalazine ($C_{18}H_{14}N_4O_5S$) dissolved by using the following method.

Mobile phase: Isopropanol, acetonitrile, water, and glacial acetic acid (11: 7: 22: 0.4)

Standard solution: 55.6 µg/mL of USP Sulfasalazine RS in 0.1 N sodium hydroxide

Sample solution: Pass about 7 mL of the solution under test through a membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for sulfasalazine is about 7.7 min.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulfasalazine ($C_{18}H_{14}N_4O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Sulfasalazine RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NMT 10% of the labeled amount of sulfasalazine ($C_{18}H_{14}N_4O_5S$) is dissolved.

Buffer stage

Medium: pH 7.5 phosphate buffer; 900 mL

Apparatus 1: 100 rpm

Time: 60 min

At the end of 60 min, determine the amount of sulfasalazine ($C_{18}H_{14}N_4O_5S$) dissolved by using the chromatographic method as described in *Acid stage*.

Tolerances: NLT 85% (*Q*) of the labeled amount of sulfasalazine ($C_{18}H_{14}N_4O_5S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

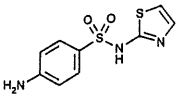
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfasalazine RS

Sulfathiazole



$C_9H_9N_3O_2S_2$ 255.32

Benzenesulfonamide, 4-amino-*N*-2-thiazolyl-. *N*¹-2-Thiazolylsulfanilamide [72-14-0].

» Sulfathiazole contains not less than 99.0 percent and not more than 100.5 percent of $C_9H_9N_3O_2S_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Sulfathiazole RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: To 100 mg add 5 mL of 3 N hydrochloric acid, and boil gently for about 5 minutes. Cool in an ice bath, add 4 mL of sodium nitrite solution (1 in 100), dilute with water to 10 mL, and place the mixture in an ice bath for 10 minutes. To 5 mL of the cooled mixture add a solution of 50 mg of 2-naphthol

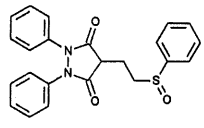
USP Monographs

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in 2 mL of sodium hydroxide solution (1 in 10): an orange-red precipitate is formed, and it darkens on standing.
Melting range, *Class I* ⟨741⟩: between 200° and 204°.
Acidity—Digest 2 g with 100 mL of water at about 70° for 5 minutes, cool at once to about 20°, and filter. Titrate 25 mL of the filtrate with 0.10 N sodium hydroxide, using 2 drops of phenolphthalein TS as the indicator: not more than 0.5 mL is required to produce a pink color. (Retain the remainder of the filtrate for the tests for *Chloride* ⟨221⟩ and for *Sulfate* ⟨221⟩.)
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chloride ⟨221⟩—A 25-mL portion of the filtrate prepared as directed in the test for *Acidity* shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).
Sulfate ⟨221⟩—A 25-mL portion of the filtrate prepared as directed in the test for *Acidity* shows no more sulfate than corresponds to 0.2 mL of 0.02 N sulfuric acid (0.04%).

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Eluant: a mixture of 1-propanol and 1 N ammonium hydroxide (22:3).
Visualization: 1.
Assay—Proceed with Sulfathiazole as directed under *Nitrite Titration* ⟨451⟩. Each mL of 0.1 M sodium nitrite is equivalent to 25.53 mg of $C_9H_9N_3O_2S_2$.

Sulfinpyrazone



$C_{23}H_{20}N_2O_3S$ 404.48
3,5-Pyrazolidinedione, 1,2-diphenyl-4-[2-(phenylsulfinyl) ethyl]-;
1,2-Diphenyl-4-[2-(phenylsulfinyl) ethyl]-3,5-pyrazolidinedione [57-96-5].

DEFINITION
Sulfinpyrazone contains NLT 98.5% and NMT 101.5% of $C_{23}H_{20}N_2O_3S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**

Sample: 600 mg of Sulfinpyrazone
Analysis: Dissolve the *Sample* in 50 mL of neutralized alcohol, with slight heating. Add phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 40.45 mg of $C_{23}H_{20}N_2O_3S$.
Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES
- **Procedure**

Solution A: Acetonitrile and tetrahydrofuran (4:1)
Mobile phase: *Solution A* and dilute phosphoric acid (3 in 1000) (7:13)
Solution B: 0.1 N acetic acid, 0.1 N potassium hydroxide, and water (6:5:14). Adjust to a pH of 5.0.
Diluent: Acetonitrile and *Solution B* (4:1)
Standard solution: 10 µg/mL of USP Sulfinpyrazone RS in *Diluent*
Sample solution: 2 mg/mL of Sulfinpyrazone in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 3 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for sulfinpyrazone is about 4.0–4.6 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses of the major peaks.
Acceptance criteria: The sum of the peak responses, other than that of the sulfinpyrazone peak, from the *Sample solution* is NMT four times the sulfinpyrazone peak response from the *Standard solution* (2.0%), and no single peak response is greater than twice the sulfinpyrazone peak response from the *Standard solution* (1.0%).

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfinpyrazone RS

Sulfinpyrazone Capsules

DEFINITION
Sulfinpyrazone Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$).

IDENTIFICATION
- **A.**

Solution A: 5% of methanol in dehydrated alcohol
Sample: Equivalent to 400 mg of sulfinpyrazone from Capsules
Analysis: Boil the *Sample* in 20 mL of *Solution A*, and filter. Add water to the filtrate until the solution becomes turbid, allow to stand until crystals form (1–2 h), remove the crystals by filtration, and dry at 105° for 1 h.

USP Monographs

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Acceptance criteria: The sulfinpyrazone obtained melts between 128° and 134°.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Use the sulfinpyrazone crystals obtained from *Identification* test *A* under *Sulfinpyrazone*.
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Solution A: Acetonitrile and tetrahydrofuran (4:1)
Solution B: Diluted phosphoric acid (3 in 1000)
Mobile phase: *Solution A* and *Solution B* (35:65)
Internal standard solution: 2 mg/mL of benzoic acid in acetonitrile
Standard solution: 0.5 mg/mL of USP Sulfinpyrazone RS prepared as follows. Transfer 100 mg of USP Sulfinpyrazone RS to a 200-mL volumetric flask. Add 100 mL of acetonitrile, mix, add 10.0 mL of *Internal standard solution*, and dilute with acetonitrile to volume. Filter, discarding the first 5 mL of the filtrate.
Sample solution: Nominally 0.5 mg/mL of sulfinpyrazone prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules, weigh, and mix. Transfer a portion of the powder, equivalent to 100 mg of sulfinpyrazone, to a 200-mL volumetric flask. Add 100 mL of acetonitrile, mix, add 10.0 mL of *Internal standard solution*, and dilute with acetonitrile to volume. Filter, discarding the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 3 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzoic acid and sulfinpyrazone are about 0.2 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 13 between the benzoic acid and sulfinpyrazone peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of sulfinpyrazone to the internal standard from the *Sample solution*
R_S = peak response ratio of sulfinpyrazone to the internal standard from the *Standard solution*
C_S = concentration of USP Sulfinpyrazone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulfinpyrazone in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: *pH 6.8 Phosphate Buffer* (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: Prepare a solution having a known concentration of USP Sulfinpyrazone RS in *Medium*.
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Absorption maximum at about 259 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfinpyrazone RS

Sulfinpyrazone Tablets

DEFINITION
Sulfinpyrazone Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$).

IDENTIFICATION
• **A.**
Sample: Equivalent to 400 mg of sulfinpyrazone from finely powdered Tablets
Analysis: Boil the *Sample* with 20 mL of alcohol, and filter. Add water to the filtrate until the solution becomes turbid, allow to stand until crystals form (1–2 h), remove the crystals by filtration, and dry at 105° for 1 h.
Acceptance criteria: The sulfinpyrazone obtained melts between 128° and 134°.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Use the sulfinpyrazone crystals obtained from *Identification* test *A* under *Sulfinpyrazone*.
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Solution A: Acetonitrile and tetrahydrofuran (4:1)
Solution B: Diluted phosphoric acid (3 in 1000)
Mobile phase: *Solution A* and *Solution B* (35:65)
Internal standard solution: 2 mg/mL of benzoic acid in acetonitrile
Standard solution: 0.5 mg/mL of USP Sulfinpyrazone RS prepared as follows. Transfer 100 mg of USP Sulfinpyrazone RS to a 200-mL volumetric flask. Add 100 mL of acetonitrile, mix, add 10.0 mL of *Internal standard solution*, and dilute with acetonitrile to volume. Filter, discarding the first 5 mL of the filtrate.

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Sample solution: Nominally 0.5 mg/mL of sulfinpyrazone prepared as follows. Transfer 100 mg of sulfinpyrazone from NLT 20 finely powdered Tablets to a 200-mL volumetric flask. Add 100 mL of acetonitrile, mix, add 10.0 mL of *Internal standard solution*, and dilute with acetonitrile to volume. Filter, discarding the first 5 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 3 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzoic acid and sulfinpyrazone are about 0.2 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 13 between the benzoic acid and sulfinpyrazone peaks
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of sulfinpyrazone to the internal standard from the *Sample solution*
R_S = peak response ratio of sulfinpyrazone to the internal standard from the *Standard solution*
C_S = concentration of USP Sulfinpyrazone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulfinpyrazone in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: *pH 6.8 Phosphate Buffer* (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: Prepare a solution having a known concentration of USP Sulfinpyrazone RS in *Medium*.
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Absorption maximum at about 259 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$) is dissolved.

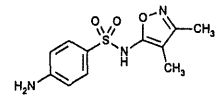
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulfinpyrazone RS

Sulfisoxazole



$C_{11}H_{13}N_3O_3S$ 267.30
Benzenesulfonamide, 4-amino-*N*-(3,4-dimethyl-5-isoxazolyl)-.
N[1]-(3,4-Dimethyl-5-isoxazolyl)sulfanilamide [127-69-5].

» Sulfisoxazole contains not less than 99.0 percent and not more than 101.0 percent of $C_{11}H_{13}N_3O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Sulfisoxazole RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The UV absorption spectrum of a 1 in 100,000 solution in pH 7.5 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), prepared by dissolving about 100 mg of Sulfisoxazole, accurately weighed, in 10 mL of sodium hydroxide solution (1 in 250) and adding sufficient amounts of the buffer solution to make 100 mL, then diluting 10 mL of the resulting solution with the buffer solution to 1000 mL, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Sulfisoxazole RS, concomitantly measured.

C: Dissolve about 10 mg in 2 mL of 3 N hydrochloric acid with the aid of heat, cool for 5 minutes in an ice bath, add 3 drops of sodium nitrite solution (1 in 100), and dilute with water to 4 mL: a yellow solution is produced. Add 1 mL of sodium hydroxide solution (1 in 10) containing 10 mg of 2-naphthol: an orange-red precipitate is formed.

Melting range ⟨741⟩: between 194° and 199°.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Selenium ⟨291⟩: 0.003%, a 200-mg test specimen being used.

Ordinary impurities ⟨466⟩—
Test solution: ethyl acetate.
Standard solution: ethyl acetate.
Eluant: a mixture of acetone, cyclohexane, and glacial acetic acid (5:4:1).
Visualization: 1.

Assay—Place about 800 mg of Sulfisoxazole, accurately weighed, in a 250-mL conical flask, add 50 mL of dimethylformamide, shake thoroughly to dissolve the solid, add 5 drops of a 1 in 100 solution of thymol blue in dimethylformamide, and titrate with 0.1 N lithium methoxide in toluene VS to a blue endpoint, taking precautions against absorption of atmospheric carbon dioxide. Perform a blank

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determination, and make any necessary correction. Each mL of 0.1 N lithium methoxide is equivalent to 26.73 mg of $C_{11}H_{13}N_3O_3S$.

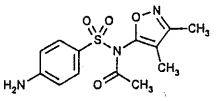
Sulfisoxazole Tablets

» Sulfisoxazole Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{11}H_{13}N_3O_3S$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Sulfisoxazole RS
Identification—Extract a quantity of powdered Tablets, equivalent to about 1 g of sulfisoxazole, with 50 mL of alcohol by boiling on a steam bath for 3 minutes, then immediately filter into a beaker. Allow to stand until a quantity of fine, needle-like crystals form. Cool, filter off the crystals, recrystallize from a small volume of alcohol, and dry at 105°: the crystals respond to *Identification* test *A* under *Sulfisoxazole.*

Dissolution ⟨711⟩—
Medium: dilute hydrochloric acid (1 in 12.5); 900 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure— [NOTE—Because of the pH-dependent nature of the UV absorption spectrum, prepare the standard and specimen solutions in the same strength of acid at approximately equal concentrations.] Determine the amount of $C_{11}H_{13}N_3O_3S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 267 nm of filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Sulfisoxazole RS in the same medium.
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{11}H_{13}N_3O_3S$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 800 mg of sulfisoxazole, transfer to a 250-mL conical flask, and proceed as directed in the *Assay* under *Sulfisoxazole,* beginning with "add 50 mL of dimethylformamide."

Sulfisoxazole Acetyl



$C_{13}H_{15}N_3O_4S$ 309.34
Acetamide, *N*-[(4-aminophenyl)sulfonyl]-*N*-(3,4-dimethyl-5-isoxazolyl)-.
N-(3,4-Dimethyl-5-isoxazolyl)-*N*-sulfanilylacetamide [80-74-0].

» Sulfisoxazole Acetyl contains not less than 98.0 percent and not more than 100.5 percent of $C_{13}H_{15}N_3O_4S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Sulfisoxazole Acetyl RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: alcohol.
Absorptivities at 290 nm, calculated on the dried basis, do not differ by more than 3.0%.
Melting range ⟨741⟩: between 192° and 195°.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Eluant: a mixture of toluene and acetone (1:1).
Visualization: 1.
Other requirements—It meets the requirements for *Residue on ignition,* and *Selenium* under *Sulfisoxazole.*
Assay—Transfer about 1 g of Sulfisoxazole Acetyl, accurately weighed, to a 250-mL beaker. Add 15 mL of glacial acetic acid, swirl to dissolve, then add 25 mL of hydrochloric acid and 80 mL of water. Proceed as directed under *Nitrite Titration* ⟨451⟩, beginning with "Cool to about 15°." Each mL of 0.1 M sodium nitrite is equivalent to 30.94 mg of $C_{13}H_{15}N_3O_4S$.

Sulfisoxazole Acetyl Oral Suspension

» Sulfisoxazole Acetyl Oral Suspension contains an amount of Sulfisoxazole Acetyl equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of sulfisoxazole ($C_{11}H_{13}N_3O_3S$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Sulfisoxazole Acetyl RS
Identification—Centrifuge a portion of it, wash the separated solid by centrifugation with several portions of water, and recrystallize from hot alcohol: the crystals so obtained respond to *Identification* tests *A* and *B* under *Sulfisoxazole Acetyl.*

Uniformity of dosage units ⟨905⟩—
FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.
pH ⟨791⟩: between 5.0 and 5.5.
Assay—Transfer an accurately measured volume of Oral Suspension, previously mixed, equivalent to about 1 g of

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sulfisoxazole, to a 250-mL beaker. Add 40 mL of hydrochloric acid and 25 mL of glacial acetic acid, and swirl to dissolve. Cautiously add 100 mL of water, and proceed as directed under *Nitrite Titration* ⟨451⟩, beginning with "Cool to about 15°." Each mL of 0.1 M sodium nitrite is equivalent to 26.73 mg of sulfisoxazole ($C_{11}H_{13}N_3O_3S$).

Precipitated Sulfur

S 32.07
Sulfur [7704-34-9].

DEFINITION
Precipitated Sulfur contains NLT 99.5% and NMT 100.5% of sulfur (S), calculated on the anhydrous basis.

IDENTIFICATION
• **A.** It burns in the air to sulfur dioxide, which can be recognized by its characteristic odor.

ASSAY
• **PROCEDURE**
Sample: 60 mg of Precipitated Sulfur
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Proceed as directed in *Oxygen Flask Combustion* ⟨471⟩, using a 1000-mL flask and using a mixture of 10 mL of water and 5.0 mL of hydrogen peroxide TS as the absorbing liquid. When the combustion is complete, fill the lip of the flask with water; loosen the stopper; then rinse the stopper, the sample holder, and the sides of the flask with water; and remove the stopper assembly. Heat the contents of the flask to boiling, and boil for 2 min. Cool to room temperature, add phenolphthalein TS, and titrate with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 1.603 mg of sulfur (S).
Acceptance criteria: 99.5%–100.5% on the anhydrous basis

OTHER COMPONENTS
• **OTHER FORMS OF SULFUR**
Sample: 1.0 g
Analysis: Shake the *Sample* with 5 mL of carbon disulfide.
Acceptance criteria: It dissolves quickly, with the exception of a small quantity of insoluble matter that is usually present.

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.3%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%
• **REACTION**
Sample: 2.0 g
Analysis: Agitate the *Sample* with 10 mL of water, and filter.
Acceptance criteria: The filtrate is neutral to litmus.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Sulfur Ointment

DEFINITION
Sulfur Ointment contains NLT 9.5% and NMT 10.5% of sulfur (S).

Precipitated Sulfur	100 g
Mineral Oil	100 g
White Ointment	800 g
To make	1000 g

Levigate the *Precipitated Sulfur* with the *Mineral Oil* to a smooth paste, and then incorporate with the *White Ointment*.

ASSAY
• **PROCEDURE**
Sample: 500 mg in a suitable conical flask
Analysis: Add 5 mL of nitric acid and 3 mL of bromine to the *Sample*, warm slightly, and allow to stand overnight. Heat gently on a steam bath until the excess bromine has been dissipated. Add 30 mL of water and then 30 mL of ether, and swirl to dissolve most of the ointment base. Transfer the mixture to a separator, with the aid of a 20-mL and a 10-mL portion of ether, followed by two 10-mL portions of water. Shake the mixture, and draw off the water layer into a suitable beaker or flask. Extract the ether layer with two 40-mL portions of water each containing 1 mL of hydrochloric acid. Heat the combined water extracts on a steam bath to remove all traces of ether. Dilute the solution with water to about 200 mL, heat to boiling, and add slowly, with constant stirring, about 20 mL of hot barium chloride TS. Heat on a steam bath for 1 h, then collect the precipitate on a filter, wash it well with hot water, dry, and ignite to constant weight. The weight of the barium sulfate so obtained, multiplied by 0.1374, represents the weight of sulfur (S).
Acceptance criteria: 9.5%–10.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in well-closed containers, and avoid prolonged exposure to excessive heat.

Sublimed Sulfur

S 32.07
Sulfur [7704-34-9].

DEFINITION
Sublimed Sulfur, dried over phosphorus pentoxide for 4 h, contains NLT 99.5% and NMT 100.5% of sulfur (S).

IDENTIFICATION
• **A.** It burns in the air to sulfur dioxide, which can be recognized by its characteristic odor.

ASSAY
• **PROCEDURE**
Sample: 60 mg of Sublimed Sulfur previously dried over phosphorous pentoxide for 4 h
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Proceed as directed in *Oxygen Flask Combustion* ⟨471⟩, using a 1000-mL flask and using a mixture of 10 mL

USP Monographs

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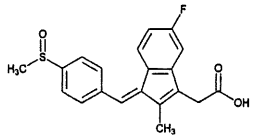
of water and 5.0 mL of hydrogen peroxide TS as the absorbing liquid. When the combustion is complete, fill the lip of the flask with water; loosen the stopper; then rinse the stopper, sample holder, and sides of the flask with water; and remove the stopper assembly. Heat the contents of the flask to boiling, and boil for about 2 min. Cool to room temperature, add phenolphthalein TS, and titrate with *Titrant*. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 1.603 mg of sulfur (S).
Acceptance criteria: 99.5%–100.5% dried over phosphorous pentoxide for 4 h

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
- **ARSENIC,** *Method I* ⟨211⟩
Test preparation: Digest 750 mg of Sublimed Sulfur with 20 mL of 6 N ammonium hydroxide for 3 h, filter, and evaporate the clear filtrate on a steam bath to dryness. Add 15 mL of 2 N sulfuric acid and 1 mL of 30% hydrogen peroxide solution, evaporate to strong fumes of sulfur trioxide, and cool. Add cautiously 10 mL of water, and again evaporate to strong fumes, repeating, if necessary, to remove any trace of hydrogen peroxide. Cool, and dilute cautiously with water to 35 mL.
Acceptance criteria: NMT 4 ppm

SPECIFIC TESTS
- **SOLUBILITY IN CARBON DISULFIDE**
Sample: 1 g
Acceptance criteria: The *Sample* dissolves slowly and usually incompletely in about 2 mL of carbon disulfide.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Sulindac



$C_{20}H_{17}FO_3S$ 356.41
1*H*-Indene-3-acetic acid, 5-fluoro-2-methyl-1-[[4-(methylsulfinyl) phenyl]methylene]-, (*Z*)-;
cis-5-Fluoro-2-methyl-1-[(*p*-methylsulfinyl)benzylidene] indene-3-acetic acid [38194-50-2].

DEFINITION
Sulindac contains NLT 98.0% and NMT 102.0% of sulindac ($C_{20}H_{17}FO_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelength: 284 nm
Medium: Hydrochloric acid in methanol (1 in 120)
Sample solution: 15 µg/mL in *Medium*
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: 0.1% Formic acid in water
Solution B: 0.1% Formic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	70	30
8.0	70	30
15.0	10	90
18.0	10	90
18.1	70	30
20.0	70	30

Diluent: Acetonitrile and water (50:50)
System suitability solution: 0.003 mg/mL each of USP Sulindac RS, USP Sulindac Related Compound A RS, USP Sulindac Related Compound B RS, and USP Sulindac Related Compound C RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Sulindac RS in *Diluent*
Sample solution: 0.2 mg/mL of Sulindac in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 330 nm
Column: 2.1-mm × 15-cm; 1.7-µm packing L1
Column temperature: 45°
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for sulindac related compound A, sulindac related compound B, and sulindac related compound C are 1.25, 1.34, and 1.67, respectively.]
Suitability requirements
Resolution: NLT 4.0 between sulindac and sulindac related compound A and between sulindac related compound A and sulindac related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulindac ($C_{20}H_{17}FO_3S$) in the portion of Sulindac taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulindac RS in the *Standard solution* (mg/mL)
C_U = concentration of Sulindac in the *Sample solution* (mg/mL)

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Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **RESIDUAL SOLVENTS** ⟨467⟩

Solvent: Dimethylsulfoxide

Acceptance criteria: Meets the requirements except that chloroform is NMT 500 ppm

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.6 µg/mL of USP Sulindac RS and 3 µg/mL each of USP Sulindac Related Compound A RS, USP Sulindac Related Compound B RS, and USP Sulindac Related Compound C RS in *Diluent*. Sonicate for 2–5 min.

Sample solution: 600 µg/mL of Sulindac in *Diluent*. Sonicate for 2–5 min.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between sulindac and sulindac related compound A and between sulindac related compound A and sulindac related compound B

Relative standard deviation: NMT 2.0% for any peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of sulindac related compound A, sulindac related compound B, and sulindac related compound C in the portion of Sulindac taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the specified impurity from the *Sample solution*

r_S = peak response of the corresponding related compound from the *Standard solution*

C_S = concentration of the corresponding related compound in the *Standard solution* (µg/mL)

C_U = concentration of Sulindac in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Sulindac taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*

r_S = peak response of sulindac from the *Standard solution*

C_S = concentration of USP Sulindac RS in the *Standard solution* (µg/mL)

C_U = concentration of Sulindac in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulindac	1.0	—
Sulindac related compound A[a]	1.25	0.5
Sulindac related compound B[b]	1.34	0.5
Sulindac related compound C[c]	1.67	0.5
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] (*E*)-2-{5-Fluoro-2-methyl-1-[4-(methylsulfinyl)benzylidene]-1*H*-inden-3-yl} acetic acid.

[b] (*Z*)-2-{5-Fluoro-2-methyl-1-[4-(methylsulfonyl)benzylidene]-1*H*-inden-3-yl} acetic acid.

[c] (*Z*)-2-{5-Fluoro-2-methyl-1-[4-(methylthio)benzylidene]-1*H*-inden-3-yl}acetic acid.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 100° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulindac RS

USP Sulindac Related Compound A RS
(*E*)-2-{5-Fluoro-2-methyl-1-[4-(methylsulfinyl) benzylidene]-1*H*-inden-3-yl}acetic acid.
$C_{20}H_{17}FO_3S$ 356.41

USP Sulindac Related Compound B RS
(*Z*)-2-{5-Fluoro-2-methyl-1-[4-(methylsulfonyl) benzylidene]-1*H*-inden-3-yl}acetic acid.
$C_{20}H_{17}FO_4S$ 372.41

USP Sulindac Related Compound C RS
(*Z*)-2-{5-Fluoro-2-methyl-1-[4-(methylthio)benzylidene]-1*H*-inden-3-yl}acetic acid.
$C_{20}H_{17}FO_2S$ 340.41

Sulindac Tablets

DEFINITION

Sulindac Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of sulindac ($C_{20}H_{17}FO_3S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV-Vis spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% formic acid in water

Solution B: 0.1%formic acid in acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	70	30
8.0	70	30
15.0	10	90
18.0	10	90
18.1	70	30
20.0	70	30

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Diluent: Acetonitrile and water (50:50)
System suitability solution: 0.018 mg/mL each of USP Sulindac RS, USP Sulindac Related Compound A RS, USP Sulindac Related Compound B RS, and USP Sulindac Related Compound C RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Sulindac RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of sulindac in *Diluent* prepared as follows. Dissolve a quantity nominally equivalent to 20 mg of sulindac from finely powdered Tablets in 70 mL of *Diluent* in a 100-mL volumetric flask. Shake by mechanical means for 60 min and dilute with *Diluent* to volume. Shake for another 5 min. Centrifuge the solution for 10 min and inject the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 330 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 15-cm; 1.7-µm packing L1
Column temperature: 45°
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between sulindac and sulindac related compound A; NLT 4.0 between sulindac related compound A and sulindac related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sulindac ($C_{20}H_{17}FO_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sulindac RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulindac in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 M pH 7.2 phosphate buffer prepared as directed in *Reagents, Indicators, and Solutions—Buffer Solutions*, except to use twice the stated quantities of the monobasic potassium phosphate solution and of the sodium hydroxide solution; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 20 µg/mL of USP Sulindac RS in *Medium*
Sample solution: Pass 20 mL of the solution under test through a suitable filter, transfer 10 mL of the filtrate to a 100-mL volumetric flask, and dilute with *Medium* to volume.
Blank: *Medium*
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 326 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sulindac ($C_{20}H_{17}FO_3S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Sulindac RS in the *Standard solution* (mg/mL)
D = dilution volume
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of sulindac ($C_{20}H_{17}FO_3S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.018 mg/mL each of USP Sulindac RS, USP Sulindac Related Compound A RS, USP Sulindac Related Compound B RS, and USP Sulindac Related Compound C RS in *Diluent*. Sonicate for 2–5 min and mix with inversion.
Sample solution: Nominally 0.6 mg/mL of sulindac in *Diluent* prepared as follows. Dissolve a quantity nominally equivalent to 60 mg of sulindac from finely powdered Tablets in 70 mL of *Diluent* in a 100-mL volumetric flask. Shake by mechanical means for 60 min and dilute with *Diluent* to volume. Shake for another 5 min. Centrifuge the solution for 10 min. Collect and inject the supernatant.
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between sulindac and sulindac related compound A; NLT 4.0 between sulindac related compound A and sulindac related compound B
Relative standard deviation: NMT 2.0% for sulindac, sulindac related compound B, and sulindac related compound C
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulindac related compound B or sulindac related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sulindac related compound B or sulindac related compound C from the *Sample solution*
r_S = peak response of sulindac related compound B or sulindac related compound C from the *Standard solution*
C_S = concentration of the corresponding related compound in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulindac in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of sulindac from the *Standard solution*
C_S = concentration of USP Sulindac RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sulindac in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

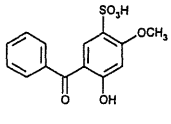
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulindac	1.0	—
Sulindac related compound A	1.26	—[a]
Sulindac related compound B	1.36	0.5
Sulindac related compound C	1.70	0.5
Individual unspecified impurity	—	0.1
Total impurities	—	3.0

[a] Sulindac related compound A is controlled in the API. Therefore, no individual acceptance criteria is needed. Sulindac related compound B and sulindac related compound C are both degradants and process impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sulindac RS
  USP Sulindac Related Compound A RS
  (*E*)-2-{5-Fluoro-2-methyl-1-[4-(methylsulfinyl) benzylidene]-1*H*-inden-3-yl}acetic acid.
  $C_{20}H_{17}FO_3S$ 356.41
  USP Sulindac Related Compound B RS
  (*Z*)-2-{5-Fluoro-2-methyl-1-[4-(methylsulfonyl) benzylidene]-1*H*-inden-3-yl}acetic acid.
  $C_{20}H_{17}FO_4S$ 372.41
  USP Sulindac Related Compound C RS
  (*Z*)-2-{5-Fluoro-2-methyl-1-[4-(methylthio)benzylidene]-1*H*-inden-3-yl}acetic acid.
  $C_{20}H_{17}FO_2S$ 340.41

Sulisobenzone



$C_{14}H_{12}O_6S$ 308.31
Benzenesulfonic acid, 5-benzoyl-4-hydroxy-2-methoxy-.
5-Benzoyl-4-hydroxy-2-methoxybenzenesulfonic acid [4065-45-6].

» Sulisobenzone contains not less than 97.0 percent and not more than 103.0 percent of $C_{14}H_{12}O_6S$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Sulisobenzone RS

Change to read:

▲***Spectroscopic Identification Tests*** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: water.
Absorptivities, calculated on the anhydrous basis, do not differ by more than 3.0%.

Water Determination, *Method I* ⟨921⟩: not more than 2.0%.

Assay—Accurately weigh about 0.25 g of Sulisobenzone, and dissolve in 5 mL of water in a conical flask. Add 50 mL of dehydrated isopropyl alcohol, titrate with 0.1 N tetrabutylammonium hydroxide VS, and determine the two endpoints potentiometrically. V_1 and V_2 are the volumes, in mL, of titrant needed to reach the first and second endpoints, respectively. To determine the blank value caused by tertiary amines present as impurities in the titrant, dissolve $V_1/5$ mL of 0.5 N hydrochloric acid VS in 50 mL of dehydrated isopropyl alcohol, and subsequently add $(5 - V_1/5)$ mL of water. Titrate with 0.1 N tetrabutylammonium hydroxide VS, and determine the two endpoints potentiometrically. V_{1B} and V_{2B} are the volumes, in mL, of titrant needed to reach the first and second endpoints, respectively. The blank value V_{BV}, in mL, is obtained by the formula:

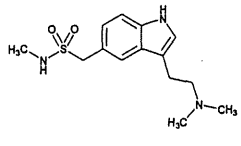
$$V_{2B} - V_{1B}$$

Calculate the percentage of $C_{14}H_{12}O_6S$ in the portion of Sulisobenzone taken by the formula:

$$308.31C[2V_1 - (V_2 - V_{BV})](1/W)(0.001)100$$

in which 308.31 is the molecular weight of Sulisobenzone; C is the concentration, in moles per L, of the 0.1 N tetrabutylammonium hydroxide VS; W is the weight, in g, of Sulisobenzone taken; 0.001 is the conversion factor for converting mL to L; and 100 is the conversion factor to percentage.

Sumatriptan



$C_{14}H_{21}N_3O_2S$ 295.40
1*H*-Indole-5-methanesulfonamide, 3-[2-(dimethylamino) ethyl]-*N*-methyl-;
3-[2-(Dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methanesulfonamide [103628-46-2].

DEFINITION
Sumatriptan contains NLT 98.0% and NMT 102.0% of sumatriptan ($C_{14}H_{21}N_3O_2S$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

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• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Dissolve 3.9 g of monobasic sodium phosphate dihydrate in water. Adjust with a solution of 50% (w/v) sodium hydroxide to a pH of about 6.5, and dilute with water to 1 L. Mix 750 mL of the this solution with 250 mL of acetonitrile.

Buffer: Dissolve 1.7 mL of dibutylamine, 0.6 mL of phosphoric acid, and 3.9 g of monobasic sodium phosphate dihydrate in water. Adjust with a solution of 50% (w/v) sodium hydroxide to a pH of about 6.5, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (25:75)

System suitability solution: 0.28 mg/mL of USP Sumatriptan Succinate RS and 0.14 mg/mL of USP Sumatriptan Succinate Related Compound C RS in *Diluent*

Standard solution: 0.14 mg/mL of USP Sumatriptan Succinate RS in *Diluent*

Sample solution: 0.1 mg/mL of Sumatriptan in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for sumatriptan succinate related compound C and sumatriptan are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between sumatriptan succinate related compound C and sumatriptan, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sumatriptan ($C_{14}H_{21}N_3O_2S$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sumatriptan Succinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Sumatriptan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sumatriptan, 295.4
M_{r2} = molecular weight of sumatriptan succinate, 413.5

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **PROCEDURE 1: LIMIT OF SUMATRIPTAN RELATED COMPOUND A**

Buffer: Dissolve 77.1 g of ammonium acetate in 100 mL of water.

Mobile phase: Methanol and *Buffer* (90:10)

Standard solution: 6.25 µg/mL of USP Sumatriptan Succinate Related Compound A RS in *Mobile phase*

Sample solution: 2 mg/mL of Sumatriptan in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sumatriptan related compound A in the portion of Sumatriptan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sumatriptan Succinate Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Sumatriptan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sumatriptan related compound A, 495.7
M_{r2} = molecular weight of sumatriptan succinate related compound A, 613.8

Acceptance criteria: NMT 0.6%

• **PROCEDURE 2**

Diluent and **System suitability solution:** Prepare as directed in the *Assay*.

Buffer: Dissolve 1.7 mL of dibutylamine, 0.6 mL of phosphoric acid, and 3.9 g of monobasic sodium phosphate dihydrate in water. Adjust with a solution of 50% (w/v) sodium hydroxide to a pH of about 7.5, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (25:75)

Identification solution: 3 mg/mL of USP Sumatriptan Succinate Related Impurities RS in *Mobile phase*

Sample solution: 2 mg/mL of Sumatriptan in *Diluent*

Chromatographic system: Prepare as directed in the *Assay*. After making sure that the resolution criteria are met, chromatograph the *Identification solution*, and record the peak responses as directed in the *Analysis*. Identify the peaks according to *Table 1*.

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between sumatriptan succinate related compound C and sumatriptan

Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Sumatriptan taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria: See *Table 1*.

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Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[3-[2-(Dimethylamino-*N*-oxide)ethyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide	0.3	0.2
[3-[2-(Aminoethyl)]-1*H*-indol-5-yl]-*N*-methylmethane sulfonamide	0.4	0.1
[3-[2-(Methylamino)ethyl]-1*H*-indol-5-yl]-*N*-methyl methanesulfonamide	0.6	0.5
Sumatriptan succinate related compound C	0.9	0.5
Sumatriptan	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities[a]	—	1.5

[a] Includes the amount of sumatriptan related compound A determined in the test for *Limit of Sumatriptan Related Compound A*.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Protect from freezing, and store below 30°.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sumatriptan RS
  USP Sumatriptan Succinate RS
  USP Sumatriptan Succinate Related Compound A RS
  [3-[2-(Dimethylamino)ethyl]-2-[[3-[2-(dimethylamino) ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide succinate salt.
  $C_{27}H_{37}N_5O_2S \cdot C_4H_6O_4$ 613.77
  USP Sumatriptan Succinate Related Compound C RS
  [3-[2-(Dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide succinate salt.
  $C_{15}H_{23}N_3O_3S \cdot 0.5C_4H_6O_4$ 384.47
  USP Sumatriptan Succinate Related Impurities RS
  Mixture of sumatriptan succinate, ▲[3-[2-(methylamino) ethyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide,▲ (ERR 1-Jun-2019) sumatriptan succinate related compound C, [3-[2-(dimethylamino-*N*-oxide)ethyl]-1*H*-indol- 5-yl]-*N*-methylmethanesulfonamide, and [3-[2-(aminoethyl)]-1*H*-indol-5-yl]-*N*-methyl methanesulfonamide.

Sumatriptan Injection

DEFINITION

Sumatriptan Injection is a sterile solution of Sumatriptan Succinate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$).

IDENTIFICATION

- **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Add 1.7 mL of butylamine, 0.66 mL of phosphoric acid, and 3.7 g of monobasic sodium phosphate to 900 mL of water. Mix, and adjust with 1 N sodium hydroxide to a pH of 7.5 ± 0.1. Dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (17:83)
Diluent: Acetonitrile and water (50:50)
Standard solution: 0.14 mg/mL of USP Sumatriptan Succinate RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of sumatriptan from the Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
Run time: About 3 times the retention time of sumatriptan
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sumatriptan Succinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of sumatriptan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sumatriptan free base, 295.40
M_{r2} = molecular weight of sumatriptan succinate, 413.49

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 4.2–5.3
- **OSMOLALITY AND OSMOLARITY** ⟨785⟩**:** 270–330 mOsmol/kg
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 29.2 USP Endotoxin Units/mg of sumatriptan.
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements under *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type 1 glass. Store between 2° and 30°, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sumatriptan Succinate RS

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Sumatriptan Nasal Spray

DEFINITION

Sumatriptan Nasal Spray is an aqueous, buffered solution of Sumatriptan. It is supplied in a form suitable for nasal administration. It contains NLT 90.0% and NMT 110.0% of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Sample: To the contents of one vial of Nasal Spray add 1 mL of a saturated sodium chloride solution. Add 1 mL of a saturated solution of sodium carbonate, and shake vigorously for about 30 s. To the solution so obtained add 2 mL of isopropyl alcohol, shake vigorously for about 30 s, and allow to stand until the phases separate. Transfer the organic phase to a suitable glass vial. Repeat the extraction with a second 2-mL portion of isopropyl alcohol, and transfer the organic phase to the same vial. Evaporate the solution under a stream of nitrogen. Dry the residue in an oven at 100° for 30 min, allow to cool to room temperature in a desiccator, and prepare a mull by the addition of 1–2 drops of mineral oil.

Acceptance criteria: Meets the requirements

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 1S (USP41)

ASSAY

Change to read:

• **PROCEDURE**

Buffer: Dissolve 1.7 mL of dibutylamine, 0.6 mL of phosphoric acid, and 3.9 g of monobasic sodium phosphate dihydrate in water. Adjust with a solution of 50% (w/v) sodium hydroxide to a pH of 6.5, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (25:75)

Diluent: Dissolve 3.9 g of monobasic sodium phosphate dihydrate in water. Adjust with a solution of 50% (w/v) sodium hydroxide to a pH of 6.5, and dilute with water to 1 L. Mix 750 mL of the resulting solution with 250 mL of acetonitrile.

System suitability solution: 0.14 mg/mL of USP Sumatriptan Succinate RS and 0.07 mg/mL of USP Sumatriptan Succinate Related Compound C RS in *Diluent*

Standard solution: 0.14 mg/mL of USP Sumatriptan Succinate RS in *Diluent*

Sample solution: Nominally equivalent to 0.1 mg/mL of sumatriptan in *Diluent* from an appropriate volume of Nasal Spray

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 282 nm

Column: 4.6-mm × 20-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

▲Run time: NLT 4 times the retention of sumatriptan▲ 1S (USP41)

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for sumatriptan succinate related compound C and sumatriptan are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between sumatriptan succinate related compound C and sumatriptan, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of ▲USP Sumatriptan Succinate RS▲ (ERR 1-Oct-2018) in the *Standard solution* (mg/mL)

C_U = nominal concentration of sumatriptan in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of sumatriptan, ▲295.40▲ 1S (USP41)

M_{r2} = molecular weight of sumatriptan succinate, ▲413.49▲ 1S (USP41)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME**

Analysis: Test 10 vials separately. Weigh each vial before and after actuation, and calculate the individual volume delivered, in µL, then calculate the mean volume delivered:

$$\text{Result} = (W_1 - W_2)/D$$

W_1 = weight of the individual vial before actuation (mg)

W_2 = weight of the individual vial after actuation (mg)

D = density of the nasal solution

Acceptance criteria: The volume of each spray delivered is between 80 and 120 µL, and the mean volume is between 90 and 110 µL.

IMPURITIES

Change to read:

• **LIMIT OF SUMATRIPTAN RELATED COMPOUND A**

Buffer: Dissolve 77.1 g of ammonium acetate in 100 mL of water.

Mobile phase: Methanol and *Buffer* (90:10)

Diluent: Prepare as directed in the *Assay*.

Standard solution: ▲0.007 mg/mL▲ 1S (USP41) of USP Sumatriptan Succinate Related Compound A RS in *Diluent*

Sample solution: Nominally equivalent to 1.0 mg/mL of sumatriptan in *Diluent* from an appropriate volume of Nasal Spray

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 282 nm

Column: 4.6-mm × 20-cm; packing L3

Flow rate: 2 mL/min

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Injection volume: 20 µL
▲Run time: NLT 2 times the retention of sumatriptan related compound A▲ 1S (USP41)
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sumatriptan succinate related compound A in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sumatriptan related compound A from the *Sample solution*
r_S = peak response of sumatriptan related compound A from the *Standard solution*
C_S = concentration of ▲USP Sumatriptan Succinate Related Compound A RS▲ 1S (USP41) in the *Standard solution* (mg/mL)
C_U = nominal concentration of sumatriptan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sumatriptan related compound A, ▲495.68▲ 1S (USP41)
M_{r2} = molecular weight of sumatriptan succinate related compound A, ▲613.77▲ 1S (USP41)

Acceptance criteria: NMT 1.5%

Change to read:

- **ORGANIC IMPURITIES**

Buffer: Dissolve 1.7 mL of dibutylamine, 0.6 mL of phosphoric acid, and 3.9 g of monobasic sodium phosphate dihydrate in water. Adjust with a solution of 50% (w/v) sodium hydroxide to a pH of 7.5, and dilute with water to 1 L.
Mobile phase: Acetonitrile and *Buffer* (25:75)
Diluent: Prepare as directed in the *Assay*.
System suitability solution: 1.4 mg/mL of USP Sumatriptan Succinate RS and 1 µg/mL of USP Sumatriptan Succinate Related Compound C RS in *Diluent*
Identification solution: 3 mg/mL of USP Sumatriptan Succinate Related Impurities RS in *Diluent*
Sample solution: Nominally equivalent to 1 mg/mL of sumatriptan in *Diluent* from an appropriate volume of Nasal Spray
Chromatographic system: Proceed as directed in the *Assay*. ▲▲ 1S (USP41)
System suitability
Sample: *System suitability solution*
▲[NOTE—See *Table 1* for the relative retention times.]▲ 1S (USP41)
Suitability requirements
Resolution: NLT 1.5 between sumatriptan succinate related compound C and sumatriptan
Analysis
Samples: ▲*Identification solution* and▲ 1S (USP41) *Sample solution*
Calculate the percentage of each ▲degradation product▲ 1S (USP41) in the portion of Nasal Spray taken:

$$\text{▲Result} = (r_U/r_T) \times (1/F) \times 100\text{▲ 1S (USP41)}$$

r_U = peak response of each ▲degradation product▲ 1S (USP41) from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

▲Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sumatriptan *N*-oxide[a]	0.3	1.0	1.5
Sumatriptan amino[b]	0.4	1.0	1.5
3-Hydroxy-2-oxo sumatriptan[c]	0.46	0.35	1.5
Sumatriptan monomethyl[d]	0.6	1.0	1.5
Sumatriptan pyrroloindolium analog[e]	0.64	0.22	1.5
Sumatriptan succinate related compound C	0.9	1.0	1.5
Sumatriptan	1.0	1.0	—
Total degradation products[f]	—	—	4.0

[a] *N*-Methyl-1-{3-[2-(dimethylamino *N*-oxide)ethyl]-1*H*-indol-5-yl} methanesulfonamide.
[b] [3-(2-Aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide.
[c] 1-{3-[2-(Dimethylamino)ethyl]-3-hydroxy-2-oxoindolin-5-yl}-*N*-methylmethanesulfonamide.
[d] *N*-Methyl-1-{3-[2-(methylamino)ethyl]-1*H*-indol-5-yl}methanesulfonamide.
[e] 3a-Hydroxy-1,1-dimethyl-5-(*N*-methylsulfamoylmethyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-1-ium sulfate.
[f] Includes the amount of sumatriptan related compound A determined in the test for *Limit of Sumatriptan Related Compound A*.▲ 1S (USP41)

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL, and it meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in 1 mL.
- **pH** ⟨791⟩**:** 5.0–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store between 2° and 30°.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sumatriptan Succinate RS
USP Sumatriptan Succinate Related Compound A RS
[3-[2-(Dimethylamino)ethyl]-2-({3-[2-(dimethylamino) ethyl]-1*H*-indol-5-yl}methyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide succinate salt.
$C_{27}H_{37}N_5O_2S \cdot C_4H_6O_4$ 613.77
USP Sumatriptan Succinate Related Compound C RS
{3-[2-(Dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl}-*N*-methylmethanesulfonamide succinate salt.
$C_{15}H_{23}N_3O_3S \cdot \frac{1}{2}C_4H_6O_4$ 384.47
USP Sumatriptan Succinate Related Impurities RS
▲Mixture of sumatriptan succinate, sumatriptan monomethyl, sumatriptan succinate related compound C, sumatriptan *N*-oxide, and sumatriptan amino.▲ 1S (USP41)

USP Monographs

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Sumatriptan Tablets

DEFINITION

Sumatriptan Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K.**▲ (CN 1-May-2020) The IR spectrum exhibits main bands at or near (±1) wavenumbers (cm^{-1}) 1708, 1567, 1339, 1300, 1235, 1207, 989, and 844.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer solution A: 2.9 g/L of monobasic sodium phosphate, 1.3 mL/L of dibutylamine, and 0.4 mL/L of phosphoric acid in water. Adjust with 10 N sodium hydroxide to a pH of 6.5.
Buffer solution B: 3.9 g/L of monobasic sodium phosphate. Adjust with 10 N sodium hydroxide to a pH of 6.5 before dilution.
Mobile phase: Acetonitrile and *Buffer solution A* (1:3)
Diluent: Acetonitrile and *Buffer solution B* (1:3)
Standard solution: Equivalent to 0.1 mg/mL of sumatriptan from USP Sumatriptan Succinate RS in *Diluent*
Sample stock solution: Nominally 1–2 mg/mL of sumatriptan from NLT 5 Tablets prepared as follows. Transfer the Tablets to a suitable volumetric flask. Add *Diluent* to fill 60%–80% of the flask volume. Sonicate for 15–20 min with intermittent shaking. Dilute with *Diluent* to volume.
Sample solution: Nominally 0.1 mg/mL of sumatriptan in *Diluent* from the *Sample stock solution*. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 1.7 times the retention time of sumatriptan
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sumatriptan Succinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sumatriptan, 295.4
M_{r2} = molecular weight of sumatriptan succinate, 413.5

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 30 rpm
Time: 15 min
Standard solution: 0.025 mg/mL of USP Sumatriptan Succinate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium* if necessary.
Instrumental conditions
Mode: UV
Detector: about 282 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor
M_{r1} = molecular weight of sumatriptan, 295.4
M_{r2} = molecular weight of sumatriptan succinate, 413.5
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of sumatriptan is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Standard solution: (L/900) mg/mL of USP Sumatriptan Succinate RS in *Medium*, where L is the Tablet label claim in mg
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Detector: About 282 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of sumatriptan, 295.4
M_{r2} = molecular weight of sumatriptan succinate, 413.5
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of sumatriptan is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min

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Standard solution: 0.035 mg/mL of USP Sumatriptan Succinate RS in *Medium*
Sample solution: Dilute the solution if necessary and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Detector: About 283 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor
M_{r1} = molecular weight of sumatriptan, 295.4
M_{r2} = molecular weight of sumatriptan succinate, 413.5
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of sumatriptan is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent: 0.1 N hydrochloric acid
Buffer: 1.9 g/L of ammonium acetate. Adjust with acetic acid to a pH of 4.1.
Solution A: Acetonitrile and methanol (90:10)
Solution B: *Solution A* and *Buffer* (5:95)
Solution C: *Solution A* and *Buffer* (22:78)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
14.5	100	0
21	0	100
28	0	100
29	100	0
37	100	0

System suitability solution: 0.1 mg/mL each of USP Sumatriptan Succinate RS and USP Sumatriptan Succinate Related Compound C RS in *Diluent*
Sensitivity solution: 0.7 µg/mL of USP Sumatriptan Succinate Related Compound C RS in *Diluent*
Sample solution: Nominally 1.25 mg/mL of sumatriptan from NLT 5 Tablets in *Diluent* prepared as follows. Transfer the Tablets to a suitable volumetric flask. Add *Diluent* to fill 80% of the flask volume. Sonicate for 5 min to disperse the Tablets completely. Allow to cool to room temperature for NLT 15 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution at NLT 3000 RM for 10 min. Alternatively, the *Sample solution* may be filtered using a suitable filter. Use the supernatant or filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between sumatriptan and sumatriptan succinate related compound C, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity as well as any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of peak responses for all peaks in the *Sample solution*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
3-Hydroxy-2-oxo sumatriptan[a]	0.23	0.40	0.5
Sumatriptan amino[b,c]	0.86	—	—
Sumatriptan monomethyl[c,d]	0.98	—	—
Sumatriptan	1.0	—	—
Sumatriptan related compound C	1.22	1.0	0.5
Sumatriptan *N*-oxide[e]	1.56	1.0	0.5
Sumatriptan related compound A[f]	1.75	1.67	0.75
Sumatriptan *N*-dimer[g]	1.86	1.0	0.3
Any individual, unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

[a] 1-{3-[2-(Dimethylamino)ethyl]-3-hydroxy-2-oxoindolin-5-yl}-*N*-methylmethanesulfonamide.
[b] [3-[2-Aminoethyl)]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide.
[c] Process impurity, included for peak identification purposes only, controlled in the drug substance.
[d] *N*-Methyl-1-{3-[2-(methylamino)ethyl]-1*H*-indol-5-yl}methanesulfonamide.
[e] *N*-Methyl-1-{3-[2-(dimethylamino *N*-oxide)ethyl]-1*H*-indol-5-yl} methanesulfonamide.
[f] [3-[2-(Dimethylamino)ethyl]-2-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl] methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide.
[g] {3-[2-(Dimethylamino)ethyl]-1-({3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl} methyl)-1*H*-indol-5-yl}-*N*-methylmethanesulfonamide.

ADDITIONAL REQUIREMENTS

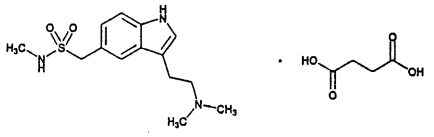
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store between 2° and 30°.

USP Monographs

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- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sumatriptan Succinate RS
  USP Sumatriptan Succinate Related Compound C RS
  [3-[2-(Dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide succinate salt.
  $C_{15}H_{23}N_3O_3S \cdot 0.5C_4H_6O_4$ 384.47

Sumatriptan Succinate



$C_{14}H_{21}N_3O_2S \cdot C_4H_6O_4$ 413.49
1*H*-Indole-5-methanesulfonamide, 3-[2-(dimethylamino) ethyl]-*N*-methyl-, butanedioate (1:1);
3-[2-(Dimethylamino)ethyl]-*N*-methylindole-5-methanesulfonamide succinate (1:1) [103628-48-4].

DEFINITION
Sumatriptan Succinate contains NLT 98.0% and NMT 102.0% of sumatriptan succinate ($C_{14}H_{21}N_3O_2S \cdot C_4H_6O_4$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Dissolve 1.7 mL of dibutylamine, 0.6 mL of phosphoric acid, and 3.9 g of sodium dihydrogen phosphate dihydrate in 750 mL of water. Adjust with a solution of 50% (w/v) sodium hydroxide to a pH of 6.5, and dilute with water to 1 L.
Diluent: Dissolve 2.97 g of monobasic sodium phosphate in 600 mL of water, adjust with a solution of 50% (w/v) sodium hydroxide to a pH of 6.5, dilute with water to 750 mL, and add 250 mL of acetonitrile.
Mobile phase: Acetonitrile and *Buffer* (200:800)
System suitability solution: 0.28 mg/mL of USP Sumatriptan Succinate RS and 0.14 mg/mL of USP Sumatriptan Succinate Related Compound C RS in *Diluent*
Standard solution: 0.14 mg/mL of USP Sumatriptan Succinate RS in *Diluent*
Sample solution: 0.14 mg/mL of Sumatriptan Succinate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for sumatriptan succinate related compound C and sumatriptan are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between sumatriptan succinate related compound C and sumatriptan, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sumatriptan succinate ($C_{14}H_{21}N_3O_2S \cdot C_4H_6O_4$) in the portion of Sumatriptan Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Sumatriptan Succinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Sumatriptan Succinate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES
- **PROCEDURE 1: LIMIT OF SUMATRIPTAN SUCCINATE RELATED COMPOUND A**

Buffer: Dissolve 77.1 g of ammonium acetate in 100 mL of water.
Mobile phase: Methanol and *Buffer* (90:10)
Standard solution: 6.25 µg/mL of USP Sumatriptan Succinate Related Compound A RS in *Mobile phase*
Sample solution: 2.8 mg/mL of Sumatriptan Succinate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sumatriptan related compound A free base in the portion of sumatriptan free base taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (M_{r3}/M_{r4}) \times 100$$

r_U = peak response of sumatriptan succinate related compound A from the *Sample solution*
r_S = peak response of sumatriptan succinate related compound A from the *Standard solution*
C_S = concentration of USP Sumatriptan Succinate Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Sumatriptan Succinate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sumatriptan related compound A, 495.7

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M_{r2} = molecular weight of sumatriptan succinate related compound A, 613.8
M_{r3} = molecular weight of sumatriptan succinate, 413.5
M_{r4} = molecular weight of sumatriptan, 295.4

Acceptance criteria: NMT 0.6%

- **PROCEDURE 2**

Diluent and **System suitability solution:** Prepare as directed in the *Assay*.

Buffer: Dissolve 1.7 mL of dibutylamine, 0.6 mL of phosphoric acid, and 3.9 g of monobasic sodium phosphate dihydrate in water. Adjust with a solution of 50% (w/v) sodium hydroxide to a pH of about 7.5, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (20:80)

Identification solution: 3 mg/mL of USP Sumatriptan Succinate Related Impurities RS in *Mobile phase*

Sample solution: 2.8 mg/mL of Sumatriptan Succinate in *Diluent*

Chromatographic system: Prepare as directed in the *Assay*. After making sure that the resolution criteria are met, chromatograph the *Identification solution*, and record the peak responses as directed in the *Analysis*. Identify the peaks according to *Table 1*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Sumatriptan Succinate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[3-[2-(Dimethylamino-*N*-oxide) ethyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide	0.3	0.2
[3-[2-(Aminoethyl)]-1*H*-indol-5-yl]-*N*-methylmethane sulfonamide	0.4	0.1
[3-[2-(Methylamino)ethyl]-1*H*-indol-5-yl]-*N*-methyl methanesulfonamide	0.6	0.5
Sumatriptan succinate related compound C	0.9	0.5
Sumatriptan	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities[a]	—	1.5

[a] Includes the amount of sumatriptan related compound A determined in the test for *Limit of Sumatriptan Succinate Related Compound A*.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Protect from freezing, and store at a temperature below 30°.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sumatriptan Succinate RS
USP Sumatriptan Succinate Related Compound A RS
[3-[2-(Dimethylamino)ethyl]-2-[[3-[2-(dimethylamino) ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide succinate salt.
$C_{27}H_{37}N_5O_2S \cdot C_4H_6O_4$ 613.77
USP Sumatriptan Succinate Related Compound C RS
[3-[2-(Dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide succinate salt.
$C_{15}H_{23}N_3O_3S \cdot 0.5C_4H_6O_4$ 384.47
USP Sumatriptan Succinate Related Impurities RS
Mixture of sumatriptan succinate, ▲[3-[2-(methylamino) ethyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide,▲ (ERR 1-Jun-2019) sumatriptan succinate related compound C, [3-[2-(dimethylamino-*N*-oxide)ethyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide, and [3-[2-(aminoethyl)]-1*H*-indol-5-yl]-*N*-methyl methanesulfonamide.

Sumatriptan Compounded Oral Suspension

DEFINITION

Sumatriptan Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of sumatriptan ($C_{14}H_{21}N_3O_2S$).

Prepare Sumatriptan Compounded Oral Suspension containing 5 mg/mL of sumatriptan as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Sumatriptan (as Sumatriptan Succinate)	500 mg (700 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar, and comminute to a fine powder, or add *Sumatriptan Succinate* powder to the mortar. Add 25 mL of *Vehicle* in portions, mixing thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 0.01 M dibutylamine in 0.025 M aqueous monobasic sodium phosphate dihydrate (25:75), adjusted with 1 N sodium hydroxide to a pH of 8.0. Filter and degas.

Standard stock solution: 4.0 mg/mL of USP Sumatriptan Succinate RS in *Mobile phase*

Standard solution: 0.12 mg/mL of sumatriptan prepared with *Standard stock solution* and *Mobile phase*

Sample solution: 0.15 mg/mL of sumatriptan prepared from Oral Suspension and 0.1 M hydrochloric acid. Pass through a 0.22-µm syringe filter into a 0.3-mL polypropylene sample vial.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for sumatriptan is about 11 min.]
Suitability requirements
Relative standard deviation: NMT 1.5% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sumatriptan ($C_{14}H_{21}N_3O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of sumatriptan in the *Standard solution* (mg/mL)
C_U = nominal concentration of sumatriptan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.6–4.6

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.
• **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored in a refrigerator
• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sumatriptan Succinate RS

Absorbable Surgical Suture

DEFINITION
Absorbable Surgical Suture is a sterile, flexible strand prepared from collagen derived from healthy mammals or from a synthetic polymer. Suture prepared from synthetic polymer may be in either monofilament or multifilament form. It is capable of being absorbed by living mammalian tissue, but may be treated to modify its resistance to absorption. Its diameter and tensile strength correspond to the size designation indicated on the label, within the limits prescribed herein. It may be modified with respect to body or texture. It may be impregnated or treated with a suitable coating, softening, or antimicrobial agent. It may be colored by a color additive approved by the FDA. The collagen suture is designated as either Plain Suture or Chromic Suture. Both types consist of processed strands of collagen, but Chromic Suture is processed by physical or chemical means so as to provide greater resistance to absorption in living mammalian tissue.

IMPURITIES
• **SOLUBLE CHROMIUM COMPOUNDS**
Standard solution: 2.83 µg/mL of potassium dichromate in water
Sample solution: 5 mL of the fluid prepared as directed in the *Extractable Color* test
Analysis: To 5 mL each of the *Standard solution* and *Sample solution* add 2 mL of a solution (1 in 100) of diphenylcarbazide in alcohol and 2 mL of 2 N sulfuric acid.
Acceptance criteria: Any color that develops in the *Sample solution* is not more intense than that in the *Standard solution* [1 ppm of chromium (Cr)].

SPECIFIC TESTS
[NOTE—If the Suture is packaged with a fluid, make the required measurements for the first four of the following tests within 2 min after removing it from the fluid.]
• **LENGTH**
Sample: A length of Suture
Analysis: Determine the length of the *Sample* without stretching.
Acceptance criteria: The length of each strand is NLT 95.0% of the length stated on the label.

Change to read:

• **SUTURES—DIAMETER** ⟨861⟩
Sample: 10 strands of Suture
Analysis: Determine the diameter as directed in the chapter.
Acceptance criteria
Collagen suture: The average diameter, and NLT 20, of the 30 measurements on the 10-strand *Sample* are within the limits on average diameter in *Table 1* for the size stated on the label. None of the individual ▲observed measurements should be less than or greater than the limits on individual diameter in *Table 1*.▲ (USP 1-Dec-2019)
Synthetic suture: The average diameter of the strands being measured is within the tolerances prescribed in *Table 2* for the size stated on the label. None of the ▲individual observed measurements should be less than or greater than the limits on individual diameter in *Table 2*.▲ (USP 1-Dec-2019)
• **TENSILE STRENGTH** ⟨881⟩, *Surgical Suture*
Sample: NLT 10 strands of Suture
Analysis: Determine the tensile strength of the *Sample* as directed in the chapter.
Acceptance criteria
Collagen suture: The tensile strength, determined as the minimum strength for each individual strand tested, and calculated as the average strength from any one lot, is as set forth in *Table 1*. If NMT 1 strand fails to meet the limit on individual strands, repeat the test with NLT 20 additional strands: the requirements of the test are met if none of the additional strands falls below the limit on individual strands, and if the average strength of all the strands tested does not fall below the stated limit in *Table 1*.
Synthetic suture: The minimum tensile strength of each size of synthetic suture, calculated as the average strength from any one lot, is as set forth in *Table 2*.
• **SUTURES—NEEDLE ATTACHMENT** ⟨871⟩: Suture on which eyeless needles are swaged meets the requirements.

Change to read:

• **STERILITY TESTS** ⟨71⟩: ▲Suture that is claimed to be sterile▲ (USP 1-Dec-2019) meets the requirements.

Change to read:

• **EXTRACTABLE COLOR** (if Suture is dyed)
Matching solutions: Prepare by combining the colorimetric solutions (CS) in the proportions indicated in *Table 3*, and adding water, if necessary, to make 10.0 parts. [See

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Reagents, Indicators, and Solutions—Solutions, Colorimetric Solutions (CS) for composition.]

Table 3. Matching Solutions

Color of Suture (Extractable Color)	Parts of Each CS per 10 Parts of Total Volume		
	Cobaltous Chloride CS	Ferric Chloride CS	Cupric Sulfate CS
Yellow-brown	0.2	1.2	—
Pink-red	1.0	—	—
Green-blue	—	—	2.0
Violet	1.6	—	8.4

Sample: NLT 250 mg of Suture

Analysis: Place the *Sample* in a conical flask containing 1.0 mL of water for each 10 mg of the *Sample.* ▲For microsutures use 2.5 mL of water for each 25 mg of the *Sample.*▲ (USP 1-Dec-2019) Close the flask, and allow it to stand at 37 ± 0.5° for 24 h. Cool. Decant the water from the Suture, and compare it with the *Matching solution.* ▲Where fibrous extraneous material may affect the color, filter the solution before performing the color determination.▲ (USP 1-Dec-2019)

Acceptance criteria: Any color present is not more intense than that of the appropriate *Matching solution.* ▲Where fibrous extraneous material may affect the color, filter the solution before performing the color determination.▲ (USP 1-Dec-2019)

ADDITIONAL REQUIREMENTS

- **▲PACKAGING AND STORAGE:▲** Preserve dry or in fluid, in containers (packets) so designed that sterility is maintained until the container is opened. A number of such containers may be placed in a box.

Change to read:

- **▲LABELING:▲** The label of each individual container (packet) of Suture indicates the size, length, type of Suture, ▲including the material composition as well as the physical configuration (e.g., monofilament/multifilament, twisted/braided),▲ (USP 1-Dec-2019) kind of needle (if a needle is included), number of Sutures (if multiple), lot number, and name of the manufacturer or distributor. If removable needles are used, the labeling so indicates. Suture size is designated by the metric size (gauge number) and the

Table 1. Collagen Suture (Absorbable Surgical Suture)

▲USP Size▲a▲ (USP 1-Dec-2019)	Metric Size (Gauge No.)	Limits on Average Diameter (mm)		▲Limits on Individual Diameter (mm)▲ (USP 1-Dec-2019)		▲▲ (USP 1-Dec-2019)		Knot-Pull Tensile Strength (in N)▲b▲ (USP 1-Dec-2019)	
		Min.	Max.	▲Min.	Max.▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	Limit on Average Min.	Limit on Individual Strand Min.
9-0	0.4	0.040	0.049	▲—▲	0.060▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	—	—
8-0	0.5	0.050	0.069	▲0.045▲	0.085▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	0.44	0.24
7-0	0.7	0.070	0.099	▲0.060▲	0.125▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	0.69	0.54
6-0	1	0.10	0.149	▲0.085▲	0.175▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲1.77▲ (USP 1-Dec-2019)	0.98
5-0	1.5	0.15	0.199	▲0.125▲	0.250▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	3.73	1.96
4-0	2	0.20	0.249	▲0.175▲	0.325▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	7.55	3.92
3-0	3	0.30	▲0.349▲	0.250	0.375▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲12.3▲ (USP 1-Dec-2019)	6.67
2-0	3.5	0.35	0.399	▲0.250▲	0.450▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	19.6	10.2
0	4	0.40	0.499	▲0.375▲	0.550▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	27.2	14.2
1	5	0.50	0.599	▲0.450▲	0.650▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	37.3	19.1
2	6	0.60	0.699	▲0.550▲	0.750▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	44.2	25.5
3	7	0.70	0.799	▲0.650▲	0.850▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲57.9▲ (USP 1-Dec-2019)	29.3
4	8	0.80	0.899	▲0.750▲	—▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	68.6	34.2

▲a USP sizes may not represent all sizes commercially available. For sizes other than those listed in this table, suture manufacturers must generate appropriate diameters and tensile strength data to support use.

b To convert from N to kg, divide N by 1.0197 and round to one significant figure.▲ (USP 1-Dec-2019)

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Table 2. Synthetic Suture (Absorbable Surgical Suture)

USP Size ▲[a]▲ (USP 1-Dec-2019)	Metric Size (Gauge No.) ▲▲ (USP 1-Dec-2019)	Limits on Average Diameter (mm)		▲Limits on Individual Diameter (mm)▲ (USP 1-Dec-2019)		▲▲ (USP 1-Dec-2019)	Knot-Pull Tensile Strength (in N) ▲[b]▲ (USP 1-Dec-2019) (except where otherwise specified)
		Min.	Max.	▲Min.	Max.▲ (USP 1-Dec-2019)		
12-0	0.01	0.001	0.009	▲—	0.015▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	—
11-0	0.1	0.010	0.019	▲0.005	0.025▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	—
10-0	0.2	0.020	0.029	▲0.015	0.035▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲0.25▲ (USP 1-Dec-2019)[c]
9-0	0.3	0.030	0.039	▲0.025	0.045▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	0.49[c]
8-0	0.4	0.040	0.049	▲0.035	0.060▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	0.69
7-0	0.5	0.050	0.069	▲0.045	0.085▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	1.37
6-0	0.7	0.070	0.099	▲0.060	0.125▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	2.45
5-0	1	0.10	0.149	▲0.085	0.175▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	6.67
4-0	1.5	0.15	0.199	▲0.125	0.250▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	9.32
3-0	2	0.20	0.249	▲0.175	0.325▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	17.4
2-0	3	0.30	0.339	▲0.250	0.375▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	26.3
0	3.5	0.35	0.399	▲0.325	0.450▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	38.2
1	4	0.40	0.499	▲0.375	0.550▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	49.8
2	5	0.50	0.599	▲0.450	0.650▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	62.3
3 and 4	6	0.60	0.699	▲0.550	0.750▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	71.5
5	7	0.70	0.799	▲0.650	—▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	—

▲[a] USP sizes may not represent all sizes commercially available. For sizes other than those listed in this table, suture manufacturers must generate appropriate diameters and tensile strength data to support use.

[b] To convert from N to kg, divide N by 1.0197 and round to one significant figure.▲ (USP 1-Dec-2019)

[c] The tensile strength of the specified USP size is measured by straight pull.

corresponding USP size. The label of the box indicates also the address of the manufacturer, packer or distributor, and the composition of any packaging fluids used.

Nonabsorbable Surgical Suture

DEFINITION

Change to read:

Nonabsorbable Surgical Suture is a flexible strand of material that is ▲not absorbed in▲ (USP 1-Dec-2019) living mammalian tissue. It may be in either monofilament or multifilament form. If it is a multifilament strand, the individual filaments may be combined by ▲▲ (USP 1-Dec-2019) twisting, braiding, or any combination thereof. It may be either sterile or nonsterile. Its diameter and tensile strength correspond to the size designation indicated on the label, within the limits prescribed herein. It may be modified with respect to body or texture, or to reduce capillarity, and may be suitably bleached. It may be impregnated or treated with a suitable coating, softening, or antimicrobial agent. It may be colored by a color additive approved by the FDA.

Nonabsorbable Surgical Suture is classed and typed as follows. Class I Suture is composed of silk or synthetic fibers of monofilament, twisted, or braided construction where the coating, if any, does not significantly affect thickness (e.g., braided silk, polyester, or nylon; monofilament nylon or polypropylene). Class II Suture is composed of cotton or linen fibers or coated natural or synthetic fibers where the coating significantly affects thickness but does not contribute significantly to strength (e.g., virgin silk sutures). Class III Suture is composed of monofilament or multifilament metal wire.

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SPECIFIC TESTS

[NOTE—If the Suture is packaged with a fluid, make the required measurements for the first four of the following tests within 2 min after removing it from the fluid.]

- **LENGTH**

Sample: A length of Suture

Analysis: Lay the *Sample* out smooth and without tension on a plane surface and determine the length of the *Sample*.

Acceptance criteria: The length of each strand is NLT 95.0% of the length stated on the label.

Change to read:

- **SUTURES—DIAMETER** ⟨861⟩

Sample: 10 strands of Suture

Analysis: Determine the diameter as directed in the chapter.

Acceptance criteria: The average diameter on the 10-strand *Sample* is within the limits ▲on average diameter (for all 30 measurements)▲ (USP 1-Dec-2019) in *Table 1* for the size stated on the label. ▲None of the observed individual measurements should be less than or greater than the limits on individual diameter in *Table 1*.▲ (USP 1-Dec-2019)

Table 1. Average Knot-Pull Limits of Various Sizes and Diameters of Sutures (Nonabsorbable Surgical Suture)

		Limits on Average Diameter (mm)		▲Limits on Individual Diameter (mm)▲ (USP 1-Dec-2019)		▲ (USP 1-Dec-2019)			Limits on Average Knot-Pull (except where otherwise specified)[a], Tensile Strength (in N)[b,▲c▲] (USP 1-Dec-2019)		
USP Size[▲d▲] (USP 1-Dec-2019)	Metric Size (Gauge No.)	Min.	Max.	▲Min.	Max.▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	Class I Min.	Class II Min.	Class III Min.
12-0	0.01	0.001	0.009	▲—	0.015▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	0.01[a]	—	0.02[a]
11-0	0.1	0.010	0.019	▲0.005	0.025▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	0.06[a]	0.05[a]	0.20[a]
10-0	0.2	0.020	0.029	▲0.015	0.035▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲0.186▲ (USP 1-Dec-2019)[a]	0.14[a]	0.59[a]
9-0	0.3	0.030	0.039	▲0.025	0.045▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲0.422▲ (USP 1-Dec-2019)[a]	0.28[a]	0.68[a]
8-0	0.4	0.040	0.049	▲0.035	0.060▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	0.59	0.39	1.08
7-0	0.5	0.050	0.069	▲0.045	0.085▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	1.08	0.59	1.57
6-0	0.7	0.070	0.099	▲0.060	0.125▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	1.96	1.08	2.65
5-0	1	0.10	0.149	▲0.085	0.175▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	3.92	2.26	5.30
4-0	1.5	0.15	0.199	▲0.125	0.250▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	5.88	4.51	8.04
3-0	2	0.20	0.249	▲0.175	0.275▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	9.41	6.47	13.3
▲2.5-0	2.5	0.25	0.299	0.225	0.325▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲13.6	7.28	15.2▲ (USP 1-Dec-2019)
2-0	3	0.30	▲0.349	0.250	0.375▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	14.1	10.0	▲17.7▲ (USP 1-Dec-2019)
0	3.5	0.35	0.399	▲0.325	0.450▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	21.2	14.2	33.3[a]
1	4	0.40	0.499	▲0.375	0.550▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	26.7	▲17.7▲ (USP 1-Dec-2019)	46.7[a]
2	5	0.50	0.599	▲0.450	0.650▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	34.5	24.9	▲57.9▲ (USP 1-Dec-2019)[a]
3 and 4	6	0.60	0.699	▲0.550	0.750▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲47.9▲ (USP 1-Dec-2019)	36.1	89.3[a]
5	7	0.70	0.799	▲0.650	0.850▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	60.4	—	112.0[a]
6	8	0.80	0.899	▲0.750	0.950▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	71.4	—	133.0[a]

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Table 1. Average Knot-Pull Limits of Various Sizes and Diameters of Sutures (Nonabsorbable Surgical Suture) *(continued)*

		Limits on Average Diameter (mm)		▲Limits on Individual Diameter (mm)▲ (USP 1-Dec-2019)		▲▲ (USP 1-Dec-2019)			Limits on Average Knot-Pull (except where otherwise specified)[a], Tensile Strength (in N)[b,▲c]▲ (USP 1-Dec-2019)		
USP Size[▲d]▲ (USP 1-Dec-2019)	Metric Size (Gauge No.)	Min.	Max.	▲Min.	Max.▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	Class I Min.	Class II Min.	Class III Min.
7	9	0.90	0.999	▲0.850	1.050▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	88.6	—	156.0[a]
8	10	1.00	1.099	▲0.950	1.150▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	—	—	178.0[a]
9	11	1.100	1.199	▲1.050	1.250▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	—	—	201.0[a]
10	12	1.200	1.299	▲1.150	▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	—	—	224.0[a]

[a] The tensile strength of sizes smaller than USP size 8-0 (metric size 0.4) is measured by straight pull. The tensile strength of sizes larger than USP size 2-0 (metric size 3) of monofilament Class III (metallic) Nonabsorbable Surgical Suture is measured by straight pull. Silver wire ▲(silver-coated fabric)▲ (USP 1-Dec-2019) meets the tensile strength values of Class I Sutures but is tested in the same manner as Class III Sutures.

[b] The limits on knot-pull tensile strength apply to Nonabsorbable Surgical Suture that has been sterilized. For nonsterile Sutures of Class I and Class II, the limits are 25% higher.

[▲c] To convert from N to kg, divide N by 1.0197 and round to one significant figure.

[d] USP sizes may not represent all sizes commercially available. For sizes other than those listed in the table, suture manufacturers must generate appropriate diameters and tensile strength data to support use.▲ (USP 1-Dec-2019)

- **TENSILE STRENGTH** ⟨881⟩, *Surgical Suture*
  Sample: NLT 10 strands of Suture
  Analysis: Determine the tensile strength of the *Sample* as directed in the chapter. Average all observations obtained.
  Acceptance criteria: The average tensile strength is NLT that set forth in *Table 1* for the class and the size stated on the label.
- **SUTURES—NEEDLE ATTACHMENT** ⟨871⟩**:** Suture on which eyeless needles are swaged meets the requirements.
- **STERILITY TESTS** ⟨71⟩**:** Suture that is claimed to be sterile meets the requirements.
- **EXTRACTABLE COLOR** (if Suture is dyed)
  Matching solutions: Prepare by combining the colorimetric solutions (CS) in the proportions indicated in *Table 2*, and adding water, if necessary, to make 10.0 parts. [See *Reagents, Indicators, and Solutions—Solutions, Colorimetric Solutions (CS)* for composition.]

Table 2. Matching Solutions

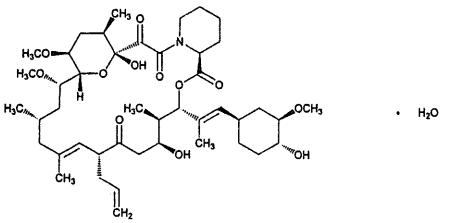
	Parts of Each CS per 10 Parts of Total Volume		
Color of Suture (Extractable Color)	Cobaltous Chloride CS	Ferric Chloride CS	Cupric Sulfate CS
Yellow-brown	0.2	1.2	—
Pink-red	1.0	—	—
Green-blue	—	—	2.0
Violet	1.6	—	8.4

Sample: NLT 250 mg of Suture
Analysis: Place the *Sample* in a conical flask containing 1.0 mL of water for each 10 mg of the *Sample*. Cover the flask with a short-stemmed funnel, heat the contents of the flask at the boiling point for 15 min, cool, and restore the volume by the addition of water, if necessary, to replace that lost by evaporation. Decant the water from the Suture, and compare it with the *Matching solution*.
Acceptance criteria: Any color present is not more intense than that of the appropriate *Matching solution*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve nonsterilized Suture in well-closed containers. Preserve sterile Suture dry or in fluid, in containers (packets) so designed that sterility is maintained until the container is opened. A number of such containers may be placed in a box.
- **LABELING:** The label of each individual container (packet) of Suture indicates the material from which the Suture is made, the size, construction, and length of the Suture, whether it is sterile or nonsterile, kind of needle (if a needle is included), number of sutures (if multiple), lot number, and name of the manufacturer or distributor. If removable needles are used, the labeling so indicates. Suture size is designated by the metric size (gauge number) and the corresponding USP size. The label of the box indicates also the address of the manufacturer, packer or distributor, and the composition of any packaging fluids used.

Tacrolimus



$C_{44}H_{69}NO_{12} \cdot H_2O$ 822.03

15,19-Epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3*S*-[3*R**,*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26a*R**]]-;

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(−)-(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone monohydrate [109581-93-3].

DEFINITION

Tacrolimus contains NLT 98.0% and NMT 102.0% of tacrolimus ($C_{44}H_{69}NO_{12}$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect solutions containing tacrolimus from light.
Solution A: 6 mM phosphoric acid
Solution B: Acetonitrile and *tert*-butyl methyl ether (81:19)
Solution C: *Solution A* and *Solution B* (4:1)
Solution D: *Solution A* and *Solution B* (1:4)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution C (%)	Solution D (%)
0	72	28
30	72	28
53	15	85
54	72	28
60	72	28

Diluent: Acetonitrile and water (7:3)
System suitability solution: 3 mg/mL of USP Tacrolimus System Suitability Mixture RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.
Standard solution: 3 mg/mL of USP Tacrolimus RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.
Sample solution: 3 mg/mL of Tacrolimus in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Temperatures
Column: 60°
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 3* for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between ascomycin and tacrolimus, *System suitability solution*
Relative standard deviation: NMT 1.0% for the sum of the responses of tacrolimus, tacrolimus open ring, and tacrolimus 19-epimer, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tacrolimus ($C_{44}H_{69}NO_{12}$) in the portion of Tacrolimus taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of tacrolimus open ring, tacrolimus 19-epimer, and tacrolimus from the *Sample solution*
r_S = sum of the peak responses of tacrolimus open ring, tacrolimus 19-epimer, and tacrolimus from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
C_U = concentration of Tacrolimus in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES, PROCEDURE 1**

Use *Organic Impurities, Procedure 1* when the impurity profile includes tacrolimus methylacrylaldehyde and tacrolimus diene. It is suggested that new columns be conditioned with about 500 mL of alcohol before use to meet the resolution criterion.
Mobile phase: Hexane, *n*-butyl chloride, and acetonitrile (7:2:1). Add *n*-butyl chloride to hexane, and mix well before adding acetonitrile. After adding acetonitrile, mix the *Mobile phase* for 2 h to get a clear solution. Any deviations from the ratio of components in the *Mobile phase* and the order of mixing will result in a two-phase solution.
System suitability solution: 0.1 mg/mL each of USP Tacrolimus RS and USP Tacrolimus Related Compound A RS in *Mobile phase*
Sample solution: 2.0 mg/mL of Tacrolimus in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: Two 4.6-mm × 25-cm columns; 5-µm packing L20
Column temperature: 28 ± 2°
Flow rate: 1.5 mL/min. Adjust the *Flow rate* so that the retention time of tacrolimus is approximately 15 min.
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.1 between tacrolimus and tacrolimus related compound A
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tacrolimus taken:

$$\text{Result} = (r_U/F_i) \times \{1/[r_T + \Sigma(r_U/F_i)]\} \times 100$$

r_U = peak response of each impurity from the *Sample solution*
F_i = relative response factor for each corresponding impurity (see *Table 2*)

USP Monographs

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r_T = peak response of tacrolimus from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus methylacryl aldehyde[a]	0.55	16.7	0.2
Tacrolimus diene[b]	0.79	2.2	0.2
Tacrolimus impurity 1[c]	0.96	1.0	0.2
Tacrolimus related compound A[d]	0.96	—	—
Tacrolimus	1.0	1.0	—
Tacrolimus 19-epimer[d, e]	1.1	—	—
Tacrolimus open ring[d, f]	1.3	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities[g]	—	—	0.3

[a] (*E*)-3-[[(1*R*,3*R*,4*R*)-4-Hydroxy-3-methoxycyclohexyl]-2-methylacrylaldehyde.
[b] (14*E*,18*E*)-17-Allyl-1-hydroxy-12-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}] octacosa-14,18-diene-2,3,10,16-tetrone.
[c] Specified unidentified impurity.
[d] For informational purposes only; not to be reported.
[e] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a,hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16,dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
[f] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4] oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.
[g] Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Use *Organic Impurities, Procedure 2* when the impurity profile includes ascomycin, desmethyl tacrolimus, tacrolimus 8-epimer, and tacrolimus 8-propyl analog. Protect solutions containing tacrolimus from light.

Solution A, Solution B, Solution C, Solution D, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 30 µg/mL of USP Tacrolimus RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.

Reporting threshold solution: 1.5 µg/mL of USP Tacrolimus RS in *Diluent*

Peak identification solution 1: 10 µg/mL of USP Tacrolimus 8-epimer RS in acetonitrile

Peak identification solution 2: 10 µg/mL of USP Tacrolimus 8-propyl Analog RS in acetonitrile

System suitability

[NOTE—Identify the related compounds by the relative retention times provided in *Table 3*.]

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between tacrolimus and ascomycin, *System suitability solution*

Relative standard deviation: NMT 10.0% for the sum of the responses of tacrolimus and tacrolimus 19-epimer, *Standard solution*

Analysis

Samples: *Sample solution, Standard solution, Reporting threshold solution, Peak identification solution 1,* and *Peak identification solution 2*

Calculate the percentage of each impurity in the portion of Tacrolimus taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = sum of the peak responses of tacrolimus 19-epimer and tacrolimus from the *Standard solution*

C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)

C_U = concentration of Tacrolimus in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Identify tacrolimus 8-epimer and tacrolimus 8-propyl analog using *Peak identification solution 1* and *Peak identification solution 2*. Report impurity peaks with responses NLT that of the peak in the *Reporting threshold solution* (0.05%). Disregard peaks with retention times less than 3 min.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tacrolimus open ring[a, b]	0.52	—
Ascomycin 19-epimer (if present)[c, d]	0.54	0.1
Tacrolimus 19-epimer[b, e]	0.63	—
Ascomycin[f]	0.87	0.50
Desmethyl tacrolimus (if present)[d, g]	0.94	0.1
Tacrolimus	1.00	—
Tacrolimus 8-epimer[h]	1.28	0.15
Tacrolimus 8-propyl analog[i]	1.33	0.15
Any individual unspecified impurity	—	0.1

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Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities[j]	—	1.0

[a] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.

[b] Tacrolimus open ring and tacrolimus 19-epimer are isomers of tacrolimus, which are present in equilibrium with the active ingredient. They are not to be reported as degradation products.

[c] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

[d] If possible from the manufacturing process.

[e] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

[f] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

[g] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,12,18-trimethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

[h] (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

[i] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

[j] Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in *N,N*-dimethylformamide
  Acceptance criteria: −110° to −115° on the "as-is" basis
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 4.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which test for *Organic Impurities* the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tacrolimus RS
  15,19-Epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3*S*-[3*R**,*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**, 18*S**,19*S**,26a*R**]]-.
  $C_{44}H_{69}NO_{12} \cdot H_2O$ 822.03
  USP Tacrolimus Related Compound A RS
  (*E*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
  $C_{43}H_{69}NO_{12}$ 792.01
  USP Tacrolimus 8-epimer RS
  (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
  $C_{44}H_{69}NO_{12}$ 804.02
  USP Tacrolimus 8-propyl Analog RS
  (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
  $C_{44}H_{71}NO_{12}$ 806.03
  USP Tacrolimus System Suitability Mixture RS
  This is a mixture of tacrolimus, ascomycin (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24, 25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
  $C_{43}H_{69}NO_{12}$ 792.01
  and tacrolimus 8-propyl analog (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*, 19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24, 25,26,26a-Hexadecahydro-5,19-dihydroxy-3-{(*E*)-2- [(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4] oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
  $C_{44}H_{71}NO_{12}$ 806.03

Tacrolimus Capsules

DEFINITION

Tacrolimus Capsules contain NLT 93.0% and NMT 105.0% of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectrum of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Allow the *Standard solution* and *Sample solution* to stand for 3 h at ambient temperature before use. Protect solutions containing tacrolimus from light.
  Solution A: 6 mM phosphoric acid

USP Monographs

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Solution B: 50 g/L of polyoxyethylene (23) lauryl ether. [NOTE—Polyoxyethylene (23) lauryl ether is also called Brij-35.]
Solution C: Acetonitrile and *Solution B* (7:3)
Mobile phase: Acetonitrile, *tert*-butyl methyl ether, and *Solution A* (335:55:600)
Standard solution: 50 µg/mL of USP Tacrolimus RS in *Solution C*
Sample solution: Equivalent to 50 µg/mL of tacrolimus from NLT 10 Capsules in *Solution C*. [NOTE—Sonicate, and stir with a magnetic stirrer.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm. When this procedure is used for *Identification* test *B*, use a diode array detector set at 200–400 nm.
Column: 4.0-mm × 5.5-cm; 3-µm packing L1
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tacrolimus 19-epimer and tacrolimus are 0.67 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0% for the sum of the tacrolimus and tacrolimus 19-epimer peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Sample solution*
r_S = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Hydroxypropylcellulose in water (1:2 × 10^4), adjusted with 6% phosphoric acid to a pH of 4.5; 900 mL
Apparatus 2: 50 rpm with sinker (see *Dissolution* ⟨711⟩, *Figure 2a*)
Time: 90 min
Mobile phase: Acetonitrile, methanol, water, and 6% phosphoric acid (46: 18: 36: 0.1)
Standard stock solution: (*L*/360) mg/mL in acetonitrile, where *L* is the Capsule label claim in mg
Standard solution: To 20.0 mL of the *Standard stock solution* add 50.0 mL of *Medium*, and mix to obtain solutions with known concentrations as indicated in *Table 1*. Allow the solution to stand for NLT 6 h at 25° before use.
Sample solution: Pass 10 mL of the solution under test through a G4 glass filter. To 5.0 mL of the filtrate add 2.0 mL of acetonitrile, and mix. Allow the solution to stand for NLT 1 h at 25° before use.

Table 1

Capsule Strength (mg)	Final Concentration (µg/mL)
0.5	0.4
1	0.8
5	4

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 50°
Flow rate: Adjust the flow rate so that the retention time of tacrolimus is approximately 14 min.
Injection volume: See *Table 2*.

Table 2

Capsule Strength (mg)	Injection Volume (µL)
0.5	800
1	400
5	80

[NOTE—For products with strengths other than those listed in *Table 2*, adjust the *Injection volume* to deliver an equivalent amount of tacrolimus into the column.]
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between tacrolimus 19-epimer and tacrolimus
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times (100/L)$$

r_U = peak response of tacrolimus from the *Sample solution*
r_S = peak response of tacrolimus from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (*Q*) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
[NOTE—Allow the *Standard solution* to stand for 3 h at ambient temperature before use. Protect solutions containing tacrolimus from light.]

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Buffer: Dissolve 6 g of sodium dodecyl sulfate and 8.28 g of monobasic sodium phosphate in 6000 mL of water. Adjust with 2 N sodium hydroxide to a pH of 7.0.
Medium: *Buffer*; 900 mL
Apparatus 2: 50 rpm, with sinkers
Time: 60 min
Standard stock solution: 0.2 mg/mL of USP Tacrolimus RS in alcohol and *Medium* (3:7). [NOTE—Dissolve USP Tacrolimus RS in alcohol using 30% of the final volume. Sonicate until dissolved, and dilute with *Medium* to volume.]
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 5 µg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Solution A: 6 mM phosphoric acid
Mobile phase: Acetonitrile, *tert*-butyl methyl ether, and *Solution A* (335:50:600)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.0-mm × 5.5-cm; 3-µm packing L1
Column temperature: 60°
Flow rate: 1.2 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tacrolimus 19-epimer and tacrolimus are 0.67 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5.0% for the sum of the areas of tacrolimus and tacrolimus 19-epimer
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Sample solution*
r_S = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 50 mg/L of hydroxypropylcellulose in water. Adjust with phosphoric acid to a pH of 4.5; 900 mL.
Apparatus 2 (without sinker) and **Time:** Proceed as directed in *Test 1*.
Buffer: 3.6 g/L of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 2.5.
Mobile phase: *Buffer* and acetonitrile (1:1)
Standard stock solution: 0.1 mg/mL of USP Tacrolimus RS in acetonitrile
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/900) mg/mL, where *L* is the Capsule label claim in mg.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 60°
Flow rate: 1.3 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tacrolimus 19-epimer, tacrolimus open ring, and tacrolimus are 0.67, 0.79, and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the *Sample solution*
r_S = sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.
Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: Hydroxypropylcellulose in water (1 in 20,000), adjusted with phosphoric acid to a pH of 4.5. See *Table 3* for the volume.

Table 3

Capsule Strength (mg)	Volume of Medium (mL)
0.5	500
1	900
5	900

Apparatus 2: 50 rpm, with sinkers
Time: 120 min
Diluent: 1 mg/mL of hydroxypropylcellulose in water. Sonicate as needed to dissolve.
Buffer: To a solution of 1 g/L of sodium 1-hexanesulfonate in water add 0.1 mL/L of trifluoroacetic acid.
Mobile phase: Acetonitrile, methanol, and *Buffer* (550:50:400)
Standard stock solution: Dissolve USP Tacrolimus RS in acetonitrile. See *Table 4* for the concentrations (*L* is the Capsule label claim in mg).

Table 4

Capsule Strength (mg)	Concentration (mg/mL)
0.5	*L*/25
1	*L*/45
5	*L*/45

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Standard solution: Dilute the *Standard stock solution* with *Diluent*. See *Table 5* for the concentrations (*L* is the Capsule label claim in mg).

Table 5

Capsule Strength (mg)	Concentration (mg/mL)
0.5	*L*/500
1	*L*/900
5	*L*/900

Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium* (mL) (see *Table 3*)

Tolerances: NLT 75% (*Q*) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: 0.05 g/L hydroxypropylcellulose in water. Adjust with phosphoric acid to a pH of 4.5; 900 mL.
Apparatus 2: 50 rpm, with sinkers
Time: 90 min
Solution A: 0.1 mL/L of trifluoroacetic acid in water
Mobile phase: Acetonitrile and *Solution A* (50:50)
Standard stock solution: 0.22 mg/mL of USP Tacrolimus RS in acetonitrile
Standard solution: (*L*/900) mg/mL of USP Tacrolimus RS from the *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Capsule
Sample solution: Centrifuge a portion of the solution under test. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 2.1-mm × 15-cm; 3.5-µm packing L7
Column temperature: 60°
Flow rate: 0.8 mL/min
Injection volume: 750 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tacrolimus 19-epimer (tautomer 1), tacrolimus open-ring (tautomer 2), and tacrolimus are 0.55, 0.79, and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 4.0% for the peaks due to tautomer 1, tautomer 2, and tacrolimus.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = sum of the peak responses of tacrolimus, tacrolimus open-ring, and tacrolimus 19-epimer from the *Sample solution*
r_S = sum of the peak responses of tacrolimus, tacrolimus open-ring, and tacrolimus 19-epimer from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.

▲**Test 6:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Dilute phosphoric acid: Transfer 7.1 mL of phosphoric acid to a 100 mL volumetric flask, and dilute with water to volume.
Medium: 50 mg/L of hydroxypropyl cellulose in water. Adjust with *Dilute phosphoric acid* to a pH of 4.5; 900 mL.
Apparatus 2: 50 rpm
Time: 60 min
Buffer: 3.6 g/L of monobasic potassium phosphate in water. Adjust with *Dilute phosphoric acid* to a pH of 2.5.
Mobile phase: Acetonitrile and *Buffer* (1:1)
Standard stock solution: 0.11 mg/mL of USP Tacrolimus RS in acetonitrile
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/900) mg/mL, where *L* is the label claim in mg/Capsule.
Sample solution: Centrifuge a portion of the solution under test. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 60°
Flow rate: 1.3 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tacrolimus 19-epimer, tacrolimus open ring, and tacrolimus are 0.77, 0.89, and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for tacrolimus
Relative standard deviation: NMT 2.0% for the sum of tacrolimus 19-epimer, tacrolimus open ring, and tacrolimus
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the *Sample solution*
r_S = sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved. ▲ (RB 1-Aug-2018)

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities, Procedure 1**

Use *Organic Impurities, Procedure 1* when the impurity profile includes tacrolimus diene and tacrolimus regioisomer. It is suggested that new columns be conditioned with about 500 mL of ethanol before use to meet the resolution criterion.

Mobile phase: Hexane, *n*-butyl chloride, and acetonitrile (7:2:1). Add *n*-butyl chloride to hexane, and mix well before adding acetonitrile. After adding acetonitrile, mix the *Mobile phase* for 2 h to get a clear solution. Any deviations from the ratio of components in the *Mobile phase* and the order of mixing will result in a two-phase solution.

System suitability solution: 0.1 mg/mL each of USP Tacrolimus RS and USP Tacrolimus Related Compound A RS in *Mobile phase*

Sample solution: Transfer the contents of a suitable number of Capsules (equivalent to about 5 mg of tacrolimus for 0.5-mg Capsules or 10 mg of tacrolimus for 1-mg and 5-mg Capsules) into a centrifuge tube. Add 1.5 mL of a mixture of *n*-butyl chloride and acetonitrile (2:1), sonicate in an ultrasonic bath for 2 min, add 3.5 mL of *n*-hexane, and mix. Centrifuge this solution, and collect the supernatant or pass the solution through a 0.5-µm membrane filter. Use the solution within 30 min of preparation.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Columns: Two 4.6-mm × 25-cm columns; 5-µm packing L20
Column temperature: 28 ± 2°
Flow rate: 1.5 mL/min. Adjust the *Flow rate* so that the retention time of tacrolimus is approximately 15 min.
Injection volume: 20 µL
Run time: 3 times the retention time of tacrolimus

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.1 between tacrolimus and tacrolimus related compound A
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/F_i) \times \{1/[r_T + \Sigma(r_U/F_i)]\} \times 100$$

r_U = peak response of each impurity from the *Sample solution*
F_i = relative response factor for each corresponding impurity (see *Table 6*)
r_T = peak response of tacrolimus from the *Sample solution*

Acceptance criteria: See *Table 6*. Disregard peaks due to the solvent.

Table 6

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus diene[a]	0.79	2.2	0.3
Tacrolimus regioisomer[b]	0.88	1.0	0.5
Tacrolimus impurity 1[c]	0.96	1.0	0.3
Tacrolimus related compound A[d]	0.96	—	—
Tacrolimus	1.0	—	—
Tacrolimus 19-epimer[e,f]	1.1	—	—
Tacrolimus open ring[e,g]	1.3	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.0

[a] (14*E*,18*E*)-17-Allyl-1-hydroxy-12-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}] octacosa-14,18-diene-2,3,10,16-tetrone.
[b] (4*E*,11*E*)-10-Allyl-7,8,10,13,14,15,16,17,18,19,20,21,26,22,28,28a-hexadecahydro-7,21-dihydroxy-3-(4-hydroxy-3-methoxycyclohexyl)-16,18-dimethoxy-4,6,12,14,20-pentamethyl-17,21-epoxy-3*H*-pyrido[2,1-*c*][1,4] oxaazacyclopentacosine-1,9,22,23(6*H*,25*H*)-tetrone.
[c] Tacrolimus impurity 1 is a specified, unidentified impurity.
[d] Tacrolimus related compound A is listed here to indicate the relative retention time of this compound. It is used in the procedure to evaluate system suitability and is not to be reported. It is not to be included in total impurities.
[e] Tacrolimus open ring and tacrolimus 19-epimer are isomers of tacrolimus, which are present in equilibrium with the active ingredient. They are not to be reported as degradation products and are not included in total impurities.
[f] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
[g] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4] oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.

• **Organic Impurities, Procedure 2**

Use *Organic Impurities, Procedure 2* when the impurity profile includes tacrolimus hydroxy acid and tacrolimus 8-epimer. It is suggested to equilibrate the column overnight with a mixture of *Solution C* and *Solution D* (17:3) before performing this procedure. Allow the *System suitability solution*, *Standard solution*, and *Sample solution* to stand for 3 h at ambient temperature before use. Protect solutions containing tacrolimus from light.

Solution A: 6 mM phosphoric acid
Solution B: Acetonitrile and *tert*-butyl methyl ether (81:19). [NOTE—The ratio of acetonitrile to *tert*-butyl methyl ether is critical.]
Solution C: *Solution A* and *Solution B* (4:1)
Solution D: *Solution A* and *Solution B* (1:4)

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Mobile phase: See *Table 7*.

Table 7

Time (min)	Solution C (%)	Solution D (%)
0	74	26
45	74	26
60	15	85
75	15	85
76	74	26
85	74	26

Solution E: 50 g/L of polyoxyethylene (23) lauryl ether in *Solution A*. [NOTE—Polyoxyethylene (23) lauryl ether is also called Brij-35.]
Diluent: Acetonitrile and *Solution E* (7:3)
System suitability solution: 1.5 mg/mL of USP Tacrolimus System Suitability Mixture RS in *Diluent*
Standard solution: 7.5 µg/mL of USP Tacrolimus RS in *Diluent*
Sensitivity solution: 1.5 µg/mL of USP Tacrolimus RS in *Diluent* from *Standard solution*
Peak identification solution 1: 10 µg/mL of USP Tacrolimus 8-epimer RS in *Diluent*
Peak identification solution 2: 10 µg/mL of USP Tacrolimus 8-propyl Analog RS in *Diluent*
Sample solution: Equivalent to 1.5 mg/mL of tacrolimus in *Diluent*. [NOTE—Shake the mixture on a mechanical shaker for 30 min, and pass through a suitable filter.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 60°
Flow rate: 1.5 mL/min
Injection volume: 40 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 3.0 between tacrolimus and ascomycin, *System suitability solution*
Relative standard deviation: NMT 10.0% for the sum of the responses of tacrolimus and tacrolimus 19-epimer, *Standard solution*
Signal-to-noise ratio: NLT 10.0, *Sensitivity solution*
Analysis
Samples: *Standard solution*, *Peak identification solution 1*, *Peak identification solution 2*, and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = sum of the peak responses of tacrolimus 19-epimer and tacrolimus from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tacrolimus in the *Sample solution* (mg/mL)
P = potency of tacrolimus in USP Tacrolimus RS (mg/mg)
F = relative response factor (see *Table 8*)

Acceptance criteria: See *Table 8*. Identify tacrolimus 8-epimer and tacrolimus 8-propyl analog using *Peak identification solution 1* and *Peak identification solution 2*. Disregard peaks that are smaller than the tacrolimus peak in the *Sensitivity solution*.

Table 8

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus hydroxy acid[a]	0.18	1.5	0.5
Tacrolimus open ring[b, c]	0.49	—	—
Ascomycin 19-epimer[d, e]	0.52	—	—
Tacrolimus 19-epimer[b, f]	0.62	—	—
Ascomycin[e, g]	0.84	—	—
Desmethyl tacrolimus[e, h]	0.91	—	—
Tacrolimus	1.0	—	—
Tacrolimus 8-epimer[i]	1.28	1.0	0.5
Tacrolimus 8-propyl analog[e, j]	1.30	—	—
Any individual unspecified impurity	—	1.0	0.2

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Table 8 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	1.5

[a] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,25a*S*,*E*)-8-Allyl-5,15,19-trihydroxy-3-{(*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl}-14,16-dimethoxy-4,10,12,18-tetramethyl-1,7,20-trioxo-1,3,4,5,6,7,8,11,12,13,14,15,16,17,18,19,20,22,23,24,25,25a-docosahydropyrido[2,1-*c*][1]oxa[4]azacyclodocosine-19-carboxylic acid.

[b] Tacrolimus open ring and tacrolimus 19-epimer are isomers of tacrolimus, which are present in equilibrium with the active ingredient. They are not to be reported as degradation products and are not included in total impurities.

[c] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.

[d] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

[e] These are process impurities that are controlled in the drug substance. They are not to be reported in the drug product.

[f] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

[g] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

[h] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,12,18-trimethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

[i] (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

[j] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which test for *Organic Impurities* the article complies. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Tacrolimus RS

USP Tacrolimus Related Compound A RS
(*E*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
$C_{43}H_{69}NO_{12}$ 792.01

USP Tacrolimus 8-epimer RS
(3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
$C_{44}H_{69}NO_{12}$ 804.02

USP Tacrolimus 8-propyl Analog RS
(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
$C_{44}H_{71}NO_{12}$ 806.03

USP Tacrolimus System Suitability Mixture RS
It contains tacrolimus, ascomycin (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
$C_{43}H_{69}NO_{12}$ 792.01
and tacrolimus 8-propyl analog (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.
$C_{44}H_{71}NO_{12}$ 806.03

Tacrolimus Compounded Oral Suspension

DEFINITION

Tacrolimus Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$).

Prepare Tacrolimus Compounded Oral Suspension 0.5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Tacrolimus capsules[a] equivalent to	50 mg of tacrolimus
Vehicle: a 1:1 mixture of Ora-Plus[b] and Syrup, *NF*, a sufficient quantity to make	100 mL

[a] Prograf 5-mg capsules, Astellas Pharma US, Inc., Deerfield, IL.

[b] Paddock Laboratories, Minneapolis, MN.

Calculate the quantity of each ingredient required for the total amount to be prepared. Empty the required number of *Tacrolimus capsules* in a suitable mortar. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tacrolimus liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

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Tacrolimus powder is not interchangeable with *Tacrolimus capsules* and should not be used.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and deionized distilled water (65:35). Filter and degas.

Standard stock solution: 0.5 mg/mL of USP Tacrolimus RS in acetonitrile

Standard solution: Pipet 1.0 mL of *Standard stock solution* into a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a nominal concentration of 50 µg/mL of tacrolimus. [NOTE—The *Standard solution* is relatively unstable, and the *Assay* should proceed immediately.]

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 µg/mL of tacrolimus. [NOTE—The *Sample solution* is relatively unstable, and the *Assay* should proceed immediately.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 70°

Flow rate: 1.7 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for tacrolimus is about 6.4 min.]

Suitability requirements

Column efficiency: NLT 2500 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Tacrolimus RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of tacrolimus in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.1–5.1

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.

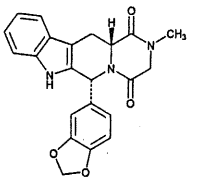
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Tacrolimus RS

Tadalafil



$C_{22}H_{19}N_3O_4$ 389.40

Pyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-, (6*R*-12a*R*)-;

(6*R*,12a*R*)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl] pyrazino[1′,2′:1,6]pyrido[3,4-*b*] indole-1,4-dione [171596-29-5].

DEFINITION

Tadalafil contains NLT 97.5% and NMT 102.5% of tadalafil ($C_{22}H_{19}N_3O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Identification solution*, as obtained in the test for *Enantiomeric and Diastereomeric Purity*.

ASSAY

• **PROCEDURE**

Solution A: Add 1.0 mL of trifluoroacetic acid to 1 L of water.

Mobile phase: Acetonitrile and *Solution A* (45:55)

Standard solution: 0.1 mg/mL of USP Tadalafil RS in acetonitrile and *Solution A* (1:1); prepare by first dissolving the standard in acetonitrile, and then diluting with *Solution A* to final volume.

Sample solution: 0.1 mg/mL of Tadalafil in acetonitrile and *Solution A* (1:1); prepare by first dissolving the sample in acetonitrile, and then diluting with *Solution A* to final volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: 285 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tadalafil ($C_{22}H_{19}N_3O_4$) in the portion of Tadalafil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Tadalafil RS in the *Standard solution* (mg/mL)

USP Monographs

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C_U = concentration of Tadalafil in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.5% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.10%, using a 1-g sample

• **ORGANIC IMPURITIES**

[NOTE—Do not use sonication during the preparation of analyte solutions.]

Solution A: Add 1.0 mL of trifluoroacetic acid to 1 L of water.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the column.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
3	85	15
30	5	95
33	5	95

Standard solution: 0.4 µg/mL of USP Tadalafil RS in acetonitrile and *Solution A* (1:1); prepare by first dissolving the standard in acetonitrile, and then diluting with *Solution A* to final volume.

Sensitivity solution: 0.2 µg/mL of USP Tadalafil RS in acetonitrile and *Solution A* (1:1) from the *Standard solution*

System suitability stock solution: To generate the 6*R*,12a*S* diastereomer of tadalafil, dissolve 4.0 mg of Tadalafil in 50 mL of a mixture of isopropyl alcohol and acetonitrile (1:1). Add 1.0 mL of 1.0 M tetrabutylammonium hydroxide in methanol, and allow to stand at room temperature for 40 min. Add 1.0 mL of trifluoroacetic acid, and dilute with a mixture of isopropyl alcohol and acetonitrile (1:1) to 100 mL.

System suitability solution: Dissolve 40 mg of Tadalafil in 50 mL of acetonitrile. Add 2.0 mL of the *System suitability stock solution*, and dilute with *Solution A* to 100 mL.

Sample solution: 0.4 mg/mL of Tadalafil in acetonitrile and *Solution A* (1:1); prepare by first dissolving the sample in acetonitrile, and then diluting with *Solution A* to final volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution, Sensitivity solution,* and *System suitability solution*

[NOTE—The relative retention times for tadalafil and the 6*R*,12a*S* diastereomer of tadalafil are about 1.0 and 1.03, respectively.]

Suitability requirements

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Peak-to-valley ratio: The ratio of the height of the 6*R*, 12a*S* diastereomer peak to the height of the valley between the 6*R*,12a*S* diastereomer peak and tadalafil is NLT 3.3, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tadalafil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of tadalafil from the *Standard solution*

C_S = concentration of USP Tadalafil RS in the *Standard solution* (mg/mL)

C_U = concentration of Tadalafil in the *Sample solution* (mg/mL)

Acceptance criteria

[NOTE—Disregard peaks due to the 6*R*,12a*S* and 6*S*, 12a*R* diastereomers of tadalafil, which co-elute at a retention time of about 1.03 relative to tadalafil. The diastereomers are controlled in the test for *Enantiomeric and Diastereomeric Purity*.]

Individual impurities: NMT 0.1%

Total impurities: NMT 0.3%

Reporting level for impurities: 0.05%

• **ENANTIOMERIC AND DIASTEREOMERIC PURITY**

Mobile phase: Hexanes and isopropyl alcohol (50:50)

Diluent: Hexanes, isopropyl alcohol, and acetonitrile (40:40:20)

Identification solution: 0.5 mg/mL of USP Tadalafil RS in *Diluent*. [NOTE—This solution is used for *Identification* test *B*.]

Standard stock solution: 50 µg/mL of USP Tadalafil RS in *Diluent*

Standard solution: 0.5 µg/mL of USP Tadalafil RS in *Diluent* from the *Standard stock solution*

System suitability stock solution: To generate the 6*R*,12a*S* diastereomer of tadalafil, dissolve 25 mg of Tadalafil in 40 mL of *Diluent*. Add 1.0 mL of 1.0 M tetrabutylammonium hydroxide in methanol, and allow to stand at room temperature for 20 min. Add 1.0 mL of trifluoroacetic acid, and dilute with *Diluent* to 50 mL.

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* and 10 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Sensitivity solution: 0.25 µg/mL of USP Tadalafil RS in *Diluent* from *Standard solution*

Sample solution: 0.5 mg/mL of Tadalafil in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: 222 nm

Column: 4.6-mm × 25-cm; 10-µm packing L51

Column temperature: 30°

Flow rate: 0.75 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0, between the 6*R*,12a*S* diastereomer and tadalafil, *System suitability solution*

Tailing factor: NLT 0.8 and NMT 1.5, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Signal-to-noise ratio: NLT 20, *Sensitivity solution*

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Analysis
Samples: *Identification solution, Standard solution,* and *Sample solution*
Calculate the percentage of each stereoisomer impurity in the portion of Tadalafil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each stereoisomer impurity from the *Sample solution*
r_S = peak response of tadalafil from the *Standard solution*
C_S = concentration of USP Tadalafil RS in the *Standard solution* (mg/mL)
C_U = concentration of Tadalafil in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2.*

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
6*R*,12a*S* diastereomer[a]	0.79	0.1
Tadalafil	1.0	—
6*S*,12a*S* enantiomer[b]	1.4	0.1
6*S*,12a*R* diastereomer[c]	1.7	0.1

[a] (6*R*,12a*S*)-6-(1,3-Benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methylpyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione.
[b] (6*S*,12a*S*)-6-(1,3-Benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methylpyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione.
[c] (6*S*,12a*R*)-6-(1,3-Benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methylpyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample under vacuum at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tadalafil RS

Tadalafil Tablets

DEFINITION
Tadalafil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of tadalafil ($C_{22}H_{19}N_3O_4$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy* **197D** or **197K**▲ (CN 1-May-2020)
Standard: Add 10 mg of USP Tadalafil RS to 15 mL of water. Shake for 20 min, centrifuge for 10 min, and discard the supernatant. Suspend the precipitate in 8 mL of ethyl acetate, and shake for 5 min. Centrifuge for 10 min, and collect the supernatant. Dry the supernatant under a stream of nitrogen. The supernatant may be heated up to 70° to aid evaporation of the ethyl acetate. [NOTE—Ethyl acetate must be completely removed to prevent interference in the spectrum.]
Sample: Transfer a quantity of Tablets, equivalent to 10–20 mg of tadalafil, into a suitable container. Add 15 mL of water, and shake for 10 min, or until the Tablets are completely dispersed. Centrifuge for 10 min, and discard the supernatant. Suspend the precipitate in 8 mL of ethyl acetate, and shake for 5 min. Centrifuge for 10 min, and collect the supernatant. Dry the supernatant under a stream of nitrogen. The supernatant may be heated up to 70° to aid evaporation of the ethyl acetate. [NOTE—Ethyl acetate must be completely removed to prevent interference in the spectrum.]
Acceptance criteria: Meets the requirements over the range from 1700–400 cm^{-1}
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, water, and trifluoroacetic acid (35: 65: 0.1)
Diluent: Acetonitrile and water (1:1)
Standard solution: 0.25 mg/mL of USP Tadalafil RS in *Diluent*
System suitability solution: To partially convert tadalafil to the 6*R*,12a*S* diastereomer, transfer 25 mL of the *Standard solution* into a suitable container. Add 0.25 mL of 5 N sodium hydroxide, mix well, and let stand for 30 min. Neutralize the solution to pH 7 by drop-wise addition of trifluoroacetic acid. [NOTE—This solution is stable for 1 month when stored in a refrigerator.]
Sample solution: Place NLT 20 Tablets into an appropriate size volumetric flask. Fill the flask about halfway with *Diluent,* and shake the mixture for about 15 min to disintegrate the Tablets. If any large fragments remain, sonicate the solution for 2 min or until fragments are dispersed. Dilute with *Diluent* to volume, and mix. Allow the solution to stand for at least 1 h to further aid Tablet dissolution. If necessary, shake the solution and perform a secondary dilution to obtain a final nominal concentration of 0.25 mg/mL. Centrifuge or filter the solution. [NOTE—The initial concentration before a secondary dilution step should not exceed 6 mg/mL.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for tadalafil and the 6*R*,12a*S* diastereomer of tadalafil are about 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 3 between tadalafil and the 6*R*,12a*S* diastereomer peak, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tadalafil ($C_{22}H_{19}N_3O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

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r_S = peak response from the *Standard solution*
C_S = concentration of USP Tadalafil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tadalafil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.5% sodium dodecyl sulfate; 1000 mL
Apparatus 2: 50 rpm
Time: 10 and 30 min
Mobile phase: Methanol and water (50:50)
Standard stock solution: 0.25 mg/mL of USP Tadalafil RS in acetonitrile and water (1:1)
Standard solution: 0.0075 mg/mL of USP Tadalafil RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 5.0-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tadalafil ($C_{22}H_{19}N_3O_4$) dissolved at each time point (Q_i):

$$Q_{10} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_{30} = (Q_{10} \times v/V) + [(r_U/r_S) \times (C_S/L) \times (V - v) \times 100]$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tadalafil RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL
v = volume of the sample withdrawn at initial time point (mL)

Tolerances: NLT 40% *(Q)* of the labeled amount of tadalafil is dissolved in 10 min and NLT 80% *(Q)* of the labeled amount of tadalafil is dissolved in 30 min.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Diluent: Acetonitrile and water (1:1)
Standard solution: 0.1–0.2 mg/mL of USP Tadalafil RS in *Diluent*
Sample solution: Add 1 Tablet to a suitable volumetric flask to prepare a solution having a nominal concentration of 0.1–0.2 mg/mL of tadalafil. Add a volume of *Diluent* equivalent to 50% of the volume of the flask, and mechanically shake for 15 min. Dilute with *Diluent* to volume, and pass a portion of the solution through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Cell: 0.1 cm
Analytical wavelength: Absorption maximum at about 285 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tadalafil ($C_{22}H_{19}N_3O_4$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tadalafil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tadalafil in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements for coated Tablets

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, Diluent, Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.25 µg/mL of USP Tadalafil RS in *Diluent* from the *Standard solution*
System suitability
Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
[NOTE—The relative retention times for tadalafil and the 6*R*,12a*S* diastereomer of tadalafil are about 1.0 and 1.2, respectively.]
Suitability requirements
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Resolution: NLT 3 between tadalafil and the 6*R*,12a*S* diastereomer peak, *System suitability solution*
Signal-to-noise ratio: NLT 20, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the peak responses from the *Sample solution*

Acceptance criteria
Individual impurities: NMT 0.2%
Total impurities: NMT 0.3%
Reporting level for impurities: 0.05%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tadalafil RS

USP Monographs

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Tadalafil Compounded Oral Suspension

DEFINITION

Tadalafil Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tadalafil ($C_{22}H_{19}N_3O_4$).

Prepare Tadalafil Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Tadalafil tablets[a] equivalent to	500 mg
Vehicle: A 1:1 mixture of Ora-Plus[b] and Ora-Sweet[b], a sufficient quantity to make	100 mL

[a] Adcirca 20-mg tablets, Eli Lilly and Company, Indianapolis, IN.
[b] Perrigo Pharmaceuticals, Allegan, MI.

Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Wet the powder with a small amount of the *Vehicle*, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tadalafil liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Solution A: 10 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0. Pass through a nylon filter of 0.45-µm pore size.

Solution B: Water adjusted with phosphoric acid to a pH of 3.0

Mobile phase: Acetonitrile and *Solution A* (60:40)

Diluent: Acetonitrile and *Solution B* (50:50)

Standard stock solution: 5 mg/mL of tadalafil prepared from USP Tadalafil RS in acetonitrile. Mix well, and store at 2°–8°.

Standard solution: Transfer 2.0 mL of the *Standard stock solution* into a 500-mL volumetric flask, and dilute with *Diluent* to volume. Mix well. Centrifuge an aliquot for 5 min at 14,000 rpm. Protect from light, and store at 2°–8°.

Sample solution: Transfer 2.0 mL of Oral Suspension into a 500-mL volumetric flask, and dilute with *Diluent* to volume. Mix well. Centrifuge an aliquot for 5 min at 14,000 rpm. Protect from light, and store at 2°–8°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 5°

Column: 30°

Flow rate: 0.8 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for tadalafil is about 4.5 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tadalafil ($C_{22}H_{19}N_3O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tadalafil from the *Sample solution*

r_S = peak response of tadalafil from the *Standard solution*

C_S = concentration of tadalafil in the *Standard solution* (mg/mL)

C_U = nominal concentration of tadalafil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.3–5.3

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Tadalafil RS

Talc

DEFINITION

Talc is a powdered, selected, natural, hydrated magnesium silicate. Pure talc has the formula $Mg_3Si_4O_{10}(OH)_2$. It may contain variable amounts of associated minerals among which chlorites (hydrated aluminum and magnesium silicates), magnesite (magnesium carbonate), calcite (calcium carbonate), and dolomite (calcium and magnesium carbonate) are predominant.

IDENTIFICATION

- **A. INFRARED ABSORPTION:** The IR spectrum of a potassium bromide dispersion of it exhibits maxima at 3677 ± 2 cm^{-1}, at 1018 ± 2 cm^{-1}, and at 669 ± 2 cm^{-1}.
- **B. PROCEDURE**

Sample: 100 mg

Analysis: Mix about 200 mg of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate, and melt in a platinum crucible. To the melt add the *Sample*, and continue heating until fusion is complete. Cool, and transfer the fused mixture to a dish or beaker with the aid of about 50 mL of hot water. Add hydrochloric acid to the liquid until effervescence ceases, then add 10 mL more of the acid, and evaporate the mixture on a steam bath to dryness. Cool, add 20 mL of water, boil, and filter the mixture. Save the insoluble residue for use in *Identification* test *C*.

To 5 mL of the filtrate add 1 mL of 6 N ammonium hydroxide and 1 mL of ammonium chloride TS. Filter, if necessary, and add 1 mL of dibasic sodium phosphate TS to the filtrate.

Acceptance criteria: A white, crystalline precipitate of magnesium ammonium phosphate is formed.

- **C. PROCEDURE**

Sample: 100 mg of the insoluble residue from *Identification* test *B*

Analysis: In a lead or platinum crucible and using a copper wire, mix the *Sample* with about 10 mg of sodium fluoride and a few drops of sulfuric acid to give a thin slurry. Cover the crucible with a thin transparent plate of plastic under which a drop of water is suspended, and warm gently.

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Acceptance criteria: Within a short time, a white ring is rapidly formed around the drop of water.

ASSAY

• **CONTENT OF MAGNESIUM**

Lanthanum chloride solution: To 5.9 g of lanthanum oxide slowly add 10 mL of hydrochloric acid, and heat to boiling. Allow to cool, and dilute with water to 100 mL.

Magnesium standard stock solution: 10 µg/mL of magnesium, prepared by diluting an 8.365 mg/mL solution of magnesium chloride in diluted hydrochloric acid with water (1 in 100)

Magnesium standard solutions: Into four identical 100-mL volumetric flasks, each containing 10.0 mL of hydrochloric acid and 10 mL of *Lanthanum chloride solution*, transfer respectively 2.5, 3.0, 4.0, and 5.0 mL of *Magnesium standard stock solution*, and dilute with water to volume.

Sample stock solution: [**CAUTION**—Perchlorates mixed with heavy metals are known to be explosive. Take proper precautions while performing this analysis.]

Weigh 500 mg of Talc in a 100-mL polytetrafluoroethylene dish. Add 5 mL of hydrochloric acid, 5 mL of lead-free nitric acid, and 5 mL of perchloric acid. Stir gently, then add 35 mL of hydrofluoric acid, and evaporate slowly on a hot plate to moist dryness (until about 0.5 mL remains). To the residue, add 5 mL of hydrochloric acid, cover with a watch glass, heat to boiling, and allow to cool. Rinse the watch glass and the dish with water, transfer to a 50-mL volumetric flask, and dilute with water to volume.

Sample solution: Dilute the *Sample stock solution* with water (1 in 200). Transfer 4.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of hydrochloric acid and 10 mL of *Lanthanum chloride solution*, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Magnesium emission line at 285.2 nm

Lamp: Magnesium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Magnesium standard solutions* and *Sample solution*

Concomitantly determine the absorbance of the solutions.

Acceptance criteria: 17.0%–19.5%

IMPURITIES

• **WATER-SOLUBLE SUBSTANCES**

Sample: 10.0 g

Analysis: To the *Sample* add 50 mL of carbon dioxide-free water, heat to boiling, and boil under a reflux condenser for 30 min. Allow to cool, filter, and dilute with carbon dioxide-free water to 50.0 mL. Test with litmus paper. Evaporate 25.0 mL of the filtrate to dryness, dry at 105° for 1 h, and weigh the residue.

Acceptance criteria: The filtrate is neutral to litmus paper, and the weight of the residue is NMT 5 mg (0.1%).

• **LIMIT OF IRON**

Iron standard stock solution: 250 µg/mL of iron from 4.840 g ferric chloride in a 150 g/L solution of hydrochloric acid in water. Prepare immediately before use.

Iron standard solutions: Into four 100-mL volumetric flasks, each containing 50.0 mL of 0.5 N hydrochloric acid, transfer respectively 2.0, 2.5, 3.0, and 4.0 mL of the *Standard iron stock solution*, and dilute each flask with water to volume.

Sample stock solution: Transfer 10.0 g of Talc to a conical flask fitted with a reflux condenser. Gradually add 50 mL of 0.5 N hydrochloric acid while stirring, and heat on a water bath for 30 min. Allow to cool. Transfer the mixture to a beaker, and allow the undissolved material to settle. Filter the supernatant into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the residue and the beaker with three 10-mL portions of hot water. Wash the filter with 15 mL of hot water, allow the filtrate to cool, and dilute with water to 100.0 mL.

Sample solution: Transfer 2.5 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 50.0 mL of 0.5 N hydrochloric acid, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Iron emission line at 248.3 nm

Lamp: Iron hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Iron standard solutions* and *Sample solution*

Concomitantly determine the absorbance of the solutions. Make any correction using a deuterium lamp.

Acceptance criteria: NMT 0.25%

• **LIMIT OF LEAD**

Sample solution: Use the *Sample stock solution* as directed in the test for *Limit of Iron*.

Diluent: Nitric acid in water (1 in 100)

Lead standard stock solution: 10 µg/mL of lead prepared as follows. Dissolve 160 mg of lead nitrate in 100 mL of *Diluent*, and dilute with water to 1000 mL. Dilute this solution with water (1 in 10).

Lead standard solutions: Into four identical 100-mL volumetric flasks, each containing 50.0 mL of 0.5 N hydrochloric acid, transfer respectively 5.0, 7.5, 10.0, and 12.5 mL of *Lead standard stock solution*, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Lead emission line at 217.0 nm

Lamp: Lead hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Lead standard solutions* and *Sample solution*

Concomitantly determine the absorbance of the solutions.

Acceptance criteria: NMT 10 ppm

• **LIMIT OF CALCIUM**

Lanthanum chloride solution: Prepare as directed in the *Assay*.

Calcium standard stock solution: 100 µg/mL of calcium, prepared immediately before use by diluting a 3.67 mg/mL solution of calcium chloride dihydrate in diluted hydrochloric acid with water (1 in 10)

Calcium standard solutions: Into four identical 100-mL volumetric flasks, each containing 10.0 mL of hydrochloric acid and 10 mL of *Lanthanum chloride solution*, transfer respectively 1.0, 2.0, 3.0, and 4.0 mL of *Calcium standard stock solution*, and dilute each solution with water to volume.

Sample stock solution: Prepare as directed in the *Assay*.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 10.0 mL of hydrochloric acid and 10 mL of *Lanthanum chloride solution*, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

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Lamp: Calcium hollow-cathode
Flame: Nitrous oxide–acetylene
Analysis
Samples: *Calcium standard solutions* and *Sample solution*
Concomitantly determine the absorbance of the solutions.
Acceptance criteria: NMT 0.9%

• **LIMIT OF ALUMINUM**
Cesium chloride solution: 25.3 mg/mL of cesium chloride in water.
Aluminum standard stock solution: 100 µg/mL of aluminum, prepared immediately before use by diluting an 8.947 mg/mL solution of aluminum chloride in water with water (1 in 10)
Aluminum standard solutions: Into four identical 100-mL volumetric flasks, each containing 10.0 mL of hydrochloric acid and 10 mL of *Cesium chloride solution*, transfer respectively 5.0, 10.0, 15.0, and 20.0 mL of *Aluminum standard stock solution*, and dilute with water to volume.
Sample stock solution: Proceed as directed in the *Assay*. Transfer 5 mL of the *Cesium chloride solution* to the 50-mL flask prior to transfer of the residue, and dilute with water to volume.
Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 10 mL of the *Cesium chloride solution* and 10.0 mL of hydrochloric acid, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Aluminum emission line at 309.3 nm
Lamp: Aluminum hollow-cathode
Flame: Nitrous oxide–acetylene
Analysis
Samples: *Aluminum standard solutions* and *Sample solution*
Concomitantly determine the absorbance of the solutions.
Acceptance criteria: NMT 2.0%

• **ABSENCE OF ASBESTOS**
[NOTE—Suppliers of Talc may use one of the following methods to determine the absence of asbestos.]
Proceed as directed for *Procedure 1* or *Procedure 2*. If either test is positive, perform *Procedure 3*.
Procedure 1: Infrared Absorption
The IR absorption spectrum of a potassium bromide dispersion of Talc at the absorption band at 758 ± 1 cm^{-1}, using scale expansion, may indicate the presence of tremolite or chlorite. If the absorption band remains after ignition of the substance at 850° for at least 30 min, it indicates the presence of tremolite. In the range 600 cm^{-1} to 650 cm^{-1} using scale expansion, any absorption band or shoulder may indicate the presence of serpentines.
Procedure 2: Use the following conditions (see *X-Ray Diffraction* ⟨941⟩):
Cu Kα monochromatic 40 kV radiation, 24–30 mA; the incident slit is set at 1°; the detection slit is set at 0.2°; the goniometer speed is 1/10° 2Θ/min; the scanning range is 10°–13° 2Θ and 24°–26° 2Θ; the sample is not oriented. Prepare a random sample, and place on the sample holder. Pack and smooth its surface with a polished glass microscope slide. Record the diffractograms: the presence of amphiboles is detected by a diffraction peak at 10.5 ± 0.1° 2Θ, and the presence of serpentines is detected by diffraction peaks at 24.3 ± 0.1° 2Θ to 12.1 ± 0.1° 2Θ.
Procedure 3: The presence of asbestos (see *Optical Microscopy* ⟨776⟩) is shown if there is a range of length to width ratios of 20:1 to 100:1, or higher for fibers longer than 5 µm; if there is a capability of splitting into very thin fibrils; and if there are two or more of the following four criteria: (1) parallel fibers occurring in bundles, (2) fiber bundles displaying frayed ends, (3) fibers in the form of thin needles, and (4) matted masses of individual fibers and/or fibers showing curvature.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩
Intended for topical administration
Total aerobic microbial count: NMT 100 cfu/g
Total combined molds and yeasts count: NMT 50 cfu/g
Intended for oral administration
Total aerobic microbial count: NMT 1000 cfu/g
Total combined molds and yeasts count: NMT 100 cfu/g

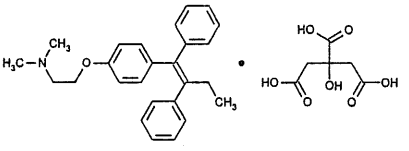
• **ACIDITY AND ALKALINITY:** Boil 2.5 g of Talc with 50 mL of carbon dioxide-free water under reflux. Filter under vacuum. To 10 mL of the filtrate, add 0.1 mL of bromothymol blue TS. Add 0.01 N hydrochloric acid until the indicator changes color. To a second 10 mL of the filtrate, add 0.1 mL of phenolphthalein TS. Add 0.01 N sodium hydroxide until the indicator turns pink.
Acceptance criteria: NMT 0.4 mL of 0.01 N hydrochloric acid is required to change the color of the bromothymol blue indicator. NMT 0.3 mL of 0.01 N sodium hydroxide is required to change the color of the phenolpthalein indicator to pink.

• **LOSS ON IGNITION** ⟨733⟩
Sample: 1 g
Analysis: Ignite at 1075 ± 25° to constant weight.
Acceptance criteria: It loses NMT 7.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.
• **LABELING:** The label states, where applicable, that the substance is suitable for oral or topical administration. The certificate of analysis states the absence of asbestos. It also indicates which method specified in the test for *Absence of Asbestos* was used for analysis.

Tamoxifen Citrate



$C_{26}H_{29}NO \cdot C_6H_8O_7$ 563.64
Ethanamine, 2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethyl, (*Z*)-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1).
(*Z*)-2-[*p*-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine citrate (1:1) [54965-24-1].

» Tamoxifen Citrate contains not less than 99.0 percent and not more than 101.0 percent of $C_{26}H_{29}NO \cdot C_6H_8O_7$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

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USP Reference standards ⟨11⟩—
USP Tamoxifen Citrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy, 197K*▲ (CN 1-May-2020): a single band in the 1700 to 1740 cm^{-1} region of the spectrum.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: methanol.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Limit of *E*-isomer—
Mobile phase—Prepare a methanol solution containing, in each liter, 320 mL of water, 2 mL of glacial acetic acid, and 1.08 g of sodium 1-octanesulfonate.
Standard preparation—Dissolve a suitable quantity, accurately weighed, of USP Tamoxifen Citrate RS in *Mobile phase* to obtain a solution having a known concentration of about 600 µg per mL.
Test preparation—Using about 30 mg of Tamoxifen Citrate, accurately weighed, proceed as directed under *Standard preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 0.7 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the responses of the major peak: the relative standard deviation is not more than 3.0%, and the relative retention time of the minor *E*-isomer peak to that of the *Z*-isomer peak is not greater than 0.93.
Procedure—Separately introduce equal volumes (about 20 µL) of the *Test preparation* and the *Standard preparation* into the liquid chromatograph by means of a suitable sampling valve. Measure the minor peak responses for the *E*-isomer obtained from the *Standard preparation* and the *Assay preparation*. Calculate the quantity, in mg, of *E*-isomer ($C_{26}H_{29}NO \cdot C_6H_8O_7$) in the portion of Tamoxifen Citrate taken by the formula:

$$0.05C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of the *E*-isomer as the citrate, based on its declared content in USP Tamoxifen Citrate RS in the *Standard preparation*, and the r_U and r_S are the minor peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. The *E*-isomer content is not more than 0.3% of tamoxifen citrate ($C_{26}H_{29}NO \cdot C_6H_8O_7$).

Iron ⟨241⟩—Accurately weigh 1.0 g, and transfer to a suitable crucible. Add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 10 mL of warm 0.1 N hydrochloric acid, and digest for about 5 minutes. Transfer the contents of the crucible with the aid of small portions of water to a 50-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL from the volumetric flask into a color-comparison tube, dilute with water to 45 mL, add 2 mL of hydrochloric acid, and mix. The limit is 0.005%.

Related impurities—
Test preparation A—Disperse about 3 g in 100 mL of water in a separator. Over a 10-minute period add 50 mL of 0.5 N sodium hydroxide, with mixing. Extract with two 50-mL portions of ether, and combine the extracts. Wash with 20 mL of water, remove the water layer, and dry the ether layer over anhydrous sodium sulfate. Evaporate the ether layer under nitrogen, and dry in vacuum at room temperature for 2 hours. Accurately weigh 1.5 g of the residue into a 10-mL volumetric flask, add 5.0 mL of a mixture of 5 volumes of acetic anhydride and 95 volumes of pyridine, and heat at 60° for 10 to 15 minutes. Cool, dilute with the same solvent mixture to volume, and mix.
Test preparation B—Using the same acetic anhydride-pyridine mixture, prepare a 1:200 dilution of *Test preparation A*.
Chromatographic system (see *Chromatography* ⟨621⟩)—Typically, the gas chromatograph is equipped with a flame-ionization detector, and contains a 4-mm × 1-m glass column packed with 5% liquid phase G17 on 100- to 120-mesh support S1AB conditioned at 300° for 24 hours. The column and injection port temperatures are maintained at about 260° and the detector temperature at about 300°. Dry helium is used as the carrier gas at a flow rate of about 60 mL per minute. In a suitable chromatogram, five replicate injections of *Test preparation B* show a relative standard deviation of not more than 3.0%.
Procedure—Inject equal portions (about 2 µL), accurately measured, of *Test preparation A* and *Test preparation B* into the chromatograph, and record the chromatograms from 0.1 to 5.0, relative to the retention time of the major peak. Measure the individual areas of the peaks other than those produced by the solvent and the tamoxifen on the chromatograms obtained from *Test preparation A*, and calculate their sum. No single peak area is greater than the total area of the tamoxifen peak on the chromatogram obtained from *Test preparation B* (0.5%), and the sum of the peak areas is not greater than twice the total area of the tamoxifen peak on the chromatogram obtained from *Test preparation B* (1.0%).

Assay—Weigh accurately about 1 g of Tamoxifen Citrate, and dissolve in 150 mL of glacial acetic acid. Titrate the solution with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass indicator electrode and a silver-silver chloride reference electrode. Each mL of 0.1 N perchloric acid is equivalent to 56.36 mg of $C_{26}H_{29}NO \cdot C_6H_8O_7$.

Tamoxifen Citrate Tablets

» Tamoxifen Citrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of tamoxifen ($C_{26}H_{29}NO$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Tamoxifen Citrate RS

Identification—
A: The UV absorption spectrum of the *Test preparation*, obtained as directed in the test for *Content uniformity*, exhibits

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maxima and minima at the same wavelengths as that of the *Standard preparation,* concomitantly measured.

B: To 1 Tablet contained in a 15-mL tube add 4 mL of pyridine and 2 mL of acetic anhydride: an immediate yellow color is produced on shaking. Then heat gently on a steam bath: a rose-pink to a deep red color develops, indicating the presence of citrate ion.

Dissolution ⟨711⟩—

Medium: 0.02 N hydrochloric acid; 1000 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of tamoxifen ($C_{26}H_{29}NO$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 275 nm of filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Tamoxifen Citrate RS in the same *Medium.*

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{26}H_{29}NO$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Standard solution—Dissolve an accurately weighed quantity of USP Tamoxifen Citrate RS in methanol to obtain a solution having a known concentration of about 15 µg per mL.

Test solution—Place 1 Tablet in a 100-mL volumetric flask, and crush with a stirring rod. Add about 75 mL of methanol, and shake for about 5 minutes. Dilute with methanol to volume, mix, and filter the solution through paper. Pipet 10 mL of the filtrate into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells at the wavelength of maximum absorbance at about 275 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of tamoxifen ($C_{26}H_{29}NO$) in the Tablets taken by the formula:

$$(371.51/563.64)(TC/D)(A_U/A_S)$$

in which 371.51 and 563.64 are the molecular weights of tamoxifen and tamoxifen citrate, respectively; T is the labeled quantity, in mg, of tamoxifen in the Tablet; C is the concentration, in µg per mL, of USP Tamoxifen Citrate RS in the *Standard solution;* D is the concentration, in µg per mL, of tamoxifen in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the *Test solution* and the *Standard solution,* respectively.

Assay—

Mobile phase—Prepare a methanol solution containing, in each liter, 320 mL of water, 2 mL of glacial acetic acid, and 1.08 g of sodium 1-octanesulfonate.

Standard preparation—Dissolve a suitable quantity, accurately weighed, of USP Tamoxifen Citrate RS in *Mobile phase* to obtain a solution having a known concentration of about 200 µg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of tamoxifen, to a stoppered, 50-mL centrifuge tube. Pipet 30 mL of *Mobile phase* into the tube, and shake by mechanical means for not less than 15 minutes. Centrifuge at about 1000 rpm, pipet 5 mL of the clear supernatant into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

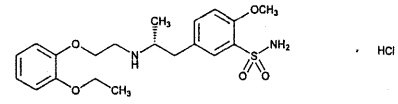
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph by means of a suitable sampling valve, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of tamoxifen ($C_{26}H_{29}NO$) in the portion of Tablets taken by the formula:

$$0.15C(371.51/563.64)(r_U/r_S)$$

in which 371.51 and 563.64 are the molecular weights of tamoxifen and tamoxifen citrate, respectively; C is the concentration, in µg per mL, of USP Tamoxifen Citrate RS in the *Standard preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Tamsulosin Hydrochloride



$C_{20}H_{28}N_2O_5S \cdot HCl$ 444.97

Benzenesulfonamide, 5-[2-[[2-(2-ethoxyphenoxy)ethyl] amino]propyl]-2-methoxy-, monohydrochloride, (*R*)-; (–)-(*R*)-5-[2-[[2-(*o*-Ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide monohydrochloride [106463-17-6].

DEFINITION

Tamsulosin Hydrochloride contains NLT 98.0% and NMT 102.0% of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K.▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
  Sample solution: 7.5 mg/mL in water, using heat to dissolve the sample. In an ice bath, cool 5 mL of the solution. Add 3 mL of diluted nitric acid, and shake until mixed thoroughly. Allow to stand for 30 min at room temperature, and filter.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

  Buffer: Transfer 0.1 g of octanesulfonic acid sodium salt and 1.0 mL of phosphoric acid into 1 L of water, and mix.

  Mobile phase: Acetonitrile and *Buffer* (20:80)

  Diluent: Acetonitrile and water (20:80)

  Standard solution: 0.5 mg/mL of USP Tamsulosin Hydrochloride RS in *Diluent*

  Sample solution: 0.5 mg/mL of Tamsulosin Hydrochloride in *Diluent*

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *System Suitability.*)

  Mode: LC

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Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.85% for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) in the portion of Tamsulosin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tamsulosin from the *Sample solution*
r_S = peak response of tamsulosin from the *Standard solution*
C_S = concentration of USP Tamsulosin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Tamsulosin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
- **Organic Impurities, Procedure 1:** Use for impurities eluting before tamsulosin

Buffer: Dissolve 8.7 mL of perchloric acid (70%) and 3.0 g of sodium hydroxide in 1900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 2.0, and add sufficient water to make 2000 mL.
Mobile phase: Acetonitrile and *Buffer* (3:7)
System suitability solution: 25 µg/mL of Tamsulosin Hydrochloride and 50 µg/mL of propylparaben in *Mobile phase*
Sample solution: 5.0 mg/mL of Tamsulosin Hydrochloride in *Mobile phase*
Standard solution: 10 µg/mL of Tamsulosin Hydrochloride from the *Sample solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.3 mL/min
Injection volume: 10 µL
[Note—Record the chromatogram for NLT 1.5 times the retention time of tamsulosin.]
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 12 between tamsulosin and propylparaben, *System suitability solution*. [Note—The elution order is tamsulosin followed by propylparaben.]
Relative standard deviation: NMT 4% for six replicate injections, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of any individual impurity eluting before the tamsulosin peak in the portion of Tamsulosin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity eluting before tamsulosin from the *Sample solution*
r_S = peak response of tamsulosin from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: The reporting level for impurities is 0.05%.
Any individual impurity: NMT 0.10%. [Note—If present, the des-ethoxy and methoxy impurities eluting at the relative retention time of about 0.8 are not separated by this method, and should be integrated together to determine conformance. (Des-ethoxy impurity is 2-methoxy-5-[(2*R*)-2-[(2-phenoxyethyl)amino]propyl] benzenesulfonamide, and methoxy impurity is 2-methoxy-5-[(2*R*)-2-[[2-(2-methoxyphenoxy)ethyl] amino]propyl]benzenesulfonamide.) NMT 0.15% of the sum of des-ethoxy and methoxy impurities is found.]

- **Organic Impurities, Procedure 2:** Use for impurities eluting after tamsulosin

Buffer, Sample solution, and **Standard solution:** Prepare as directed in *Procedure 1*.
[Note—Use the *Mobile phase* in *Procedure 1* to prepare the *Sample solution* and *Standard solution*.]
Mobile phase: Acetonitrile and *Buffer* (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
[Note—Record the chromatogram for NLT 5 times the retention time of tamsulosin.]
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: Use a column that meets the resolution requirements in *Procedure 1*.
Relative standard deviation: NMT 4% for six replicate injections
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of any individual impurity eluting after the tamsulosin peak in the portion of Tamsulosin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity eluting after tamsulosin from the *Sample solution*
r_S = peak response of tamsulosin from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: The reporting level for impurities is 0.05%.
Any individual impurity: NMT 0.10%
Total impurities: NMT 0.2%, including all impurities in *Procedure 1* and *Procedure 2*

- **Enantiomeric Purity**

Mobile phase: Hexane, dehydrated alcohol, methanol, and diethylamine (650:200:150:1)

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System suitability solution: 40 µg/mL of USP Racemic Tamsulosin Hydrochloride RS in methanol
Sample solution: 2.0 mg/mL of Tamsulosin Hydrochloride in methanol
Standard solution: 2 µg/mL of Tamsulosin Hydrochloride from the *Sample solution* in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L51
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for the *S*-enantiomer and tamsulosin are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2 between the *S*-enantiomer and tamsulosin
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the *S*-enantiomer in the portion of Tamsulosin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *S*-enantiomer from the *Sample solution*
r_S = peak response of tamsulosin from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.3% of the *S*-enantiomer

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tamsulosin Hydrochloride RS
USP Racemic Tamsulosin Hydrochloride RS

Tamsulosin Hydrochloride Capsules

DEFINITION
Tamsulosin Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE 1**
Solution A: Dilute 20 mL of hydrochloric acid with water to 1000 mL.
Solution B: Dissolve 8.7 mL of perchloric acid and 3.0 g of sodium hydroxide in 1900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 2.0, and add sufficient water to make 2000 mL.
Mobile phase: Acetonitrile and *Solution B* (3:7)
Internal standard solution: 0.4 mg/mL of propylparaben in a mixture of acetonitrile and water (3:7)
Standard stock solution: 0.5 mg/mL of USP Tamsulosin Hydrochloride RS in a mixture of acetonitrile and water (3:7)
Standard solution: Transfer 2.0 mL of the *Standard stock solution* to a suitable container, add 5.0 mL of the *Internal standard solution*, and add *Mobile phase* to make 40 mL.
Sample solution: Weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the powder, equivalent to about 1 mg of tamsulosin hydrochloride based on the label claim, into a Teflon-lined, screw-capped centrifuge tube. Place approximately 100 glass balls with a diameter of about 5 mm into the tube, add 20 mL of 0.05 N sodium hydroxide, heat at 50° for 10 min, and shake well for 30 min. Add to the solution 15 mL of a mixture of acetonitrile and *Solution A* (2:1), and shake well. Add 5.0 mL of the *Internal standard solution*, and shake well. Centrifuge at 1500 rpm for 10 min, and use the supernatant, passing it, if necessary, through a membrane filter of 0.5-µm or smaller pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.0-mm × 15-cm or 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min for the 4.0-mm column and 1.3 mL/min for the 4.6-mm column. [NOTE—The flow rate can be adjusted as needed to achieve a recommended retention time of approximately 6 min for tamsulosin.]
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 12 between tamsulosin and propylparaben. [NOTE—The elution order is tamsulosin hydrochloride followed by propylparaben.]
Relative standard deviation: NMT 2.0% for the ratios of the peak areas for tamsulosin and the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times [C_S \times (V_S/W)] \times 100$$

R_U = peak area ratio of tamsulosin to the internal standard from the *Sample solution*
R_S = peak area ratio of tamsulosin to the internal standard from the *Standard solution*
C_S = concentration of USP Tamsulosin Hydrochloride RS in the *Standard stock solution* (mg/mL)
V_S = volume of the *Standard stock solution* taken to prepare the *Standard solution* (mL)
W = amount of tamsulosin hydrochloride, based on the label claim, taken to prepare the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%
• **PROCEDURE 2:** Use this *Procedure* for Capsules labeled to meet the requirements of *Dissolution Test 2*.

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Solution B and **Mobile phase:** Proceed as directed for *Procedure 1*.

Buffer: Dissolve 3.4 g of monobasic potassium phosphate in 1 L of water. Adjust with 2 N sodium hydroxide to a pH of 5.80 ± 0.05.

Standard solution: Prepare a solution containing 1.2 mg/mL of USP Tamsulosin Hydrochloride RS in methanol. Dilute with *Buffer* to obtain a solution containing 3.2 µg/mL.

Sample solution: Weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the Capsule contents, equivalent to 1.6 mg of tamsulosin hydrochloride, into a 100-mL volumetric flask. Add 20 mL of methanol, stir for 30 min, sonicate for 30 min, and stir again for 30 min. Add 40 mL of methanol, sonicate for another 30 min, and stir for another 60 min. Dilute with methanol to volume, mix well, and allow the solution to stand for 5 min. Dilute 5 mL of this solution with *Buffer* to 25 mL, and allow the solution to stand for 5 min. Pass through a PVDF filter of 0.45-µm pore size, discarding the first 5 mL of the filtrate.

Chromatographic system: Proceed as directed for *Procedure 1*, except use an *Injection volume* of 50 µL.

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **PROCEDURE 3:** Use this *Procedure* for Capsules labeled to meet the requirements of *Dissolution Test 3*.

Solution B and **Mobile phase:** Proceed as directed for *Procedure 1*.

Buffer: Dissolve 6.9 g of monobasic sodium phosphate monohydrate in 1 L of water, and adjust with 5 N sodium hydroxide to a pH of 7.2 ± 0.05.

Standard solution: Prepare a solution containing 0.5 mg/mL of USP Tamsulosin Hydrochloride RS in a mixture of methanol and water (1:1), and dilute a portion of this solution with methanol to obtain a solution containing 0.03 mg/mL.

Sample solution: Weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the Capsule contents, equivalent to 0.8 mg of tamsulosin hydrochloride, into a 25-mL volumetric flask. Add 5 mL of *Buffer*, shake for 15 min, add 10 mL of methanol, shake for 1 h, and dilute with methanol to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system: Proceed as directed for *Procedure 1*, except use an *Injection volume* of 20 µL.

System suitability and **Analysis:** Proceed as directed for *Procedure 2*.

Acceptance criteria: 90.0%–110.0%

• **PROCEDURE 4:** For Capsules labeled to meet the requirements of *Dissolution Test 4*, proceed as directed for *Procedure 1*.

• **PROCEDURE 5:** Use this *Procedure* for Capsules labeled to meet the requirements of *Dissolution Test 5*.

Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed for *Procedure 1*.

Diluent: Methanol and water (75:25)

Standard solution: Prepare a solution containing 0.016 mg/mL of USP Tamsulosin Hydrochloride RS in *Diluent*.

Sample solution: Transfer the contents of 10 Capsules (equivalent to 4 mg of tamsulosin hydrochloride) into a 250-mL volumetric flask. Add 200 mL of *Diluent*, stir, and sonicate simultaneously for at least 2 h. Cool, and dilute with *Diluent* to volume. Pass through a suitable filter.

System suitability and **Analysis:** Proceed as directed for *Procedure 2*.

Acceptance criteria: 90.0%–110.0%

• **PROCEDURE 6:** Use this *Procedure* for Capsules labeled to meet the requirements of *Dissolution Test 6*.

Solution B and **Mobile phase:** Proceed as directed for *Procedure 1*.

Buffer: Dissolve 6.8 g of monobasic potassium phosphate and 0.9 g of sodium hydroxide in 1 L of water. Adjust with sodium hydroxide solution to a pH of 6.8 ± 0.05.

Diluent A: Acetonitrile and *Buffer* (1:1)

Diluent B: Acetonitrile and *Solution B* (1:1)

Standard solution: Prepare a solution containing 0.4 mg/mL of USP Tamsulosin Hydrochloride RS in *Diluent A*, using sonication as necessary. Dilute 2 mL of this solution with *Diluent B* to 100 mL.

Sample solution: Weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the Capsule contents, equivalent to 4 mg of tamsulosin hydrochloride, into a 100-mL volumetric flask. Add 60 mL of *Diluent A*, and sonicate with intermittent shaking to disperse the pellets completely. Cool, and dilute with *Diluent A* to volume. Centrifuge, transfer 5 mL of supernatant solution to a 25-mL volumetric flask, and dilute with *Diluent B* to volume. Pass through a nylon membrane filter of 0.45-µm pore size.

Chromatographic system: Proceed as directed for *Procedure 1*, except use an *Injection volume* of 20 µL.

System suitability and **Analysis:** Proceed as directed for *Procedure 2*.

Acceptance criteria: 90.0%–110.0%

• **PROCEDURE 7:** Use this *Procedure* for Capsules labeled to meet the requirements of *Dissolution Test 7*.

Solution B and **Mobile phase:** Proceed as directed for *Procedure 1*.

Buffer: Dissolve 76 g of tribasic sodium phosphate in 1 L of water.

Diluent: 0.1 N hydrochloric acid and *Buffer* (3:1), adjusted with diluted hydrochloric acid or sodium hydroxide solution to a pH of 7.0

Standard solution: 0.0016 mg/mL of USP Tamsulosin Hydrochloride RS in *Diluent*

Sample solution: Weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the Capsule contents, equivalent to 0.4 mg of tamsulosin hydrochloride, into a 250-mL volumetric flask, and dilute with *Diluent* to volume. Stir for 24 h by mechanical means at 40° protected from light. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system: Proceed as directed for *Procedure 1*, except use an *Injection volume* of 50 µL.

System suitability and **Analysis:** Proceed as directed for *Procedure 2*.

Acceptance criteria: 90.0%–110.0%

• **PROCEDURE 8:** Use this *Procedure* for Capsules labeled to meet the requirements of *Dissolution Test 8*.

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Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed for *Procedure 1*.

Standard solution: Prepare a solution containing 0.1 mg/mL of USP Tamsulosin Hydrochloride RS in methanol. Dilute 5 mL of this solution with *Mobile phase* to 20 mL.

Sample solution: Weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the Capsule contents, equivalent to 2.5 mg of tamsulosin hydrochloride, into a 100-mL volumetric flask. Add 25 mL of 0.1 N sodium hydroxide, and sonicate for 30 min. Add 30 mL of *Mobile phase*, shake by mechanical means for 30 min, and dilute with *Mobile phase* to volume. Centrifuge, and pass through a PVDF membrane filter of 0.45-µm pore size, discarding the first few mL of the filtrate.

System suitability and **Analysis:** Proceed as directed for *Procedure 2*.

Acceptance criteria: 90.0%–110.0%

• **PROCEDURE 9:** For Capsules labeled to meet the requirements of *Dissolution Test 9*, proceed as directed for *Procedure 1*.

• **PROCEDURE 10:** Use this *Procedure* for Capsules labeled to meet the requirements of *Dissolution Test 10*.

Solution A, Solution B, and **Mobile phase:** Proceed as directed for *Procedure 1*.

Diluent: Acetonitrile and water (3:7)

Standard stock solution: 0.62 mg/mL of USP Tamsulosin Hydrochloride RS in *Diluent*

Internal standard solution: 0.5 mg/mL of propylparaben in *Diluent*

Standard solution: Transfer 2.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, add 5.0 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.

Sample solution: Weigh and crush the contents of NLT 20 Capsules and transfer the powder equivalent to 1.2 mg of tamsulosin hydrochloride into a 50-mL volumetric flask. Add 25 mL of 0.05 N sodium hydroxide solution, heat at 50° for 15 min, and shake mechanically for 60 min. Sonicate for 30 min with intermittent shaking and add 15 mL of a mixture of *Solution A* and acetonitrile (1:2). Sonicate for another 15 min with intermittent shaking. Add 5 mL of *Internal standard solution*, dilute with a mixture of *Solution A* and acetonitrile (1:2) to volume, and mix well. Centrifuge the solution for 10 min and pass the solution through a suitable glass fiber filter.

Chromatographic system: Proceed as directed for *Procedure 1*.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 12 between tamsulosin and propylparaben. [NOTE—The elution order is tamsulosin hydrochloride followed by propylparaben.]

Relative standard deviation: NMT 2.0% for each peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of tamsulosin to the internal standard from the *Sample solution*

R_S = peak area ratio of tamsulosin to the internal standard from the *Standard solution*

C_S = concentration of USP Tamsulosin Hydrochloride RS in the *Standard stock solution* (mg/mL)

C_U = nominal concentration of tamsulosin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Acid stage medium: Dissolve 2.0 g of sodium chloride in 5.7 mL of hydrochloric acid, and add water to make up to 1000 mL. To 500 mL of this fluid add 1 mL of polysorbate 80 aqueous solution (3 g in 200 mL of water) just before the test; 500 mL.

Buffer stage medium: pH 7.2 phosphate buffer (dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, add 90 mL of 0.2 N sodium hydroxide and 500 mL of water, adjust with 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of 7.2 ± 0.05, and dilute with water to 1000 mL); 500 mL

Apparatus 2: 100 rpm, with sinker (see *Dissolution* ⟨711⟩, *Figure 2a*)

Times: 2, 3, and 8 h

Procedure: Perform the test using *Acid stage medium*. At 2 h after the start of the test, withdraw 10.0 mL of the solution under test (T1). Drain the *Acid stage medium* immediately by suction through a tube capped with a 60-mesh stainless wire screen. Rinse the drain tube while adding the *Buffer stage medium* previously warmed, and continue the test. At 3 h after the start of the test (1 h after replacement of the *Medium*), withdraw 10.0 mL of the solution under test (T2), replace the same volume with warmed *Buffer stage medium*, and continue the test. At 8 h after the start of the test (6 h after the replacement of the *Medium*), withdraw 10.0 mL of the solution under test (T3).

Internal standard solution: 0.008 mg/mL of propylparaben in acetonitrile and water (3:7)

Standard solution: Prepare a solution containing 0.5 mg/mL of USP Tamsulosin Hydrochloride RS in acetonitrile and water (3:7). Transfer 4.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Acid stage medium* to volume. Transfer 4.0 mL of this dilution to another 100-mL volumetric flask, and dilute with *Acid stage medium* to volume. This solution has a known concentration (C_S) of about 0.8 µg/mL of tamsulosin hydrochloride. Transfer 10.0 mL of this last dilution to a test tube, and add 2.0 mL of the *Internal standard solution*.

Sample solutions: Add 2.0 mL of the *Internal standard solution* to T1, mix well, and pass through a suitable filter of 0.5-µm pore size, discarding the first 5 mL. Add 1.0 mL of 0.5 N hydrochloric acid and 2.0 mL of *Internal standard solution* to T2, mix well, and pass through a suitable filter of 0.5-µm pore size, discarding the first 5 mL. Add 1.0 mL of 0.5 N hydrochloric acid and 2.0 mL of *Internal standard solution* to T3. Mix well, and pass through a suitable filter of 0.5-µm pore size, discarding the first 5 mL.

Chromatographic system: Proceed as directed in the *Assay, Procedure 1*, but use the *Standard solution* described for *Dissolution*, and an *Injection volume* of 250 µL instead of 10 µL.

Analysis: Calculate the peak area ratios (R_{T1}, R_{T2}, R_{T3}, and R_S) of tamsulosin to the internal standard for all *Sample solutions* and the *Standard solution*. Calculate the percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each of the following time points.

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At 2 h:

$$D_1 = [(C_S \times V)/L] \times (R_{T1}/R_S) \times 100$$

At 3 h:

$$D_2 = [(C_S \times V)/L] \times [(R_{T2}/R_S) + (R_{T1}/R_S)] \times 100$$

At 8 h:

$$D_3 = [(C_S \times V)/L] \times [(R_{T3}/R_S) + (R_{T2}/R_S \times V_{T2}/V) + (R_{T1}/R_S)] \times 100$$

C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Capsule)
V_{T2} = volume of the withdrawn aliquot of T2, 10 mL

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
2	13–34
3	47–68
8	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Acid stage medium: 0.003% Polysorbate 80, pH 1.2 (dilute 8.5 mL of hydrochloric acid with water to 900 mL, add 0.03 mL of polysorbate 80, adjust with 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of 1.2 ± 0.05, and dilute with water to 1000 mL); 500 mL
Buffer stage medium: pH 7.2 phosphate buffer (proceed as directed for *Test 1*); 500 mL
Apparatus 2: 100 rpm, with sinkers[1]
Times: 2 h for the *Acid stage medium* and 6 h for the *Buffer stage medium*; 8 h total test time
Procedure: Perform the test using *Acid stage medium*. At 2 h after the start of the test, withdraw a sample of the solution under test. Carefully discard the *Acid stage medium* and replace it with the *Buffer stage medium* previously warmed, and continue the test. At 6 h after the replacement of the *Medium*, withdraw a sample of the solution under test.
Standard stock solution: Transfer about 25.0 mg of USP Tamsulosin Hydrochloride RS to a 50-mL volumetric flask. Add 25 mL of methanol. Dilute with *Buffer stage medium* to volume. Sonicate until dissolved.
Standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a final concentration of 0.8 µg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Buffer solution: 3.4 g/L of monobasic potassium phosphate in water. Adjust with 2 N sodium hydroxide to a pH of 5.80 ± 0.05.
Mobile phase: Acetonitrile and *Buffer solution* (1:3)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis: Calculate the concentration of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) in the *Medium* at each time point (C_i).
At 2 h:

$$C_1 = (r_U/r_S) \times C_S$$

At 6 h in the *Buffer stage medium* (8 h total test time):

$$C_2 = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (Q_i).
At 2 h:

$$Q_1 = (C_1 \times V) \times (100/L)$$

At 6 h in the *Buffer stage medium* (8 h total test time):

$$Q_2 = (C_1 + C_2) \times (V/L) \times 100$$

V = volume of *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: NMT 25% of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) is dissolved in 2 h. NLT 85% of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) is dissolved in 8 h.
The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Acid stage
Acid stage medium: 0.003% Polysorbate 80, pH 1.2 (proceed as directed for *Test 2*); 500 mL
Apparatus 2: 100 rpm, with sinkers
Time: 2 h
Procedure: Pass a portion of the solution under test through a suitable filter. Leave the remaining *Acid stage medium* in the vessel and proceed with the *Buffer stage*.
Tolerances: NMT 10% (Q) of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) is dissolved.
Buffer stage
Buffer stage concentrate: 0.1 M phosphate buffer, pH 11.6 (138 g of sodium phosphate monohydrate and 320 mL of 5 N sodium hydroxide in 10 L of water. Adjust with 5 N sodium hydroxide to a pH of 11.6 ± 0.05.)

[1] A suitable sinker is available as catalog number CAPWHT-2S from www.qla-llc.com.

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Buffer stage medium: Add 500 mL of the *Buffer stage concentrate* to the remaining *Acid stage medium* in each vessel. The final pH is about 7.2.
Apparatus 2: 100 rpm, with sinkers
Times: 3 and 8 h, including the 2 h in the *Acid stage medium*
Standard stock solution: 0.5 mg/mL of USP Tamsulosin Hydrochloride RS in water and methanol (1:1)
Standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a final concentration of 0.3 µg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter.
pH 5.5 buffer solution: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 2 N sodium hydroxide to a pH of 5.5 ± 0.05.
Mobile phase: Acetonitrile and *pH 5.5 buffer solution* (2:3)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.5%
Analysis: Calculate the concentration of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (C_i).
At 3 h:

$$C_1 = (r_U/r_S) \times C_S$$

At 8 h:

$$C_2 = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (Q_i):

$$Q_i = (C_1V_1 + \sum_{i=2}^{n-1} C_iV_S + C_nV) \times (100/L)$$

V_1 = volume of *Acid stage medium*, 500 mL
V_S = volume of the sample taken (mL)
C_n = concentration of tamsulosin hydrochloride at each time point (mg/mL)
V = volume of *Buffer stage medium*, 1000 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
3	65–85
8	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Acid stage medium: 0.003% Polysorbate 80, pH 1.2 (proceed as directed for *Test 2*); 500 mL
Buffer stage medium: pH 7.2 phosphate buffer (proceed as directed for *Test 1*); 500 mL
Apparatus 2: 100 rpm
Times: 2 h for the *Acid stage medium*, and 3 and 8 h for the *Buffer stage medium* (including the 2 h in the *Acid stage medium*)
Procedure: Perform the test using the *Acid stage medium*. At 2 h after the start of the test, withdraw a sample of the solution under test. Carefully discard the *Acid stage medium*, replace it with the *Buffer stage medium* previously warmed, and continue the test. At 1 and 6 h after the replacement of the *Medium*, withdraw a sample of the solution under test. Replace the volume of *Medium* withdrawn with the same volume of *Buffer stage medium*, previously warmed.
50 mM sodium perchlorate solution: Dissolve 7.0 g of monohydrate sodium perchlorate in 1 L of water, and add 5 mL of phosphoric acid.
Mobile phase: *50 mM sodium perchlorate solution* and acetonitrile (3:2)
Acid stage standard stock solution: Transfer 20 mg of USP Tamsulosin Hydrochloride RS to a 500-mL volumetric flask, add 25 mL of methanol, and sonicate until dissolved. Dilute with *Acid stage medium* to volume.
Acid stage standard solution: Dilute the *Acid stage standard stock solution* with *Acid stage medium* to obtain a final concentration of about 0.08 µg/mL.
Buffer stage standard stock solution: Transfer 20 mg of USP Tamsulosin Hydrochloride RS to a 250-mL volumetric flask, add 25 mL of methanol, and sonicate until dissolved. Dilute with *Buffer stage medium* to volume.
Buffer stage standard solution: Dilute the *Buffer stage standard stock solution* with *Buffer stage medium* to obtain a final concentration of about 0.8 µg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Columns
Guard: 3-mm × 4-cm; packing L1
Analytical: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 200 µL
System suitability
Samples: *Acid stage standard solution* and *Buffer stage standard solution*
Suitability requirements
Column efficiency: NLT 1600 theoretical plates, *Acid stage standard solution*; NLT 1300, *Buffer stage standard solution*
Relative standard deviation: NMT 3.0%, *Acid stage standard solution*; NMT 1.5%, *Buffer stage standard solution*
Analysis: Calculate the concentration of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (C_i).
At 2 h:

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$$C_1 = (r_U/r_S) \times C_S$$

At 3 h:

$$C_2 = (r_U/r_S) \times C_S$$

At 8 h:

$$C_3 = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (Q_i):

$$Q_i = (C_1V_1 + \sum_{i=2}^{n-1} C_iV_S + C_nV) \times (100/L)$$

V_1 = volume of *Acid stage medium*, 500 mL
V_S = volume of the sample taken (mL)
C_n = concentration of tamsulosin hydrochloride at each time point (mg/mL)
V = volume of *Buffer stage medium*, 500 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
2	0–10
3	45–68
8	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified in the *Buffer stage* conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Acid stage medium: 0.003% Polysorbate 80, pH 1.2 (proceed as directed for *Test 2*); 500 mL
Buffer stage medium: pH 7.2 phosphate buffer (proceed as directed for *Test 1*); 500 mL
Apparatus 2: 50 rpm, with sinkers
Times: 2 h for the *Acid stage medium*, and 3 and 5 h for the *Buffer stage medium* (including the 2 h in the *Acid stage medium*)
Buffer solution: Dissolve 1.0 g of octanesulfonic acid sodium salt and 1.4 g of monobasic potassium phosphate in 1 L of water. Adjust with potassium hydroxide to a pH of 6.5 ± 0.05.
Mobile phase: *Buffer solution* and acetonitrile (3:2)
Standard stock solution: 0.04 mg/mL of USP Tamsulosin Hydrochloride RS in *Buffer stage medium*
Standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a final concentration of 0.8 µg/mL.
Sample solution: Centrifuge a portion of the solution under test at NMT 3000 rpm for NLT 20 min.
Procedure: Perform the test using *Acid stage medium*. At 2 h after the start of the test, withdraw a sample of the solution under test. Carefully discard the *Acid stage medium*, replace it with the *Buffer stage medium* previously warmed, and continue the test. At 1 and 3 h after the replacement of the *Medium*, withdraw a sample of the solution under test. Replace the volume of *Medium* withdrawn with the same volume of *Buffer stage medium*, previously warmed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 3.9-mm × 7.5-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 4.0%
Analysis: Calculate the concentration of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (C_i).
At 2 h:

$$C_1 = (r_U/r_S) \times C_S$$

At 3 h:

$$C_2 = (r_U/r_S) \times C_S$$

At 5 h:

$$C_3 = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (Q_i):

$$Q_i = (C_1V_1 + \sum_{i=2}^{n-1} C_iV_S + C_nV) \times (100/L)$$

V_1 = volume of *Acid stage medium*, 500 mL
V_S = volume of the sample taken (mL)
C_n = concentration of tamsulosin hydrochloride at each time point (mg/mL)
V = volume of *Buffer stage medium*, 500 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (%)
2	15–35
3	60–80
5	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

USP Monographs

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Acid stage medium: 0.003% Polysorbate 80, pH 1.2 (proceed as directed for *Test 2*); 500 mL
Buffer stage medium: pH 7.2 phosphate buffer (proceed as directed for *Test 1*); 500 mL
Apparatus 2: 100 rpm using a 40-mesh basket as a sinker, and the paddle height adjusted at 4.5 cm from the bottom of the vessel
Times: 2 h for the *Acid stage medium*, and 3 and 8 h for the *Buffer stage medium* (including the 2 h in the *Acid stage medium*)
Procedure: Perform the test using *Acid stage medium*. At 2 h after the start of the test, withdraw a sample of the solution under test. Carefully discard the *Acid stage medium*, replace it with the *Buffer stage medium* previously warmed, and continue the test. At 1 and 6 h after the replacement of the *Medium*, withdraw a sample of the solution under test. Replace the volume of *Medium* withdrawn with the same volume of *Buffer stage medium*, previously warmed.
Buffer solution: Dissolve 3 g of sodium hydroxide and 8.7 mL of perchloric acid in 1900 mL of water, adjust with 0.5 M sodium hydroxide to a pH of 2.0 ± 0.05, and dilute with water to 2000 mL.
Mobile phase: *Buffer solution* and acetonitrile (7:3)
Standard stock solution: 0.5 mg/mL of USP Tamsulosin Hydrochloride RS in methanol
Acid stage standard solution: Dilute the *Standard stock solution* with *Acid stage medium* to obtain a final concentration of 0.8 µg/mL.
Buffer stage standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a final concentration of 0.8 µg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm, 3-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Samples: *Acid stage standard solution* and *Buffer stage standard solution*
Suitability requirements
Column efficiency: NLT 7000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis: Calculate the concentration of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (C_i).
At 2 h:

$$C_1 = (r_U/r_S) \times C_S$$

At 3 h:

$$C_2 = (r_U/r_S) \times C_S$$

At 8 h:

$$C_3 = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (Q_i):

$$Q_i = (C_1V_1 + \sum_{i=2}^{n-1} C_iV_S + C_nV) \times (100/L)$$

V_1 = volume of *Acid stage medium*, 500 mL
V_S = volume of the sample taken (mL)
C_n = concentration of tamsulosin hydrochloride at each time point (mg/mL)
V = volume of *Buffer stage medium*, 500 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
2	0–20
3	47–68
8	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Acid stage medium: 0.003% Polysorbate 80, in simulated gastric fluid without enzyme (pH 1.2) (prepare a solution containing 3.00 g of polysorbate 80 in 200 mL of water, and dilute 2 mL of this solution to 1 L with simulated gastric fluid without enzyme); 500 mL
Buffer stage medium: pH 7.2 phosphate buffer (proceed as directed for *Test 1*); 500 mL, deaerated
Apparatus 2: 100 rpm, with wire helix sinkers
Times: 2 h in the *Acid stage medium*, and 4 and 12 h in the *Buffer stage medium* (including the 2 h in the *Acid stage medium*)
Standard stock solution: 1 mg/mL of USP Tamsulosin Hydrochloride RS in alcohol
Acid stage standard solution: Dilute the *Standard stock solution* with *Acid stage medium* to obtain a final concentration of about 0.2 µg/mL.
Buffer stage standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a final concentration of about 0.8 µg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Procedure: Perform the test using *Acid stage medium*. At 2 h after the start of the test, withdraw a sample of the solution under test. Carefully discard the *Acid stage medium*, replace it with the *Buffer stage medium* previously warmed, and continue the test. At 2 and 10 h after the replacement of the *Medium*, withdraw a sample of the solution under test. Replace the volume of *Medium* withdrawn with the same volume of *Buffer stage medium*, previously warmed.
Mobile phase A: 2.76 g/L of monobasic sodium phosphate monohydrate in water. Adjust with phosphoric acid to a pH of 2.5 ± 0.05.
Mobile phase B: Acetonitrile
Gradient program: See *Table 6*.

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Table 6

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	86	14
3	86	14
4.5	30	70
5	86	14

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 2-mm × 5-cm; packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Samples: *Acid stage standard solution* and *Buffer stage standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis: Calculate the concentration of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (C_i).
At 2 h:

$$C_1 = (r_U/r_S) \times C_S$$

At 4 h:

$$C_2 = (r_U/r_S) \times C_S$$

At 12 h:

$$C_3 = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (Q_i):

$$Q_i = (C_1V_1 + \sum_{i=2}^{n-1} C_iV_S + C_nV) \times (100/L)$$

V_1 = volume of *Acid stage medium*, 500 mL
V_S = volume of the sample taken (mL)
C_n = concentration of tamsulosin hydrochloride at each time point (mg/mL)
V = volume of *Buffer stage medium*, 500 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved (%)
2	5–25
4	46–66
12	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.
Acid stage medium: 0.003% Polysorbate 80, pH 1.2 (proceed as directed for *Test 2*); 500 mL
Buffer stage medium: pH 7.2 phosphate buffer (proceed as directed for *Test 1*); 500 mL
Apparatus 2: 100 rpm, with sinkers
Times: 2 h in the *Acid stage medium*, and 3 and 8 h in the *Buffer stage medium* (including the 2 h in the *Acid stage medium*)
Standard stock solution: 0.4 mg/mL of USP Tamsulosin Hydrochloride RS in methanol
Acid stage standard solution: Dilute the *Standard stock solution* with *Acid stage medium* to obtain a final concentration of 0.8 µg/mL.
Buffer stage standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a final concentration of 0.8 µg/mL.
Procedure: Perform the test using *Acid stage medium*. At 2 h after the start of the test, withdraw a sample of the solution under test. Carefully discard the *Acid stage medium*, replace it with the *Buffer stage medium* previously warmed, and continue the test. At 1 and 6 h after the replacement of the *Medium*, withdraw a sample of the solution under test. Replace the volume of *Medium* withdrawn with the same volume of *Buffer stage medium*, previously warmed.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Buffer solution: Dissolve 6.8 g of dibasic ammonium phosphate in 800 mL of water, and add 2 mL of triethylamine. Adjust with phosphoric acid to a pH of 6.5. Dilute with water to 1000 mL.
Mobile phase: *Buffer solution* and Acetonitrile (7:3)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 100 µL
System suitability
Sample: *Buffer stage standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis: Calculate the concentration of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (C_i).
At 2 h:

$$C_1 = (r_U/r_S) \times C_S$$

At 3 h:

$$C_2 = (r_U/r_S) \times C_S$$

At 8 h:

$$C_3 = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

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C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (Q_i):

$$Q_i = (C_1V_1 + \sum_{i=2}^{n-1} C_iV_S + C_nV) \times (100/L)$$

V_1 = volume of *Acid stage medium*, 500 mL
V_S = volume of the sample taken (mL)
C_n = concentration of tamsulosin hydrochloride at each time point (mg/mL)
V = volume of *Buffer stage medium*, 500 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved (%)
2	0–10
3	50–70
8	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.
Acid stage medium: 0.003% Polysorbate 80, pH 1.2 (proceed as directed for *Test 2*); 500 mL
Buffer stage medium: pH 7.2 phosphate buffer (proceed as directed for *Test 1*); 500 mL
Apparatus 2: 100 rpm, with helix wire coil sinker
Times: 2 h in *Acid stage medium*, and 3 and 8 h in *Buffer stage medium* (including the 2 h in *Acid stage medium*)
Standard stock solution: 0.05 mg/mL of USP Tamsulosin Hydrochloride RS in methanol
Acid stage standard solution: Dilute the *Standard stock solution* with *Acid stage medium* to obtain a final concentration of 0.8 µg/mL.
Buffer stage standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a final concentration of 0.8 µg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Procedure: Perform the test using *Acid stage medium*. At 2 h after the start of the test, withdraw a sample of the solution under test. Carefully discard the *Acid stage medium*, replace it with the *Buffer stage medium* previously warmed, and continue the test. At 1 and 6 h after the replacement of the *Medium*, withdraw a sample of the solution under test. Replace the volume of *Medium* withdrawn with the same volume of *Buffer stage medium*, previously warmed.
Buffer: 3.45 g/L of monobasic ammonium phosphate in water. Adjust with triethylamine to a pH of 6.5 ± 0.05.
Mobile phase: *Buffer* and Acetonitrile (3:2). Add 1 g of sodium 1-pentanesulfonate per L of the mixture.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 100 µL
System suitability
Samples: *Acid stage standard solution* or *Buffer stage standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis: Calculate the concentration of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (C_i).
At 2 h:

$$C_1 = (r_U/r_S) \times C_S$$

At 3 h:

$$C_2 = (r_U/r_S) \times C_S$$

At 8 h:

$$C_3 = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the appropriate *Standard solution*
C_S = concentration of the appropriate *Standard solution* (mg/mL)

Calculate the cumulative percentage of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (Q_i):

$$Q_i = (C_1V_1 + \sum_{i=2}^{n-1} C_iV_S + C_nV) \times (100/L)$$

V_1 = volume of *Acid stage medium*, 500 mL
V_S = volume of the sample taken (mL)
C_n = concentration of tamsulosin hydrochloride at each time point (mg/mL)
V = volume of *Buffer stage medium*, 500 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 9*.

Table 9

Time (h)	Amount Dissolved (%)
2	0–20
3	45–65
8	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 10: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*.
Acid stage medium: Dissolve 2.0 g of sodium chloride and 5.7 mL of hydrochloric acid in 1000 mL of water. To 500 mL of this solution add 1 mL of polysorbate 80 aqueous solution (3 g in 200 mL of water) just before the test; 500 mL.
Buffer stage medium: pH 7.2 phosphate buffer (dissolve 68 g of monobasic potassium phosphate and 13.8 g of

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sodium hydroxide in 10 L of water, and adjust with dilute sodium hydroxide solution or dilute phosphoric acid to a pH of 7.2); 500 mL

Apparatus 2: 100 rpm, with a suitable sinker

Times: 2, 3, and 8 h

Procedure: Perform the test using *Acid stage medium*. At 2 h after the start of the test, withdraw 10.0 mL of the solution under test. Drain the *Acid stage medium* immediately using a 60-mesh stainless wire screen. Add the *Buffer stage medium* previously warmed, and continue the test. At 3 h after the start of the test (1 h after replacement of the *Medium*), withdraw 10.0 mL of the solution under test, replace the same volume with warmed *Buffer stage medium*, and continue the test. At 8 h after the start of the test (6 h after the replacement of the *Medium*), withdraw 10.0 mL of the solution under test.

Standard stock solution: Prepare a solution containing 0.5 mg/mL of USP Tamsulosin Hydrochloride RS in acetonitrile and water (3:7).

Acid stage standard solution: Dilute the *Standard stock solution* with *Acid stage medium* to obtain a final concentration of about 0.8 µg/mL.

Buffer stage standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a final concentration of about 0.8 µg/mL.

Sample solution: Pass a portion of the solution under test through a suitable glass fiber filter, discarding the first 5 mL of the filtrate.

Chromatographic system: Proceed as directed in the *Assay, Procedure 10*, but use the *Injection volume* of 100 µL instead of 10 µL.

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis: Calculate the concentration (C_i) of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*

r_S = peak response from the appropriate *Standard solution*

C_S = concentration of the appropriate *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V_1 \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_1 \times V_1) + (C_2 \times V)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_1 \times V_1) + (C_3 \times V) + (C_2 \times V_S)] \times (1/L) \times 100$$

C_i = concentration of tamsulosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

V_1 = volume of the *Acid stage medium*, 500 mL

L = label claim (mg/Capsule)

V = volume of the *Buffer stage medium*, 500 mL

V_S = volume of the *Sample solution* withdrawn and replaced with *Medium*, 10 mL

Tolerances: See *Table 10*.

Table 10

Time (h)	Amount Dissolved (%)
2	7–27
3	47–68
8	NLT 80

The percentages of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

[NOTE—Use the following *Procedure for content uniformity* if *Procedure 1* is used in the *Assay*. For all other formulations, proceed as directed in *Uniformity of Dosage Units* ⟨905⟩.]

Solution A, Solution B, Mobile phase, and **Standard stock solution:** Prepare as directed in the *Assay, Procedure 1*.

Internal standard solution: 0.16 mg/mL of propylparaben in a mixture of acetonitrile and water (3:7)

Standard stock solution 1: Transfer 5.0 mL of the *Standard stock solution* to a 25-mL volumetric flask, and dilute with a mixture of acetonitrile and water (3:7) to volume.

Standard solution: Transfer 4.0 mL of *Standard stock solution 1* to a suitable container. Add 5.0 mL of the *Internal standard solution*, and add the *Mobile phase* to make 40 mL.

Sample solution: Place the contents of 1 Capsule into a Teflon-lined, screw-capped centrifuge tube. Place approximately 100 glass balls with a diameter of about 5 mm into the tube, add 20 mL of 0.05 N sodium hydroxide, heat at 50° for 10 min, and shake well for 30 min. Add 15 mL of a mixture of acetonitrile and *Solution A* (2:1) to the solution, and shake well. Add 5.0 mL of the *Internal standard solution*, and shake well. Centrifuge at 1500 rpm for 10 min, and use the supernatant, passing it if necessary through a membrane filter of 0.5-µm or smaller pore size.

Chromatographic system: Proceed as directed in the *Assay, Procedure 1* except use an *Injection volume* of 25 µL.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$) in the Capsule taken:

$$\text{Result} = (R_U/R_S) \times [C_S \times (V_S/L)] \times 100$$

R_U = peak area ratio of tamsulosin to the internal standard from the *Sample solution*

R_S = peak area ratio of tamsulosin to the internal standard from the *Standard solution*

C_S = concentration of USP Tamsulosin Hydrochloride RS in *Standard stock solution 1* (mg/mL)

V_S = volume of *Standard stock solution 1* taken to prepare the *Standard solution* (mL)

L = label claim (mg/Capsule)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

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• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tamsulosin Hydrochloride RS
(–)-(*R*)-5-[2-[[2-(*o*-Ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide monohydrochloride.
$C_{20}H_{28}N_2O_5S \cdot HCl$ 444.97

Tannic Acid

Tannin.
Tannic acid; Tannin [1401-55-4].

» Tannic Acid is a tannin usually obtained from nutgalls, the excrescences produced on the young twigs of *Quercus infectoria* Oliver, and allied species of *Quercus* Linné (Fam. Fagaceae), from the seed pods of Tara (*Caesalpinia spinosa*), or from the nutgalls or leaves of sumac (any of a genus *Rhus*).

Packaging and storage—Preserve in tight, light-resistant containers.

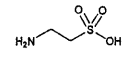
Identification—
A: To 2 mL of a solution (1 in 10) add 1 drop of ferric chloride TS: a bluish black color or precipitate results.
B: To a solution (1 in 10) add an equal volume of gelatin solution (1 in 100): a precipitate is formed.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 12.0% of its weight.
Residue on ignition ⟨281⟩: not more than 1.0%.
Arsenic, *Method II* ⟨211⟩: 3 ppm.
Gum or dextrin—Dissolve 2 g in 10 mL of hot water: the solution is not more than slightly turbid. Cool, filter, and divide the filtrate into two equal portions. To one portion add 10 mL of alcohol: no turbidity is produced.
Resinous substances—To a portion of the filtrate obtained in the test for *Gum or dextrin* add 10 mL of water: no turbidity is produced.

Adhesive Tape

» Adhesive Tape consists of fabric and/or film evenly coated on one side with a pressure-sensitive, adhesive mixture. Its length is not less than 98.0 percent of that declared on the label, and its average width is not less than 95.0 percent of the declared width. If Adhesive Tape has been rendered sterile, it is protected from contamination by appropriate packaging.

Packaging and storage—Preserve in well-closed containers, and prevent exposure to excessive heat and to sunlight. Tape that has been rendered sterile is so packaged that the sterility of the contents of the package is maintained until the package is opened for use.
Labeling—The package label of Tape that has been rendered sterile indicates that the contents may not be sterile if the package bears evidence of damage or previously has been opened. The package label indicates the length and width of the Tape, and the name of the manufacturer, packer, or distributor.
Dimensions—Measure its length: it is not less than 98.0% of the labeled length. Measure its width at 5 locations evenly spaced along the center line of the Tape: the average of 5 measurements is not less than 95% of the labeled width of the Tape.
Tensile strength—Determine the tensile strength of Tape, after previously unrolling and conditioning it for not less than 4 hours in a standard atmosphere of 65 ± 2% relative humidity, at 21 ± 1.1° (70 ± 2°F), with a pendulum-type testing machine, as described under *Tensile Strength* ⟨881⟩. The Tape made from fabric has a tensile strength, determined warpwise, of not less than 20.41 kg (45 pounds) per 2.54 cm of width. The Tape made from film has a tensile strength of not less than 3 kg per 2.54 cm of width.
Adhesive strength—Determine the adhesive strength of Tape that is made from fabric by cutting a strip of the Tape 2.54 cm wide and approximately 15 cm long, and applying 12.90 sq cm, 2.54 cm by 5.08 cm, of one end of the strip to a clean plastic or glass surface by means of a rubber roller under a pressure of 850 g, passing the roller twice over the Tape at a rate of 30 cm per minute. Adjust the temperature of the plastic or glass surface and the Tape to 37°, and conduct the test immediately thereafter as directed under *Tensile Strength* ⟨881⟩, using a pendulum-type testing machine, the pull being exerted parallel with the warp and the plastic or glass surface: the average of not less than 10 tests is not less than 18 kg.
Sterility Tests ⟨71⟩ —Tape that has been rendered sterile meets the requirements.

Taurine



$C_2H_7NO_3S$ 125.15
Taurine
Ethanesulfonic acid, 2-amino-;
2-Aminoethane-1-sulfonic acid [107-35-7].

DEFINITION
Taurine contains NLT 98.0% and NMT 102.0% of taurine ($C_2H_7NO_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solutions*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Water adjusted with acetic acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Solution A* (80:20)
Standard stock solution: Prepare a solution containing 1.0 mg/mL of USP Taurine RS in *Mobile phase*.

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Standard solutions: Using the *Standard stock solution* prepare three *Standard solutions* with concentrations of 0.4, 0.5, and 0.6 mg/mL of USP Taurine RS in *Mobile phase.*
Sample solution: 0.5 mg/mL of Taurine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Evaporative light-scattering detector (ELSD)
Drift tube temperature: 55° or optimize according to the manufacturer's recommendations to achieve optimal signal-to-noise ratio
Nebulizer gas: Nitrogen
Nebulizer gas flow rate: 1.5 L/min or optimize according to the manufacturer's recommendations to achieve optimal signal-to-noise ratio
Column: 3-mm × 10-cm; 2.5-µm packing L104
Column temperature: 25°
Flow rate: 0.4 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution* at 0.5 mg/mL
Suitability requirements
Column efficiency: NLT ▲6000▲ (RB 1-Mar-2019) theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT ▲3%▲ (RB 1-Mar-2019)
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the logarithms of the taurine peak areas against the logarithms of taurine concentrations, in mg/mL, in the three *Standard solutions* and establish a calibration curve by least-squares regression. The correlation coefficient for the regression line is NLT ▲0.99. Inject the *Sample solution* three times and obtain the average value of taurine peak areas.▲ (RB 1-Mar-2019) Using the logarithm of the ▲average▲ (RB 1-Mar-2019) taurine peak area from the *Sample solution*, calculate the logarithm of taurine concentration from the calibration curve and calculate the concentration, *C*, in mg/mL, of taurine in the *Sample solution* by taking the antilog of the result.
Calculate the percentage of taurine ($C_2H_7NO_3S$) in the portion of Taurine taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of Taurine in the *Sample solution* (mg/mL), determined from the calibration curve
C_U = concentration of Taurine in the *Sample solution* (mg/mL)

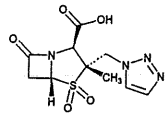
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.3%
• **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*
Standard: 0.50 mL of 0.020 N hydrochloric acid
Sample: 0.7 g of Taurine
Acceptance criteria: NMT 0.05%
• **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*
Standard: 0.25 mL of 0.020 N sulfuric acid
Sample: 0.8 g of Taurine
Acceptance criteria: NMT 0.03%
• **IRON** ⟨241⟩**:** NMT 30 ppm
• **RELATED COMPOUNDS**
Sample solution: 10 mg/mL of Taurine in water
Standard solution: 0.05 mg/mL of USP Taurine RS in water, an equivalent concentration of about 0.5% of the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)
Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
Analysis: Dry the plate at 80° for 30 min. Spray the plate with *Spray reagent*, and heat at 80° for about 10 min. Examine the plate under white light. [NOTE—The R_F value for the taurine spots should be about 0.2.]
Acceptance criteria: No secondary spot of the *Sample solution* is larger or more intense than the principal spot of the *Standard solution.*
Individual impurities: NMT 0.5%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Taurine RS

Tazobactam



$C_{10}H_{12}N_4O_5S$ 300.29
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-, 4,4-dioxide, [2*S*-(2α,3β,5α)]-;
(2*S*,3*S*,5*R*)-3-Methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 4,4-dioxide [89786-04-9].

$C_{10}H_{12}N_4O_5S \cdot ½H_2O$ 309.30
[428863-55-2].

DEFINITION
Tazobactam contains NLT 98.0% and NMT 102.0% of $C_{10}H_{12}N_4O_5S$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Mobile phase: Dissolve 1.32 g of dibasic ammonium phosphate in 750 mL of water. Adjust with 5% v/v phosphoric acid to a pH of 2.5, and dilute with water to

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1000 mL. Add 30 mL of acetonitrile, mix, and pass through a filter of 0.2-µm pore size.

System suitability solution: 16 µg/mL of L-phenylalanine, 50 µg/mL of USP Tazobactam RS, and 8 µg/mL of USP Tazobactam Related Compound A RS in *Mobile phase*. Maintain the *System suitability solution* at 3° until injection. Prepare fresh daily. If an autosampler is used, replace the plastic tubing connected to the injection needle with a stainless steel assembly, and maintain at 3°. If a chilled autosampler is not used, then this solution should be injected immediately after preparation.

Standard solution: 0.5 mg/mL of USP Tazobactam RS in *Mobile phase*. Cool, and maintain the *Standard solution* at 3° until injection. If an autosampler is used, replace the plastic tubing connected to the injection needle with a stainless steel assembly, and maintain at 3°. If a chilled autosampler is not used, then this solution should be injected immediately after preparation.

Sample solution: 0.5 mg/mL of Tazobactam in *Mobile phase*. Cool, and maintain the *Sample solution* at 3° until injection. If an autosampler is used, replace the plastic tubing connected to the injection needle with a stainless steel assembly, and maintain at 3°. If a chilled autosampler is not used, then this solution should be injected immediately after preparation.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 6.0 between tazobactam and L-phenylalanine, *System suitability solution*

Tailing factor: NMT 1.8, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tazobactam ($C_{10}H_{12}N_4O_5S$) in the portion of Tazobactam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Tazobactam RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Blank: *Mobile phase*. Cool, and maintain the *Blank* at 3° until injection. If an autosampler is used, replace the plastic tubing connected to the injection needle with a stainless steel assembly, and maintain at 3°. If a chilled autosampler is not used, then this solution should be injected immediately after preparation.

Sample solution: Prepare as directed in the *Assay*. Cool, and maintain the *Sample solution* at 3° until injection. If an autosampler is used, replace the plastic tubing connected to the injection needle with a stainless steel assembly, and maintain at 3°. If a chilled autosampler is not used, then this solution should be injected immediately after preparation.

Analysis

Samples: *Blank* and *Sample solution*

Ignore any peaks of the *Sample solution* that correspond to any peaks of the *Blank*.

Calculate the percentage of each impurity in the portion of Tazobactam taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity in the *Sample solution*

r_T = sum of all the peak responses in the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tazobactam related compound A	0.29	1.0
L-Phenylalanine	0.71	—
Tazobactam	1.0	—
Any other individual impurity	—	0.1
Total impurities[a]	—	0.3

[a] Total of all impurities other than tazobactam related compound A.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** The level of bacterial endotoxins is such that the requirements of the relevant dosage form monograph(s) in which Tazobactam is used can be met.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL, in dimethylformamide

Acceptance criteria: +160° to +167° (t = 20°)

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.
- **pH** ⟨791⟩

Sample solution: 2.5 mg/mL

Acceptance criteria: 1.8–2.8

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.6% for the anhydrous form; 2.2–3.8% for the hemihydrate form

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** Where it is the hemihydrate form, the label so indicates.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Tazobactam RS

USP Tazobactam Related Compound A RS

(2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.

$C_7H_{12}N_4O_4S$ 248.26

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Technetium Tc 99m Albumin Aggregated Injection

Albumins, blood serum, metastable technetium-99 labeled.

» Technetium Tc 99m Albumin Aggregated Injection is a sterile, aqueous suspension of Albumin Human that has been denatured to produce aggregates of controlled particle size that are labeled with ^{99m}Tc. It is suitable for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as aggregated albumin expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. It may contain antimicrobial, reducing, chelating, and stabilizing agents, buffers, and nonaggregated albumin human. Other chemical forms of radioactivity do not exceed 10 percent of the total radioactivity. Its production and distribution are subject to federal regulations (see *Biologics* ⟨1041⟩ and *Radioactivity* ⟨821⟩).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, at a temperature between 2° and 8°.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as aggregated albumin expressed as total megabecquerels (millicuries or microcuries) and concentration as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours. In addition, the labeling states that it is not to be used if clumping of the albumin is observed and directs that the container be agitated before the contents are withdrawn into a syringe.
Particle size—Shake the Injection well, and determine the dimension of not less than 100 particles of a representative test specimen, using a suitable counting chamber, such as a hemacytometer grid, by optical microscopy. Not less than 90.0% of the observed aggregated particles have a diameter between 10 µm and 90 µm, and none of the observed particles have a diameter greater than 150 µm.
Bacterial Endotoxins Test ⟨85⟩—The limit of endotoxin content is not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩: between 3.8 and 8.0.
Radiochemical purity—Place a measured volume of Injection, appropriately diluted, such that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩), and allow to dry. Develop the chromatogram over a period of about 3 to 4 hours by ascending chromatography, using dilute methanol (7 in 10), and air-dry. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. Not less than 90% of the total radioactivity is found as aggregated albumin (at the point of application).

Place a measured volume of Injection in a centrifuge tube, and determine the net radioactivity in a suitable counting assembly. Centrifuge at approximately 2000 rpm for 5 to 10 minutes. Separate the supernatant by aspiration, and determine its net radioactivity in a suitable counting assembly. Determine the percentage of radioactivity in the supernatant taken by the formula:

$$100(A/B)$$

in which *A* is the net radioactivity, in counts per minute, in the supernatant aliquot, and *B* is the total radioactivity, in counts per minute, in the tube prior to centrifugation. Not more than 10.0% of the radioactivity is found in the supernatant, which contains soluble and dispersed radiochemical impurities, following centrifugation.

Protein concentration—
Test preparation—Transfer 2.0 mL of Injection to a suitable centrifuge tube, and centrifuge at about 2000 rpm for 5 to 10 minutes. Decant the supernatant, and add 2.0 mL of Sodium Chloride Injection to wash the centrifuged aggregate. Centrifuge again at 2000 rpm for 5 to 10 minutes, decant the supernatant, and add 2.0 mL of Sodium Chloride Injection.
Standard preparation—To a second test tube add 2.0 mL of a solution containing 2.0 mg of Albumin Human per mL in 0.9 percent sodium chloride solution.
Procedure—To a third test tube add 2.0 mL of Sodium Chloride Injection to provide a blank. To each of the three tubes containing the *Test preparation*, *Standard preparation*, and blank, add 4.0 mL of biuret reagent TS, mix, and allow to stand for 30 minutes, accurately timed, for maximum color development. Additional mixing or slight heating may be required to dissolve the aggregated albumin completely, but the *Test preparation*, *Standard preparation*, and blank are to be treated identically. Determine the absorbances of the solutions from the *Test preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 540 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of aggregated albumin in each mL of the Injection taken by the formula:

$$2(A_U/A_S)$$

in which A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively. The protein concentration is not more than 1 mg, as aggregated albumin, per 37 MBq (1 mCi) of Tc 99m at the time of administration.
Biological distribution—Inject intravenously between 0.075 MBq and 0.75 MBq (2 µCi and 20 µCi) of Injection, in a volume not exceeding 0.2 mL, into the caudal vein of each of three 20- to 25-g mice. [NOTE—Other animal species, such as Sprague-Dawley rats (weighing 100 g to 175 g), may be used.] Five to 10 minutes after the injection, sacrifice the animals, and carefully remove the liver and lungs of each by dissection. Place each organ and the remaining carcass in separate, suitable counting containers, and determine the radioactivity, in counts per minute, in each container with an appropriate detector, using the same counting geometry. Determine the percentage of radioactivity in the liver and the lungs taken by the formula:

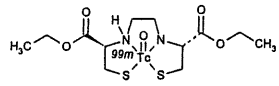
$$100(A/B)$$

in which *A* is the net radioactivity, in counts per minute, in the organ, and *B* is the total radioactivity, in counts per minute, in the lungs, liver, and carcass. Not less than 80.0% of the radioactivity is found in the lungs, and not more than 5.0% of

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the radioactivity is found in the liver, in not less than two of the animals.
Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.
Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (μCi) per mL, of Injection by use of a calibrated system.

Technetium Tc 99m Bicisate Injection



» Technetium Tc 99m Bicisate Injection is a sterile, clear, colorless solution, suitable for intravenous administration, of bicisate dihydrochloride complexed to radioactive technetium (^{99m}Tc). It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as a complex with bicisate, expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 10 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or multiple-dose containers, at controlled room temperature.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as labeled bicisate expressed as total megabecquerels (or millicuries) per mL at the time of calibration; the expiration date and time; the lot number; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 175/*V* USP Endotoxin Units per mL, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.
Radiochemical purity—Prepare four vials of Injection and perform the test on each vial. Apply about 5 μL of Injection 2 cm from the bottom of a 2.5-cm × 7.5-cm thin-layer chromatographic silica gel sheet. Allow the spot to dry for 5 to 10 minutes. Position the plate in a pre-equilibrated chromatographic chamber containing HPLC grade ethyl acetate, and develop the chromatogram until the solvent front has moved 5 cm from the origin (see *Chromatography* ⟨621⟩). Remove the plate from the chamber, and allow to dry. Cut the chromatographic sheet 4.5 cm from the bottom. Separately count the activity on each piece in a dose calibrator or a gamma counter. The activity on the upper portion contains the ^{99m}Tc bicisate complex, and the activity on the lower section contains all radioimpurities. Calculate the percentage of radiochemical purity of the ^{99m}Tc Bicisate Injection by the formula:

$$100P/(P + C)$$

in which *P* is the count from the top part of the plate, and *C* is the count from the bottom part of the plate: not less than 90% of the total radioactivity is found as Tc^{99m} bicisate. Calculate the mean percent radiochemical purity of the four test vials.
Related radiochemical compounds— [NOTE—This test is performed on the same test specimen used to perform the *Radiochemical purity* test. Perform the tests in parallel with a minimal delay in spotting of the chromatographic media following the 30-minute Injection incubation period.] Apply about 2 μL of the Injection 1 cm from the bottom of a 2.5-cm × 7.5-cm reverse-phase thin-layer chromatographic plate (or equivalent), and allow the spot to air-dry thoroughly. Place the plate into a developing tank containing a mixture of acetone and 0.5 M ammonium acetate (60:40), develop to 7 cm, remove the plate, and air-dry (see *Chromatography* ⟨621⟩). Using a suitable calibrated scanner, determine the compounds present by calculating the retention factors for all peaks present. Compounds and approximate R_F values are as follows:

^{99m}Tc bicisate	0.15-0.44
^{99m}Tc(IV) bicisate	0.3-0.4
Tc99m Bicisate and Tc ^{99m}(IV) Bicisate	0.15-0.44
Hydrolyzed reduced Tc	0.00-0.14
Free pertechnetate and ^{99m}Tc ethylene cisteinate monomer	0.70-0.84
^{99m}Tc EDTA	0.95-1.0

Calculate the quantity of ^{99m}Tc(IV) ligand in the Injection by subtracting the Tc 99m Bicisate percentage obtained in the test for *Radiochemical purity* from the combined ^{99m}Tc Bicisate and ^{99m}Tc(IV) Bicisate Area percentage obtained in the *Related radiochemical compounds* test. The sum of the impurities is not greater than 10%.
Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It meets also the requirements for *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of manufacture.
Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in Mbq (or in mCi) per mL, of the Injection by use of a calibrated system.

Technetium Tc 99m Disofenin Injection

» Technetium Tc 99m Disofenin Injection is a sterile, aqueous solution, suitable for intravenous administration, of disofenin that is labeled with ^{99m}Tc. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as a disofenin complex, expressed in megabecquerels (microcuries or millicuries) per

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mL at the time indicated on the labeling. It contains a suitable reducing agent.

Packaging and storage—Preserve in single-dose or in multiple-dose containers sealed under a suitable inert atmosphere.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of preparation; the amount of ^{99m}Tc as expressed as total megabecquerels (microcuries or millicuries) and concentration as megabecquerels (microcuries or millicuries) per mL at the time of preparation; the expiration date and time; and a statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.
pH ⟨791⟩: between 4.0 and 5.0.
Radiochemical purity—The determination of radiochemical purity for this Injection requires the use of two separate chromatography systems.

System A—Apply a measured volume of Injection, appropriately diluted, such that it provides a count rate of about 20,000 counts per minute, about 13 mm from one end of a 10- × 51-mm thin-layer chromatographic strip impregnated with silica gel (see *Chromatography* ⟨621⟩), and immediately develop the chromatogram over a suitable period by ascending chromatography, using methanol as the solvent. Allow the chromatogram to dry. Cut the chromatogram in two along a line about 13 mm from the top. Place the top and bottom strips into separate counting containers. Determine the radioactivity of each, using a suitable ionization chamber. Hydrolyzed Tc 99m and technetium-tin colloid are located at the origin of the bottom strip (R_F 0 to 0.1).

System B—Pretreat a 1.3- × 51-mm strip of chromatographic paper by soaking it for 1 minute in a 0.3 M carbonate-bicarbonate buffer, pH 9.0, prepared by dissolving 0.3 g of anhydrous sodium carbonate and 1.9 g of anhydrous sodium bicarbonate in 100 mL of water. Remove the paper strip, blot lightly with absorbent paper, and dry in an oven for about 45 minutes at 85°. Proceed as directed for *System A* using the pretreated chromatographic paper and a developing solvent of methyl ethyl ketone. Free pertechnetate is located at the solvent front (top strip). The sum of the percentage of radioactivity at the origin in *System A* and the percentage of radioactivity at the solvent front in *System B* is not greater than 10.0%.
Biological distribution—Inject between 75 MBq and 111 MBq (2 mCi and 3 mCi) of Injection, in a volume not exceeding 0.1 mL, into the caudal vein of each of three 25- to 40-g male albino mice. One hour after the injection, anesthetize and then decapitate the animals. Drain the blood of each separately into pre-weighed counting containers. Dissect the animals, and place the kidneys, liver, stomach (exclusive of duodenum), and gallbladder, and intestines, of each mouse into separate counting containers. Using a suitable counting assembly, determine the radioactivity of each container. Correct all radioactivity measurements for decay. Determine the percentage of radioactivity in the kidneys, liver, stomach, and gallbladder and intestines taken by the formula:

$$100(A/B)$$

in which A is the radioactivity, in counts per minute, in the organ, and B is the total radioactivity, in counts per minute, injected. Determine the percentage of radioactivity in the blood taken by the formula:

$$[100(C/W_S)0.07(W_R)]/B$$

in which C is the radioactivity, in counts per minute, in the specimen of blood, W_S is the weight, in g, of the blood specimen, 0.07 is the assumption that the total blood weight of the mouse is 7% of the total body weight, and W_R is the weight, in g, of the mouse. Not less than 70% of the injected radioactivity is present in the gallbladder and intestines, not more than 10.0% of the injected radioactivity is present in the liver, not more than 10.0% of the injected radioactivity is present in the kidneys, not more than 3.0% of the injected radioactivity is present in the stomach, and not more than 3.0% of the injected radioactivity is present in the blood, in not less than two mice.
Other requirements—It meets the requirements of the tests for *Radionuclide identification*, *Radionuclidic purity*, and *Bacterial endotoxins* under *Sodium Pertechnetate Tc 99m Injection*. It also meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the date of preparation, and except that it is not subject to the recommendation on *Container Content*.
Assay for radioactivity(see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (μCi or mCi) per mL, of Injection by the use of a calibrated system.

Technetium Tc 99m Exametazime Injection

2-Butanone, 3,3′-[(2,2-dimethyl-1,3-propanediyl)diimino]bis-dioxime-^{99m}Tc.
d,1-Hexamethylpropylene amine oxime-^{99m}Tc [105613-48-7].

» Technetium Tc 99m Exametazime Injection is a sterile, aqueous solution, suitable for intravenous administration, composed of the primary lipophilic complex of exametazime that is labeled with radioactive ^{99m}Tc. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as primary lipophilic exametazime complex expressed in megabecquerels (or in microcuries or millicuries) per mL at the date and time indicated in the labeling. It may contain antioxidants, reducing agents, and buffers. A stabilizing solution containing methylene blue and phosphate buffer may be added to the final radio labeled product to extend the expiration time. Other chemical forms of radioactivity (^{99m}Tc pertechnetate, ^{99m}Tc hydrolyzed reduced species, and ^{99m}Tc secondary exametazime complex) do not exceed 20.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or multiple-dose containers, at controlled room temperature.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the

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amount of ^{99m}Tc as labeled exametazime expressed as total megabecquerels (or as total microcuries or millicuries) and concentration as megabecquerels (or as microcuries or millicuries) per mL at the time of calibration; the expiration date and time; and a statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours. [NOTE—The label states that, upon constitution with Sodium Pertechnetate Tc 99m Injection, the beyond-use time is 30 minutes for the unstabilized Injection and between 4 hours and 6 hours for the stabilized Injection.]

pH ⟨791⟩: between 9.0 and 9.8 for unstabilized Injection, and between 6.5 and 7.5 for stabilized Injection.

Radiochemical purity—Prepare three 12- × 75-mm chromatographic tubes by packing with about 0.3 mL of fresh methyl ethyl ketone, 0.9% nonbacteriostatic sodium chloride solution, and a 50% acetonitrile solution, prepared with nonbacteriostatic water, respectively. To each of two 6- × 0.7-cm instant thin-layer chromatographic strips[1] and one 6- × 0.7-cm strip of chromatographic paper,[2] apply 5 µL of freshly prepared (within 2 minutes of reconstitution) Injection about 1 cm from the bottom of the strip. Do not allow to dry. Place one instant thin-layer chromatographic strip into the tube containing methyl ethyl ketone, the second instant thin-layer chromatographic strip into the tube containing the 0.9% sodium chloride solution, and the paper strip into the tube containing the 50% acetonitrile solution. The strips must not adhere to the sides of the tubes. Allow the chromatograms to develop until the solvent front has moved to the top of the strips. Remove the strips from the tubes, and allow the solvents to evaporate. Determine the radioactive distribution by scanning the strip sections, using a suitable collimated radiation detector. The difference between the radioactivity detected at the origin of the 0.9% sodium chloride strip and at the origin of the methyl ethyl ketone strip represents the primary lipophilic ^{99m} Tc exametazime complex. The radioactivity at the solvent front of the 0.9% sodium chloride strip represents the ^{99m} Tc pertechnetate radiochemical impurity. The radioactivity at the origin of the 50% acetonitrile strip represents the ^{99m} Tc hydrolyzed, reduced radiochemical impurity. The difference between the radioactivity detected at the origin of the methyl ethyl ketone strip and the origin of the 50% acetonitrile strip represents the secondary ^{99m} Tc exametazime complex radiochemical impurity. Not less than 80% of the total radioactivity is found as primary lipophilic ^{99m} Tc exametazime complex. [NOTE—Complete the measurement within 30 minutes after constitution of the unstabilized Injection and between 4 hours and 6 hours after constitution of the stabilized Injection.]

Biological distribution—Inject between 18.5 MBq and 37 MBq (between 0.5 mCi and 1 mCi) of Injection in a volume not exceeding 0.1 mL into the caudal vein of each of three 130- to 190-g Sprague-Dawley rats. Five to ten minutes after the injection, sacrifice the animals and carefully remove the brain, liver, and intestines (including gall bladder). Place each organ and the remaining carcass in separate, suitable counting containers, and determine the radioactivity, in counts per minute, in each container with an appropriate detector, using the same counting geometry. Determine the percentage of radioactivity in the brain, liver, intestines, and remaining carcass by the formula:

$$100(A/B)$$

in which A is the net radioactivity, in counts per minute, in the organ, and B is the total radioactivity, in counts per minute in the brain, liver, intestines, and carcass. Not less than 1.5% of the radioactivity is found in the brain. Not more than 20% of the radioactivity is found in the intestines, and not more than 15% of the radioactivity is found in the liver, in not less than two of the animals.

Other requirements—It meets the requirements of the tests for *Radionuclidic identification* and *Radionuclide purity* and *Bacterial endotoxins* under *Sodium Pertechnetate Tc 99m Injection*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (or µCi) per mL, of Injection by use of a calibrated system.

[1] ITLC/SG, 6- × 0.7-cm strip available from Biodex Medical Systems, Shirley, NY.

[2] Whatman Grade 31ET Chr, available from Whatman LabSales, Hillsboro, OR.

Technetium Tc 99m Mebrofenin Injection

» Technetium Tc 99m Mebrofenin Injection is a sterile aqueous solution of Stannous Fluoride and Mebrofenin labeled with radioactive technetium Tc 99m suitable for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc, as the complex with mebrofenin, expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. It may contain antimicrobial agents and buffers. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, at controlled room temperature.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as labeled mebrofenin expressed as total megabecquerels (or millicuries) and the concentration as megabecquerels per mL (or as millicuries per mL) on the date and time of calibration; the expiration date and time; and the statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

pH ⟨791⟩: between 4.2 and 5.7.

Radiochemical purity— [NOTE—The determination of radiochemical purity for this product requires the use of two separate chromatographic methods.]

Method 1—

pH 6.8 PHOSPHATE BUFFER—Mix 64 mL of 0.2 M monobasic sodium phosphate and 61 mL of 0.1 M dibasic sodium phosphate in a 1-liter volumetric flask, dilute with water to volume, and mix.

MOBILE PHASE —Prepare a filtered and degassed mixture of methanol and *pH 6.8 phosphate buffer* (625:375). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

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CHROMATOGRAPHIC SYSTEM (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 3.9-mm × 30-cm column that contains 10-µm packing L1. It is also equipped with a flow-through gamma ray detector having a cell volume of about 10 µL. A constant flow rate of about 1 mL per minute is maintained, and counts are recorded and charted at about 5-second intervals.

PROCEDURE —Constitute the Injection, and allow to stand for 15 minutes. Chromatograph a volume of Injection, with activity of 0.6 to 15 MBq (16 to 400 µCi), and record the chromatogram. The resolution between the secondary characteristic peak, eluting at about 3.5 minutes, and the principal peak, eluting at about 6 minutes, is not less than 1.5. The time interval between the elution of the Tc 99m pertechnetate peak, at about 2 minutes, and the principal peak is not less than 3.0 minutes. If the foregoing criteria are met, record the counts for the pertechnetate Tc 99m peak, the two complex peaks, a representative baseline segment, and the total counts for the chromatogram. Calculate the percentage of complexed radioactivity in the two characteristic peaks taken by the formula:

$$100C/(T - P)$$

in which C is the count of the complex peak of interest, T is the total count, and P is the pertechnetate peak count, each being corrected for the corresponding baseline count. The complexed radioactivity is not less than 90.0% in the principal peak and between 1% and 6% in the second characteristic peak. Calculate the percentage of all other complexed radioactivity (this excludes the main, secondary, and free pertechnetate peaks) taken by the formula:

$$100K/(T - P)$$

in which K is the sum of all other peaks, and T and P are as defined above. Record the result for use in the calculation as directed under *Method 2*.

Method 2—

CHROMATOGRAPHIC SYSTEM A (see *Chromatography* ⟨621⟩)—Prepare a 1.0- × 12.5-cm silicic acid-impregnated glass microfiber strip by dipping it in a 10% sodium chloride solution and removing the excess liquid by blotting. Dry the strip by heating in an oven at about 80°. Apply about 5 µL of the Injection with activity of about 3.7 MBq (100 µCi) about 1.5 cm from one end of the strip, and immediately develop the chromatogram in a chromatographic chamber containing saturated sodium chloride solution until the solvent front reaches a level about 1 cm from the top of the strip. Remove the strip from the chamber, and allow to air-dry. Separate the top and bottom portions of the chromatogram by cutting the strip in half, and record the counts separately for each portion. Calculate the percentage of unreduced pertechnetate taken by the formula:

$$100P/(P + C)$$

in which P is the count from the top half of the strip, and C is the count from the bottom half of the strip.

CHROMATOGRAPHIC SYSTEM B —Proceed as directed under *Chromatographic system A* except to use a 1.0- × 12.5-cm silica gel-impregnated glass microfiber strip, without preliminary treatment, and a mixture of acetonitrile and water (3:1) as the developing solvent. Calculate the percentage of reduced hydrolyzed Tc 99m taken by the formula:

$$100R/(R + D)$$

in which R is the count from the bottom half of the strip, and D is the count from the top half of the strip. The sum of the percentage of unreduced pertechnetate from *System A*, the percentage of reduced hydrolyzed Tc 99m from *System B*, and the percentage of other complexed radioactivity determined from *Method 1* is not greater than 10.0%.

Biological distribution—Inject 0.25 mL of Injection containing between 15 and 19 MBq (0.4 and 0.5 mCi) into the external jugular vein of each of three anesthetized male rats weighing between 150 and 350 g, and from which food has been withheld for 15 to 24 hours prior to the test. Thirty minutes after the injection, sacrifice the animals and carefully remove the liver, kidneys, and gastrointestinal tract of each rat. Place the organs in separate counting containers. Using a suitable counting assembly, determine the radioactivity in each container. Correct radioactivity measurements for decay. Determine the percentages of radioactivity in the liver, kidneys, and gastrointestinal tract taken by the formula:

$$100(A/B)$$

in which A is the radioactivity, in counts per minute, in the organ, and B is the average injected radioactivity, in counts per minute, in the corresponding standards. Not less than 75% of the injected radioactivity is found in the gastrointestinal tract, not more than 10% in the liver, and not more than 5% in the kidneys in not less than 2 of the rats.

Other requirements—It meets the requirements of the tests for *Radionuclide identification*, *Radionuclidic purity*, and *Bacterial endotoxins* under *Sodium Pertechnetate Tc 99m Injection*. It also meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed and dispensed prior to the completion of the test for *Sterility*, the latter being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (or in mCi) per mL, of Injection by use of a calibrated system.

Technetium Tc 99m Medronate Injection

» Technetium Tc 99m Medronate Injection is a sterile, aqueous solution, suitable for intravenous administration, of sodium medronate and stannous chloride or stannous fluoride that is labeled with radioactive Tc 99m. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Tc 99m as stannous medronate complex expressed in megabecquerels (microcuries or millicuries) per mL at the date and time indicated in the labeling. It may contain antimicrobial agents, antioxidants, and buffers. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or multiple-dose containers at a temperature specified in the labeling.

Bacterial Endotoxins Test ⟨85⟩ —The limit of endotoxin content is not more than $175/V$ USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in

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which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 4.0 and 7.8.

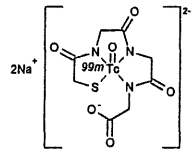
Radiochemical purity—Not more than 10.0% of unbound Tc 99m (free pertechnetate), hydrolyzed Tc 99m, and technetium-tin colloid is present, determined as follows.

System A—Under an atmosphere of nitrogen, place a measured volume of Injection, such that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a paper chromatographic strip (see *Chromatography* ⟨621⟩). Immediately develop the chromatogram over a suitable period by ascending chromatography, using sodium chloride solution (0.9 in 100), and dry it under nitrogen. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. Hydrolyzed Tc 99m and technetium-tin colloid are located at the origin (R_F 0 to 0.1).

System B—Proceed as directed for *System A*, except to develop the chromatogram in dilute methanol (85 in 100). Free pertechnetate is located at an R_F of 0.6 to 0.8. The sum of the percentage of radioactivity at the origin in *System A* plus the percentage of radioactivity at an R_F of 0.6 to 0.8 in *System B* is not greater than 10.0%.

Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*, and meets the requirements for *Labeling*, *Biological distribution*, and *Assay for radioactivity* under *Technetium Tc 99m Pyrophosphate Injection*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content*.

Technetium Tc 99m Mertiatide Injection



$C_8H_8N_3Na_2O_6S^{99m}Tc$

Technetate(2-)-^{99m}*Tc*,[*N*-[*N*-[*N*-(mercaptoacetyl)-glycyl] glycyl]glycinato(5-)-*N*,*N*′,*N*″,*S*]-oxo-, disodium, (*SP*-5-25)-.

Disodium [*N*-[*N*-[*N*-(mercaptoacetyl)glycyl]glycyl]glycinato (5-)-*N*,*N*′,*N*″,*S*]-oxo[^{99m}Tc]technetate(V) [125224-05-7].

» Technetium Tc 99m Mertiatide Injection is a sterile aqueous solution, suitable for intravenous injection, that contains ^{99m}Tc in the form of a chelate of mertiatide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as mertiatide complex expressed in megabecquerels (or in microcuries or millicuries) per mL at the date and time indicated in the labeling. It contains uncomplexed betiatide, a suitable ^{99m}Tc reducing agent, a transfer ligand, and stabilizers.

Packaging and storage—Preserve in single-dose or multiple-dose containers.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as labeled mertiatide expressed as total megabecquerels (or millicuries) and the concentration as megabecquerels per mL (or as millicuries per mL) on the date and time of calibration; the expiration date and time; and a statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 175/*V* USP Endotoxin Unit per mL, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 5.0 and 6.0.

Radiochemical purity—

Method 1 (Determination of hydrolyzed reduced technetium)—Apply about 5 to 10 µL (100 to 250 µCi) 15 mm from the bottom of a 25-mm × 20-cm strip of chromatographic paper (see *Chromatography* ⟨621⟩). Immediately develop the chromatogram by ascending chromatography using a solvent system consisting of a mixture of acetonitrile and water (60:40) until the solvent front has moved about 13 cm from the origin. Remove the strip, and allow to dry. Determine the radioactivity distribution by scanning the chromatogram using a suitable collimated radiation detector. Calculate the percentage of hydrolyzed reduced technetium by the formula:

$$100(A_{ht}/B_s)$$

in which A_{ht} is the sum of all the peaks at or near the origin, where R_F is less than 0.25, and B_s is the sum of all of the peaks: not more than 2.0% of hydrolyzed reduced technetium is found.

Method 2 (Simultaneous determination of free pertechnetate and ^{99m}Tc mertiatide)—

SOLUTION A —Prepare a filtered and degassed solution of monobasic potassium phosphate in water to obtain a solution containing 1.36 mg per mL. To each L of this solution, add 1.0 mL of triethylamine, and adjust with 1.0 N hydrochloric acid to a pH of between 4.9 and 5.1. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

SOLUTION B —Prepare a filtered and degassed solution of monobasic potassium phosphate in 900 mL of water, and add 100 mL of tetrahydrofuran to obtain a solution containing 1.36 mg per mL. To each L of this solution, add 1.0 mL of triethylamine, and adjust with 1.0 N hydrochloric acid to a pH of between 4.9 and 5.1. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

MOBILE PHASE —Mix specified portions of freshly prepared and degassed *Solution A* and *Solution B*.

TEST SOLUTION —Immediately before testing, dilute a portion of the Injection with *Water for Injection* to obtain a concentration of between 400 to 600 µCi. [NOTE—The extent to which the sample is diluted is determined by the sensitivity of the radiometric detector.]

CHROMATOGRAPHIC SYSTEM (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 3.9-mm × 15-cm column that contains packing L1 and a flow-through gamma-ray detector and is programmed to provide a *Mobile phase* consisting of variable mixtures of *Solution A* and *Solution B*. The system is equilibrated for 15 minutes with a *Mobile phase* consisting of a mixture of 90% *Solution A* and 10% *Solution B*. After injecting the *Test solution*, change the composition of the *Mobile phase* linearly over the next 30 minutes so that the final *Mobile phase* composition consists of

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20% *Solution A* and 80% *Solution B*. Hold with this composition for 5 minutes and then change in reverse to a composition of 90% *Solution A* and 10% *Solution B* over a 5-minute period. The flow rate is about 1 mL per minute.

PROCEDURE —Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatograms by gradient elution (see the *Chromatographic system*). The retention time is between 1.8 and 2.2 minutes for ^{99m}Tc pertechnetate and between 10 and 14 minutes for ^{99m}Tc mertiatide. Calculate the percentage of ^{99m}Tc pertechnetate by the formula:

$$100(r_{pt}/r_s)$$

in which r_{pt} is the peak response of ^{99m}Tc pertechnetate, and r_s is the sum of all peak responses: not more than 6.0% of ^{99m}Tc pertechnetate is found.

Calculate the percentage of ^{99m}Tc mertiatide by the formula:

$$100(r_{mt}/r_s')$$

in which r_{mt} is the peak response of ^{99m}Tc mertiatide, and r_s' is the sum of all peak responses: not less than 90.0% of ^{99m}Tc mertiatide is found.

Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It also meets the requirement under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility Tests* ⟨71⟩, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity ⟨821⟩—Using a suitable counting assembly, determine the radioactivity, in Mbq (or millicuries) per mL, of the Injection by use of a calibrated system.

Technetium Tc 99m Oxidronate Injection

» Technetium Tc 99m Oxidronate Injection is a sterile, clear, colorless solution, suitable for intravenous administration, of radioactive technetium (^{99m}Tc) in the form of a chelate of oxidronate sodium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as chelate expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. It may contain buffers, preservatives, reducing agents, and stabilizers. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

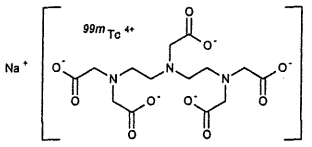
Packaging and storage—Preserve in single-dose or multiple-dose containers.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as labeled oxidronate expressed as total megabecquerels (microcuries or millicuries) and concentration as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

pH ⟨791⟩: between 2.5 and 7.0.

Other requirements—It meets the requirements for *Bacterial endotoxins, Radiochemical purity, Biological distribution, Other requirements*, and *Assay for radioactivity* under *Technetium Tc 99m Pyrophosphate Injection*.

Technetium Tc 99m Pentetate Injection



$C_{14}H_{18}N_3NaO_{10}{}^{99m}Tc$

Technetate(1-) ^{99m} Tc, [*N*,*N*-bis[2-[bis(carboxymethyl)amino] ethyl]glycinato(5-)]-, sodium.

Sodium [*N*,*N*-bis[2-[bis(carboxymethyl)amino]ethyl] glycinato(5-)]-technetate(1-)- ^{99m} Tc [65454-61-7].

» Technetium Tc 99m Pentetate Injection is a sterile solution of pentetic acid that is complexed with ^{99m}Tc in Sodium Chloride Injection. It is suitable for intravenous administration and may contain buffers. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as the pentetic acid complex expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, at a temperature between 2° and 8°.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as labeled pentetic acid complex expressed as total megabecquerels (millicuries or microcuries) and concentration as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

Bacterial Endotoxins Test ⟨85⟩ —The limit of endotoxin content is not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 3.8 and 7.5.

Radiochemical purity—

Phosphate buffer—Dissolve 2.6 g of monobasic sodium phosphate and 3.0 g of anhydrous dibasic sodium phosphate in water to make 1000 mL. Adjust, if necessary, by the addition of 0.2 M phosphoric acid or 0.2 M sodium hydroxide, to a pH of 6.8 ± 0.05.

Procedure —Soak a 2.5- × 17.0-cm cellulose polyacetate strip in 100 mL of *Phosphate buffer* for 10 to 60 minutes.

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Remove the strip with forceps, taking care to handle the outer edges only. Place the strip between two absorbent pads, and blot to remove excess solution. Attach the strip to the support bridge of an electrophoresis chamber containing *Phosphate buffer* in each side of the chamber. Ensure that each end of the strip is in contact with *Phosphate buffer*. Mark the application line near the cathode end of the strip. Apply as a spot 1 µL of a 1 in 1000 solution of amaranth in *Phosphate buffer*. Adjacent to this, apply as a narrow streak 5 µL of Injection having an activity of about 1 mCi per mL, previously diluted with saline TS, if necessary. Attach the chamber cover, and perform the electrophoresis at 300 volts until the amaranth (red dye) has moved about four-fifths of the length of the strip. Remove the strip from the chamber, and blot the ends on paper towels. Using a suitable scanner and counting assembly, determine the radioactivity distribution. Labeled technetium pentetate migrates a distance that is 0.9 relative to that of amaranth. Hydrolyzed technetium Tc 99m is located at the origin and pertechnetate migrates a distance that is 0.7 relative to that of amaranth: the sum of the percentages of radioactivity of hydrolyzed technetium Tc 99m and pertechnetate is not greater than 10.0% of the total radioactivity on the strip.

Biological distribution—Inject intravenously between 0.075 MBq and 0.75 MBq (2 µCi and 20 µCi) of Injection, in a volume not exceeding 0.2 mL, into the caudal vein of each of three 20- to 25-g mice. Approximately 10 to 15 minutes after injection, place each animal in a suitable container and count the radioactivity of each with an appropriate radiation detector, using the same counting geometry, in order to determine the total activity injected. Approximately 24 hours after injection, repeat the radioactivity counting of each animal, as above. After correcting for decay, determine the percentage of radioactivity retained in each animal 24 hours after injection by the formula:

$$100(A/B)$$

in which A is the net radioactivity, in counts per minute (corrected for decay), in the animal at 24 hours, and B is the total radioactivity, in net counts per minute, at the time of injection. The percentage of injected radioactivity retained 24 hours after administration is not greater than 5.0% of the injected activity in any of the animals.

Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (µCi) per mL, of Injection by use of a calibrated system.

Sodium Pertechnetate Tc 99m Injection

Pertechnetic acid ($H^{99m}TcO_4$), sodium salt.
Sodium pertechnetate ($Na^{99m}TcO_4$) [23288-60-0].

» Sodium Pertechnetate Tc 99m Injection is a sterile solution, suitable for intravenous or oral administration, containing radioactive technetium (^{99m}Tc) in the form of sodium pertechnetate and sufficient Sodium Chloride to make the solution isotonic. Technetium 99m is a radioactive nuclide formed by the radioactive decay of molybdenum 99. Molybdenum 99 is a radioactive isotope of molybdenum and may be formed by the neutron bombardment of molybdenum 98 or as a product of uranium fission.

Sodium Pertechnetate Tc 99m Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc at the date and hour stated on the label. Other chemical forms of ^{99m}Tc do not exceed 5 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or multiple-dose containers.

Labeling—If intended for intravenous use, label it with the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. Label it also to include the following: the time and date of calibration; the amount of ^{99m}Tc as sodium pertechnetate expressed as total megabecquerels (millicuries) and as megabecquerels (millicuries) per mL on the date and at the time of calibration; a statement of the intended use, whether oral or intravenous; the expiration date; and the statement "Caution—Radioactive Material." If the Injection has been prepared from molybdenum 99 produced from uranium fission, the label so states. The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{99m}Tc that exhibits a major photopeak having an energy of 0.140 MeV.

Bacterial Endotoxins Test ⟨85⟩—The limit of endotoxin content is not more than $175/V$ USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 4.5 and 7.5.

Radiochemical purity—Place a volume of Injection, appropriately diluted, such that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩). Develop the chromatogram over a suitable period of time by ascending chromatography, using a mixture of acetone and 2 N hydrochloric acid (80:20). Allow the chromatogram to air-dry. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. The radioactivity of the pertechnetate band is not less than 95% of the total radioactivity in the test specimen. The R_F value for the pertechnetate band (approximately 0.9) falls within ±10.0% of the value found for a known sodium pertechnetate Tc 99m specimen when determined under identical conditions.

Radionuclidic purity—Using a suitable counting assembly, determine the radioactivity of each radionuclidic impurity, in kBq per MBq (µCi per mCi) of technetium 99m, in the Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

For Injection prepared from technetium 99m derived from parent molybdenum 99 formed as a result of neutron bombardment of stable molybdenum—

MOLYBDENUM 99—The presence of molybdenum 99 in the Injection is shown by its characteristic gamma-ray spectrum. The most prominent photopeaks of this radioactive nuclide have energies of 0.181, 0.740, and 0.780 MeV. Molybdenum

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99 decays with a radioactive half-life of 66.0 hours. The amount of molybdenum 99 is not greater than 0.15 kBq per MBq (0.15 µCi per mCi) of technetium 99m per administered dose in the Injection, at the time of administration.

OTHER GAMMA-EMITTING RADIONUCLIDIC IMPURITIES —The total amount of other gamma-emitting radionuclidic impurities does not exceed 0.5 kBq per MBq (0.5 µCi per mCi) of technetium 99m, and does not exceed 92 kBq (2.5 µCi) per administered dose of the Injection, at the time of administration.

For Injection prepared from technetium 99m derived from parent molybdenum 99 formed as a result of uranium fission—*Gamma- and beta-emitting impurities*—

MOLYBDENUM 99—The Injection meets the requirements set forth for the Injection prepared by neutron irradiation of stable molybdenum (see foregoing).

IODINE 131—The most prominent photopeak of this radioactive nuclide has an energy of 0.364 MeV. Iodine 131 decays with a radioactive half-life of 8.08 days. The concentration of iodine 131 is not more than 0.05 kBq per MBq (0.05 µCi per mCi) of technetium 99m, at the time of administration.

RUTHENIUM 103—The most prominent photopeak of this radioactive nuclide has an energy of 0.497 MeV. Ruthenium 103 decays with a radioactive half-life of 39.5 days. The concentration of ruthenium 103 is not more than 0.05 kBq per MBq (0.05 µCi per mCi) of technetium 99m, at the time of administration.

STRONTIUM 89—Determine the presence of strontium 89 in the Injection by a counting system appropriate for the detection of particulate radiations. Strontium 89 decays by a beta emission with a maximum energy of 1.463 MeV, and a radioactive half-life of 52.7 days. Strontium 89 may be present in a concentration of not more than 0.0006 kBq per MBq (0.0006 µCi per mCi) of technetium 99m, at the time of administration.

STRONTIUM 90—Determine the presence of strontium 90 in the Injection by a counting system appropriate for the detection of particulate radiations. Strontium 90 decays by a beta emission with a maximum energy of 0.546 MeV, and a radioactive half-life of 27.7 years. Strontium 90 may be present in a concentration of not more than 0.00006 kBq per MBq (0.00006 µCi per mCi) of technetium 99m, at the time of administration.

ALL OTHER RADIONUCLIDIC IMPURITIES—Not more than 0.01% of all other beta and gamma emitters is present at the time of administration. Not more than 0.001 Bq of gross alpha impurity per 1 MBq (or 0.001 nCi of gross alpha impurity per 1 mCi) of technetium 99m is present at the time of administration.

Chemical purity—

Aluminum (To be determined if separation is accomplished by an alumina column in the preparation of the Injection)—

ALUMINUM STANDARD SOLUTION —Dissolve 35.17 mg, accurately weighed, of aluminum potassium sulfate dodecahydrate in water to make 1000.0 mL. Each mL of this solution contains 2 µg of Al.

PROCEDURE—Pipet 10 mL of *Aluminum standard solution* into each of two 50-mL volumetric flasks. To each flask add 3 drops of methyl orange TS and 2 drops of 6 N ammonium hydroxide, then add 0.5 N hydrochloric acid, dropwise, until the solution turns red. To one flask add 25 mL of sodium thioglycolate TS, and to the other flask add 1 mL of edetate disodium TS. To each flask add 5 mL of eriochrome cyanine TS and 5 mL of acetate buffer TS, and add water to volume. Immediately determine the absorbance of the solution containing sodium thioglycolate TS at the wavelength of maximum absorbance at about 535 nm, with a suitable spectrophotometer, using the solution containing the edetate disodium TS as a blank. Repeat the procedure using two 1.0-mL aliquots of Injection. Calculate the quantity, in µg per mL, of aluminum in the Injection taken by the formula:

$$20(T_U/T_S)$$

in which T_U and T_S are the absorbances of the solution from the Injection and the solution containing the aluminum standard, respectively. The concentration of aluminum ion in the Injection is not greater than 10 µg per mL.

Methyl ethyl ketone (To be determined if separation is accomplished by liquid-liquid extraction in the preparation of the Injection)—Place 1.0 mL of the Injection in a suitable container, and dilute with water to 20.0 mL. Add 2.0 mL of 1 N sodium hydroxide, mix, then add 2.0 mL of 0.1 N iodine, dropwise, and again mix. At the same time, prepare a standard by placing 1.0 mL of a solution of methyl ethyl ketone (1 in 1000) in a similar container and diluting with water to 20.0 mL. Add 2.0 mL of 1 N sodium hydroxide, mix, then add 2.0 mL of 0.1 N iodine, dropwise, and again mix. After 2 minutes, the turbidity of the test specimen does not exceed that of the standard (0.1%).

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (mCi) per mL, in Injection by use of a calibrated system.

Technetium Tc 99m Pyrophosphate Injection

» Technetium Tc 99m Pyrophosphate Injection is a sterile, aqueous solution, suitable for intravenous administration, of pyrophosphate that is labeled with ^{99m}Tc. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as pyrophosphate expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. It may contain antimicrobial agents, buffers, reducing agents, and stabilizers. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or multiple-dose containers, at a temperature between 2° and 8°.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as labeled tetrasodium pyrophosphate expressed as total megabecquerels (microcuries or millicuries) and concentration as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

Bacterial Endotoxins Test ⟨85⟩ —The limit of endotoxin content is not more than 175/*V* USP Endotoxin Unit per mL of

USP Monographs

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the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 4.0 and 7.5.

Radiochemical purity—The determination of radiochemical purity for this product requires the use of two separate chromatography systems.

System A—Under an atmosphere of nitrogen, place a measured volume of Injection, appropriately diluted, such that it provides a count rate of about 20,000 counts per minute, about 20 mm from one end of a thin-layer chromatographic strip impregnated with silica gel (see *Chromatography* ⟨621⟩), and allow to dry. Develop the chromatogram over a suitable period by ascending chromatography, using saline TS, and dry it under nitrogen. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. Hydrolyzed Tc 99m and technetium-tin colloid are located at the origin (R_f 0 to 0.1).

System B—Proceed as directed for *System A*, except to develop the chromatogram in a mixture of methanol and acetone (1:1). Free pertechnetate is located at the solvent front. The sum of the percentage of radioactivity at the origin in *System A* plus the percentage of radioactivity at the solvent front in *System B* is not greater than 10.0%.

Biological distribution—Inject intravenously between 0.075 MBq and 75 MBq (2 µCi and 2 mCi) of Injection, in a volume not exceeding 0.2 mL, into the caudal or external jugular vein of each of three 175- to 250-g rats. Approximately 1 hour after the injection, sacrifice the animals, and carefully remove the liver, both kidneys, and one femur of each by dissection, freeing the femur from soft tissue. Remove the tail 20 to 30 mm above the injection site, and discard. Place each organ, both kidneys, and the remaining carcass in separate, suitable counting containers, and determine the radioactivity, in counts per minute, in each container with an appropriate detector, using the same counting geometry. Determine the percentage of radioactivity in the liver, kidneys, and femur taken by the formula:

$$100(A/B)$$

in which *A* is the net radioactivity, in counts per minute, in the organ, and *B* is the total radioactivity, in counts per minute, in the liver, kidneys, femur, and carcass. Not more than 5.0% of the total radioactivity is found in the liver or in the kidneys, and not less than 1.0% of the total radioactivity is found in the femur, in not fewer than 2 of the rats.

Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (µCi) per mL, of the Injection by use of a calibrated system.

Technetium Tc 99m (Pyro- and trimeta-) Phosphates Injection

» Technetium Tc 99m (Pyro- and trimeta-) Phosphates Injection is a sterile, aqueous solution, suitable for intravenous administration, composed of sodium pyrophosphate, sodium trimetaphosphate, and stannous chloride labeled with radioactive Tc 99m. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as phosphate expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

pH ⟨791⟩: between 4.0 and 7.0.

Radiochemical purity—Place a measured volume of Injection, appropriately diluted, such that it provides a count rate of about 20,000 counts per minute, about 10 mm from one end of a 10- × 50-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩), and immediately develop the chromatogram over a period of 3 to 5 minutes by ascending chromatography, using saline TS, and air-dry. Determine the radioactivity distribution by scanning with a suitable collimated radiation detector. Not more than 10.0% of the total radioactivity is found at the point of application. Similarly prepare a second strip, and thoroughly dry the spot with nitrogen. Develop the chromatogram over a period of 35 to 45 seconds by ascending chromatography, using freshly distilled methyl ethyl ketone, and allow to dry. Determine the radioactivity distribution as previously directed. Not less than 90.0% of the total radioactivity is found at the point of application.

Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*, and the requirements of the tests for *Packaging and storage*, *Labeling*, *Bacterial endotoxins*, *Biological distribution*, and *Assay for radioactivity* under *Technetium Tc 99m Pyrophosphate Injection*. It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content*.

Technetium Tc 99m Red Blood Cells Injection

» Technetium Tc 99m Red Blood Cells Injection is a preparation of anticoagulated whole blood that is labeled with ^{99m}Tc. The cells are prepared for labeling by collection of an autologous sample of whole blood, which is anticoagulated with Heparin Sodium or anticoagulant dextrose solution.

Technetium Tc 99m Red Blood Cells Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of ^{99m}Tc as labeled blood cells expressed in megabecquerels (or in microcuries or millicuries) per mL at the time indicated in the labeling. It may contain anticoagulants, such as heparin or anticoagulant citrate solution, chelating agents, stannous chloride, and sodium hypochlorite. Other chemical forms of radioactivity do not exceed 10.0

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percent of the total radioactivity. When derived from donor blood, its production and distribution derived from donor blood are subject to federal regulations (see *Biologics* ⟨1041⟩ and *Radioactivity* ⟨821⟩).

Caution— A strict aseptic technique must be followed for collection of the blood sample, along with the processing steps required to label it with Technetium Tc 99m. The blood samples must be labeled with the name of the patient and patient's identification code to prevent administration of the sample to other than the intended patient. In the event that donor blood is used, it must first be tested for viral contaminants and carefully typed and cross-matched to ensure compatibility with that of the recipient.

Packaging and storage—Preserve in adequately shielded single-dose or multiple-dose containers, at controlled room temperature.

Labeling—Label it to include the following in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the patient's name and identification number; the type of anticoagulant used; the time and date of calibration; the amount expressed as total megabecquerels (or microcuries or millicuries) and concentration as megabecquerels (or microcuries or millicuries) per mL at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

Clarity and color of solution—Observe the appearance and color of the supernatant diluted plasma obtained in the *Radiochemical purity* test procedure. The diluted plasma is clear and has a colorless to a very slight pink or yellow appearance. Samples that produce a distinctive red coloration are not acceptable for administration.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 5.5 and 8.0.

Radiochemical purity—Transfer 0.2 mL of the Injection to a centrifuge tube containing 2 mL of 0.9% sodium chloride solution. Centrifuge for five minutes, and carefully withdraw the diluted plasma by pipet. Measure the radioactivity in the plasma and red blood cells separately in a suitable counter. Calculate labeling efficiency by the formula:

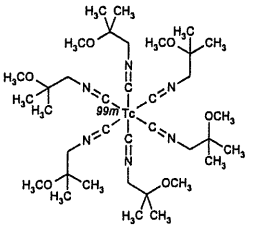
$$100[A_{RBC}/(A_{RBC} + A_P)]$$

in which A_{RBC} is the activity in the red blood cells, and A_P is the activity in the plasma. Not less than 90% of the ^{99m}Tc present in the Injection is bound to the red blood cells.

Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (or μCi) per mL, of the Injection by use of a calibrated system.

Technetium Tc 99m Sestamibi Injection



$C_{36}H_{66}N_6O_6{}^{99m}Tc$

Technetium(1+)-^{99m}*Tc*,hexakis(1-isocyano-2-methoxy-2-methylpropane)-, (*OC*-6-11)-.

Hexakis(2-methoxy-2methylpropyl isocyanide)[^{99m}Tc] technetium(1+) [109581-73-9].

» Technetium Tc 99m Sestamibi Injection is a sterile, aqueous solution of tetrakis(2–methoxy-isobutyl isonitrile) copper (I) tetrafluoroborate that is labeled with ^{99m} Tc suitable for intravenous administration. It contains not less than 90 percent and not more than 110 percent of the labeled amount of ^{99m} Tc as a complex with sestamibi, expressed in megabecquerels (or in milicuries) per mL at the time indicated in the labeling. It contains reducing agents, a buffer, and an inert filler. Other chemical forms of radioactivity do not exceed 10 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or multiple-dose containers.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of constitution; the volume of constitution; the amount of ^{99m}Tc as labeled sestamibi expressed as total megabecquerels (or millicuries) per mL at the time of constitution; the expiration date and time; the lot number; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 175/*V* USP Endotoxin Units per mL, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 5.0 and 6.0.

Radiochemical purity—

Preparation of the test vials—Constitute each of 4 vials with 1 mL (1875 ± 187.5 MBq, or 50 ± 5 mCi) of *Sodium Pertechnetate Tc 99m Injection*. Heat the vials in boiling water for 10 minutes. After heating, allow the vials to cool to room temperature for 15 minutes.

Method 1 (Thin-layer chromatography)—Apply 1 to 2 μL of Injection about 1 cm from the bottom of a 25-mm × 7.75-cm reverse-phase chromatographic plate, and allow to dry (see *Chromatography* ⟨621⟩). Position the plate in a chromatographic chamber and develop the chromatograms

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in a freshly prepared (not more than 4 hours) solvent system consisting of a mixture of acetonitrile, methanol, 3.85% ammonium acetate, and tetrahydrofuran (4:3:2:1) until the solvent front has moved about 6 cm from the origin. Remove the plate and allow it to air-dry. Determine the radioactivity distribution by scanning the chromatogram with a suitable radiation detector. A mean of not less than 90% (area %) of the radioactivity is found at an R_F value between 0.3 and 0.6. Free pertechnetate is located at about the R_F 0.8 to 1.0, and radiocolloid is located at about R_F 0 to 0.1. The sum of the mean percentages of free pertechnetate and colloid does not exceed 10%.

Method 2 (High-pressure liquid chromatography)—

MOBILE PHASE—Prepare a filtered and degassed mixture of methanol, 0.05 M ammonium sulfate solution, and acetonitrile (45:35:20). Make adjustments if necessary (see *System Suitablity* under *Chromatography* ⟨621⟩).

CHROMATOGRAPHIC SYSTEM (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 3.9-mm × 30-cm column that contains 10-µm packing L1. It is also equipped with a flow-through gamma-ray detector. The flow rate is about 2 mL per minute. If ^{99m}Tc pentamibi dimethylvinyl isonitrile is present, the relative retention between the ^{99m}Tc sestamibi peak and the ^{99m}Tc pentamibi dimethylvinyl isonitrile peak is 1.3 to 1.5.

PROCEDURE—Inject about 5 µL (9.375 MBq or 250 µCi) of *Sodium Pertechnetate Tc 99m Injection* into the chromatograph, and adjust the integrator/recording device so that the peak is 25% to 100% of full scale. Separately inject equal volumes (about 5 µL, 9.375 MBq, or 250 µCi) of the injection under test into the chromatograph, record the chromatograms, and measure the area percentage for all of the peaks present. The retention time for ^{99m}Tc sestamibi is about 5 to 10 minutes. The retention time for ^{99m}Tc pentamibi dimethylvinyl isonitrile is about 6 to 13 minutes. Correct for the presence of colloid, which is not measured by this method, taken by the equation:

$$C_f = [(100\%) - (A_c)]/100$$

in which C_f is the correction factor, and A_c is the mean area percentage for the colloid obtained from *Method 1*. Obtain corrected area percentage by multiplying the correction factor (C_f) by the area percentage of the peaks present in the chromatogram. A mean of not less than 90% (corrected area percentage) of the total radioactivity is represented by ^{99m}Tc sestamibi, and a mean of not more than 5% (corrected area percentage) of the total radioactivity is present as ^{99m}Tc pentamibi dimethylvinyl isonitrile.

Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It meets also the requirements for *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed and dispensed prior to the completion of the test for *Sterility*, the latter being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (or in mCi) per mL, of the Injection by use of a calibrated system.

Technetium Tc 99m Succimer Injection

meso-2,3-Dimercaptosuccinic acid, ^{99m}Tc complex.

» Technetium Tc 99m Succimer Injection is a sterile, clear, colorless, aqueous solution of succimer complexed with ^{99m}Tc. It is suitable for intravenous administration. It contains not less than 85.0 percent of the labeled amount of ^{99m}Tc as the succimer complex expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. It may contain reducing agents. Other chemical forms of radioactivity do not exceed 15.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose containers, at a temperature between 15° and 30°. Do not freeze or store above 30°. Protect from light.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as labeled succimer expressed as total megabecquerels (microcuries or millicuries) and concentration as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours. In addition, the labeling states that it is not to be used if discoloration or particulate matter is observed. [NOTE—A beyond-use time of 30 minutes shall be stated on the label upon constitution with Sodium Pertechnetate Tc 99m Injection.]

Bacterial Endotoxins Test ⟨85⟩ —The limit of endotoxin content is not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 2.0 and 3.0.

Radiochemical purity—Activate a 65- × 95-mm silicic acid thin-layer chromatographic plate by heating at 100-110° for 30 minutes. Cool over silica gel and immediately apply 1 µL of Injection, appropriately diluted, if necessary, to a radioactive concentration of 18.5 to 370 MBq (0.5 to 10 mCi) per mL, about 17 mm from one end of the chromatographic plate, and allow to dry. Develop the chromatogram over a period of about 30 to 45 minutes by ascending chromatography, using *n*-butanol saturated with 0.3 N hydrochloric acid, and air-dry. Determine the radioactive distribution by scanning the chromatogram with a suitable collimated radiation detector. Not less than 85% of the total radioactivity is found as succimer at an R_F between 0.45 and 0.70. Hydrolyzed ^{99m}Tc is located at the origin (R_F 0 to 0.15) and the unbound ^{99m}Tc is located at the solvent front (R_F 1.0).

Biological distribution—Inject intravenously between 3.7 MBq and 92.5 MBq (100 µCi and 2500 µCi) of Injection, in a volume of 0.2 to 0.25 mL, into the caudal vein of each of three 125-g to 225-g anesthetized Sprague-Dawley female rats. Clamp the opening of the urethra with a hemostat. Sacrifice the animals 1 hour after the injection, and carefully remove the kidneys, bladder, and liver and spleen of each as *three* separate organs by dissections. Place each organ and the remaining carcass (excluding the tail) in separate, suitable counting containers, and determine the radioactivity, in counts per minute, in each container with an appropriate detector, using the same counting geometry. Determine the percentage of administered radioactive dose in each organ: not less than 40% of the administered radioactive dose is found in the kidneys and a ratio of not less than 6:1 of the

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administered dose is found in the ratio kidneys/(liver and spleen), in not fewer than two of the animals.
Other requirements—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.
Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (μCi) per mL, of Injection by use of a calibrated system.

Technetium Tc 99m Sulfur Colloid Injection

Sulfur, colloidal, metastable technetium-99 labeled [7704-34-9].

» Technetium Tc 99m Sulfur Colloid Injection is a sterile, colloidal dispersion of sulfur labeled with radioactive ^{99m}Tc, suitable for intravenous administration.

Technetium Tc 99m Sulfur Colloid Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of ^{99m}Tc as sulfur colloid expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. It may contain chelating agents, buffers, and stabilizing agents. Other chemical forms of radioactivity do not exceed 8 percent of the total radioactivity. [NOTE—Agitate the container before withdrawing the Injection into a syringe.]

Packaging and storage—Store in single-dose or multiple-dose containers.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as sulfur colloid expressed as total megabecquerels (microcuries or millicuries) and concentration as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours; in addition, the labeling states that it is not to be used if flocculent material is visible and directs that the container be agitated before the Injection is withdrawn into a syringe.
Radionuclide identification (see *Radioactivity* ⟨821⟩)—It meets the requirements of the test for *Radionuclide identification* under *Sodium Pertechnetate Tc 99m Injection*.
Bacterial Endotoxins Test ⟨85⟩ —The limit of endotoxin content is not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩: between 4.5 and 7.5.
Radionuclidic purity—It meets the requirements of the test for *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*.
Radiochemical purity—Place a measured volume of Injection, appropriately diluted, such that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩), and allow to dry. Develop the chromatogram over a suitable period by ascending chromatography, using dilute methanol (8.5 in 10), and air-dry. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. Not less than 92% of the total radioactivity is found as sulfur colloid (at the point of application).
Biological distribution—Inject intravenously between 0.075 MBq and 0.75 MBq (2 μCi and 20 μCi) of the Injection in a volume not exceeding 0.2 mL into the caudal vein of each of three 20-g to 25-g mice. Ten to 30 minutes after the injection, sacrifice the animals, and carefully remove the liver and lungs of each by dissection. Place each organ and remaining carcass in separate, suitable counting containers, and determine the radioactivity, in counts per minute, in each container in an appropriate scintillation well counter, using the same counting geometry. Determine the percentage of radioactivity in the liver and the lungs taken by the formula:

$$100(A/B)$$

in which *A* is the net radioactivity, in counts per minute, in the organ, and *B* is the total radioactivity, in counts per minute, in the lungs, liver, and carcass. Not less than 80% of the radioactivity is found in the liver and not more than 5% of the radioactivity is found in the lungs, in two of the mice.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.
Assay for radioactivity(see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity, in MBq (μCi) per mL, of Injection by use of a calibrated system.

Technetium Tc 99m Tetrofosmin Injection

» Technetium Tc 99m Tetrofosmin Injection is a sterile aqueous solution, suitable for intravenous injection, that contains ^{99m}Tc in the form of a complex of tetrofosmin. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{99m}Tc as tetrofosmin complex expressed in MBq (or mCi) per mL at the date and time indicated in the labeling. It may contain reducing agents, stabilizers, and buffers. It contains no antimicrobial agents. Other chemical forms of radioactivity do not exceed 10.0 percent of the total.

Packaging and storage—Preserve in adequately shielded single-dose or in multiple-dose containers. Protect from light. Store at a temperature not exceeding 25°.

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Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{99m}Tc as labeled tetrofosmin expressed as total MBq (or mCi) and the concentration as megabecquerels per mL (or as mCi per mL) on the date and time of calibration; the expiration date and time; and a statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{99m}Tc is 6.0 hours.

Bacterial Endotoxins Test ⟨85⟩ —The limit of endotoxin content is not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

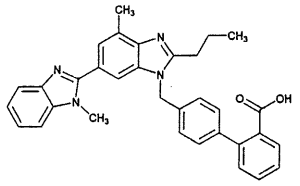
pH ⟨791⟩: between 8.3 and 9.1.

Radiochemical purity—To determine the amount of free technetium, apply a 10- to 20-µL volume of Injection about 3.0 cm from the bottom of a 2- × 20-cm instant thin-layer chromatographic silica gel strip (see *Chromatography* ⟨621⟩). Immediately develop the chromatogram by ascending chromatography to a height of 15 cm using a solvent system consisting of a mixture of acetone and dichloromethane (35:65). Allow the chromatogram to air-dry. Determine the radioactivity distribution of the chromatogram by scanning with a suitable radiation detector. The R_F value of the technetium Tc 99m tetrofosmin spot is approximately 0.5; the sum of radioactivity at the solvent front (unbound pertechnetate) and the origin (reduced hydrolyzed technetium and hydrophilic impurities) is not more than 10%.

Other requirements—It meets the requirements for *Radionuclide identification* and *Radionuclidic purity* under *Sodium Pertechnetate Tc 99m Injection*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the date of manufacture.

Assay for radioactivity ⟨see *Radioactivity* ⟨821⟩⟩—Using a suitable counting assembly, determine the radioactivity, in MBq (µCi) per mL, of the Injection by use of a calibrated system.

Telmisartan



$C_{33}H_{30}N_4O_2$ 514.62

[1,1′-Biphenyl]-2-carboxylic acid, 4′-[(1,4′-dimethyl-2′-propyl[2,6′-bi-1*H*-benzimidazol]-1′-yl)methyl-];
4′-[[4-Methyl-6-(1-methyl-2-benzimidazolyl)-2-propyl-1-benzimidazolyl]methyl]-2-biphenylcarboxylic acid [144701-48-4].

DEFINITION

Telmisartan contains NLT 98.0% and NMT 101.0% of $C_{33}H_{30}N_4O_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)**:** USP Telmisartan RS[NOTE—Heating the solution may be necessary for complete dissolution.] Cool the solution in an ice bath, filter the crystals, and dry at 105°.
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY

- **PROCEDURE**

(See *Titrimetry* ⟨541⟩.)

Sample solution: 190 mg of Telmisartan in 5 mL of anhydrous formic acid. Dilute with 75 mL of acetic anhydride.

Analysis: Titrate with 0.1 M perchloric acid versus a blank determination under the same conditions. Each mL of 0.1 M perchloric acid is equivalent to 25.73 mg of $C_{33}H_{30}N_4O_2$.

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%. A 1-g sample is used.
- **ORGANIC IMPURITIES**

[NOTE—Freshly prepare sample solutions, and protect from light.]

Solution A: 2.0 g of monobasic potassium phosphate and 3.8 g of sodium 1-pentanesulfonate in 1 L of water. Adjust with 1 M phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile and methanol (4:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
27	20	80
32	20	80
32.1	70	30
37	70	30

System suitability solution: Dissolve USP Telmisartan RS and USP Telmisartan Related Compound B RS in methanol (0.2 mL/mg of USP Telmisartan RS) and 100 µL of 1 M sodium hydroxide solution. Sonicate to dissolve. The final concentration is 2.5 mg/mL of the USP Telmisartan RS and 2.5 µg/mL of USP Telmisartan Related Compound B RS in methanol.

Standard solution: Dissolve USP Telmisartan RS in methanol (0.2 mL/mg of USP Telmisartan RS) and 100 µL of 1 M sodium hydroxide solution. Sonicate to dissolve. The final concentration is 0.025 mg/mL.

Sample solution: Dissolve Telmisartan in methanol (0.2 mL/mg of Telmisartan) and 100 µL of 1 M sodium hydroxide solution. Sonicate to dissolve. The final concentration is 2.5 mg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.0-mm × 12.5-cm; 5-µm packing L1

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Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 2 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between telmisartan and telmisartan related compound B, *System suitability solution*
Tailing factor: Between 0.9 and 1.5 for telmisartan related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0% for the telmisartan peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Telmisartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of telmisartan from the *Standard solution*
C_S = concentration of USP Telmisartan RS in the *Standard solution*
C_U = concentration of Telmisartan in the *Sample solution*

Acceptance criteria: See *Table 2*. [NOTE—Calculate the total impurities from the sum of all impurity peaks greater than or equal to 0.05%.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Telmisartan related compound A[a]	0.3	0.1
Telmisartan amide[b]	0.7	0.1
Telmisartan related compound B[c]	0.9	0.1
Telmisartan diacid[d]	0.67	0.1
Telmisartan *tert*-butyl ester[e]	1.7	0.2
Telmisartan unknown impurity	1.8	0.2
Any other individual impurity	—	0.1
Total impurities	—	1.0

[a] 1,7′-Dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[*d*]imidazole.
[b] 4′-[(1,7′-Dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[*d*]imidazol-3′-yl)methyl] biphenyl-2-carboxamide.
[c] 4′-[(1,7′-Dimethyl-2′-propyl-1*H*,1′*H*-2,5′-bibenzo[*d*]imidazol-1′-yl)methyl] biphenyl-2-carboxylic acid.
[d] 1-[(2′-Carboxybiphenyl-4-yl)methyl]-4-methyl-2-propyl-1*H*-benzimidazole-6-carboxylic acid.
[e] *tert*-Butyl 4′-[(1,7′-dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[*d*]imidazol-3′-yl) methyl]biphenyl-2-carboxylate.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩: Dry 1.0 g of the sample at 105° to constant weight: it loses NMT 1.5%.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Telmisartan RS
  USP Telmisartan Related Compound B RS
  4′-[(1,7′-Dimethyl-2′-propyl-1*H*,1′*H*-2,5′-bibenzo[*d*] imidazol-1′-yl)methyl]biphenyl-2-carboxylic acid.
  $C_{33}H_{30}N_4O_2$ 514.62

Telmisartan Tablets

DEFINITION
Telmisartan Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet Spectroscopy*: **197U**▲ (CN 1-May-2020): The spectrum of the solution under test corresponds to that of the *Standard solution* as obtained in *Dissolution Test 1*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Diluent: 0.005 N methanolic solution of sodium hydroxide
Buffer: 2.0 g/L of ammonium dihydrogen phosphate. Adjust with 1 M phosphoric acid to a pH of 3.0.
Mobile phase: Methanol and *Buffer* (70:30)
Standard stock solution: 0.8 mg/mL of USP Telmisartan RS and 0.1 mg/mL of USP Telmisartan Related Compound A RS in *Diluent*
Standard solution: 0.11 mg/mL of USP Telmisartan RS and 0.013 mg/mL of USP Telmisartan Related Compound A RS in *Mobile phase* from the *Standard stock solution*. Pass the solution through a membrane filter of 0.45-µm pore size.
Sample solution: Transfer NLT 20 Tablets into a suitable volumetric flask, and add about 80% of the volume of *Diluent*. Swirl to disperse, and sonicate for about 10 min. Allow to cool to room temperature, dilute with *Diluent* to volume, and mix. Pass the resulting solution through a membrane filter of 0.45-µm pore size. Further dilute quantitatively in *Mobile phase* to prepare a solution having a concentration of 0.11 mg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 298 nm
Column: 4.0-mm × 4-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.7 mL/min
Injection volume: 5 µL

System suitability
Sample: *Standard solution*
Suitability requirements
[NOTE—The relative retention times for telmisartan related compound A and telmisartan are 0.53 and 1.00, respectively.]
Resolution: NLT 3 between telmisartan and telmisartan related compound A
Tailing factor: NMT 2.0 for the telmisartan peak
Capacity factor: NLT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) in the portion of Tablets taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of telmisartan from the *Sample solution*
r_S = peak response of telmisartan from the *Standard solution*
C_S = concentration of USP Telmisartan RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of telmisartan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: pH 7.5 phosphate buffer (prepared by dissolving 13.61 g of potassium dihydrogen phosphate in about 800 mL of water, adjusting with 2 M sodium hydroxide to a pH of 7.5, and diluting with water to 1000 mL); 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Standard solution: Transfer about 44 mg of USP Telmisartan RS to a 100-mL volumetric flask. Add 1 mL of 0.1 M sodium hydroxide, and dilute with methanol to volume. Dilute this solution quantitatively with *Medium* to obtain a solution having a final concentration of about 0.011 mg/mL.
Sample solution
For Tablets labeled to contain 20 mg: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Further dilute the filtrate with *Medium* (1:2).
For Tablets labeled to contain 40 mg: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Further dilute the filtrate with *Medium* (1:4).
For Tablets labeled to contain 80 mg: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Further dilute the filtrate with *Medium* (1:8).
Instrumental conditions
Mode: UV
Analytical wavelength: 296 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of telmisartan ($C_{33}H_{30}N_4O_2$) dissolved:

$$\text{Result} = (A_U \times C_S \times V \times 100)/(A_S \times D \times L)$$

A_U = absorbance of the *Sample solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
A_S = absorbance of the *Standard solution*
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Buffer: 2.72 g/L of potassium dihydrogen phosphate. Add 2 mL of triethylamine per liter of solution and adjust with phosphoric acid to a pH of 2.4.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Standard stock solution: 0.44 mg/mL of USP Telmisartan RS prepared as follows. To a suitable amount of USP Telmisartan RS in a suitable volumetric flask add methanol, about 50% of the total volume. Sonicate to dissolve, cool to room temperature, and dilute with *Medium* to volume.
Standard solution: (L/900) mg/mL of USP Telmisartan RS in *Medium* from *Standard stock solution*, where L is the label claim in mg/Tablet
Sample solution: Pass portions of the solution under test through a suitable filter of 0.45-µm pore size and discard the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 298 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.6 times the retention time of telmisartan
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Telmisartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium, Apparatus 2, and **Instrumental conditions:** Proceed as directed in *Dissolution Test 1*.
Time: 20 min
Standard solution: 0.011 mg/mL of USP Telmisartan RS prepared as follows. Transfer about 44 mg of USP Telmisartan RS to a 100-mL volumetric flask. Add 1 mL of 0.1 M sodium hydroxide, and sonicate to dissolve. Dilute with methanol to volume and mix. Dilute this solution quantitatively with *Medium* to obtain a solution of the required final concentration.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first few milliliters. Dilute quantitatively with *Medium* as needed.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL

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D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Buffer, Mobile phase, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of telmisartan from the *Sample solution*

Acceptance criteria: NMT 0.2% of any individual impurity

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Telmisartan RS

USP Telmisartan Related Compound A RS

1,7′-Dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[*d*]imidazole ▲monohydrate.

$C_{19}H_{20}N_4 \cdot H_2O$ 322.41 ▲ (ERR 1-Jun-2019)

Telmisartan and Amlodipine Tablets

DEFINITION

Telmisartan and Amlodipine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount each of telmisartan ($C_{33}H_{30}N_4O_2$) and amlodipine ($C_{20}H_{25}ClN_2O_5$).

IDENTIFICATION

• **A.** The retention times of the two major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

• **B.** The UV spectra of the two major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 0.022 M monobasic sodium phosphate dihydrate and 2 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 6.0.

Mobile phase: Acetonitrile and *Buffer* (40:60)

Diluent: Add 5 mL of triethylamine to 500 mL of water. Add 500 mL of acetonitrile and mix.

Standard stock solution 1: 0.4 mg/mL of USP Telmisartan RS in *Diluent*

Standard stock solution 2: 0.4 mg/mL of USP Amlodipine Besylate RS in *Diluent*

Standard solution: 0.08 mg/mL of USP Telmisartan RS and 0.03 mg/mL of USP Amlodipine Besylate RS in *Diluent* from *Standard stock solution 1* and *Standard stock solution 2*

Sample stock solution: Nominally 0.4 mg/mL of telmisartan and 0.1 mg/mL of amlodipine from Tablets prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask. Add acetonitrile to about 20% of the volume of the flask, and sonicate for 5 min with intermittent shaking. Add *Diluent* to about 80% of the volume of the flask and sonicate until the Tablets are completely dispersed. Dilute with *Diluent* to the volume. Centrifuge and use the supernatant.

Sample solution: Nominally 0.08 mg/mL of telmisartan and 0.02 mg/mL of amlodipine from *Sample stock solution* in *Diluent*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 257 nm. For *Identification B*, use a diode array detector in the range of 200–350 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 10°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 20 min

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for amlodipine and telmisartan are 0.5 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for telmisartan; NMT 2.5 for amlodipine

Relative standard deviation: NMT 2.0% for telmisartan and amlodipine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of telmisartan from the *Sample solution*
r_S = peak response of telmisartan from the *Standard solution*
C_S = concentration of USP Telmisartan RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of telmisartan in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amlodipine besylate in the *Sample solution* (mg/mL)

USP Monographs

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M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: 90.0%–110.0% each of telmisartan and amlodipine

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Test for telmisartan
Medium: pH 7.5 phosphate buffer (0.05 M monobasic potassium phosphate and 0.038 M sodium hydroxide in 1 L of water; adjusted with diluted sodium hydroxide solution to a pH of 7.5); 900 mL
Apparatus 2: 75 rpm
Time: 20 min
Buffer: 0.022 M monobasic sodium phosphate dihydrate and 2 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Diluent: Add 5 mL of triethylamine to 500 mL of water. Add 500 mL of acetonitrile and mix.
Standard stock solution: 0.9 mg/mL of USP Telmisartan RS in *Diluent*. [NOTE—Sonication may be required to aid dissolution.]
Standard solution: 0.045 mg/mL of USP Telmisartan RS from *Standard stock solution* in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter with suitable pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 12 min
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D) \times V \times (1/L) \times 100$$

r_U = peak response of telmisartan from the *Sample solution*
r_S = peak response of telmisartan from the *Standard solution*
C_S = concentration of USP Telmisartan RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 900 mL
L = label claim of telmisartan (mg/Tablet)

Test for amlodipine
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 75 rpm
Time: 20 min
Mobile phase and **Chromatographic system:** Proceed as directed in *Test for telmisartan*.
Standard stock solution: 0.7 mg/mL of USP Amlodipine Besylate RS in *Medium*. [NOTE—Sonication may be required to aid dissolution.]
Standard solution: 0.03 mg/mL of USP Amlodipine Besylate RS from *Standard stock solution* in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of suitable pore size.
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D) \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 500 mL
L = label claim of amlodipine (mg/Tablet)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Tolerances: NLT 80% (*Q*) of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) is dissolved. NLT 80% (*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Test for telmisartan
Medium: pH 7.5 phosphate buffer (6.805 g/L of monobasic potassium phosphate and 1.6 g/L of sodium hydroxide in water; adjusted with 5 N sodium hydroxide solution or phosphoric acid to a pH of 7.5); 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: 1.54 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.0.
Mobile phase: Acetonitrile and *Buffer* (50:50)
Diluent: 0.01 N hydrochloric acid
Standard stock solution: 0.56 mg/mL of USP Telmisartan RS, prepared as follows. Transfer a quantity of USP Telmisartan RS to a suitable volumetric flask. Add 40% of the total volume of both methanol and *Diluent*. Sonicate to dissolve. Dilute with *Diluent* to volume and mix well.
Standard solution
For Tablets labeled to contain 80 mg of telmisartan: 0.09 mg/mL of USP Telmisartan RS in *Medium* from the *Standard stock solution*
For Tablets labeled to contain 40 mg of telmisartan: 0.045 mg/mL of USP Telmisartan RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm

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Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperatures
Autosampler: 10°
Column: 35°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of the telmisartan peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for amlodipine and telmisartan are 0.69 and 1.00, respectively.]
Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of telmisartan from the *Sample solution*
r_S = peak response of telmisartan from the *Standard solution*
C_S = concentration of USP Telmisartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of telmisartan (mg/Tablet)

Test for amlodipine
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: 1.54 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.0.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Standard stock solution: 0.35 mg/mL of USP Amlodipine Besylate RS prepared as follows. Transfer a quantity of USP Amlodipine Besylate RS to a suitable volumetric flask. Add 5% of the total volume of methanol. Sonicate to dissolve. Dilute with water to volume and mix well.
Standard solution
For Tablets labeled to contain 10 mg of amlodipine: 0.028 mg/mL of USP Amlodipine Besylate RS in *Medium* from the *Standard stock solution*
For Tablets labeled to contain 5 mg of amlodipine: 0.014 mg/mL of USP Amlodipine Besylate RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperatures
Autosampler: 10°
Column: 35°
Flow rate: 1.5 mL/min
Injection volume: 40 µL
Run time: NLT 2.5 times the retention time of the amlodipine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for amlodipine and telmisartan are 1.0 and 1.9, respectively.]
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim of amlodipine (mg/Tablet)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Tolerances: NLT 80% (*Q*) of the labeled amount each of telmisartan ($C_{33}H_{30}N_4O_2$) and amlodipine ($C_{20}H_{25}ClN_2O_5$) are dissolved.
▲**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Test for telmisartan
Medium: pH 7.5 phosphate buffer (dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water; adjusted with sodium hydroxide to a pH of 7.5); 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.4.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Standard stock solution: 0.89 mg/mL of USP Telmisartan RS in methanol. Sonication may be needed to aid dissolution.
Standard solution
For Tablets labeled to contain 80 mg of telmisartan: 0.089 mg/mL of USP Telmisartan RS in *Medium* from the *Standard stock solution*
For Tablets labeled to contain 40 mg of telmisartan: 0.045 mg/mL of USP Telmisartan RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of the telmisartan peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for amlodipine and telmisartan are 0.78 and 1.00, respectively.]

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Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of telmisartan from the *Sample solution*
r_S = peak response of telmisartan from the *Standard solution*
C_S = concentration of USP Telmisartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of telmisartan (mg/Tablet)

Test for amlodipine
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 75 rpm
Time: 15 min
Buffer and **Mobile phase:** Prepare as directed in the *Test for telmisartan*.
Standard stock solution: 0.28 mg/mL of USP Amlodipine Besylate RS prepared as follows. Transfer a quantity of USP Amlodipine Besylate RS to a suitable volumetric flask. Add about 3% of the total volume of methanol. Sonicate to dissolve. Dilute with *Medium* to volume and mix well.
Standard solution
For Tablets labeled to contain 10 mg of amlodipine: 0.028 mg/mL of USP Amlodipine Besylate RS in *Medium* from the *Standard stock solution*
For Tablets labeled to contain 5 mg of amlodipine: 0.014 mg/mL of USP Amlodipine Besylate RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system: Proceed as directed in the *Test for telmisartan* except for the *Run time*.
Run time: NLT 2 times the retention time of the amlodipine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for amlodipine and telmisartan are 1.00 and 1.28, respectively.]
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim of amlodipine (mg/Tablet)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Tolerances: NLT 80% (Q) of the labeled amount each of telmisartan ($C_{33}H_{30}N_4O_2$) and amlodipine ($C_{20}H_{25}ClN_2O_5$) are dissolved. ▲ (RB 1-Feb-2019)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer 1: 0.023 M ammonium acetate in water. Adjust with phosphoric acid to a pH of 5.5.
Solution A: Acetonitrile and *Buffer 1* (20:80)
Solution B: Acetonitrile and *Buffer 1* (65:35)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
5	95	5
15	70	30
35	45	55
50	5	95
65	0	100
70	0	100
75	95	5
80	95	5

Buffer 2: 0.023 M ammonium acetate in water. Adjust with phosphoric acid to a pH of 2.0.
Diluent: Acetonitrile and *Buffer 2* (40:60)
Standard stock solution 1: 0.5 mg/mL of USP Telmisartan RS in *Diluent*
Standard stock solution 2: 0.17 mg/mL of USP Amlodipine Besylate RS in *Diluent*
Standard solution: 25 µg/mL of USP Telmisartan RS from *Standard stock solution 1* and 4.25 µg/mL of USP Amlodipine Besylate RS from *Standard stock solution 2* in *Diluent*
Sensitivity solution: 0.25 µg/mL of USP Telmisartan RS from *Standard stock solution 1* and 0.11 µg/mL of USP Amlodipine Besylate RS from *Standard stock solution 2* in *Diluent*
Sample solution: Nominally 0.25 mg/mL of amlodipine prepared as follows. Transfer a suitable quantity, nominally equivalent to 25 mg of amlodipine from finely powdered Tablets (NLT 10), to a suitable volumetric flask. Add *Diluent* to 70% of the volume of the flask. Sonicate in cold water for 15 min with intermittent shaking. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The relative retention times for amlodipine and telmisartan are 0.74 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.5, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

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Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amlodipine related compound D or amlodipine mannitol adduct in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine related compound D or amlodipine mannitol adduct from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of amlodipine in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of each individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual unspecified degradation product from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of amlodipine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Besylate[a]	0.08	—	—
Amlodipine related compound D[b]	0.59	0.39	1.0
Amlodipine mannitol adduct	0.67	1.00	0.50
Amlodipine	0.74	—	—
Telmisartan related compound A[c, d]	0.78	—	—
Telmisartan related compound B[d, e]	0.86	—	—
Telmisartan	1.0	—	—
Any individual unspecified degradation product	—	—	0.2
Total degradation products	—	—	2.0

[a] Peak due to besylate (benzenesulfonic acid).
[b] 3-Ethyl 5-methyl 4-(2-chlorophenyl)-2-{[2-(1,3-dioxoisoindolin-2-yl)ethoxy]methyl}-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.
[c] 1,7′-Dimethyl-2′-propyl-1*H*,3′*H*-2,5′-bibenzo[*d*]imidazole.
[d] Process impurities controlled in the drug substance.
[e] 4′-[(1,7′-Dimethyl-2′-propyl-1*H*,1′*H*-2,5′-bibenzo[*d*]imidazol-1′-yl)methyl]biphenyl-2-carboxylic acid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amlodipine Besylate RS
  USP Telmisartan RS

Telmisartan and Hydrochlorothiazide Tablets

DEFINITION

Telmisartan and Hydrochlorothiazide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) and NLT 90.0% and NMT 107.5% of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U.▲** (CN 1-May-2020) The spectrum of the solution under test corresponds to that of the *Standard solution*, as obtained by the *Assay*.
- **B.** The retention time of the two major peaks of the *Sample solution* corresponds to that of the two major peaks in *Standard solution A*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: 0.005 M methanolic solution of sodium hydroxide

Buffer: 2.0 g/L of ammonium dihydrogen phosphate. Adjust with phosphoric acid to a pH of 3.0.

Solution A: Methanol and acetonitrile (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	85	15
3.50	85	15
3.51	45	55
7.70	45	55
7.71	20	80
12.0	20	80
12.1	85	15
15.5	85	15

Standard stock solution 1: 0.025 mg/mL of USP Benzothiadiazine Related Compound A RS in *Diluent*

Standard stock solution 2: 1.6 mg/mL or 3.2 mg/mL (required for analyzing the Tablet strength of 80 mg/12.5 mg) of USP Telmisartan RS, 0.5 mg/mL of USP Hydrochlorothiazide RS, and 2.5 µg/mL of USP Benzothiadiazine Related Compound A RS (from *Standard stock solution 1*) in *Diluent*

Standard solution A: Dilute *Standard stock solution 2* with a 1:1 solution of *Buffer* and *Solution A* to prepare 0.32 mg/mL of telmisartan, 0.1 mg/mL of hydrochlorothiazide, and 0.5 µg/mL of benzothiadiazine related compound A for Tablet

USP Monographs

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strengths of 80 mg/25 mg and 40 mg/12.5 mg. The final concentrations for analyzing the Tablet strength of 80 mg/12.5 mg are 0.32 mg/mL of telmisartan, 0.05 mg/mL of hydrochlorothiazide solution, and 0.25 µg/mL of benzothiadiazine related compound A.

Sample stock solution: Transfer NLT 10 Tablets to a suitable volumetric flask, add 0.1 N sodium hydroxide solution (5% of the total volume of the flask), and shake until the Tablets have completely disintegrated. Add methanol (80% of the total volume of the flask). Sonicate for 10 min and stir vigorously for 30 min. Allow to cool to room temperature, dilute with methanol to volume, and mix. The concentration of the *Sample stock solution* is about 1.6 mg/mL of telmisartan. [NOTE—The hydrochlorothiazide concentration may vary depending on the ratio of telmisartan to hydrochlorothiazide in the Tablet.] Centrifuge a portion of the solution at 4000 rpm. [NOTE—To prevent heat from degrading the sample, do not extend the sonication time and maintain the bath temperature at NMT 22° by adding ice.]

Sample solution: Dilute 1 mL of the *Sample stock solution* to 5 mL in a 1:1 solution of *Buffer* and *Solution A*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector
UV 270 nm: For hydrochlorothiazide
UV 298 nm: For telmisartan
Column: 4.0-mm × 12.5-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution A*
Suitability requirements
Resolution: NLT 2.0 between hydrochlorothiazide and benzothiadiazine related compound A
Relative standard deviation: NMT 2.0% for both the telmisartan and hydrochlorothiazide peaks

Analysis
Samples: *Standard solution A* and *Sample solution*
Calculate the percentages of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of telmisartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response of telmisartan or hydrochlorothiazide from *Standard solution A*
C_S = concentration of USP Telmisartan RS or USP Hydrochlorothiazide RS in *Standard solution A* (mg/mL)
C_U = nominal concentration of telmisartan or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% of telmisartan and 90.0%–107.5% of hydrochlorothiazide

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Telmisartan
Medium: pH 7.5 phosphate buffer prepared as follows. 13.61 g/L of monobasic potassium phosphate in water. Adjust with 2 M sodium hydroxide to a pH of 7.5; 900 mL.
Apparatus 2: 75 rpm
Time: 30 min
Hydrochlorothiazide
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Analysis: Determine the amounts of telmisartan and hydrochlorothiazide dissolved by the following method.
Solution A: 5.0 g/L of ammonium dihydrogen phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile
Standard stock solution: Appropriate amounts of USP Telmisartan RS and USP Hydrochlorothiazide RS in methanol
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discard the first few mL, and dilute with appropriate *Medium*, if necessary.
Telmisartan standard solution: Dilute the *Standard stock solution* with *Telmisartan Medium* to obtain a solution having a known concentration of telmisartan similar to that expected in the *Sample solution*.
Hydrochlorothiazide standard solution: Dilute the *Standard stock solution* with *Hydrochlorothiazide Medium* to obtain a solution having a known concentration of hydrochlorothiazide similar to that expected in the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector
UV 270 nm: For hydrochlorothiazide
UV 298 nm: For telmisartan
Column: 3.0-mm × 6-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 0.6 mL/min from 0 to 5.00 min and 1.0 mL/min from 5.01 to 6.20 min. The flow rate goes back to 0.6 ▲mL/min▲ (ERR 1-Jan-2019) from 6.21 to 9.70 min.
Injection volume: 4 µL
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	85	15
1.50	85	15
1.51	60	40
5.00	60	40
5.01	20	80
6.20	20	80
6.21	85	15
9.70	85	15

System suitability
Samples: *Telmisartan standard solution* and *Hydrochlorothiazide standard solution*
[NOTE—The relative retention times for hydrochlorothiazide and telmisartan are 0.33 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.5 for both telmisartan and hydrochlorothiazide
Relative standard deviation: NMT 2%

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Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of telmisartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response of telmisartan from the *Telmisartan standard solution* or hydrochlorothiazide from the *Hydrochlorothiazide standard solution*
C_S = concentration of telmisartan in the *Telmisartan standard solution* or hydrochlorothiazide in the *Hydrochlorothiazide standard solution* (mg/mL)
L = label claim for telmisartan or hydrochlorothiazide (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of telmisartan and hydrochlorothiazide is dissolved.

Test 2

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Dissolve 6.8 g of monobasic potassium phosphate and 1.56 g of sodium hydroxide in 1 L of water; adjust with 10% sodium hydroxide solution to a pH of 7.5; 900 mL.

Apparatus 2: 75 rpm

Time: 30 min

Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 1 L of water. Add 2 mL of triethylamine per liter of solution and adjust with phosphoric acid to a pH of 2.4.

Mobile phase: Acetonitrile and *Buffer* (35:65)

Telmisartan standard stock solution: 0.45 mg/mL of USP Telmisartan RS prepared as follows. To a suitable amount of USP Telmisartan RS in a suitable volumetric flask, add methanol to about 50% of the total volume. Sonicate to dissolve, cool to room temperature, and dilute with *Medium* to volume.

Hydrochlorothiazide standard stock solution: 0.28 mg/mL of USP Hydrochlorothiazide RS prepared as follows. To a suitable amount of USP Hydrochlorothiazide RS in a suitable volumetric flask, add methanol to about 50% of the total volume. Sonicate to dissolve, cool to room temperature, and dilute with *Medium* to volume.

Standard solution: (L/900) mg/mL each of USP Telmisartan RS and USP Hydrochlorothiazide RS in *Medium*, from *Telmisartan standard stock solution* and *Hydrochlorothiazide standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: NLT 1.7 times the retention time of telmisartan

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for both telmisartan and hydrochlorothiazide

Column efficiency: NLT 1500 theoretical plates for both telmisartan and hydrochlorothiazide

Relative standard deviation: NMT 2.0% for both telmisartan and hydrochlorothiazide

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of telmisartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response of telmisartan or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Telmisartan RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) each of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Test 3

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Telmisartan

Medium: pH 7.5 phosphate buffer (dissolve 13.61 g of monobasic potassium phosphate in 1 L of water. Adjust with 2 N sodium hydroxide to a pH of 7.5); 900 mL

Apparatus 2: 75 rpm

Time: 20 min

Hydrochlorothiazide

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 15 min

Solution A: Transfer 10 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	70	30
12	30	70
13	70	30
17	70	30

Telmisartan standard stock solution: 0.9 mg/mL of USP Telmisartan RS prepared as follows. To a suitable amount of USP Telmisartan RS in a suitable volumetric flask, add methanol to 60% of the total volume. Sonicate to dissolve. Dilute with methanol to volume.

Hydrochlorothiazide standard stock solution: 0.35 mg/mL of USP Hydrochlorothiazide RS prepared as follows. To a suitable amount of USP Hydrochlorothiazide RS in a suitable volumetric flask, add methanol to about 10% of the total volume. Sonicate to dissolve. Dilute with *Hydrochlorothiazide Medium* to volume.

Telmisartan standard solution: Dilute the *Telmisartan standard stock solution* with *Telmisartan Medium* to obtain a solution having a known concentration of telmisartan similar to that of the *Sample solution*.

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Hydrochlorothiazide standard solution: Dilute the *Hydrochlorothiazide standard stock solution* with *Hydrochlorothiazide Medium* to obtain a solution having a known concentration of hydrochlorothiazide similar to that of the *Sample solution*.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector

UV 270 nm: For hydrochlorothiazide

UV 298 nm: For telmisartan

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of telmisartan

System suitability

Samples: *Telmisartan standard solution* and *Hydrochlorothiazide standard solution*

[NOTE—The relative retention times for hydrochlorothiazide and telmisartan are 0.44 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for both telmisartan and hydrochlorothiazide

Relative standard deviation: NMT 2.0% for both telmisartan and hydrochlorothiazide

Analysis

Samples: *Telmisartan standard solution*, *Hydrochlorothiazide standard solution*, and *Sample solution*

Calculate the percentage of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of telmisartan or hydrochlorothiazide from the *Sample solution*

r_S = peak response of telmisartan or hydrochlorothiazide from the *Telmisartan standard solution* or *Hydrochlorothiazide standard solution*

C_S = concentration of USP Telmisartan RS or USP Hydrochlorothiazide RS in the *Telmisartan standard solution* or *Hydrochlorothiazide standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) each of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Buffer, Solution A, Mobile phase, Standard stock solution 1, Standard solution A, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution 3: 1.6 mg/mL of USP Telmisartan RS and 0.5 mg/mL of USP Hydrochlorothiazide RS in *Diluent* for Tablet strengths of 40 mg/12.5 mg and 80 mg/25 mg. For the Tablet strength of 80 mg/12.5 mg, the concentrations are 1.6 mg/mL of USP Telmisartan RS and 0.25 mg/mL of USP Hydrochlorothiazide RS in *Diluent*.

Standard solution B: 1.25 µg/mL of USP Benzothiadiazine Related Compound A RS in *Diluent* from *Standard stock solution 1*. Dilute further with a 1:1 solution of *Buffer* and *Solution A* to prepare a 0.25-µg/mL solution for a Tablet strength of 80 mg/12.5 mg, and a 0.5-µg/mL solution for Tablet strengths of 40 mg/12.5 mg and 80 mg/25 mg.

Sensitivity solution: Dilute 10 mL of *Standard stock solution 3* with *Diluent* to 100 mL. Combine 1.0 mL of this solution with 2.0 mL of *Standard stock solution 1* and dilute with *Diluent* to 100 mL. Dilute 1 mL of this solution to 5 mL with a 1:1 solution of *Buffer* and *Solution A*.

System suitability

Samples: *Standard solution A* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between hydrochlorothiazide and benzothiadiazine related compound A, *Standard solution A*

Relative standard deviation: NMT 2.0% for both the telmisartan and hydrochlorothiazide peaks, *Standard solution A*

Signal-to-noise ratio: NLT 3.0 for the telmisartan, hydrochlorothiazide, and benzothiadiazine related compound A peaks from the *Sensitivity solution*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*

r_S = peak response of benzothiadiazine related compound A from *Standard solution B*

C_S = concentration of USP Benzothiadiazine Related Compound A RS in *Standard solution B* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation impurity related to hydrochlorothiazide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation impurity at 270 nm from the *Sample solution*

r_S = peak response of hydrochlorothiazide from *Standard solution A*

C_S = concentration of USP Hydrochlorothiazide RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation impurity related to telmisartan in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation impurity at 298 nm from the *Sample solution*

r_S = peak response of telmisartan from *Standard solution A*

C_S = concentration of USP Telmisartan RS in *Standard solution A* (mg/mL)

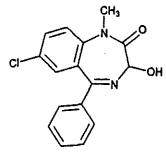
C_U = nominal concentration of telmisartan in the *Sample solution* (mg/mL)

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Acceptance criteria
Individual impurities: NMT 1.0% of benzothiadiazine related compound A and NMT 0.2% of each individual unspecified degradation impurity related to telmisartan or hydrochlorothiazide
Total impurities: NMT 0.2% of the sum of all degradation products related to telmisartan and NMT 1.5% of the sum of all hydrochlorothiazide degradation products

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzothiadiazine Related Compound A RS
  4-Amino-6-chloro-1,3-benzenedisulfonamide.
  $C_6H_8ClN_3O_4S_2$ 285.73
  USP Hydrochlorothiazide RS
  USP Telmisartan RS

Temazepam



$C_{16}H_{13}ClN_2O_2$ 300.74
2*H*-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-;
7-Chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one [846-50-4].

DEFINITION
Temazepam contains NLT 98.0% and NMT 102.0% of temazepam ($C_{16}H_{13}ClN_2O_2$), calculated on the dried basis. [**CAUTION**—Temazepam is a potent sedative; its powder should not be inhaled.]

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 3.9 g/L of ammonium acetate in water
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*. [NOTE—Gradient based on a dwell volume of 1.0 mL.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
13	70	30
25	30	70
28	30	70
33	70	30
35	70	30

Diluent: Acetonitrile and *Solution A* (30:70)
System suitability solution: 0.1 mg/mL each of USP Temazepam RS, USP Temazepam Related Compound A RS, USP Temazepam Related Compound F RS, and USP Temazepam Related Compound G RS prepared as follows. Transfer suitable quantities of each USP Reference Standard to a suitable volumetric flask. Dissolve in 30% of the flask volume of acetonitrile. Dilute with *Solution A* to volume.
Standard stock solution: 0.5 mg/mL of USP Temazepam RS prepared as follows. Transfer a suitable quantity of USP Temazepam RS to a suitable volumetric flask. Dissolve in 30% of the flask volume of acetonitrile. Dilute with *Solution A* to volume.
Standard solution: 0.1 mg/mL from *Standard stock solution* in *Diluent*
Sample stock solution: 0.5 mg/mL of Temazepam prepared as follows. Transfer a suitable quantity of Temazepam to a suitable volumetric flask. Dissolve in 30% of the flask volume of acetonitrile. Dilute with *Solution A* to volume.
Sample solution: 0.1 mg/mL from *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm or 2.6-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between temazepam related compound F and temazepam; NLT 1.5 between temazepam and temazepam related compound G, *System suitability solution*
Relative standard deviation: NMT 0.73% for temazepam, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of temazepam ($C_{16}H_{13}ClN_2O_2$) in the portion of Temazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Temazepam RS in the *Standard solution* (mg/mL)
C_U = concentration of Temazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and **Standard stock solution:** Proceed as directed in the *Assay*.

USP Monographs

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Standard solution: 0.005 mg/mL from *Standard stock solution* in *Diluent*
Sensitivity solution: 0.2 µg/mL of USP Temazepam RS in *Diluent* from *Standard stock solution*
Sample solution: 1 mg/mL of Temazepam prepared as follows. Dissolve in acetonitrile using 30% of the final volume, and dilute with *Solution A* to volume.
Chromatographic system: Prepare as directed in the *Assay*.
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between temazepam related compound F and temazepam; NLT 1.5 between temazepam and temazepam related compound G, *System suitability solution*
Relative standard deviation: NMT 5.0% for temazepam, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Temazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of temazepam from the *Standard solution*
C_S = concentration of USP Temazepam RS in the *Standard solution* (mg/mL)
C_U = concentration of Temazepam in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting limit is 0.02% for temazepam related compound A and 0.05% for all other impurities.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxazepam[a]	0.54	1.0	0.2
Methylnordazepam *N*-oxide[b]	0.63	1.5	0.2
Temazepam related compound F	0.83	0.65	0.2
Temazepam	1.0	—	—
Temazepam related compound G	1.3	0.68	0.2
O-Methyl temazepam[c]	1.6	1.0	0.2
Diazepam[d]	1.9	1.0	0.15
O-Acetyl temazepam[e]	2.0	1.0	0.2
Temazepam related compound A	2.6	1.2	0.05
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
[b] 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.
[c] 7-Chloro-1,3-dihydro-3-methoxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
[d] 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
[e] 7-Chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl acetate.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Temazepam RS
USP Temazepam Related Compound A RS
5-Chloro-2-methylaminobenzophenone.
$C_{14}H_{12}ClNO$ 245.70
USP Temazepam Related Compound F RS
7-Chloro-1-methyl-5-phenyl-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione.
$C_{16}H_{13}ClN_2O_2$ 300.74
USP Temazepam Related Compound G RS
7-Chloro-1,4-dimethyl-5-phenyl-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione.
$C_{17}H_{15}ClN_2O_2$ 314.77

Temazepam Capsules

DEFINITION
Temazepam Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of temazepam ($C_{16}H_{13}O_2N_2Cl$).
[**CAUTION**—Temazepam is a potent sedative: its powder should not be inhaled.]

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
• **B. THIN-LAYER CHROMATOGRAPHY**
Standard solution: 5 mg/mL of USP Temazepam RS in methanol
Sample solution: Transfer a portion of Capsule contents equivalent to about 50 mg of temazepam to a 25-mL volumetric flask, add 10 mL of methanol, shake by mechanical means, and allow to settle. Use the supernatant layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Toluene, dioxane, methanol, and ammonium hydroxide (65:30:5:1)
Analysis: Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the

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solvent front, air-dry, and examine the plate under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
• **PROCEDURE**
Buffer: 2.7 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (47:53)
Internal standard solution: 0.2 mg/mL of benzophenone in a mixture of methanol and water (9:1)
Standard solution: 0.2 mg/mL of USP Temazepam RS in *Internal standard solution*
Sample solution: Nominally 0.2 mg/mL of temazepam in *Internal standard solution*, from the weighed and mixed contents of NLT 20 Capsules. Shake mechanically for 30 min. Allow the contents of the flask to settle, and then filter, discarding the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 25-cm; packing L16
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for temazepam and benzophenone are about 1.0 and 2.0, respectively.]
Suitability requirements
Resolution: NLT 1 between the temazepam peak and any other peak
Column efficiency: NLT 800 theoretical plates
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of temazepam ($C_{16}H_{13}O_2N_2Cl$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

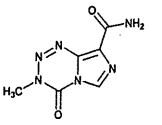
R_U = peak response ratio of temazepam to the internal standard from the *Sample solution*
R_S = peak response ratio of temazepam to the internal standard from the *Standard solution*
C_S = concentration of USP Temazepam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of temazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Buffer: Dissolve 2 g of sodium hydroxide in 450 mL of water in a 1-L volumetric flask. Adjust with acetic acid to a pH of 4.0, and dilute with water to volume. Deaerate.
Medium: *Buffer* with 0.05% polysorbate 80; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Detector: UV 310 nm
Standard solution: USP Temazepam RS in *Medium*
Sample solution: Filter a portion of the solution under test. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of temazepam ($C_{16}H_{13}O_2N_2Cl$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Temazepam RS

Temozolomide



$C_6H_6N_6O_2$ 194.15
Imidazo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamide, 3,4-dihydro-3-methyl-4-oxo-;
3,4-Dihydro-3-methyl-4-oxoimidazo[5,1-*d*]-*as*-tetrazine-8-carboxamide [85622-93-1].

DEFINITION
Temozolomide contains NLT 98.0% and NMT 102.0% of temozolomide ($C_6H_6N_6O_2$), calculated on the anhydrous basis.
[**CAUTION**—Temozolomide is cytotoxic. Great care should be taken to prevent inhaling particles of Temozolomide and exposure to the skin.]

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
[NOTE—Shake the solutions containing temozolomide to aid the dissolution. Do not sonicate.]
• **PROCEDURE**
Solution A: 0.5% (v/v) glacial acetic acid in water
Mobile phase: *Solution A* and methanol (96:4), containing 0.94 g/L of sodium 1-hexanesulfonate (0.005 M)
Diluent: Dimethyl sulfoxide. [NOTE—Use a freshly opened bottle.]
Standard solution: 1.0 mg/mL of USP Temozolomide RS in *Diluent*
Sample solution: 1.0 mg/mL of Temozolomide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.9
Relative standard deviation: NMT 1.5%

USP Monographs

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of temozolomide ($C_6H_6N_6O_2$) in the portion of Temozolomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Temozolomide RS in the *Standard solution* (mg/mL)
C_U = concentration of Temozolomide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
[NOTE—Shake the solutions containing temozolomide to aid the dissolution. Do not sonicate.]
Mobile phase, Diluent, and **Sample solution:** Proceed as directed in the *Assay*.
Standard solution: 1.0 µg/mL of USP Temozolomide RS in *Diluent*
System suitability solution: Mix 5 mL of 0.1 N hydrochloric acid and 5 mL of 1.0 mg/mL of USP Temozolomide RS in *Diluent*. Heat the container for 1 h on a steam or boiling water bath. [NOTE—The preparation forms 2-azahypoxanthine, temozolomide acid, and aminoimidazolecarboxamide.]
Chromatographic system: Proceed as directed in the *Assay*, using a run time of NLT 3.2 times the retention time of the temozolomide peak.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between temozolomide acid and temozolomide peaks
Analysis
Samples: *Sample solution*, *Standard solution*, and *System suitability solution*
Inject the *System suitability solution*, and identify the organic impurities according to the relative retention times given in *Table 1*.
Calculate the percentage of each individual impurity in the portion of Temozolomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of temozolomide from the *Standard solution*
C_S = concentration of USP Temozolomide RS in the *Standard solution* (mg/mL)
C_U = concentration of Temozolomide in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Azahypoxanthine[a]	0.42	1.6	0.2
Temozolomide related compound A[b]	0.53	1.0	0.5
Temozolomide acid[c]	0.84	1.0	0.1
Temozolomide	1.0	—	—
Aminoimidazolecarboxamide[d]	1.37[e]	2.5	0.1
Cyanotemozolomide[f,g] (if present)	2.3	1.0	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.8

[a] 4a,5-Dihydro-4*H*-imidazo[4,5-*d*][1,2,3]triazin-4-one.
[b] 4-Diazo-4*H*-imidazole-5-carboxamide.
[c] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxylic acid.
[d] 5-Aminoimidazole-4-carboxamide. Two peaks may be observed, use the sum of the peak areas for calculation.
[e] It may vary and depend on the column.
[f] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carbonitrile.
[g] If possible from the manufacturing process.

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method Ic*: NMT 0.4%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Temozolomide RS

Temozolomide Capsules

DEFINITION
Temozolomide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of temozolomide ($C_6H_6N_6O_2$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 1 mg/mL of USP Temozolomide RS in acetonitrile
Sample solution: Transfer the contents of Capsules indicated in *Table 1* to a glass beaker containing a stir bar. Add acetonitrile according to *Table 1*. Stir for NLT 20 min. Sonicate for 10 min followed by a 30-s stirring to disperse any remaining solids. Allow the contents of the flask to settle.

Table 1

Capsule Strength (mg/Capsule)	Acetonitrile (mL)	Number of Capsules
5	50	4
20	50	1
100	100	1
140	140	1
180	180	1

USP Monographs

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Table 1 *(continued)*

Capsule Strength (mg/Capsule)	Acetonitrile (mL)	Number of Capsules
250	250	1

Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 20-cm × 20-cm or 10-cm × 20-cm of 0.25-mm layer of chromatographic silica gel mixture
Application volume
Standard solution: 10 µL
Sample solution: See *Table 2.*

Table 2

Capsule Strength (mg/Capsule)	Sample Solution (µL)
5	25
20	25
100	10
140	10
180	10
250	10

Developing solvent system: Acetone and toluene (90:15)
Analysis: Spot the *Standard solution* and *Sample solution* approximately 2.5 cm from the bottom of the plate. Evaporate the solvent using a current of air. Place the plate in the developing chamber and allow the *Developing solvent system* to rise to approximately 75% of the plate height. Remove the plate from the developing chamber, mark the position of the solvent front, and allow the plate to dry in the fume hood. Observe the plate under 254 nm of UV light.
Acceptance criteria: The R_F value from the *Sample solution* corresponds to that from the *Standard solution.*

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**
Store the solutions containing temozolomide at 4°.
Solution A: 0.5% (v/v) of glacial acetic acid in water
Mobile phase: *Solution A* and methanol (96:4), containing 0.94 g/L of sodium 1-hexanesulfonate (0.005 M)
System suitability stock solution: 0.4 mg/mL of USP Temozolomide RS in *Mobile phase*
System suitability solution: Transfer 25 mL of 0.1 N hydrochloric acid and 25 mL of *System suitability stock solution* into a container. Heat the container at 80° for 4 h. [NOTE—The preparation forms 2-azahypoxanthine, temozolomide acid, and aminoimidazolecarboxamide.]
Standard solution: 0.1 mg/mL of USP Temozolomide RS in *Mobile phase* from *System suitability stock solution*
Sample stock solution: Place 10 Capsules in a suitable volumetric flask according to *Table 3.* Add 80% of the final volume of *Mobile phase* with agitation or stirring for NLT 1 h. Ensure that the Capsules are broken apart into small pieces and that contents are adequately dissolved. Dilute with *Mobile phase* to volume and mix well. Allow to sit for 30 min and mix again. Centrifuge 25 mL of this solution for 10 min or until the solution is clear. Pass the supernatant through a suitable filter of 0.45-µm pore size and discard the first few milliliters.

Table 3

Capsule Strength (mg/Capsule)	Volumetric Flask Size (mL)	Nominal Concentration (mg/mL)
5	500	0.1
20	200	1.0
100	1000	1.0
140	2000	0.7
180	2000	0.9
250	2000	1.25

Sample solution: Nominally equivalent to 0.1 mg/mL of temozolomide in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Autosampler temperature: 4°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 1.9 for the temozolomide peak, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of temozolomide from the *Sample solution*
r_S = peak response of temozolomide from the *Standard solution*
C_S = concentration of USP Temozolomide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of temozolomide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 500 mL (for 5 mg/Capsule), 900 mL (for all other strengths)
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: Prepare a solution of USP Temozolomide RS in *Medium* according to *Table 4.*

Table 4

Capsule Strength (mg/Capsule)	Nominal Concentration (mg/mL)
5	0.01
20	0.022
100	0.11

USP Monographs

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Table 4 *(continued)*

Capsule Strength (mg/Capsule)	Nominal Concentration (mg/mL)
140	0.16
180	0.20
250	0.28

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.8-µm pore size. Discard a few milliliters of the filtrate and use the remaining filtrate for analysis.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 328 nm

Cell length: See *Table 5*.

Table 5

Capsule Strength (mg/Capsule)	Cell Length (cm)
5	1
20	1
100	0.1
140	0.05
180	0.05
250	0.05

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*; 500 mL (for 5 mg/Capsule), 900 mL (for all other strengths)

L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of temozolomide ($C_6H_6N_6O_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Store the solutions containing temozolomide at 4°.

Mobile phase, System suitability stock solution, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.

Sensitivity solution: 0.1 µg/mL of USP Temozolomide RS in *Mobile phase* from *System suitability stock solution*

Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.

Run time: NLT twice the retention time of the temozolomide peak

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.5 between the temozolomide and aminoimidazolecarboxamide peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_T = sum of the relevant peak areas from the *Sample solution*

Acceptance criteria: See *Table 6*. The reporting threshold is 0.1%. Disregard any peak due to an excipient.

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)	
		5 mg/Capsule or 20 mg/Capsule	All Other Strengths
2-Azahypoxanthine[a]	0.4	0.8	0.8
Temozolomide related compound A[b, c]	0.5	—	—
Temozolomide acid[d]	0.9	0.2	0.2
Temozolomide	1.0	—	—
Aminoimidazolecarboxamide[e]	1.4	1.2	1.0
Cyanotemozolomide[c, f]	2.3	—	—
Any unspecified impurity	—	0.2	0.2
Total impurities	—	1.7	1.2

[a] 4a,5-Dihydro-4*H*-imidazo[4,5-*d*][1,2,3]triazin-4-one.

[b] 4-Diazo-4*H*-imidazole-5-carboxamide.

[c] It is a process impurity and is listed for identification only. It is controlled in the drug substance. It is not reported for the drug product and should not be included in the total impurities.

[d] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxylic acid.

[e] 5-Aminoimidazole-4-carboxamide.

[f] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carbonitrile.

SPECIFIC TESTS

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined molds and yeast count is NMT 5×10^2 cfu/g. It meets the requirements of the tests for the absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Temozolomide RS

USP Monographs

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Temozolomide for Injection

DEFINITION

Temozolomide for Injection is a sterile, lyophilized mixture of Temozolomide and suitable added substances. It contains NLT 95.0% and NMT 105.0% of the labeled amount of temozolomide ($C_6H_6N_6O_2$).

[**CAUTION**—Temozolomide is cytotoxic. Great care should be taken to prevent inhaling particles of temozolomide and exposure to the skin.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

Standard: Dissolve a suitable amount of USP Temozolomide RS in methylene chloride, using 2 mL of methylene chloride for each 5 mg of USP Temozolomide RS. Evaporate the methylene chloride under a stream of nitrogen to dryness, and use the residue.

Sample: Inject 40 mL of methylene chloride into the vial of Temozolomide for Injection and shake the vial for 30 min using a mechanical shaker. Allow the vial to sit for 15 min or until undissolved solids settle to the bottom. Pass 15 mL of the supernatant through a filter of 0.45-µm pore size. Discard the first 5 mL and collect the remaining filtrate into a 20-mL scintillation vial. Evaporate the methylene chloride under a stream of nitrogen to dryness, and use the residue.

Analysis

Samples: *Standard* and *Sample*

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE:** Store the solutions containing temozolomide at 2°–8°.

Solution A: 0.5% (v/v) glacial acetic acid in water

Mobile phase: *Solution A* and methanol (96:4), containing 0.94 g/L of sodium 1-hexanesulfonate (0.005 M)

System suitability stock solution: 0.2 mg/mL of USP Temozolomide RS in *Mobile phase*

System suitability solution: Transfer 50 mL of 0.1 N hydrochloric acid and 50 mL of the *System suitability stock solution* to a container. Heat the container at 80° for 4 h. Transfer 10 mL of this solution into a 25-mL volumetric flask and dilute with *Mobile phase* to volume. [NOTE—The preparation forms 2-azahypoxanthine, temozolomide acid, and aminoimidazolecarboxamide.]

Standard solution: 0.1 mg/mL of USP Temozolomide RS in *Mobile phase* from the *System suitability stock solution*

Sample stock solution: Nominally equivalent to 2.0 mg/mL of temozolomide in water, prepared as follows. Add 40 mL of water into each of five vials of Temozolomide for Injection and shake manually to dissolve. Combine the solutions from the 5 vials into a 250-mL volumetric flask and dilute with water to volume.

Sample solution: Nominally equivalent to 0.1 mg/mL of temozolomide in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Autosampler temperature: 2°–8°

Flow rate: 1 mL/min

Injection volume: 75 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 1.9 for the temozolomide peak, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) in the portion of Temozolomide for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of temozolomide from the *Sample solution*

r_S = peak response of temozolomide from the *Standard solution*

C_S = concentration of USP Temozolomide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of temozolomide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Weight Variation*: Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES:** Store the solutions containing temozolomide at 2°–8°.

Mobile phase, System suitability stock solution, System suitability solution, and Sample solution: Prepare as directed in the *Assay*.

Sensitivity solution: 0.1 µg/mL of USP Temozolomide RS in *Mobile phase* from the *System suitability stock solution*

Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.

Run time: NLT 2.5 times the retention time of the temozolomide peak

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.5 between the temozolomide and aminoimidazolecarboxamide peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Temozolomide for Injection taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_T = sum of relevant peak areas from the *Sample solution*

F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.1%. Disregard any peak due to an excipient.

USP Monographs

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Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Azahypoxanthine[a]	0.4	1.6	0.4
Temozolomide related compound A[b,c]	0.5	—	—
Temozolomide acid[d]	0.9	0.76	0.2
Temozolomide	1.0	—	—
Aminoimidazolecarboxamide[e]	1.4	3.5	1.0
Cyanotemozolomide[c,f]	2.3	—	—
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	2.0

[a] 4a,5-Dihydro-4*H*-imidazo[4,5-*d*][1,2,3]triazin-4-one.
[b] 4-Diazo-4*H*-imidazole-5-carboxamide.
[c] It is a process impurity and is listed for identification only. It is controlled in the drug substance. It is not reported for the drug product and should not be included in the total impurities.
[d] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxylic acid.
[e] 5-Aminoimidazole-4-carboxamide.
[f] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carbonitrile.

SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: Add 40 mL of water into a vial of Temozolomide for Injection and manually shake to dissolve the solids.
  Acceptance criteria: 3.0–4.5
- **Bacterial Endotoxins Test** ⟨85⟩: Meets the requirements
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Refrigerate at 2°–8°. After reconstitution, store the reconstituted product at room temperature. The reconstituted product must be used within 14 h, including infusion time.
- **USP Reference Standards** ⟨11⟩
  USP Temozolomide RS

Temozolomide Compounded Oral Suspension

DEFINITION

Temozolomide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of temozolomide ($C_6H_6N_6O_2$).

Prepare Temozolomide Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Temozolomide capsules,[a] equivalent to	1 g of temozolomide
Povidone K-30, *USP*	500 mg
Anhydrous Citric Acid, *USP*	25 mg
Purified Water, *USP*	1.5 mL
Vehicle: a 1:1 mixture of Ora-Plus[b] and Ora-Sweet[b] (regular or sugar-free), a sufficient quantity to make	100 mL

[a] Temodar 100-mg capsules, Schering Corporation, Whitehouse, NJ.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Empty the required number of *Temozolomide capsules* in a suitable mortar. Add the *Povidone K-30* powder to the mortar, and triturate to a fine powder. [Note—Povidone is critical for physical stability.] Dissolve the *Anhydrous Citric Acid* in *Purified Water*. Add the mixture of *Anhydrous Citric Acid* and *Purified Water* to the mortar to wet the powder. Mix thoroughly to form a uniform paste. Add the *Vehicle* in small portions, and triturate to make a smooth mixture. Add increasing volumes of the *Vehicle* to make a temozolomide liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well. It is necessary to wear appropriate personal protective equipment and to perform this procedure in a biologic safety cabinet.

[**Caution**—Temozolomide is cytotoxic. Great care should be taken to prevent inhaling particles of temozolomide and exposure to the skin.]

ASSAY

- **Procedure**
  Solution A: 10 mM ammonium phosphate adjusted to a pH of 3.25
  Mobile phase: Methanol and *Solution A* (12:88). Filter and degas.
  Standard stock solution: 0.5 mg/mL of USP Temozolomide RS in *Mobile phase*. Transfer the mixture to a centrifuge tube, and centrifuge at 1,500 × g for 10 min. Pass the supernatant through a filter of 0.45-µm pore size, into a glass vial.
  Standard solution: 0.1 mg/mL of temozolomide prepared from *Standard stock solution* and *Mobile phase*
  Sample solution: Shake thoroughly each bottle of Oral Suspension. Immediately transfer 2.5 mL of Oral Suspension to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Vortex the sample for 30 s, centrifuge for 10 min at 1,500 × g, and pass through a membrane filter of 0.22-µm pore size. Pass at least 0.5 mL of sample through the filter before collecting the sample in a vial. Transfer 1.0 mL of the resultant solution to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 0.1 mg/mL of temozolomide.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 245 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.0 mL/min
  Injection volume: 15 µL
  System suitability
  Sample: *Standard solution*
  [Note—The retention time for temozolomide is about 7.9 min.]
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0% for replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*

USP Monographs

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Calculate the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Temozolomide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of temozolomide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

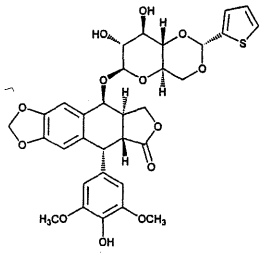
SPECIFIC TESTS

- **pH** ⟨791⟩: 3.5–4.5

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in tight, light-resistant containers. Store in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded, when stored in a refrigerator
- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Temozolomide RS

Teniposide



$C_{32}H_{32}O_{13}S$ 656.65

Furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one, 5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-9-[4,6-*O*-(2-thienylmethylene)-β-D-glucopyranosyloxy]-, [5*R*-[5α,5aβ,8aα,9β(*R**)]]-;
4′-Demethylepipodophyllotoxin 9-[4,6-*O*-(*R*)-2-thenylidene-β-D-glucopyranoside];
(5*S*,5a*R*,8a*R*,9*R*)-9-(4-Hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3′,4′:6,7]naphtho[2,3-*d*][1,3]dioxol-5-yl 4,6-*O*-(thiophen-2-ylmethylidene)-β-D-glucopyranoside [29767-20-2].

DEFINITION

Teniposide contains NLT 97.0% and NMT 103.0% of teniposide ($C_{32}H_{32}O_{13}S$), calculated on the anhydrous and solvent-free basis.

[**Caution**—Great care should be taken in handling teniposide, because it is a cytotoxic agent.]

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **Procedure**

Buffer: 5 mM monobasic potassium phosphate in water
Solution A: Acetonitrile and *Buffer* (36:64). Adjust with dibasic potassium phosphate to an apparent pH of 7.0.
Solution B: Acetonitrile and water (90:10)
Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 15 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	100	0
60	0	100
66[a]	0	100

[a] This time may be adjusted if needed to be at least 2.2 times the retention time of the teniposide peak.

Diluent: Acetonitrile and *Buffer* (1:1)
Standard solution: 0.4 mg/mL of USP Teniposide RS, prepared as follows. Transfer USP Teniposide RS to a suitable volumetric flask, add acetonitrile equivalent to 10% of the final volume, and sonicate to dissolve. Dilute with *Diluent* to volume.
Sample solution: 0.4 mg/mL of Teniposide, prepared as follows. Transfer Teniposide to a suitable volumetric flask, add acetonitrile equivalent to 10% of the final volume, and sonicate to dissolve. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 4-µm packing L11
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.85%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of teniposide ($C_{32}H_{32}O_{13}S$) in the portion of Teniposide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Teniposide RS in the *Standard solution* (mg/mL)
C_U = concentration of Teniposide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩

Analysis: Ignite the sample at 700°–800°.
Acceptance criteria: NMT 0.2%

USP Monographs

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• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Sample solution:** Proceed as directed in the *Assay*.

System suitability solution: Use the *Standard solution* in the *Assay*. [NOTE—USP Teniposide RS contains a small amount of *R*-3-thienylidene regioisomer.]

Chromatographic system: Proceed as directed in the *Assay*, except for *Detectors*.

Detectors

UV 242 nm: For thiophenealdehyde

UV 220 nm: For all other impurities

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between *R*-3-thienylidene regioisomer and teniposide peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Teniposide taken:

$$\text{Result} = r_U/\{\Sigma[r_U \times (1/F)] + r_T\} \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution* at 242 nm for thiophenealdehyde and 220 nm for all other impurities

F = relative response factor for each individual impurity (see *Table 2*)

r_T = peak area of teniposide from the *Sample solution* at 220 nm

Acceptance criteria

Individual impurities: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lignan P[a]	0.12	1.0	0.1
Teniposide related compound A[b]	0.29	1.4	0.1
Thiophenealdehyde[c]	0.30	1.0	0.1
S-3-Thienylidene regioisomer[d]	0.91	1.0	0.1
R-3-Thienylidene regioisomer[e]	0.92	1.0	0.5
Teniposide	1.0	—	—
Picroteniposide[f]	1.09	1.0	0.1
Demethylepipodophyllotoxin dimer[g]	1.39	1.3	0.1
Tetrabenzyl benzoyl lignan P[h]	2.11	0.81	0.1
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	3

[a] 4′-Demethylepipodophyllotoxin 9-β-D-glucopyranoside.

[b] 4′-Demethylepipodophyllotoxin.

[c] Thiophene-2-carbaldehyde. It is quantitated at 242 nm.

[d] 4′-Demethylepipodophyllotoxin 9-[4,6-*O*-(*S*)-3-thenylidene-β-D-glucopyranoside].

[e] 4′-Demethylepipodophyllotoxin 9-[4,6-*O*-(*R*)-3-thenylidene-β-D-glucopyranoside].

[f] (5*R*,5a*S*,8a*R*,9*S*)-9-[4,6-*O*-(*R*)-2-Thenylidene-β-D-glucopyranosyloxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one.

[g] (5*R*,5a*R*,8a*R*,9*S*,5′*R*,5a′*R*,8a′*R*,9′*S*)-9,9′-Oxybis(5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one).

[h] (5*R*,5a*R*,8a*R*,9*S*)-9-[(*O*²,*O*³,*O*⁴,*O*⁶-Tetrabenzyl)-β-D-glucopyranosyloxy]-5,8,8a,9-tetrahydro-5-(4-benzoyloxy-3,5-dimethoxyphenyl)furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1%

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 5 mg/mL in chloroform and methanol (9:1)

Acceptance criteria: −101.0° to −113.0°, at 20° on the anhydrous and solvent-free basis

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Teniposide RS

Teniposide Injection

DEFINITION

Teniposide Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of teniposide ($C_{32}H_{32}O_{13}S$). It is a sterile solution in a nonaqueous medium and may contain benzyl alcohol or other suitable preservatives.

[**CAUTION**—Great care should be taken in handling teniposide, because it is a cytotoxic agent.]

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Acetonitrile and water (25:75)

Solution B: Acetonitrile and water (56:44)

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
16	64.5	35.5
36	64.5	35.5
60	0	100

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
90	0	100

Diluent: Acetonitrile and 5 µM monobasic potassium phosphate (1:1)

Standard solution: 0.4 mg/mL of USP Teniposide RS in *Diluent*, prepared as follows. Transfer a known amount of USP Teniposide RS into a suitable volumetric flask and add acetonitrile equivalent to 10% of the final volume. Dissolve with the aid of sonication and dilute with *Diluent* to volume.

Sample solution: Nominally equivalent to 0.4 mg/mL of teniposide in *Diluent*, prepared as follows. Dilute a portion of the Injection with *Diluent* to obtain a solution containing 0.4 mg/mL of teniposide.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Flow rate: 1.3 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of teniposide ($C_{32}H_{32}O_{13}S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Teniposide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of teniposide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, and **Sample solution:** Proceed as directed in the *Assay*.

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 10 min. [NOTE—The duration of initial isocratic hold may be adjusted from 4 min to up to 10 min to meet the resolution requirements and to achieve the retention time of the teniposide peak to about 35 min.]

Benzaldehyde stock solution: 0.15 mg/mL of USP Benzaldehyde RS in *Diluent*

Teniposide related compound A stock solution: 0.15 mg/mL of USP Teniposide Related Compound A RS, prepared as follows. Transfer USP Teniposide Related Compound A RS to a suitable volumetric flask, add acetonitrile equivalent to 20% of the final volume, and sonicate to dissolve. Dilute with *Diluent* to volume.

System suitability solution 1: 15 µg/mL each of USP Teniposide Related Compound A RS and USP Benzaldehyde RS in *Diluent* from *Teniposide related compound A stock solution* and *Benzaldehyde stock solution*

System suitability solution 2: Use the *Standard solution* in the *Assay*. [NOTE—USP Teniposide RS contains a small amount of *R*-3-thienylidene regioisomer.]

Chromatographic system: Proceed as directed in the *Assay*, except for the *Detectors*.
Detectors
UV 242 nm: For thiophenealdehyde
UV 220 nm: For all other impurities

System suitability
Samples: *System suitability solution 1* and *System suitability solution 2*
[NOTE—The relative retention times for benzaldehyde, teniposide related compound A, *R*-3-thienylidene regioisomer, and teniposide are about 0.42, 0.43, 0.97, and 1.0, respectively.]
Suitability requirements
Resolution 1: NLT 0.9 between benzaldehyde and teniposide related compound A, *System suitability solution 1*
Resolution 2: NLT 1.2 between *R*-3-thienylidene regioisomer and teniposide peaks, *System suitability solution 2*

Analysis
Sample: *Sample solution*
Calculate the percentage of each specified degradation product in the portion of Injection taken:

$$\text{Result} = r_U/\{\Sigma[r_U \times (1/F)] + r_T\} \times (1/F) \times 100$$

r_U = peak area of each specified degradation product from the *Sample solution* at 242 nm for thiophenealdehyde and 220 nm for all other impurities
F = relative response factor for each individual impurity (see *Table 2*)
r_T = peak area of teniposide from the *Sample solution* at 220 nm

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lignan P[a]	0.16	1.0	2.5
Thiophenealdehyde[b]	0.28	1.2	0.5
Teniposide	1.0	—	—
Picroteniposide[c]	1.11	1.0	0.25
Total specified degradation products	—	—	3.0

[a] 4′-Demethylepipodophyllotoxin 9-β-D-glucopyranoside.
[b] Thiophene-2-carbaldehyde. It is quantitated at 242 nm.
[c] (5*R*,5a*S*,8a*R*,9*S*)-9-[4,6-*O*-(*R*)-2-Thenylidene-β-D-glucopyranosyloxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 1.5 USP Endotoxin Units/mg of teniposide.
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
• **pH** ⟨791⟩
Sample solution: Dilute 5 mL of Injection with 45 mL of water.
Acceptance criteria: 4.0–6.5
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** It meets the requirements for small-volume injections.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store under refrigeration and protect from light.
- **LABELING:** Label it to indicate that the Injection is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzaldehyde RS
  USP Teniposide RS
  USP Teniposide Related Compound A RS
  4′-Demethylepipodophyllotoxin.
  $C_{21}H_{20}O_8$ 400.38

Terazosin Capsules

DEFINITION

Terazosin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{19}H_{25}N_5O_4 \cdot HCl$, calculated as the free base.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Diluent: 0.1 N hydrochloric acid
  Standard solution: 0.005 mg/mL of terazosin hydrochloride from USP Terazosin Hydrochloride RS in *Diluent*. Sonicate for 10 min to completely dissolve. Pass the solution through a nylon filter of 0.45-µm pore size.
  Sample solution: Combine the contents from 20 Capsules and transfer 10 mg into a 100-mL flask. Fill with *Diluent* to 50% of the volume of the flask. Sonicate the flask for 10 min. Allow it to cool to room temperature. Dilute with *Diluent* to volume. Further dilute 5 mL of this solution with *Diluent* to 100 mL, and mix well. Pass 10 mL of this preparation through a PTFE filter with a 0.45-µm pore size.
  Blank: Pass the *Diluent* through a PTFE filter of 0.45-µm pore size.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  [NOTE—Use an all-glass syringe.]
  Hydrochloric acid solution: Prepare 0.1 N methanolic hydrochloric acid by adding 0.85 mL of hydrochloric acid to 1 L of methanol.
  Diluent: *Hydrochloric acid solution* and water (2:3)
  Mobile phase: Acetonitrile and water (7:3). Pass through a nylon filter of 0.45-µm pore size. Add 10 mL/L of glacial acetic acid, and degas. After degassing, pipet 0.20 mL/L of diethylamine into the solution, and mix.
  Standard stock solution: 0.55 mg/mL of terazosin hydrochloride from USP Terazosin Hydrochloride RS in *Diluent*. Sonicate for 5 min to completely dissolve.
  Standard solution: 0.055 mg/mL of terazosin hydrochloride in *Diluent* from *Standard stock solution*. Pass through a PTFE filter of 0.45-µm pore size, discarding NLT the first 8 mL of filtrate.
  Sample solution: Combine the contents of NLT 20 Capsules and weigh a quantity equivalent to 10 mg of terazosin into a 200-mL flask. Add 100 mL of *Diluent*, and sonicate for NLT 10 min. Shake the flask mechanically for NLT 10 min. Repeat until the sample is well dispersed. Allow the solution to cool, and dilute with *Diluent* to volume. Pass through a PTFE filter of 0.45-µm pore size, discarding the first 8 mL of filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 2.5 mL/min
  Injection size: 25 µL
  Run time: NLT twice the retention time of the terazosin peak
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.8 for the terazosin peak
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of terazosin ($C_{19}H_{25}N_5O_4$), based on the label claim, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U	= peak response of terazosin from the *Sample solution*
r_S	= peak response of terazosin from the *Standard solution*
C_S	= concentration of terazosin hydrochloride in the *Standard solution* (mg/mL)
C_U	= nominal concentration of terazosin in the *Sample solution* (mg/mL)
M_{r1}	= molecular weight of terazosin, 387.43
M_{r2}	= molecular weight of terazosin hydrochloride, 423.89

  Acceptance criteria: 90.0%–110.0%, calculated as the free base

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm, with sinkers
  Time: 60 min
  Standard solution: (387.44/423.89)(L/900) mg/mL of USP Terazosin Hydrochloride RS in *Medium*, where L is the Capsule label claim (terazosin), in mg
  Sample solution: At the designated time, withdraw 20 mL of the solution under test, and centrifuge at NMT 3000 rpm for 20 min.
  Capsule blank solution: Dissolve 10 empty Capsule shells in 900 mL of *Medium* heated to 37°. Centrifuge a portion of this solution for 20 min.
  Spectrometric conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.
  Mode: UV
  Analytical wavelength: UV 246 nm
  Cell length: 5 cm for Capsules labeled to contain 1 mg; 1 cm for Capsules labeled to contain 2 or 5 mg; 0.5 cm for Capsules labeled to contain 10 mg
  Blank: *Medium*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of terazosin dissolved:

$$\text{Result} = \{[A_U - (A_B/10)]/A_S\} \times C_S/L \times V \times 100$$

A_U	= absorbance of the *Sample solution*
A_B	= absorbance of the *Capsule blank solution*

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A_S = absorbance of the *Standard solution*
C_S = concentration of terazosin in the *Standard solution* (mg/mL)
L = terazosin label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of terazosin is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm, with wire helix sinker.
Time: 30 min
0.1 M phosphate buffer: 13.6 g/mL of monobasic potassium phosphate and 4 mL/L of triethylamine in water. Adjust with phosphoric acid to a pH of 2.5 ± 0.05.
Mobile phase: Acetonitrile and *0.1 M phosphate buffer* (1:3)
Standard solution: (387.44/423.89)(L/900) mg/mL of USP Terazosin Hydrochloride RS in *Medium*, where L is the Capsule label claim (terazosin), in mg
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm
Column: 3.9-mm × 30-cm; 10-µm packing L11
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of terazosin dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of terazosin in the *Standard solution* (mg/mL)
L = terazosin label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of terazosin is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

• **Procedure**

Buffer: Dissolve 4.1 g of monobasic potassium phosphate and 1.1 g of sodium 1-heptanesulfonate monohydrate in 950 mL of water. Adjust with phosphoric acid to a pH of 3.0 ± 0.10. Dilute with water to 1 L. Pass through a nylon filter of 0.45-µm pore size.
Mobile phase: Acetonitrile and *Buffer* (6:19)
Standard solution: 0.003 mg/mL of terazosin hydrochloride from USP Terazosin Hydrochloride RS in *Mobile phase*
Sample solution: Transfer 15 mg of terazosin from the contents of 20 Capsules into a 50-mL volumetric flask. Dilute with *Mobile phase* to approximately half the volume of the flask. Sonicate for NLT 10 min, and shake the flask for NLT 20 min. Dilute with *Mobile phase* to volume, and pass through a nylon or Teflon filter of 0.45-µm pore size, discarding the first 5 mL of filtrate. The final concentration of the *Sample solution* is 0.3 mg/mL.
Sensitivity solution: 0.15 µg/mL of USP Terazosin Hydrochloride RS in *Mobile phase* from the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: NLT 1.2 times the retention time of terazosin for the *Standard solution* and *Sensitivity solution*; NLT 4.5 times the retention time of terazosin in the *Sample solution*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Capacity factor, k′: NLT 1.0 for the terazosin peak, *Standard solution*
Tailing factor: NMT 2.0 for the terazosin peak, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10 for the terazosin peak, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of terazosin from the *Standard solution*
C_S = concentration of terazosin hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of terazosin in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)
M_{r1} = molecular weight of terazosin, 387.43
M_{r2} = molecular weight of terazosin hydrochloride, 423.89

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperazinyl-ADMQ[a]	0.52	1.1	0.4
Chloro ADMQ[b]	1.37	1.2	0.4
Bis-ADMQ-piperazine[c]	3.85	1.0	0.4
Any other individual, unspecified impurity	—	—	0.2
Total impurities	—	—	1.2

[a] *N*-(4-Amino-6,7 dimethoxy-2-quinazolinyl)piperazine.
[b] 2-Chloro-4-amino-6,7 dimethoxy-2-quinazoline.
[c] *N,N*-Bis-(4-amino-6,7 dimethoxy-2-quinazolinyl)piperazine.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers protected from moisture and light. Store at controlled room temperature.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Terazosin Hydrochloride RS

Terazosin Tablets

DEFINITION

Change to read:

Terazosin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ▲anhydrous▲ (USP 1-May-2019) terazosin hydrochloride ($C_{19}H_{25}N_5O_4 \cdot HCl$), calculated as the free base.

IDENTIFICATION

Change to read:

• **A.** ▲The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
▲▲ (USP 1-May-2019)
Hydrochloric acid solution: 0.01 N methanolic hydrochloric acid is prepared by adding 0.85 mL of hydrochloric acid to 1 L of methanol.
Diluent: *Hydrochloric acid solution* and water (40:60)
Mobile phase: Acetonitrile and water (70:30). Add 10.00 mL/L of glacial acetic acid, and degas. Pass through a nylon filter of 0.45-µm pore size. Pipet 0.20 mL/L of diethylamine into the solution, and mix.
Standard stock solution: 0.55 mg/mL of USP Terazosin Hydrochloride RS in *Diluent*. Sonicate for 5 min to completely dissolve.
Standard solution: 0.055 mg/mL of USP Terazosin Hydrochloride RS in *Diluent* from the *Standard stock solution*. Pass through a PTFE filter of 0.45-µm pore size, discarding the first few milliliters of filtrate.
Naproxen standard solution: 0.5 mg/mL of USP Naproxen RS, prepared as follows. Dissolve by sonication of USP Naproxen RS in 25% of the volume of the flask of acetonitrile, and dilute with water to volume.
System suitability solution: 0.05 mg/mL of USP Naproxen RS and 0.055 mg/mL of USP Terazosin Hydrochloride RS in *Diluent* from the *Naproxen standard solution* and *Standard stock solution*, respectively. Pass through a PTFE filter of 0.45-µm pore size, discarding the first few milliliters of filtrate.
Sample solution: Nominally 0.05 mg/mL of terazosin in *Diluent*, prepared as follows. ▲Transfer a quantity of NLT 20 Tablets,▲ (USP 1-May-2019) equivalent to 10 mg of terazosin into a 200-mL flask. Add 100 mL of *Diluent*, and sonicate for NLT 10 min. Shake the flask mechanically for NLT 10 min. Repeat until the sample is well dispersed. Allow the solution to cool, and dilute with *Diluent* to volume. Pass through a PTFE filter of 0.45-µm pore size, discarding the first few milliliters of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 2.5 mL/min
Injection volume: 25 µL
Run time: NLT twice the retention time of terazosin
System suitability
Samples: *Standard solution* and *System suitability solution*
▲[NOTE— The relative retention times for naproxen and terazosin are 0.7 and 1.0, respectively.]▲ (USP 1-May-2019)
Suitability requirements
Resolution: NLT 2.0 between naproxen and terazosin, *System suitability solution*
Tailing factor: NMT 1.8 for terazosin, *System suitability solution*
Relative standard deviation: NMT 2.0, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of terazosin ($C_{19}H_{25}N_5O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of terazosin from the *Sample solution*
r_S = peak response of terazosin from the *Standard solution*
C_S = concentration of ▲USP Terazosin Hydrochloride RS▲ (USP 1-May-2019) in the *Standard solution* (mg/mL)
C_U = nominal concentration of terazosin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of terazosin, ▲387.44▲ (USP 1-May-2019)
M_{r2} = molecular weight of ▲anhydrous▲ (USP 1-May-2019) terazosin hydrochloride, ▲423.90▲ (USP 1-May-2019)

Acceptance criteria: 90.0%–110.0%, calculated as the free base

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: 0.006 mg/mL of USP Terazosin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size. Dilute with *Medium* to a concentration similar to that of the *Standard solution*, assuming complete dissolution of the label claim.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: UV 245 nm
Cell length: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of terazosin ($C_{19}H_{25}N_5O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*

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A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* ▲(mg/mL)▲ (USP 1-May-2019)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of terazosin, ▲387.44▲ (USP 1-May-2019)
M_{r2} = molecular weight of ▲anhydrous▲ (USP 1-May-2019) terazosin hydrochloride, ▲423.90▲ (USP 1-May-2019)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of terazosin ($C_{19}H_{25}N_5O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Buffer: Dissolve 4.1 g of monobasic potassium phosphate and 1.1 g of sodium 1-heptanesulfonate monohydrate in 950 mL of water. Adjust with phosphoric acid to a pH of 3.0 ± 0.10. Dilute with water to 1 L. Pass through a ▲suitable▲ (USP 1-May-2019) filter of 0.45-µm pore size.

Mobile phase: Acetonitrile and *Buffer* (24:76)

Standard solution: 0.003 mg/mL of ▲▲ (USP 1-May-2019) USP Terazosin Hydrochloride RS in *Mobile phase*

Sensitivity solution: 0.15 µg/mL of USP Terazosin Hydrochloride RS in *Mobile phase* from the *Standard solution*

Sample solution: ▲Nominally 0.3 mg/mL of terazosin in *Mobile phase*, prepared as follows. Transfer a portion of the powder from the crushed Tablets (NLT 20), equivalent to 15 mg of terazosin, into a 50-mL volumetric flask.▲ (USP 1-May-2019) Dilute with *Mobile phase* to approximately half the volume of the flask. Sonicate for NLT 10 min, and shake the flask for NLT 20 min. Dilute with *Mobile phase* to volume, and pass through a ▲suitable▲ (USP 1-May-2019) filter of 0.45-µm pore size, discarding the first 5 mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 246 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1 ▲▲ (USP 1-May-2019)

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: ▲NLT 4.5 times the retention time of terazosin▲ (USP 1-May-2019)

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Capacity factor, *k'*: NLT 1.0, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each ▲specified and unspecified degradation product▲ (USP 1-May-2019) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual ▲degradation product▲ (USP 1-May-2019) from the *Sample solution*
r_S = peak response of terazosin from the *Standard solution*
C_S = concentration of ▲USP Terazosin Hydrochloride RS▲ (USP 1-May-2019) in the *Standard solution* (mg/mL)
C_U = nominal concentration of terazosin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)
M_{r1} = molecular weight of terazosin, ▲387.44▲ (USP 1-May-2019)
M_{r2} = molecular weight of ▲anhydrous▲ (USP 1-May-2019) terazosin hydrochloride, ▲423.90▲ (USP 1-May-2019)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
▲Terazosin related compound A▲ (USP 1-May-2019)[a]	0.52	1.1	0.4
▲Terazosin	1.0	1.0	—▲ (USP 1-May-2019)
▲Doxazosin related compound C▲ (USP 1-May-2019)[b]	1.37	1.2	0.4
▲Terazosin related compound C▲ (USP 1-May-2019)[c]	3.85	1.0	0.4
Any ▲unspecified degradation product▲ (USP 1-May-2019)	—	▲1.0▲ (USP 1-May-2019)	0.2
Total ▲degradation products▲ (USP 1-May-2019)	—	—	1.2

▲[a] 6,7-Dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine dihydrochloride.
[b] 2-Chloro-4-amino-6,7-dimethoxyquinazoline.
[c] 2,2′-(Piperazine-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine) dihydrochloride.▲ (USP 1-May-2019)

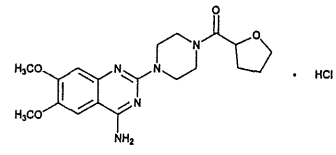
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers protected from moisture and light. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Naproxen RS
USP Terazosin Hydrochloride RS

Terazosin Hydrochloride



$C_{19}H_{25}N_5O_4 \cdot HCl \cdot 2H_2O$ 459.92

Piperazine, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2-furanyl)carbonyl]-, monohydrochloride, dihydrate.

1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(tetrahydro-2-furoyl)piperazine monohydrochloride dihydrate [70024-40-7].

Anhydrous 423.89

[63074-08-8].

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» Terazosin Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{19}H_{25}N_5O_4 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, and store at a temperature between 20° and 25°.

USP Reference standards ⟨11⟩—
USP Terazosin Hydrochloride RS
USP Terazosin Related Compound A RS
1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride.
$C_{14}H_{19}N_5O_2 \cdot 2HCl$ 362.25
USP Terazosin Related Compound B RS
1-(4-Hydroxy-6,7-dimethoxy-2-quinazolinyl))-4-[(tetrahydro-2-furanyl)carbonyl]piperazine.
$C_{19}H_{24}N_4O_5$ 388.42
USP Terazosin Related Compound C RS
1,4-Bis(4-amino-6,7-dimethoxy-2-quinazolinyl) piperazine, dihydrochloride.
$C_{24}H_{28}N_8O_4 \cdot 2HCl$ 565.45

Color and clarity of solution—Dissolve a quantity of Terazosin Hydrochloride in methanol solution (90 in 100) to obtain a 1 in 100 solution: this solution is clear and colorless to pale yellow, when compared to methanol solution (90 in 100).

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: It meets the requirements of the tests for *Chloride* ⟨191⟩, a solution prepared by dissolving 100 mg in 10 mL of methanol solution (90 in 100) being examined.

Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 3 hours: it loses not more than 9.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.2%, determined on a 1.0-g specimen.

Limit of tetrahydro-2-furancarboxylic acid—

Blank solution—Transfer 2.0 mL of glacial acetic acid to a 100-mL volumetric flask, dilute with acetone to volume, and mix. Mix 5.0 mL of this solution and 5.0 mL of acetone; pass through a nylon membrane filter having a 0.45-µm or finer porosity, previously washed with acetone; and discard the first 1 mL of the filtrate.

Internal standard solution—Transfer about 100 mg of capric acid, accurately weighed, to a 100-mL volumetric flask; dissolve in and dilute with acetone to volume; and mix. Transfer 10.0 mL of this solution and 2.0 mL of glacial acetic acid to a 100-mL volumetric flask, dilute with acetone to volume, and mix.

Standard stock solution—Dissolve an accurately weighed amount of tetrahydro-2-furancarboxylic acid in acetone to obtain a solution having a known concentration of about 1.0 mg per mL. Dilute with acetone quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 100 µg per mL.

Standard solution—Transfer 5.0 mL of the *Standard stock solution* and 5.0 mL of *Internal standard solution* to a 50-mL centrifuge tube, and mix. Pass through a nylon membrane filter having a 0.45-µm or finer porosity, previously washed with acetone; and discard the first 1 mL of the filtrate.

Test solution—Transfer about 100 mg of Terazosin Hydrochloride, accurately weighed, to a 50-mL centrifuge tube; add 5.0 mL of acetone and 5.0 mL of *Internal standard solution;* and shake for about 30 minutes. Centrifuge for about 10 minutes; pass through a nylon membrane filter having a 0.45-µm or finer porosity, previously washed with acetone; and discard the first 1 mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 10-m fused-silica capillary column coated with a 1.2-µm film of liquid phase G25. The column temperature is maintained at about 170°. The injection port is configured for splitless injection, and its temperature is maintained at about 230°. The detector temperature is maintained at about 240°. The carrier gas is helium, flowing at a rate of about 9 mL per minute. Chromatograph the *Blank solution,* and measure the peak responses as directed for *Procedure:* ensure that there are no extraneous peaks. Chromatograph the *Standard solution,* and measure the peak responses as directed for *Procedure:* the relative retention times are 1.0 for tetrahydro-2-furancarboxylic acid and 1.2 for capric acid; the resolution, *R,* between tetrahydro-2-furancarboxylic acid and capric acid is not less than 2.3; and the relative standard deviation, determined from the peak response ratios of tetrahydro-2-furancarboxylic acid to capric acid for replicate injections is not more than 6.5%.

Procedure—Separately inject equal volumes (about 0.2 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of tetrahydro-2-furancarboxylic acid in the portion of Terazosin Hydrochloride taken by the formula:

$$(C/W)(R_U/R_S)$$

in which C is the concentration, in µg per mL, of tetrahydro-2-furancarboxylic acid in the *Standard solution; W* is the weight, in mg, of Terazosin Hydrochloride taken to prepare the *Test solution;* and R_U and R_S are the peak response ratios obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.1% is found.

Limit of 1-[(tetrahydro-2-furanyl)carbonyl] piperazine—

Derivatization solution—Dissolve about 2.0 g of 3,5-dinitrobenzoyl chloride in 250 mL of acetonitrile.

Phosphate buffer solution—Transfer about 96.3 g of dibasic potassium phosphate and 3.85 g of monobasic potassium phosphate, each accurately weighed, to a 500-mL volumetric flask. Dissolve in and dilute with water to volume. Adjust with phosphoric acid solution (10 in 100) or sodium hydroxide solution (10 in 100) to a pH of 8.0 ± 0.1. Transfer 25.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume. Adjust with phosphoric acid solution (10 in 100) or sodium hydroxide solution (10 in 100) to a pH of 8.0 ± 0.1.

Solution A—Use filtered and degassed water.

Solution B—Use filtered and degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Blank solution—Use acetonitrile.

Standard solution—Dissolve an accurately weighed quantity of 1-[(tetrahydro-2-furanyl)carbonyl]piperazine in acetonitrile to obtain a solution having a known concentration of about 1.0 mg per mL. Dilute quantitatively, and stepwise if necessary, with acetonitrile, to obtain a solution having a known concentration of about 5 µg per mL.

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Test solution—Transfer about 125 mg of Terazosin Hydrochloride, accurately weighed, to a 25-mL volumetric flask; dissolve in and dilute with a mixture of acetonitrile and water (1:1) to volume; and mix.

Derivatization procedure—Transfer 5-mL portions of the *Blank solution,* the *Standard solution,* and the *Test solution,* each to a separate 100-mL volumetric flask, and proceed with each as follows. Add 5.0 mL of *Phosphate buffer solution,* and mix. Add 10.0 mL of *Derivatization solution* while swirling, allow to stand at room temperature for about 20 minutes, and mix. Dilute with a mixture of acetonitrile and water (1:1) to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm analytical column that contains packing L7. The flow rate is 1.5 mL per minute, except it is changed to 2.0 mL per minute during the period between 40 and 80 minutes. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–35	82	18	isocratic
35–40	82→10	18→90	linear gradient
40–75	10	90	isocratic
75–80	10→82	90→18	linear gradient
80–100	82	18	isocratic

Separately inject equal volumes (about 50 µL) of the derivatized *Blank solution* and the derivatized *Standard solution,* and measure the peak responses as directed for *Procedure,* ensuring that the peaks in the chromatogram of the derivatized *Standard solution* that correspond to those obtained from the derivatized *Blank solution* do not interfere with the determination: the retention time for 1-[(tetrahydro-2-furanyl)carbonyl]piperazine is more than 22 minutes; the column efficiency is not less than 3500 theoretical plates; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the derivatized *Standard solution* and the derivatized *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of 1-[(tetrahydro-2-furanyl)carbonyl]piperazine in the portion of Terazosin Hydrochloride taken by the formula:

$$2500(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of 1-[(tetrahydro-2-furanyl)carbonyl]piperazine in the *Standard solution; W* is the weight, in mg, of Terazosin Hydrochloride taken to prepare the *Test solution;* and r_U and r_S are the peak areas for 1-[(tetrahydro-2-furanyl)carbonyl]piperazine derivative obtained from the derivatized *Test solution* and the derivatized *Standard solution,* respectively: not more than 0.1% is found.

Related compounds—

pH 3.2 Citrate buffer, Standard stock preparation, and *Mobile phase*—Proceed as directed in the *Assay.*

Diluent 1—Dissolve 6.0 g of sodium citrate and 4.0 g of anhydrous citric acid in water, dilute with water to 1.0 L, and mix.

Diluent 2—Prepare a mixture of water, acetonitrile, and methanol (60:30:10).

Standard stock solution 1—Dissolve an accurately weighed quantity of USP Terazosin Related Compound A RS in *Diluent 1,* and dilute with *Diluent 1* to obtain a solution having a known concentration of about 0.5 mg per mL.

Standard stock solution 2—Dissolve an accurately weighed quantity of USP Terazosin Related Compound B RS in methanol, and dilute with methanol to obtain a solution having a known concentration of about 0.5 mg per mL.

Standard stock solution 3—Dissolve an accurately weighed quantity of USP Terazosin Related Compound C RS in *Diluent 2,* and dilute with *Diluent 2* to obtain a solution having a known concentration of about 0.1 mg per mL.

Standard solution—Transfer 5.0 mL of *Standard stock preparation,* 4.0 mL of *Standard stock solution 1,* 4.0 mL of *Standard stock solution 2,* and 20 mL of *Standard stock solution 3* to a 100-mL volumetric flask containing about 60 mL of *Diluent 2.* Dilute with *Diluent 2* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Test solution—Use the *Assay stock preparation* prepared as directed in the *Assay.*

Chromatographic system—Prepare as directed in the *Assay.* Chromatograph the *Mobile phase,* and record the peak responses as directed for *Procedure:* ensure that there are no significant interfering peaks. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.2 for terazosin related compound A, 1.0 for terazosin, 1.48 for terazosin related compound B, and 2.57 for terazosin related compound C; the resolution, *R,* between terazosin and terazosin related compound B is not less than 9.0; the column efficiency determined from the terazosin peak is not less than 12,000 theoretical plates; the tailing factor for the terazosin related compound C peak is not more than 3.0; and the relative standard deviation for replicate injections determined from the terazosin peak is not more than 2.0%, and not more than 5.0% determined from the terazosin related compound C peak.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for about 60 minutes, and measure the peak responses. Separately calculate the quantities, in mg, of terazosin related compound A and terazosin related compound C in the portion of Terazosin Hydrochloride taken by the formula:

$$200C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard solution;* and r_U and r_S are the peak responses for the corresponding related compound obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.3% of terazosin related compound A is found; and not more than 0.4% of terazosin related compound C is found. Calculate the quantity, in mg, of each impurity in the portion of Terazosin Hydrochloride taken by the formula:

$$200C(r_i/r_T)$$

in which C is the concentration, in mg per mL, of USP Terazosin Hydrochloride RS in the *Standard solution;* r_i is the peak response for each impurity, other than terazosin related compound A and terazosin related compound C, obtained from the *Test solution;* and r_T is the terazosin peak response obtained from the *Standard solution:* not more than 0.3% of any impurity eluting prior to the terazosin peak is found; not more than 0.1% of any other impurity is found; and not more than 0.6% of total impurities is found.

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Assay—

pH 3.2 Citrate buffer—Dissolve 12.0 g of sodium citrate dihydrate and 28.5 g of anhydrous citric acid in 1.95 L of water. Adjust with anhydrous citric acid or sodium citrate to a pH of 3.2 ± 0.1. Dilute with water to 2.0 L, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *pH 3.2 Citrate buffer* and acetonitrile (1685:315). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve an accurately weighed quantity of USP Terazosin Hydrochloride RS in *Mobile phase,* and dilute with *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.

Standard preparation—Transfer 10.0 mL of *Standard stock preparation* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume; and mix.

Assay stock preparation—Transfer about 100 mg of Terazosin Hydrochloride, accurately weighed, to a 200-mL volumetric flask; dissolve in and dilute with *Mobile phase* to volume; and mix.

Assay preparation—Transfer 10.0 mL of *Assay stock preparation* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer l0.0 mL of this solution to a l00-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The column temperature is maintained at about 30°. The flow rate is about 1.0 mL per minute. Chromatograph the *Mobile phase,* and record the peak responses as directed for *Procedure:* ensure that there are no significant interfering peaks. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 12,000 theoretical plates; the tailing factor is not less than 0.9 and not more than 1.3; and the relative standard deviation for replicate injections is not more than 0.9%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for about 45 minutes, and measure the peak responses. Calculate the quantity, in mg, of $C_{19}H_{25}N_5O_4 \cdot HCl$ in the portion of Terazosin Hydrochloride taken by the formula:

$$10{,}000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Terazosin Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Terbinafine Compounded Oral Suspension

DEFINITION

Terbinafine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of terbinafine ($C_{21}H_{25}N$). Prepare Terbinafine Compounded Oral Suspension containing 25 mg/mL of terbinafine as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Terbinafine (as Terbinafine Hydrochloride)	2500 mg (2810 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, *NF,* and Vehicle for Oral Suspension, *NF,* a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder or add *Terbinafine Hydrochloride* powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a terbinafine suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (2:3), with 0.15% triethylamine and 0.15% phosphoric acid. Make adjustments if necessary.

Standard stock solution: 1.0 mg/mL of USP Terbinafine Hydrochloride RS in methanol

Standard solution: Transfer 0.5 mL of *Standard stock solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume to obtain a solution containing 5 µg/mL of terbinafine, and pass through a suitable filter of 0.22-µm pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 1.0 mL to a 25-mL volumetric flask. Dilute with methanol to volume to obtain a nominal concentration of 1 mg/mL of terbinafine. Mix the sample again. Accurately pipet 0.5 mL of the diluted terbinafine solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a nominal concentration of 5 µg/mL of terbinafine.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 224 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Flow rate: 0.4 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time of the terbinafine peak is 5.1 min.]

Suitability requirements

Relative standard deviation: NMT 5.8%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of terbinafine ($C_{21}H_{25}N$) in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of terbinafine in the *Standard solution* (µg/mL)

C_U = nominal concentration of terbinafine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 5.3–5.7

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Terbinafine Hydrochloride RS

Terbinafine Tablets

DEFINITION
Terbinafine Tablets contain Terbinafine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of terbinafine ($C_{21}H_{25}N$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer solution: 0.85 g/L of monobasic potassium phosphate in water. Add 1 g of sodium 1-decanesulfonate and adjust with dilute phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer solution* (2:3)
Standard solution: 0.2 mg/mL of USP Terbinafine Hydrochloride RS in *Mobile phase* (equivalent to 0.19 mg/mL of terbinafine)
Sample stock solution: Nominally equivalent to 0.5 mg/mL of terbinafine in *Mobile phase*, from crushed, finely powdered Tablets. [NOTE—Sonicate for 20 min with intermittent shaking.]
Sample solution: Nominally equivalent to 0.2 mg/mL of terbinafine in *Mobile phase*, from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 3.9-mm × 15-cm; 5-µm packing L7
Flow rate: 1.8 mL/min
Injection volume: 5 µL
Run time: 1.5 times the retention time of the terbinafine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of terbinafine ($C_{21}H_{25}N$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Terbinafine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of terbinafine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of terbinafine, 291.44
M_{r2} = molecular weight of terbinafine hydrochloride, 327.90

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: pH 3.0 citrate buffer; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: 32 µg/mL of USP Terbinafine Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 283 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of terbinafine ($C_{21}H_{25}N$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Terbinafine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of terbinafine, 291.44
M_{r2} = molecular weight of terbinafine hydrochloride, 327.90
V = volume of *Medium*, 500 mL
D = dilution factor for the *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of terbinafine ($C_{21}H_{25}N$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Buffer solution, Mobile phase, and **Sample solution:** Proceed as directed in the *Assay*.
Standard solution: 0.7 µg/mL of USP Terbinafine Hydrochloride RS in *Mobile phase* (equivalent to 0.6 µg/mL of terbinafine)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 15-cm; 5-µm packing L7
Flow rate: 1.8 mL/min
Injection volume: 50 µL
Run time: 4 times the retention time of the terbinafine peak
System suitability
Sample: *Standard solution*

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Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of terbinafine from the *Standard solution*
C_S = concentration of USP Terbinafine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of terbinafine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)
M_{r1} = molecular weight of terbinafine, 291.44
M_{r2} = molecular weight of terbinafine hydrochloride, 327.90

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Methyl-1-(naphthalen-1-yl) methanamine	0.14	1.5	0.2
Terbinafine	1.0	—	—
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	0.7

• **LIMIT OF TERBINAFINE DIMER**
Diluent: Acetonitrile and water (4:1)
Solution A: 1 mL/L of triethylamine in water
Solution B: 1 mL/L of triethylamine in a mixture of acetonitrile and water (19:1)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	40	60
8	30	70
18	30	70
24	16	84
26	5	95
30	0	100
37	40	60
45	40	60

Standard solution: 1.4 µg/mL of USP Terbinafine Hydrochloride RS in *Diluent* (equivalent to 1.2 µg/mL of terbinafine)
Sample solution: Nominally equivalent to 2.5 mg/mL of terbinafine in *Diluent*, from crushed, finely powdered Tablets. [NOTE—Sonicate for 20 min with intermittent shaking.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 52°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the terbinafine dimer in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the terbinafine dimer from the *Sample solution*
r_S = peak response of terbinafine from the *Standard solution*
C_S = concentration of USP Terbinafine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of terbinafine in the *Sample solution* (mg/mL)
F = relative response factor for the terbinafine dimer
M_{r1} = molecular weight of terbinafine, 291.44
M_{r2} = molecular weight of terbinafine hydrochloride, 327.90

Acceptance criteria: See *Table 3*.

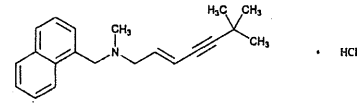
Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Terbinafine	1.0	—	—
Terbinafine dimer[a]	2.0	2.1	0.05

[a] (2*E*,4*E*)-4-(4,4-Dimethylpent-2-ynylidene)-N^1,N^5-dimethyl-N^1,N^5-bis(naphthalen-1-ylmethyl)pent-2-ene-1,5-diamine.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Terbinafine Hydrochloride RS

Terbinafine Hydrochloride



$C_{21}H_{25}N \cdot HCl$ 327.90

USP Monographs

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1-Naphthalenemethanamine, *N*-(6,6-dimethyl-2-hepten-4-ynyl)-*N*-methyl-, (*E*)-, hydrochloride;
(*E*)-*N*-(6,6-Dimethyl-2-hepten-4-ynyl)-*N*-methyl-1-naphthalenemethylamine, hydrochloride;
(2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine hydrochloride [78628-80-5].

DEFINITION

Terbinafine Hydrochloride contains NLT 98.0% and NMT 102.0% of terbinafine hydrochloride ($C_{21}H_{25}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements of the test when using dehydrated alcohol as a solvent

ASSAY

- **PROCEDURE**

Protect all solutions containing Terbinafine Hydrochloride from light.

Buffer: Prepare a solution in water containing 2.0 mL/L of triethylamine. Adjust with diluted acetic acid to a pH of 7.5.
Solution A: *Solution C* and *Buffer* (7:3)
Solution B: *Solution C* and *Buffer* (95:5)
Solution C: Methanol and acetonitrile (3:2)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
25	0	100
30	0	100
30.1	100	0
38	100	0

Diluent: Acetonitrile and water (1:1)
System suitability solution: 1 mg/mL of USP Terbinafine Hydrochloride RS and 5 µg/mL of USP Terbinafine Related Compound B RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Terbinafine Hydrochloride RS in *Diluent*
Sample solution: 0.5 mg/mL of Terbinafine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.0-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for terbinafine related compound B and terbinafine are 0.94 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between terbinafine related compound B and terbinafine, *System suitability solution*
Tailing factor: NLT 0.8 and NMT 1.5 for terbinafine, *Standard solution*
Relative standard deviation: NMT 2.0% for terbinafine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of terbinafine hydrochloride ($C_{21}H_{25}N \cdot HCl$) in the portion of Terbinafine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of terbinafine from the *Sample solution*
r_S = peak response of terbinafine from the *Standard solution*
C_S = concentration of USP Terbinafine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Terbinafine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

Protect all solutions containing Terbinafine Hydrochloride from light.

Buffer, Solution A, Solution B, Solution C, Mobile phase, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.5 µg/mL each of USP Terbinafine Hydrochloride RS, USP Terbinafine Related Compound A RS, USP Terbinafine Related Compound B RS, USP Terbinafine Related Compound C RS, and USP Terbinafine Related Compound D RS in *Diluent*
Sample solution: 0.5 mg/mL of Terbinafine Hydrochloride in *Diluent*
Sensitivity solution: 0.25 µg/mL of USP Terbinafine Hydrochloride RS in *Diluent* from the *Standard solution*
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between terbinafine related compound B and terbinafine, *System suitability solution*
Relative standard deviation: NMT 10% for terbinafine, *Standard solution*
Signal-to-noise ratio: NLT 10 for terbinafine, *Sensitivity solution*
Calculate the signal-to-noise ratio:

$$\text{Result} = (2H)/h$$

H = measured height of the terbinafine peak
h = amplitude of the average measured baseline noise

Analysis
Samples: *Standard solution* and *Sample solution*
Identify the peaks based on their relative retention times as given in *Table 2*.
Calculate the percentage of terbinafine related compound A, terbinafine related compound B, terbinafine related compound C, and terbinafine related compound D in the portion of Terbinafine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

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r_U = peak response of terbinafine related compound A, terbinafine related compound B, terbinafine related compound C, or terbinafine related compound D from the *Sample solution*
r_S = peak response of terbinafine related compound A, terbinafine related compound B, terbinafine related compound C, or terbinafine related compound D from the *Standard solution*
C_S = concentration of USP Terbinafine Related Compound A RS, USP Terbinafine Related Compound B RS, USP Terbinafine Related Compound C RS, or USP Terbinafine Related Compound D RS in the *Standard solution* (µg/mL)
C_U = concentration of Terbinafine Hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of terbinafine dimer or any other individual impurity in the portion of Terbinafine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of terbinafine dimer or any other individual impurity from the *Sample solution*
r_S = peak response of the terbinafine peak from the *Standard solution*
C_S = concentration of USP Terbinafine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Terbinafine Hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak observed in the blank, and any peak less than 0.05%.

Table 2

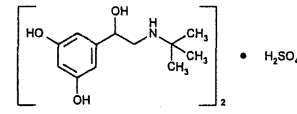
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Terbinafine related compound A[a]	0.1	—	0.1
Terbinafine related compound C[b]	0.92	—	0.1
Terbinafine related compound B[c]	0.94	—	0.1
Terbinafine	1.0	—	—
Terbinafine related compound D[d]	1.1	—	0.1
Terbinafine dimer[e]	1.7	2.5	0.05
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.3

[a] *N*-Methyl-1-(naphthalen-1-yl)methanamine, also known as *N*-methyl-*C*-(naphthalen-1-yl)methanamine.
[b] *trans*-Isoterbinafine or (2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-2-ylmethyl)hept-2-en-4-yn-1-amine.
[c] *cis*-Terbinafine or (2*Z*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine.
[d] 4-Methylterbinafine or (2*E*)-*N*,6,6-Trimethyl-*N*-((4-methylnaphthalen-1-yl)methyl)hept-2-en-4-yn-1-amine.
[e] (2*E*,4*E*)-4-(4,4-Dimethylpent-2-ynylidene)-N^1,N^5-dimethyl-N^1,N^5-bis(naphthalen-1-ylmethyl)pent-2-ene-1,5-diamine.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers, protected from light. Store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Terbinafine Hydrochloride RS
USP Terbinafine Related Compound A RS
N-Methyl-*C*-(naphthalen-1-yl)methanamine hydrochloride.
$C_{12}H_{13}N \cdot HCl$ 207.70
USP Terbinafine Related Compound B RS
(2*Z*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine hydrochloride.
$C_{21}H_{25}N \cdot HCl$ 327.89
USP Terbinafine Related Compound C RS
(2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-2-ylmethyl)hept-2-en-4-yn-1-amine hydrochloride.
$C_{21}H_{25}N \cdot HCl$ 327.89
USP Terbinafine Related Compound D RS
(2*E*)-*N*,6,6-Trimethyl-*N*-[(4-methylnaphthalen-1-yl)methyl]hept-2-en-4-yn-1-amine hydrochloride.
$C_{22}H_{27}N \cdot HCl$ 341.92

Terbutaline Sulfate



$(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$ 548.65
1,3-Benzenediol, 5-[2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-, sulfate (2:1) (salt).
(±)-α-[(*tert*-Butylamino)methyl]-3,5-dihydroxybenzyl alcohol sulfate (2:1) (salt) [23031-32-5].

» Terbutaline Sulfate contains not less than 98.0 percent and not more than 101.0 percent of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers, at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Terbutaline Sulfate RS
USP Terbutaline Related Compound A RS
3,5-Dihydroxy-ω-*t*-butylaminoacetophenone sulfate.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
Acidity—Dissolve 0.20 g in 10 mL of carbon dioxide-free water, and titrate with 0.020 N sodium hydroxide from a microburet to a pH of about 6, determining the endpoint potentiometrically, using a calomel-glass electrode system: not more than 0.50 mL of 0.020 N sodium hydroxide is required (0.3% as acetic acid).

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Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

Chromatographic purity—
Ion-pair solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Dissolve an accurately weighed quantity of USP Terbutaline Sulfate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 3 µg per mL.
Test solution—Use the *Assay preparation.*
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity, if present, in the portion of Terbutaline Sulfate taken by the formula:

$$5000(C/W)(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP Terbutaline Sulfate RS in the *Standard solution; W* is the weight, in mg, of Terbutaline Sulfate taken to prepare the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the terbutaline peak response obtained from the *Standard solution:* the sum of all impurities is not more than 1.0%.

Assay—
Ion-pair solution—Transfer 3.15 g of ammonium formate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust the solution with formic acid to a pH of about 3.0, add 5.49 g of sodium 1-hexanesulfonate, dilute with water to volume, and mix.
Mobile phase—Prepare a filtered and degassed mixture of *Ion-pair solution* and methanol (77:23). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve accurately weighed quantities of USP Terbutaline Sulfate RS and USP Terbutaline Related Compound A RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of 1.0 mg per mL and 0.4 mg per mL, respectively.
Standard preparation—Dissolve an accurately weighed quantity of USP Terbutaline Sulfate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL.
Assay preparation—Transfer about 50 mg of Terbutaline Sulfate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times for terbutaline related compound A and terbutaline are 0.9 and 1.0, respectively; the resolution, *R,* between terbutaline related compound A and terbutaline is not less than 2.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor for the terbutaline peak is not more than 2.0; and the relative standard deviation for replicate injections determined from the terbutaline peak is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$ in the portion of Terbutaline Sulfate taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Terbutaline Sulfate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Terbutaline Sulfate Injection

» Terbutaline Sulfate Injection is a sterile solution of Terbutaline Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$.
[NOTE—Do not use the Injection if it is discolored.]

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light, at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Terbutaline Sulfate RS
USP Terbutaline Related Compound A RS
3,5-Dihydroxy-ω-*t*-butylaminoacetophenone sulfate.

Identification—
A: Apply 2 µL of Injection and 2 µL of a solution of USP Terbutaline Sulfate RS in sodium chloride solution (0.9 in 100) containing 1 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a solvent system consisting of a mixture of isopropyl alcohol, cyclohexane, and formic acid (13:5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry with a current of air. Spray the plate with a 1 in 50 solution of 4-aminoantipyrine in methanol, allow to air-dry, and spray with a 2 in 25 solution of potassium ferricyanide in a solvent prepared by mixing ammonium hydroxide with water (4:1): the R_F value of the principal spot obtained from the Injection corresponds to that obtained from the Standard solution.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1250.0 USP Endotoxin Units per mg of terbutaline sulfate.
pH ⟨791⟩: between 3.0 and 5.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Terbutaline Sulfate.*
Assay preparation—Use Injection. If necessary, quantitatively dilute an accurately measured volume of Injection with water to obtain a solution having a concentration of about 1 mg per mL.

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Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$ in each mL of the Injection taken by the formula:

$$(LC/D)(r_U/r_S)$$

in which L is the labeled quantity, in mg per mL, of terbutaline sulfate in the Injection, C is the concentration, in mg per mL, of USP Terbutaline Sulfate RS in the *Standard preparation*, D is the concentration, in mg per mL, of terbutaline sulfate in the *Assay preparation*, based upon the labeled quantity, in mg per mL, of terbutaline sulfate in the Injection and the extent of dilution, and r_U and r_S are the terbutaline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Terbutaline Sulfate Compounded Oral Suspension

DEFINITION

Terbutaline Sulfate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of terbutaline sulfate $[(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4]$. Prepare Terbutaline Sulfate Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Terbutaline Sulfate	100 mg
Syrup, *NF*,[a] a sufficient quantity to make	100 mL

[a] Syrup, *NF*, containing 0.2% sodium benzoate.

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder or add *Terbutaline Sulfate* powder. Add the *Syrup, NF*, to make a terbutaline sulfate suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Syrup, NF*, to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: A solution of methanol and 20 mM monobasic potassium phosphate (2:23), adjusted with phosphoric acid to a pH of 3.6. Filter and degas.

Standard stock solution: 5 mg/mL of USP Terbutaline Sulfate RS in methanol

Standard solution: Transfer 0.2 mL of *Standard stock solution* to a 100-mL volumetric flask, dilute with water to volume to obtain a solution containing 10 µg/mL of terbutaline sulfate, and pass through a suitable filter of 0.22-µm pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 1.0 mL to a 10-mL volumetric flask. Dilute with *Mobile phase* to volume to obtain a nominal concentration of 100 µg/mL of terbutaline sulfate. Extract terbutaline sulfate from Oral Suspension with methanol. Accurately pipet 1 mL of Oral Suspension and 3 mL of *Mobile phase* in the barrel of a 5-mL plastic syringe. Shake, and pass through a suitable filter of 0.22-µm pore size into a 10-mL volumetric flask. Repeat the process with an additional 2 mL of methanol. Bring to a final volume of 10 mL with *Mobile phase* to obtain a nominal concentration of 10 µg/mL of terbutaline sulfate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 3.9-mm × 30-cm; 10-µm microphenyl packing L11

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for the terbutaline sulfate peak is 5 min.]

Suitability requirements

Relative standard deviation: NMT 2.2% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of terbutaline sulfate $[(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4]$ in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Terbutaline Sulfate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of terbutaline sulfate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.

• **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded when stored in a refrigerator

• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Terbutaline Sulfate RS

Terbutaline Sulfate Tablets

» Terbutaline Sulfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$.

Packaging and storage—Preserve in tight containers, at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Terbutaline Sulfate RS

USP Terbutaline Related Compound A RS

3,5-Dihydroxy-ω-*t*-butylaminoacetophenone sulfate.

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

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Procedure—Determine the amount of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$ dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Ion-pair solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Terbutaline Sulfate.*

Standard preparation—Dissolve an accurately weighed quantity of USP Terbutaline Sulfate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL. Transfer 10.0 mL of the solution so obtained to a 100-mL volumetric flask, add 10 mL of 0.05 N sulfuric acid, dilute with water to volume, and mix.

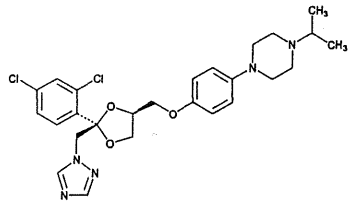
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of terbutaline sulfate, to a 100-mL volumetric flask. Add 10 mL of 0.05 N sulfuric acid and 20 mL of water, and shake for 15 minutes. Dilute with water to volume, mix, and filter.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of terbutaline sulfate $[(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4]$ in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Terbutaline Sulfate RS in the *Standard preparation;* and r_U and r_S are the terbutaline peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Terconazole



$C_{26}H_{31}Cl_2N_5O_3$ 532.46

Piperazine, 1-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)-, *cis*-.

cis-1-[*p*-[[2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-4-isopropylpiperazine [67915-31-5].

» Terconazole contains not less than 98.0 percent and not more than 102.0 percent of $C_{26}H_{31}Cl_2N_5O_3$, calculated on the dried basis.

Packaging and storage—Preserve in light-resistant containers. Store at room temperature.

USP Reference standards ⟨11⟩—

USP Terconazole RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020)

Specific rotation ⟨781S⟩: between −1° and +1° at 20°.

Test solution: 40 mg per mL solution in methylene chloride.

Loss on drying ⟨731⟩—Dry it in a vacuum at 80° for 4 hours: it loses not more than 0.75% of its weight, a 2.0-g specimen being used.

Residue on ignition ⟨281⟩: not more than 0.1%, a 2.0-g specimen being used.

Related compounds— [NOTE—Use the solutions within 24 hours if protected from light and within 1 hour if not protected from light.]

Solution A—Prepare and filter a 0.6% ammonium carbonate solution in water.

Solution B: acetonitrile.

Solution C: tetrahydrofuran.

Mobile phase—Use variable mixtures of *Solution A, Solution B,* and *Solution C* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve in and dilute with alcohol an accurately weighed quantity of USP Terconazole RS to obtain a solution having a known concentration of about 0.1 mg per mL.

Test solution—Dissolve in and dilute with alcohol an accurately weighed quantity of Terconazole to obtain a solution having a concentration of about 10 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 10-cm column that contains 3-µm packing L1. The flow rate is about 2 mL per minute. The chromatograph is programmed as shown in the table below.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	*Solution C* (%)	Elution
0–15	80→55	20→25	0→20	linear gradient
15–17	55→0	25→80	20	linear gradient
17–20	0	80	20	isocratic
20–21	0→80	80→20	20→0	step gradient
21–25	80	20	0	re-equilibration

Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor for terconazole peak is not less than 0.9 and not more than 1.3; and the relative standard deviation for replicate injection is not more than 5.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the peak responses. Identify the impurities using the relative retention times given in *Table 1.* Calculate the percentage of each terconazole related compound in the portion of Terconazole taken by the formula:

$$100(C_S/C_U)(r_U/r_S)(1/F)$$

in which C_S and C_U are the concentrations, in mg per mL, of terconazole in the *Standard solution* and the *Test solution,* respectively; r_U is the peak response of each impurity obtained from the *Test solution;* r_S is the peak response of terconazole obtained from the *Standard solution;* and F is the relative response factor for each impurity relative to terconazole.

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Table 1

Impurity	Approx. RRT	Relative Response Factor (*F*)	Limit (%)
B[a]	0.88	0.94	0.50
A[b]	0.95	0.92	0.50
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 1-[4-[[(2*RS*,4*SR*)-2-(2,4-Dichlorophenyl)-2-[(4*H*-1,2,4-triazol-4-yl]methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)piperazine.
[b] 1-[4-[[(2*RS*,4*RS*)-2-(2,4-Dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl)methoxy]phenyl]-4-(1-methylethyl)piperazine.

The limits in *Table 1* are met. Disregard any impurity that is less than 0.10%.

Assay—Dissolve about 135 mg of Terconazole, accurately weighed, in about 70 mL of previously neutralized glacial acetic acid. Titrate with 0.1 N perchloric acid VS, and determine the endpoint potentiometrically (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 17.75 mg of $C_{26}H_{31}Cl_2N_5O_3$.

Teriparatide

SVSEIQLMHN LGKHLNSMER VEWLRKKLQD VHNF—OH

$C_{181}H_{291}N_{55}O_{51}S_2$ 4117.72 (as free base)

L-Phenylalanine, L-seryl-L-valyl-L-seryl-L-α-glutamyl-L-isoleucyl-L-glutaminyl-L-leucyl-L-methionyl-L-histidyl-L-asparaginyl-L-leucylglycyl-L-lysyl-L-histidyl-L-leucyl-L-asparaginyl-L-seryl-L-methionyl-L-α-glutamyl-L-arginyl-L-valyl-L-α-glutamyl-L-tryptophyl-L-leucyl-L-arginyl-L-lysyl-L-lysyl-L-leucyl-L-glutaminyl-L-α-aspartyl-L-valyl-L-histidyl-L-asparaginyl-; L-Seryl-L-valyl-L-seryl-L-α-glutamyl-L-isoleucyl-L-glutaminyl-L-leucyl-L-methionyl-L-histidyl-L-asparaginyl-L-leucylglycyl-L-lysyl-L-histidyl-L-leucyl-L-asparaginyl-L-seryl-L-methionyl-L-α-glutamyl-L-arginyl-L-valyl-L-α-glutamyl-L-tryptophyl-L-leucyl-L-arginyl-L-lysyl-L-lysyl-L-leucyl-L-glutaminyl-L-α-aspartyl-L-valyl-L-histidyl-L-asparaginyl-L-phenylalanine. [52232-67-4].

DEFINITION

Teriparatide, also called rhPTH (1-34), is a single-chain peptide containing 34 amino acids identical to the 34 N-terminal amino acids of human parathyroid hormone. Teriparatide is produced by a method based on recombinant DNA (rDNA) technology. The host cell-derived protein content is below the limit approved by the competent authority. In addition, the host cell-derived and vector-derived DNA are below the limit approved by the competent authority. Teriparatide is a white to almost white powder. It contains NLT 95.0% and NMT 105.0% of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$), calculated on the anhydrous, acetic acid-free, and chloride-free basis.

IDENTIFICATION

- **A.** The ratio of the retention time of the teriparatide peak of the *Sample solution* to that of the *Standard solution*, as obtained in the *Assay*, is 1.00 ± 0.03.
- **B. PEPTIDE MAPPING**

Solution A: 0.1% (v/v) trifluoroacetic acid in water

Solution B: Acetonitrile, trifluoroacetic acid, and water (60: 0.1: 40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	96	4
6	96	4
20	45	55
25	0	100
25.1	96	4
35	96	4

20 mM sodium phosphate buffer: 2.30 mg/mL of anhydrous dibasic sodium phosphate and 0.60 mg/mL of sodium phosphate monobasic monohydrate in water. Adjust with sodium hydroxide or phosphoric acid to a pH of 7.8 before final dilution.

Enzyme solution: About 0.25 mg/mL of *Staphylococcus aureus* V8 protease in *20 mM sodium phosphate buffer*. Stable for 72 h when stored at 2°–8°.

Standard solution: Prepare a 1.5 ± 0.15 mg/mL solution of USP Teriparatide RS in *20 mM sodium phosphate buffer*. Combine this solution with *Enzyme solution* for a teriparatide to protease ratio of 10:1 (w/w). Mix and incubate at 37° for 18–24 h. Quench the digestion by adding the volume of *Solution A* needed to reach a final digested teriparatide concentration of approximately 0.25 mg/mL. Stable for 72 h when stored at 2°–8°.

Sample solution: Prepare a 1.5 ± 0.15 mg/mL solution of Teriparatide in *20 mM sodium phosphate buffer*. Combine this solution with *Enzyme solution* for a Teriparatide to protease ratio of 10:1 (w/w). Mix and incubate at 37° for 18–24 h. Quench the digestion by adding the volume of *Solution A* needed to reach a final digested teriparatide concentration of approximately 0.25 mg/mL. Stable for 72 h when stored at 2°–8°.

Blank: Combine *20 mM sodium phosphate buffer* with *Enzyme solution* in the same proportions used for the *Standard solution* and the *Sample solution*. Mix and incubate at 37° for 18–24 h. Quench by adding the same volume of *Solution A* as for the *Standard solution* and the *Sample solution*. Stable for 72 h when stored at 2°–8°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The *System suitability* procedure must be performed both before starting sample testing and at the end of each sample run.]

Suitability requirements

Chromatogram similarity: In the chromatogram from the *Standard solution*, identify the peaks due to digest fragments I, II, III, IV, and V. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with the USP Certificate for USP Teriparatide RS.

Resolution: NLT 1.5 between peaks indicated as fragments III and I

Tailing factor: NMT 2.3 for the peak indicated as fragment IV

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

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Record the chromatograms. For each of the five fragments, determine the ratio of the fragment retention time from the *Sample solution* to the corresponding fragment retention time from the *Standard solution* obtained from the first *System suitability* injection.

Acceptance criteria: The chromatographic profile of the *Sample solution* corresponds to that of the *Standard solution*. All five fragments, I, II, III, IV, and V, must be present. The fragment retention time ratio is 1.00 ± 0.03 for all five fragments.

• **C. BIOIDENTITY**

Basic medium: Sterile Dulbecco's modified Eagle's medium (DMEM) containing high glucose, L-glutamine, pyridoxine hydrochloride, and 25mM HEPES[1]

Growth medium: 10% fetal bovine serum (FBS) in *Basic medium* prepared as follows. To 450 mL of *Basic medium*, add 50 mL of heat inactivated irradiated FBS[2] and mix. Sterilize the solution by filtering the solution using a 0.22-µm, low-protein-binding, sterile filter unit and store at 2°–8°.

Serum starve medium: 0.1% (w/v) bovine serum albumin (BSA)-fraction V in *Basic medium* prepared as follows. Add 0.50 g of BSA-fraction V to 500 mL of *Basic medium*, and mix. Sterilize the solution by filtering the solution using a 0.22-µm, low-protein-binding, sterile filter unit and store at 2°–8°.

Vehicle: 150 mM sodium chloride, 0.1% (w/v) BSA-fraction V, and 0.001 N hydrochloric acid prepared as follows. Dissolve 1.75 g of sodium chloride and 0.2 g of BSA-fraction V in 180 mL of water. Add 20.0 mL of 0.01 N hydrochloric acid to the solution. Sterilize the solution by filtering the solution using a 0.22-µm, low-protein-binding, sterile filter unit and store at 2°–8°.

600 mM IBMX solution: Add 0.30 g of 3-isobutyl-1-methyl-xanthine (IBMX) to 2.25 mL of dimethyl sulfoxide (DMSO) and vortex to dissolve. Aliquot and store at −18° to −24°.

Cell culture preparation: Culture UMR-106 rat osteogenic sarcoma cell line[3] in *Growth medium* at 37 ± 2° and 10 ± 2% carbon dioxide (CO_2) atmosphere in a humidified incubator. Cells should be passaged when the cultures are approximately 65%–85% confluent as determined microscopically at an appropriate magnification (such as 200–400×). [NOTE—Do not allow the cells to go beyond 85% confluence for cell passage or analysis.] For cell passage, remove the media from the cells. Rinse the cells once with sterile Dulbecco's phosphate buffer saline (DPBS) without calcium and magnesium.[4] Rinse the cells with approximately 5–10 mL of 0.25% (w/v) trypsin in 1 mM EDTA solution,[5] and immediately remove all but approximately 1–2 mL of the 0.25% (w/v) trypsin with 1 mM EDTA solution from the cells. Allow the 0.25% (w/v) trypsin with 1 mM EDTA solution to remain on the cells for 1–2 min at a temperature ranging from room temperature to 37° until the cells begin to round and release from the culture surface. Resuspend the cells in an appropriate volume of *Growth medium* and count them. Cells are passaged into a T162 cm^2 flask with approximately 30 mL of *Growth medium* at 0.75–6 × 10^6 cells per flask.

Preparation of cells for analysis: Use cells that are between passages 4 and 20, 65%–85% confluent, and 2–5 days post-passage. Following the procedure described in *Cell culture preparation*, prepare an appropriate volume of cell solution at 0.75 × 10^5 viable cells per mL in *Growth medium*. To 96-well flat-bottom plates,[6] add 200 µL of cell solution per well. Mix cell solution frequently during dispensing to prevent cells from settling and to ensure consistent density throughout the plate. Incubate plates for 18–26 h at 37 ± 2° and 10 ± 2% carbon dioxide (CO_2). Following the incubation, remove media from the cells and add 200 µL of *Serum starve medium* to each well. Incubate plates for 18–26 h at 37 ± 2° and 10 ± 2% carbon dioxide (CO_2).

Diluent A: 1 mM IBMX in Hanks' balanced salt solution (HBSS) with phenol red[7] prepared as follows. Very slowly add 500 µL of warmed (30°–40°) *600 mM IBMX solution* to 300 mL of warmed HBSS with phenol red while continuously mixing on a stir plate. [NOTE—It is acceptable to substitute HBSS containing phenol red with HBSS without phenol red.[8] HBSS containing phenol red is preferred because it is easier to visualize the wells.]

Assay/lysis solution: 0.55 mM IBMX in assay/lysis buffer from a suitable cAMP immunoassay kit for 96-well plates[9] prepared as follows. Very slowly add 27.5 µL of warmed *600 mM IBMX solution* to 30 mL of warmed assay/lysis buffer.

Diluted cAMP-AP conjugate: Dilute the cAMP-alkaline phosphatase (AP) conjugate (1:100) with the conjugate dilution buffer from the same cAMP kit used for the *Assay/lysis solution*.[9] Prepare 2.5 mL of diluted conjugate per 96-well plate. Use within 4 h.

Standard stock solution: Dissolve the contents of 1 vial of USP Teriparatide RS in an appropriate volume of *Vehicle* to obtain a 250-µg/mL solution.

Standard solution: Prepare a 1-µM solution by mixing 82.4 µL of the *Standard stock solution* with 4.92 mL of *Vehicle*.

Sample stock solution: Prepare a 1-mg/mL solution of Teriparatide in *Vehicle*.

Sample solution: Prepare a 1-µM solution by mixing 20 µL of the *Sample stock solution* with 4.98 mL of *Vehicle*. [NOTE—Following the preparation of the *Standard solution* and *Sample solution*, the diluting and delivery of the samples to the cells must occur within 45 min. Dilutions must be made in borosilicate glass tubes. Allow all solutions to equilibrate to room temperature prior to use.]

Preparation of diluted standard solutions and sample solutions: Prepare three separate dilution sets from the *Standard solution* and *Sample solution* in borosilicate glass tubes at various concentrations (e.g., 3.0, 1.0, 0.333, 0.167, 0.0833, 0.0417, 0.0208, 0.0069, and 0.0023 nM) using *Diluent A*. [NOTE—Only a single standard and a single sample (three separate dilution sets for each) should be prepared and run for each assay plate. For each assay plate, a freshly prepared standard and sample must be used. Each assay consists of at least three independent runs (or three assay plates).]

Analysis: Following cell serum starvation, wash cells at least twice with 300 µL per well of HBSS without phenol red[4] at room temperature. Place 100 µL per well of each dilution prepared from the *Standard solution* and *Sample solution* into appropriate wells of the plate. [NOTE—See *Design and Development of Biological Assays* ⟨1032⟩ for helpful information on randomization of samples and plate layout.] Incubate the plates at 25 ± 2° for 20 ± 5 min with

[1] ThermoFisher catalog number 12430054 or suitable equivalent.
[2] GE Healthcare Life Sciences catalog number SH30070.03HI or suitable equivalent.
[3] American Type Culture Collection, catalog number CRL-1661.
[4] ThermoFisher catalog number 14190144 or suitable equivalent.
[5] ThermoFisher catalog number 25200056 or suitable equivalent.
[6] Corning Costar catalog number 3595 or suitable equivalent.
[7] ThermoFisher catalog number 24020117 or suitable equivalent.
[8] ThermoFisher catalog number 14025092 or suitable equivalent.
[9] ThermoFisher catalog number 4412182 or 4412183 or suitable equivalent.

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gentle shaking. Discard the solutions and wash cells twice with 300 µL per well of HBSS without phenol red at room temperature. Add 100 µL per well of *Assay/lysis solution* and incubate the plates at 37 ± 2° for 30 ± 5 min to lyse the cells. Mix cell lysate with a multi-channel pipette prior to transfer. Transfer 60 µL of cell lysate to the appropriate wells of the 96-well assay plate from the cAMP immunoassay assay kit. Add 30 µL of *Diluted cAMP-AP conjugate* to each well containing the cell lysate that is derived from the cells treated with the *Diluted standard solutions* or *Diluted sample solutions*, and mix on a plate shaker for approximately 1–2 min. Add 60 µL of anti-cAMP antibody from the cAMP kit to the wells and incubate at 25 ± 2° for 60 ± 5 min on a plate shaker with gentle shaking. Discard the solutions and wash the plates six times with 300 µL per well of wash buffer from the cAMP kit, blotting the plate between each wash. Add 100 µL of substrate/enhancer solution from the cAMP kit to each well. Mix on a plate shaker for 1–2 min. Remove the plates from the shaker and incubate the plates at room temperature (such as 20°–27°) for 40 ± 10 min. Read the plate in a suitable microtiter plate luminescence reader.

Calculations: Fit a constrained 4-parameter logistic curve to the median relative light units (RLU) at each concentration from the *Diluted standard solutions* and *Diluted sample solutions*. Calculate the relative potency of each teriparatide sample compared to the standards of each run by EC_{50}. Determine the combined weighted percent mean relative potency of the runs following *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays, Method 2*.

System suitability

Samples: *Diluted standard solutions* and *Diluted sample solutions*

Suitability requirements

Asymptote ratio: NLT 3.0 for the ratio of the upper asymptote to the lower asymptote of the 4-parameter logistic curve from each run of both the *Diluted standard solutions* and *Diluted sample solutions*

Slope: NLT 1.0 for each run

L term: NMT 0.2000 for each run. [NOTE—L term is determined by subtracting the log of the 95% lower confidence limit from the log of the 95% upper confidence limit of the relative potency.]

Combined assay L term: NMT 0.1500 [NOTE—See *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays, Method 2* for the calculation.]

Acceptance criteria: 60%–120% of the relative potency to USP Teriparatide RS on the as-is basis

ASSAY

• **PROCEDURE**

0.2 M sulfate buffer: 28.4 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.

Solution A: Acetonitrile and *0.2 M sulfate buffer* (10:90)

Solution B: Acetonitrile and *0.2 M sulfate buffer* (50:50)

Mobile phase: *Solution A* and *Solution B* (63:37). [NOTE—The *Mobile phase* composition may be adjusted to obtain the desired retention time for the teriparatide main peak.]

Diluent: Acetonitrile and *0.2 M sulfate buffer* (25:75)

Standard solutions: Prepare in triplicate 250 µg/mL of USP Teriparatide RS in *Diluent*. Stable for 72 h when stored at 2°–8° in a sealed container.

Sample solutions: Prepare in duplicate approximately 250 µg/mL of Teriparatide in *Diluent*. Stable for 72 h when stored at 2°–8° in a sealed container. [NOTE—Teriparatide should be equilibrated and weighed in a controlled humidity chamber of 25 ± 5% relative humidity, then dissolved in *Diluent*. Determine the water content within 24 h of weighing.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Autosampler: 2°–8°

Column: 40 ± 5°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Run time: 15 min

System suitability

Samples: *Standard solutions*

[NOTE—The retention time for teriparatide is 7.5–11.7 min.]

Suitability requirements

Tailing factor: NMT 1.5 for the teriparatide peak

Relative standard deviation: NMT 1.25% calculated from the injection of three separate *Standard solutions*

Analysis

Samples: *Standard solutions* and *Sample solutions*

Calculate the concentration of teriparatide (C_S), in µg/mL, in each of the *Standard solutions*:

$$C_S = (L_S / V_S)$$

L_S = content of teriparatide in USP Teriparatide RS (µg)

V_S = volume of *Diluent* used for the *Standard solutions* (mL)

For each injection of the *Standard solution*, calculate a response factor (F_R):

$$F_R = (r_S / C_S)$$

r_S = peak response of teriparatide from the *Standard solution*

C_S = concentration of USP Teriparatide RS in the *Standard solution* (µg/mL)

Calculate the mean response factor (F_M) for all three *Standard solutions*.

Calculate the percentage (P_U) of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$) in the portion of Teriparatide taken:

$$P_U = (r_U / F_M) \times [V_U / (W \times F)] \times 100$$

r_U = peak response of teriparatide from the *Sample solution*

F_M = mean response factor for all three *Standard solutions*

V_U = volume of *Diluent* used to prepare the *Sample solutions* (mL)

W = weight of Teriparatide taken to prepare the *Sample solutions* (mg)

F = conversion factor from mg to µg, 1000

Calculate the percentage of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$) corrected for water, acetate, and chloride contents:

$$\text{Result} = P_U / [(100 - \%\text{ of water} - \%\text{ of acetate} - \%\text{ of chloride})/100]$$

Acceptance criteria: 95.0%–105.0% on the anhydrous, acetic acid-free, and chloride-free basis

OTHER COMPONENTS

• **ACETATE CONTENT**

Mobile phase: 0.01 N sulfuric acid in water

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Standard solution 1: 0.072 mg/mL of acetate in *Mobile phase* prepared as follows. Weigh approximately 100 mg of anhydrous sodium acetate, place in a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Dilute 1 mL of this solution with *Mobile phase* to 10.0 mL. Stable for 72 h when stored at 2°–8°.

Standard solution 2: 0.144 mg/mL of acetate in *Mobile phase* prepared as follows. Weigh approximately 200 mg of anhydrous sodium acetate, place in a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Dilute 1 mL of this solution with *Mobile phase* to 10.0 mL. Stable for 72 h when stored at 2°–8°.

Standard solution 3: 0.216 mg/mL of acetate in *Mobile phase* prepared as follows. Weigh approximately 300 mg of anhydrous sodium acetate, place in a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Dilute 1 mL of this solution with *Mobile phase* to 10.0 mL. Stable for 72 h when stored at 2°–8°.

Sample solution: Under controlled relative humidity of 25 ± 5%, prepare in duplicate approximately 5 mg/mL of Teriparatide in *Mobile phase*. Stable for 72 h when stored at 2°–8°.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 9.0-mm × 25-cm; packing L22
Temperatures
Autosampler: 2°–8°
Column: Ambient
Flow rate: 1.0 mL/min
Injection volume: 100 µL
Run time: 12–15 min

System suitability
Samples: *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*
[NOTE—The retention time of acetate is 9.2–11.6 min.]
Suitability requirements
Tailing factor: NMT 1.5, *Standard solution 2*
Relative standard deviation: NMT 1.25% from triplicate injections, *Standard solution 2*
Percentage of deviation: NMT 2.0 for each concentration from triplicate injections, *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

$$\text{Result} = \{[(r_S - b)/a]/C_S\} \times 100$$

r_S = peak response of acetate from *Standard solution 1*, *Standard solution 2*, or *Standard solution 3*
b = *y*-intercept of the calibration curve described in the *Analysis*
a = slope of the calibration curve described in the *Analysis*
C_S = concentration of acetate in *Standard solution 1*, *Standard solution 2*, or *Standard solution 3* (mg/mL)

Analysis
Samples: *Standard solutions* and *Sample solution*
Determine the peak response of acetate.
Calculate the concentration of acetate (C_S), in mg/mL, in the *Standard solutions*:

$$C_S = (W_S/V_S) \times F \times D$$

W_S = weight of anhydrous sodium acetate (corrected for purity) (mg)
V_S = initial volume of *Mobile phase*, 100 mL
F = ratio of molecular weight of acetate (59.04 g/mol) to molecular weight of anhydrous sodium acetate (82.03 g/mol), 0.7197
D = dilution factor from 1 mL to 10 mL, 0.1

Construct a least-squares calibration curve, using the peak area of the *Standard solutions* versus their acetate concentrations (mg/mL). Determine the concentration of acetate (C_U), in mg/mL, in the *Sample solution*, using the equation of a line for the calibration curve.
Calculate the percentage of acetate in the portion of Teriparatide taken:

$$\text{Result} = C_U \times (V_U/W_U) \times 100$$

C_U = concentration of acetate in the *Sample solution* (mg/mL)
V_U = volume of the *Sample solution* (mL)
W_U = weight of Teriparatide taken (mg)

Acceptance criteria: NMT 5.0%

• **CHLORIDE CONTENT**
Mobile phase: 1.7 mM sodium bicarbonate and 1.8 mM sodium carbonate in water
Standard stock solution: Dry a sufficient quantity of sodium chloride at 105° for approximately 30 min. Weigh about 165.9 mg of previously dried sodium chloride, and place in a 100-mL volumetric flask. Dilute with water to volume. Mix until completely dissolved. This solution contains the equivalent of 1000 µg/mL of chloride.
System suitability solution: Weigh approximately 150 mg of sodium nitrite, and place in a 100-mL volumetric flask. Dilute with water to volume. Transfer 1.0 mL of this solution to a 100-mL volumetric flask. Transfer 2.5 mL of the *Standard stock solution* to the same flask. Dilute with water to volume, and mix well.
Standard solutions: Transfer 1.0, 2.0, 3.0, and 4.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. Dilute with water to volume, and mix well.
Sample solution: Under controlled relative humidity of 25 ± 5%, prepare in duplicate 1.0 mg/mL of Teriparatide in water. Stable for 72 h when stored at ambient temperature.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with anion suppressor current at 100 mA
Columns
Guard: 4.0-mm × 5.0-cm; packing L94
Analytical: 4.0-mm × 25-cm; 13-µm packing L94
Column temperature: Ambient
Flow rate: 2 mL/min
Injection volume: 50 µL
Run time: 10 min

System suitability
Sample: *System suitability solution*
[NOTE—Chloride elutes earlier than nitrite.]
Suitability requirements
Resolution: NLT 1.5 between chloride and nitrite peaks
Tailing factor: <2.0 for chloride and nitrite peaks
Relative standard deviation of standard curve: <3.0%
Relative standard deviation: <2.0% for chloride and nitrite peaks from five replicate injections

Analysis
Samples: *Standard solutions* and *Sample solution*
Inject the *Standard solutions* followed by the *Sample solution*.
Calculate the concentration of chloride (C_S), in µg/mL, in the *Standard stock solution*:

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$$C_S = (W_S/V_S) \times F \times D$$

W_S = weight of sodium chloride (mg)
V_S = volume of water, 100 mL
F = ratio of molecular weight of chloride (35.46 g/mol) to molecular weight of sodium chloride (58.45 g/mol), 0.607
D = conversion factor from mg to µg, 1000

Determine the concentrations of chloride, in µg/mL, in the *Standard solutions*.
Construct a least-squares calibration curve, using the peak area of the *Standard solutions* versus their chloride concentrations (µg/mL). Determine the concentration of chloride (C_U), in µg/mL, in the *Sample solution*, using the equation of a line for the calibration curve.
Calculate the percentage of chloride in the portion of Teriparatide taken:

$$\text{Result} = [(C_U \times V_U \times F)/W_U] \times 100$$

C_U = concentration of chloride in the *Sample solution* (µg/mL)
V_U = volume of the *Sample solution* (mL)
F = conversion factor from µg to mg, 0.001
W_U = weight of Teriparatide (mg)

Acceptance criteria: NMT 4.0%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PRODUCT-RELATED IMPURITIES**

0.2 M sulfate buffer: 28.4 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.
Solution A: Acetonitrile and *0.2 M sulfate buffer* (10:90)
Solution B: Acetonitrile and *0.2 M sulfate buffer* (50:50)
[NOTE—If the sodium sulfate precipitates, gentle heating and continuous stirring may be required. The sodium sulfate should not re-precipitate if this procedure is followed.]
Mobile phase: See *Table 2*. [NOTE—The *Mobile phase* composition may be adjusted to obtain the desired retention time of the teriparatide peak. The *Solution B* percentage at 5 and 35 min should be changed to alter the retention time, but the same gradient slope should be maintained.]

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	65	35
35	60	40
45	0	100
45.1	100	0
55	100	0

System suitability solution: Use an appropriate solution containing approximately 0.8% of the first post-main peak in *Solution A*. [NOTE—Teriparatide containing the first post-main peak may be prepared by dissolving Teriparatide in water to obtain a concentration of 2 mg/mL. Adjust with hydrochloric acid to a pH of 3.0. Incubate this solution at 50° for 9 days. The solution may be aliquoted and stored frozen. Dilute 1:1 with *Solution A* prior to injection. The first post-main peak is a degradation product resulting from this process and elutes immediately after the teriparatide peak. The relative retention times for teriparatide and this first post-main peak are 1.00 and 1.05, respectively.]
Sample solution: Approximately 0.7 mg/mL of Teriparatide in *Solution A*
Blank: *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Autosampler: 5°
Column: 40°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The retention time for teriparatide is 19.8–24.8 min.]
Suitability requirements
Peak-to-valley ratio: The ratio of the height of the first post-main peak to the valley between the teriparatide peak and the first post-main peak is NLT 1.5.
Tailing factor: NMT 2.0 for the teriparatide peak
Analysis
Sample: *Sample solution*
Measure the peak responses for all integrated peaks.
Calculate the percentage of methionyl sulfoxides of teriparatide in the portion of Teriparatide taken:

$$\text{Result} = [(r_{Met+O(8)} + r_{Met+O(18)} + r_{Met+O(8,18)})/r_T] \times 100$$

$r_{Met+O(8)}$ = peak response of *Met+O(8)* teriparatide (oxidized on Met 8)
$r_{Met+O(18)}$ = peak response of *Met+O(18)* teriparatide (oxidized on Met 18)
$r_{Met+O(8,18)}$ = peak response of *Met+O(8,18)* teriparatide (oxidized on Met 8 and Met 18)
r_T = sum of all the peak responses

Calculate the percentage of the largest other related impurity of teriparatide in the portion of Teriparatide taken:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = peak response of the largest other related impurity of teriparatide
r_T = sum of all the peak responses

Calculate the total percentage of teriparatide related impurities in the portion of Teriparatide taken:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = sum of the peak responses of the teriparatide related impurities
r_T = sum of all the peak responses

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Met+O(8,18) teriparatide	0.38	—
Met+O(8) teriparatide	0.48	—
Met+O(18) teriparatide	0.58	—

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Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Teriparatide	1.0	—
Total of methionyl sulfoxides of teriparatide [consisting of *Met+O(8)* teriparatide, *Met+O(18)* teriparatide, and *Met+O(8,18)*]	—	0.5
Largest other individual related impurities	—	0.5
Total impurities	—	2.5

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 50 USP Endotoxin Units/mg of Teriparatide drug substance
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 100 cfu/g of Teriparatide drug substance.
- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*: NMT 10.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Unless otherwise prescribed, store in an airtight container, protected from light, at a temperature lower than –10°.
- **LABELING:** Label it to indicate that the material has been produced by methods based on recombinant DNA technology.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Teriparatide RS

Teriparatide Injection

DEFINITION

Teriparatide Injection is a sterile solution of Teriparatide in Water for Injection. It contains NLT 90.0% and NMT 105.0% of the labeled amount of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$). The formulation may contain a suitable preservative.

IDENTIFICATION

- **A.** The ratio of the retention time of the teriparatide peak of the *Sample solution* to that of the *Standard solution* is 1.00 ± 0.03, as obtained in the *Assay*.
- **B. BIOIDENTITY**

Basic medium: Sterile Dulbecco's modified Eagle's medium (DMEM) containing high glucose, L-glutamine, pyridoxine hydrochloride, and 25 mM HEPES[1]

Growth medium: 10% fetal bovine serum (FBS) in *Basic medium* prepared as follows. To 450 mL of *Basic medium*, add 50 mL of heat inactivated irradiated FBS[2] and mix. Sterilize the solution by filtering the solution using a 0.22-µm, low-protein-binding, sterile filter unit and store at 2°–8°.

Serum starve medium: 0.1% (w/v) bovine serum albumin (BSA)-fraction V in *Basic medium* prepared as follows. Add 0.50 g of BSA-fraction V to 500 mL of *Basic medium*, and mix. Sterilize the solution by filtering the solution using a 0.22-µm, low-protein-binding, sterile filter unit and store at 2°–8°.

Vehicle: 150 mM sodium chloride, 0.1% (w/v) BSA-fraction V, and 0.001 N hydrochloric acid prepared as follows. Dissolve 1.75 g of sodium chloride and 0.2 g of BSA-fraction V in 180 mL of water. Add 20.0 mL of 0.01 N hydrochloric acid to the solution. Sterilize the solution by filtering the solution using a 0.22-µm, low-protein-binding, sterile filter unit and store at 2°–8°.

600 mM IBMX solution: Add 0.30 g of 3-isobutyl-1-methyl-xanthine (IBMX) to 2.25 mL of dimethyl sulfoxide (DMSO) and vortex to dissolve. Aliquot and store at –18° to –24°.

Cell culture preparation: Culture UMR-106 rat osteogenic sarcoma cell line[3] in *Growth medium* at 37 ± 2° and 10 ± 2% carbon dioxide (CO_2) atmosphere in a humidified incubator. Cells should be passaged when the cultures are approximately 65%–85% confluent as determined microscopically at an appropriate magnification (such as 200–400×). [NOTE—Do not allow the cells to go beyond 85% confluence for cell passage or analysis.] For cell passage, remove the media from the cells. Rinse the cells once with sterile Dulbecco's phosphate buffer saline (DPBS) without calcium and magnesium.[4] Rinse the cells with approximately 5–10 mL of 0.25% (w/v) trypsin in 1 mM EDTA solution,[5] and immediately remove all but approximately 1–2 mL of the 0.25% (w/v) trypsin with 1 mM EDTA solution from the cells. Allow the 0.25% (w/v) trypsin with 1 mM EDTA solution to remain on the cells for 1–2 min at a temperature ranging from room temperature to 37° until the cells begin to round and release from the culture surface. Resuspend cells in an appropriate volume of *Growth medium* and count. Cells are passaged into a T162 cm^2 flask with approximately 30 mL of *Growth medium* at 0.75–6 × 10^6 cells per flask.

Preparation of cells for analysis: Use cells that are between passages 4 and 20, 65%–85% confluent, and 2–5 days post-passage. Following the procedure described in *Cell culture preparation*, prepare an appropriate volume of cell solution at 0.75 × 10^5 viable cells per mL in *Growth medium*. To 96-well flat-bottom plates,[6] add 200 µL of cell solution per well. Mix cell solution frequently during dispensing to prevent cells from settling and to ensure consistent density throughout the plate. Incubate plates for 18–26 h at 37 ± 2° and 10 ± 2% carbon dioxide (CO_2). Following the incubation, remove media from the cells and add 200 µL of *Serum starve medium* to each well. Incubate plates for 18–26 h at 37 ± 2° and 10 ± 2% carbon dioxide (CO_2).

Diluent A: 1 mM IBMX in Hanks' balanced salt solution (HBSS) with phenol red[7] prepared as follows. Very slowly add 500 µL of warmed (30°–40°) *600 mM IBMX solution* to 300 mL of warmed HBSS with phenol red while continuously mixing on a stir plate. [NOTE—It is acceptable to substitute HBSS containing phenol red with HBSS without phenol red.[8] HBSS containing phenol red is preferred because it is easier to visualize the wells.]

Assay/lysis solution: 0.55 mM IBMX in assay/lysis buffer from a suitable cAMP immunoassay kit for 96-well plates[9] prepared as follows. Very slowly add 27.5 µL of warmed *600 mM IBMX solution* to 30 mL of warmed assay/lysis buffer.

[1] ThermoFisher catalog number 12430054 or suitable equivalent.
[2] GE Healthcare Life Sciences catalog number SH30070.03HI or suitable equivalent.
[3] American Type Culture Collection, catalog number CRL-1661.
[4] ThermoFisher catalog number 14190144 or suitable equivalent.
[5] ThermoFisher catalog number 25200056 or suitable equivalent.
[6] Corning Costar catalog number 3595 or suitable equivalent.
[7] ThermoFisher catalog number 24020117 or suitable equivalent.
[8] ThermoFisher catalog number 14025092 or suitable equivalent.
[9] ThermoFisher catalog number 4412182 or 4412183 or suitable equivalent.

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Diluted cAMP-AP conjugate: Dilute the cAMP-alkaline phosphatase (AP) conjugate (1:100) with the conjugate dilution buffer from the same cAMP kit used for the *Assay/lysis solution*.[9] Prepare 2.5 mL of diluted conjugate per 96-well plate. Use within 4 h.

Standard stock solution: Dissolve the contents of 1 vial of USP Teriparatide RS in an appropriate volume of *Vehicle* to obtain a 250-µg/mL solution.

Standard solution: Prepare a 1-µM solution by mixing 82.4 µL of the *Standard stock solution* with 4.92 mL of *Vehicle*.

Sample solution: Prepare a 1-µM solution by diluting the Injection with *Vehicle*.

[NOTE—Following the preparation of the *Standard solution* and *Sample solution*, the diluting and delivery of the samples to the cells must occur within 45 min. Dilutions must be made in borosilicate glass tubes. Allow all solutions to equilibrate to room temperature prior to use.]

Preparation of diluted standard solutions and sample solutions: Prepare three separate dilution sets from the *Standard solution* and *Sample solution* in borosilicate glass tubes at various concentrations (e.g., 3.0, 1.0, 0.333, 0.167, 0.0833, 0.0417, 0.0208, 0.0069, and 0.0023 nM) using *Diluent A*. [NOTE—Only a single standard and a single sample (three separate dilution sets for each) should be prepared and run for each assay plate. For each assay plate, a freshly prepared standard and sample must be used. Each assay consists of at least three independent runs (or three assay plates).]

Analysis: Following cell serum starvation, wash cells at least twice with 300 µL per well of HBSS without phenol red[4] at room temperature. Place 100 µL per well of each dilution prepared from the *Standard solution* and *Sample solution* into appropriate wells of the plate. [NOTE—See *Design and Development of Biological Assays* ⟨1032⟩ for helpful information on randomization of samples and plate layout.] Incubate the plates at 25 ± 2° for 20 ± 5 min with gentle shaking. Discard the solutions and wash cells twice with 300 µL per well of HBSS without phenol red at room temperature. Add 100 µL per well of *Assay/lysis solution* and incubate the plates at 37 ± 2° for 30 ± 5 min to lyse the cells. Mix cell lysate with a multi-channel pipette prior to transfer. Transfer 60 µL of cell lysate to the appropriate wells of the 96-well assay plate from the cAMP immunoassay assay kit. Add 30 µL of *Diluted cAMP-AP conjugate* to each well containing the cell lysate that is derived from the cells treated with the *Diluted standard solutions* or *Diluted sample solutions*, and mix on a plate shaker for approximately 1–2 min. Add 60 µL of anti-cAMP antibody from the cAMP kit to the wells and incubate at 25 ± 2° for 60 ± 5 min on a plate shaker with gentle shaking. Discard the solutions and wash the plates six times with 300 µL per well of wash buffer from the cAMP kit, blotting the plate between each wash. Add 100 µL of substrate/enhancer solution from the cAMP kit to each well. Mix on a plate shaker for 1–2 min. Remove the plates from the shaker and incubate the plates at room temperature (such as 20°–27°) for 40 ± 10 min. Read the plate in a suitable microtiter plate luminescence reader.

Calculations: Fit a constrained 4-parameter logistic curve to the median relative light units (RLU) at each concentration from the *Diluted standard solutions* and *Diluted sample solutions*. Calculate the relative potency of each teriparatide sample compared to the standards of each run by EC_{50}. Determine the combined weighted percent mean relative potency of the runs following *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays, Method 2*.

System suitability

Samples: *Diluted standard solutions* and *Diluted sample solutions*

Suitability requirements

Asymptote ratio: NLT 3.0 for the ratio of the upper asymptote to the lower asymptote of the 4-parameter logistic curve from each run of both the *Diluted standard solutions* and *Diluted sample solutions*

Slope: NLT 1.0 for each run

L term: NMT 0.2000 for each run. [NOTE—L term is determined by subtracting the log of the 95% lower confidence limit from the log of the 95% upper confidence limit of the relative potency.]

Combined assay L term: NMT 0.1500

[NOTE—See *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays, Method 2* for the calculation.]

Acceptance criteria: 75%–125% of the relative potency to USP Teriparatide RS on the as-is basis

ASSAY

• **PROCEDURE**

0.2 M sulfate buffer: 28.4 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.

Solution A: Acetonitrile and *0.2 M sulfate buffer* (10:90)

Solution B: Acetonitrile and *0.2 M sulfate buffer* (50:50)

Mobile phase: *Solution A* and *Solution B* (61:39). [NOTE—The *Mobile phase* composition may be adjusted to obtain the retention time of approximately 8 min for the teriparatide main peak.]

Diluent for standard solution: Acetonitrile and *0.2 M sulfate buffer* (25:75)

0.27 M sulfate buffer: 38.8 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.

Diluent for sample solution: Acetonitrile and *0.27 M sulfate buffer* (31:69)

Standard solutions: Prepare in triplicate 100 µg/mL of USP Teriparatide RS in *Diluent for standard solution*. *Standard solutions* are stable for 48 h when stored at 2°–8° in a sealed container.

Sample solutions: Prepare in duplicate approximately 50–100 µg/mL of teriparatide in *Diluent for sample solution*. *Sample solutions* are stable for 48 h when stored at 2°–8° in a sealed container.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Autosampler: 5°

Column: 40°

Flow rate: 0.8 mL/min

Injection volume: 25 µL

Run time: 20 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the teriparatide peak

Relative standard deviation: NMT 1.25% calculated from three injections of one *Standard solution*

Analysis

Samples: *Standard solutions* and *Sample solutions*

Measure the peak responses corresponding to teriparatide. Calculate the concentration of teriparatide (C_S), in µg/mL, in each of the *Standard solutions*:

$$C_S = (L_S/V_S)$$

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L_S = content of teriparatide in USP Teriparatide RS (µg)
V_S = volume of *Diluent for standard solution* used for each *Standard solution* (mL)

Determine the average concentration of teriparatide (C_M) for all three *Standard solutions*.
Calculate the mean response factor (F_M) for all three *Standard solutions*:

$$F_M = (r_M/C_M)$$

r_M = average peak response of teriparatide from the *Standard solutions*
C_M = average concentration of teriparatide in the *Standard solutions* (µg/mL)

Calculate the concentration of teriparatide (C_U), in µg/mL, in the portion of Injection taken:

$$C_U = (r_U \times F)/F_M$$

r_U = peak response of teriparatide from the *Sample solution*
F = dilution factor used to prepare the *Sample solution*
F_M = mean response factor for all three *Standard solutions*

Calculate the percentage of the labeled amount of teriparatide in the portion of Injection taken:

$$\text{Result} = (C_U/C_L) \times 100$$

C_U = concentration of teriparatide in the portion of Injection taken (µg/mL)
C_L = nominal concentration of teriparatide in the portion of Injection taken (µg/mL)

Acceptance criteria: 90.0%–105.0%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PRODUCT-RELATED IMPURITIES**

0.2 M sulfate buffer: 28.4 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.
Solution A: Acetonitrile and *0.2 M sulfate buffer* (10:90)
Solution B: Acetonitrile and *0.2 M sulfate buffer* (50:50)
[NOTE—If the sodium sulfate precipitates, gentle heating and continuous stirring may be required. The sodium sulfate should not re-precipitate if this procedure is followed.]
Mobile phase: See *Table 1*. [NOTE—The *Mobile phase* composition may be adjusted to obtain the desired retention time of the teriparatide peak. *Solution B* percentage at 8 min and 68 min may also be changed, if necessary, to obtain the desired retention time, but the same gradient slopes should be maintained. A change of 0.5% of *Solution B* will alter the retention time of the main peak approximately 100 s.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
8	76	24
68	60	40
75	0	100
80	0	100

System suitability solution: Use an appropriate solution containing approximately 0.8% of the first post-main peak in *Solution A*. [NOTE—Teriparatide containing the first post-main peak may be prepared by dissolving teriparatide in water to obtain a concentration of 2 mg/mL. Adjust with hydrochloric acid to a pH of 3.0. Incubate this solution at 50° for 9 days. The solution may be aliquoted and stored frozen. Dilute 1:3 with *Solution A* to approximately the same concentration as the Injection portion prior to injection. The first post-main peak is a degradation product resulting from this process and elutes immediately after the teriparatide peak. The relative retention times for teriparatide and this first post-main peak are 1.00 and 1.02, respectively.]
Sample solution: Use the solution from an undiluted Injection container.
Blank: *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Autosampler: 5°
Column: 40°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The retention time for teriparatide is 60.83–66.67 min.]
Suitability requirements
Peak-to-valley ratio: The ratio of the height of the first post-main peak to the valley between the teriparatide peak and the first post-main peak is NLT 1.5.
Tailing factor: NMT 2.0 for the teriparatide peak
Analysis
Sample: *Sample solution*
Measure the peak responses for all integrated peaks.
Calculate the percentage of a related impurity, rhPTH (1–30) (a cleavage product of teriparatide at Asn 30), in the portion of Injection taken:

$$\text{Result} = (r_{rhPTH(1-30)}/r_T) \times 100$$

$r_{rhPTH(1-30)}$ = peak response of rhPTH(1–30)
r_T = sum of all the peak responses excluding peaks due to added preservatives or excipients

Calculate the percentage of a related impurity, teriparatide succinimide (30) (formation of succinimide on Asn 30), in the portion of Injection taken:

$$\text{Result} = (r_{Suc}/r_T) \times 100$$

r_{Suc} = peak response of teriparatide succinimide (30)
r_T = sum of all the peak responses excluding peaks due to added preservatives or excipients

Calculate the percentage of the largest other related impurity of teriparatide in the portion of Injection taken:

$$\text{Result} = (r_i/r_T) \times 100$$

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r_i = peak response of the largest other related impurity of teriparatide
r_T = sum of all the peak responses excluding peaks due to added preservatives or excipients

Calculate the percentage of total related impurities in the portion of Injection taken:

$$\text{Result} = [(r_T - r_S)/r_T] \times 100$$

r_T = sum of all the peak responses excluding peaks due to added preservatives or excipients
r_S = peak response of teriparatide

Acceptance criteria: See *Table 2*.

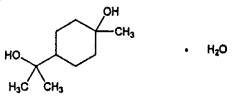
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
rhPTH (1–30)	0.77–0.78	1.2
Teriparatide succinimide (30)	0.98–0.99	1.2
Teriparatide	1.0	—
Largest other individual related impurity	—	1.0
Total impurities	—	7.0

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.8–4.5
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 100 USP Endotoxin Units/mg of teriparatide drug product
- **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Unless otherwise prescribed, store in a sterile, airtight, tamper-proof container, protected from light, at a temperature of 2°–8°. The Injection is not to be frozen.
- **Labeling:** Label it to indicate that the material has been produced by methods based on recombinant DNA technology.
- **USP Reference Standards** ⟨11⟩
  USP Endotoxin RS
  USP Teriparatide RS

Terpin Hydrate



$C_{10}H_{20}O_2 \cdot H_2O$ 190.28
$C_{10}H_{20}O_2$ 172.27
Cyclohexanemethanol, 4-hydroxy-α,α,4-trimethyl-, monohydrate;
p-Menthane-1,8-diol monohydrate [2451-01-6].
Anhydrous [80-53-5].

DEFINITION
Terpin Hydrate contains NLT 98.0% and NMT 100.5% of terpin ($C_{10}H_{20}O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Dried over a suitable desiccant for 4 h
  Acceptance criteria: Meets the requirements
- **B.**
  Analysis: Add a few drops of sulfuric acid to a hot solution.
  Acceptance criteria: The liquid becomes turbid and develops a strong aromatic odor.

ASSAY
- **Procedure**
  Internal standard solution: 20 mg/mL of biphenyl in chloroform
  Standard solution: Transfer 170 mg of USP Terpin Hydrate RS to a 100-mL volumetric flask, dissolve in 5 mL of alcohol, add 5.00 mL of *Internal standard solution*, and dilute with chloroform to volume.
  Sample solution: Transfer 170 mg of Terpin Hydrate to a 100-mL volumetric flask, dissolve in 5 mL of alcohol, add 5.00 mL of *Internal standard solution*, and dilute with chloroform to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: GC
  Detector: Flame ionization
  Column: 3.5-mm × 1.2-m glass; packed with 6% G1 on support S1A
  Temperatures
  Injection port: 260°
  Detector: 260°
  Column: 120°
  Carrier gas: Nitrogen
  Flow rate: Necessary to yield approximate retention times of 7 min for terpin and 11 min for biphenyl
  Injection volume: 1 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 2.0 between the terpin and biphenyl peaks
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of terpin ($C_{10}H_{20}O_2$) in the portion of Terpin Hydrate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of terpin to biphenyl from the *Sample solution*
R_S = peak response ratio of terpin to biphenyl from the *Standard solution*
C_S = concentration of USP Terpin Hydrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Terpin Hydrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–100.5% on the anhydrous basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS
- **Water Determination,** *Method I* ⟨921⟩: 9.0%–10.0%
- **Residual Turpentine:** No odor of turpentine oil

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Terpin Hydrate RS

Terpin Hydrate Oral Solution

DEFINITION
Terpin Hydrate Oral Solution contains, in each 100 mL, NLT 1.53 g and NMT 1.87 g of terpin hydrate ($C_{10}H_{20}O_2 \cdot H_2O$).

ASSAY
- **PROCEDURE**

Internal standard solution: 20 mg/mL of biphenyl in chloroform

Standard solution: Transfer 170 mg of USP Terpin Hydrate RS to a 100-mL volumetric flask, dissolve in 5 mL of alcohol, add 5.0 mL of *Internal standard solution*, and dilute with chloroform to volume.

Sample solution: Pipet 10 mL of Oral Solution into a separator. Add 20 mL of water and 10 mL of 5 N sodium hydroxide, and extract with three 25-mL portions of chloroform, filtering each, successively, through cotton. Rinse the cotton with chloroform. To the combined rinse and extracts add 5.0 mL of *Internal standard solution*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 3.5-mm × 1.2-m glass; packed with 6% G1 on support S1A
Temperatures
Injection port: 260°
Detector: 260°
Column: 120°
Carrier gas: Nitrogen
Flow rate: Necessary to yield approximate retention times of 7 min for terpin and 11 min for biphenyl
Injection volume: 1 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the terpin and biphenyl peaks
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of terpin hydrate ($C_{10}H_{20}O_2 \cdot H_2O$) in 100 mL of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times W_S \times V$$

R_U = peak response ratio of terpin to biphenyl from the *Sample solution*
R_S = peak response ratio of terpin to biphenyl from the *Standard solution*
W_S = weight of USP Terpin Hydrate RS, calculated on the anhydrous basis (g)
V = volume of sample, 10 mL

Acceptance criteria: 1.53–1.87 g

SPECIFIC TESTS
- **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩ : 90.0%–110.0% of the labeled amount of C_2H_5OH

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Terpin Hydrate RS

Terpin Hydrate and Codeine Oral Solution

» Terpin Hydrate and Codeine Oral Solution contains, in each 100 mL, not less than 1.53 g and not more than 1.87 g of terpin hydrate ($C_{10}H_{20}O_2 \cdot H_2O$), and not less than 180 mg and not more than 220 mg of codeine ($C_{18}H_{21}NO_3 \cdot H_2O$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Terpin Hydrate RS
USP Codeine Phosphate RS

Identification—
Developing solvent—Prepare a mixture of methylene chloride and methanol (9:1).
Standard solution A—Dissolve a suitable quantity of USP Terpin Hydrate RS in methylene chloride to obtain a solution having a concentration of about 3 mg per mL. [NOTE—A small volume of methanol may be used to aid dissolution of the terpin hydrate.]
Standard solution B—Transfer 20 mg of USP Codeine Phosphate RS to a suitable separator containing 10 mL of water, add 1 mL of 1 N sodium hydroxide, and mix. Add 10 mL of methylene chloride, and shake for 1 minute. Allow the layers to separate, and drain the lower organic layer into a suitable flask. Discard the aqueous layer.
Test solution—Transfer 10 mL of Oral Solution to a suitable separator containing 10 mL of water, and add 1 mL of 1 N sodium hydroxide. Add 10 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear lower organic layer as the *Test solution*.
Procedure—Apply separately 5 µL of *Standard solution A*, *Standard solution B*, and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a chromatographic chamber containing the *Developing solvent* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and allow the plate to dry. Examine the plate under short-wavelength UV light, and mark the location of the codeine spots. Spray the plate with phosphomolybdic acid TS, and heat at 105° for 5 minutes. The terpin hydrate spots appear blue on a yellow background. The R_F values of the spots due to terpin hydrate and codeine obtained from the *Test solution* correspond to those obtained from *Standard solutions A* and *B*, respectively.
Alcohol Determination, *Method II* ⟨611⟩: between 90.0% and 110.0% of the labeled amount of C_2H_5OH.

Assay for terpin hydrate—
Internal standard solution—Prepare a chloroform solution containing 20 mg of biphenyl and 2.6 mg of *N*-phenylcarbazole in each mL.
Standard preparation—Transfer about 26 mg of USP Codeine Phosphate RS and about 170 mg of USP Terpin Hydrate RS, both accurately weighed, to a separator, add 5 mL of alcohol, shake to dissolve the terpin hydrate, add 25 mL of water to dissolve the codeine phosphate, add 10 mL of 5 N sodium hydroxide, and extract with three 25-mL portions of

USP Monographs

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chloroform, filtering each, successively, through cotton. Rinse the cotton with chloroform. To the combined rinse and extracts add 5.00 mL of *Internal standard solution*, and mix.

Assay preparation—Pipet 10 mL of Oral Solution into a separator, add 20 mL of water and 10 mL of 5 N sodium hydroxide, and extract with three 25-mL portions of chloroform, filtering each, successively, through cotton. Rinse the cotton with chloroform. To the combined rinse and extracts add 5.00 mL of *Internal standard solution*, and mix.

Chromatographic system and *System suitability*—Proceed as directed in the *Assay* under *Terpin Hydrate*. [NOTE—Heat the column to 230° to remove the *N*-phenylcarbazole and codeine from prior injections.]

Procedure—Inject about 1 µL of the *Standard preparation* into a suitable gas chromatograph, and record the chromatogram. Similarly, inject about 1 µL of the *Assay preparation*, and record the chromatogram. Calculate the quantity, in mg, of terpin hydrate ($C_{10}H_{20}O_2 \cdot H_2O$) in each mL of the Oral Solution taken by the formula:

$$0.1(190.28/172.27)W_S(R_U/R_S)$$

in which 190.28 and 172.27 are the molecular weights of terpin hydrate ($C_{10}H_{20}O_2 \cdot H_2O$) and anhydrous terpin ($C_{10}H_{20}O_2$); respectively, W_S is the weight, in mg, of USP Terpin Hydrate RS, calculated on the anhydrous basis; and R_U and R_S are the area-ratios of terpin to biphenyl obtained from the chromatograms for the *Assay preparation* and the *Standard preparation*, respectively.

Assay for codeine—

Internal standard solution—Prepare as directed under *Assay for terpin hydrate*.

Standard preparation—Evaporate the remaining *Standard preparation* for Oral Solution from the *Assay for terpin hydrate* nearly to dryness, and dissolve the residue in about 20 mL of chloroform.

Assay preparation—Evaporate the remaining *Assay preparation* for Oral Solution from the *Assay for terpin hydrate* nearly to dryness, and dissolve the residue in about 20 mL of chloroform.

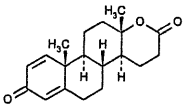
Chromatographic system and *System suitability*—Proceed as directed in the *Assay* under *Terpin Hydrate*, except to maintain the temperature of the column at 230° instead of 120°.

Procedure—Proceed as directed under *Assay for terpin hydrate*, except to maintain the temperature of the column at 230° instead of 120°. The retention times for *N*-phenylcarbazole and codeine are about 7 minutes and 10 minutes, respectively. Calculate the quantity, in mg, of codeine ($C_{18}H_{21}NO_3 \cdot H_2O$) in each mL of the Oral Solution taken by the formula:

$$0.1(317.39/397.37)W_S(R_U/R_S)$$

in which 317.39 and 397.37 are the molecular weights of codeine ($C_{18}H_{21}NO_3 \cdot H_2O$) and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4$), respectively; W_S is the weight, in mg, of USP Codeine Phosphate RS; and R_U and R_S are the area-ratios of codeine to *N*-phenylcarbazole obtained from the chromatograms for the *Assay preparation* and the *Standard preparation*, respectively.

Testolactone



$C_{19}H_{24}O_3$ 300.39

D-Homo-17a-oxaandrosta-1,4-diene-3,17-dione;
13-Hydroxy-3-oxo-13,17-secoandrosta-1,4-dien-17-oic acid δ-lactone [968-93-4].

DEFINITION

Testolactone contains NLT 95.0% and NMT 105.0% of testolactone ($C_{19}H_{24}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Solution: 10 µg/mL in methanol

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Isoniazid reagent: Dissolve 1.0 g of isoniazid in 500 mL of methanol. Add 1.25 mL of hydrochloric acid, and dilute with methanol to 1000 mL.

Standard stock solution: 30 µg/mL of USP Testolactone RS in chloroform

Standard solution: Transfer 5.0 mL of *Standard stock solution* to a 25-mL volumetric flask. Add 10.0 mL of *Isoniazid reagent*, place the flask in a water bath maintained at a temperature of 55 ± 2°, and allow to stand for 70 min. Cool, and dilute with chloroform to volume.

Sample stock solution: 30 µg/mL of Testolactone in chloroform

Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 25-mL volumetric flask. Add 10.0 mL of *Isoniazid reagent*, place the flask in a water bath maintained at a temperature of 55 ± 2°, and allow to stand for 70 min. Cool, and dilute with chloroform to volume.

Blank solution: Transfer 5.0 mL of chloroform to a 25-mL volumetric flask. Add 10.0 mL of *Isoniazid reagent*, place the flask in a water bath maintained at a temperature of 55 ± 2°, and allow to stand for 70 min. Cool, and dilute with chloroform to volume.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum absorbance of 415 nm

Cell: 1 cm

Blank: *Blank solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of testolactone ($C_{19}H_{24}O_3$) in the portion of Testolactone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Testolactone RS in the *Standard solution* (mg/mL)

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C_U = concentration of Testolactone in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Standard solution: 0.2 mg/mL of USP Testolactone RS in acetone

Sample solution: 5 mg/mL of Testolactone in acetone

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: Coat a 20- × 20-cm thin-layer chromatographic plate with a 0.25-mm layer of chromatographic silica gel mixture. Dry for 15 min at room temperature, heat at 105° for 1 h, and cool in a desiccator. Divide the area of the plate into three approximately equal sections, use the left and right sections for the *Sample solution* and the *Standard solution*, respectively, and use the center section for the blank.

Application volume: 10 µL for the *Standard solution*; 20 µL for the *Sample solution*

Developing solvent system: Butyl acetate and acetone (4:1)

Analysis: Apply the *Standard solution* and *Sample solution* 2.5 cm from the bottom of the designated sections of the plate, and dry the spots with a current of air. Develop the chromatogram in the *Developing solvent system*, until the solvent front has moved to within 1 cm of the top of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, NMT two impurities are found in the chromatogram of the *Sample solution*, and the size and color of the spot representing any impurity obtained from the *Sample solution* are not greater or more intense than those of the principal spot of the *Standard solution*.

• **ORDINARY IMPURITIES** ⟨466⟩

Solvent: Methanol

Eluant: Butyl acetate and acetone (4:1)

Visualization: 6

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 12.5 mg/mL in chloroform

Acceptance criteria: −44° to −52°

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 100° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Testolactone RS

Testolactone Tablets

DEFINITION

Testolactone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of testolactone ($C_{19}H_{24}O_3$).

IDENTIFICATION

• **A.**

Hydroxylamine reagent: Mix 5 mL of a solution of hydroxylamine hydrochloride in methanol (3.5 in 100) with 15 mL of a solution of potassium hydroxide in methanol (5.6 in 100), and use this reagent within 2 h after mixing. Store the solution of hydroxylamine hydrochloride in methanol and the solution of potassium hydroxide in methanol in a refrigerator. Discard both after 1 month.

Iron reagent: Dissolve 1.5 g of ferric chloride in 30 mL of water, add 3 mL of perchloric acid and 15 mL of nitric acid, and heat the solution until dense, white fumes are produced. Cool, add 40 mL of water and 10 mL of nitric acid, and dilute with perchloric acid to 100 mL. [NOTE—This concentrate may be stored and used for several months.] Transfer 4.0 mL of this concentrate to a 100-mL volumetric flask, add 36 mL of alcohol, and dilute with water to volume.

Analysis: Place a portion of finely powdered Tablets, equivalent to 250 mg of testolactone, in a 100-mL volumetric flask. Add 85 mL of chloroform, and shake by mechanical means for 30 min. Add chloroform to volume, and centrifuge the solution. Transfer 2 mL of the supernatant to a small test tube, and evaporate with the aid of a gentle current of air to dryness. Add 0.1 mL of *Hydroxylamine reagent*, heat in a water bath at 60° for 1 min, then add 1 mL of *Iron reagent*.

Acceptance criteria: A rose-violet color is produced.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Place a portion of finely powdered Tablets, equivalent to 100 mg of testolactone, in a glass-stoppered, 35-mL centrifuge tube. Add 20 mL of chloroform, insert the stopper, and shake by mechanical means for 10 min. Centrifuge the mixture, and filter the supernatant into a 50-mL beaker. Evaporate on a steam bath to dryness, and dry the residue under vacuum at 100° for 3 h.

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Isoniazid reagent: Dissolve 1.0 g of isoniazid in 500 mL of methanol. Add 1.25 mL of hydrochloric acid, and dilute with methanol to 1000 mL.

Standard stock solution: 30 µg/mL of USP Testolactone RS in chloroform

Standard solution: Transfer 5.0 mL of *Standard stock solution* to a 25-mL volumetric flask. Add 10.0 mL of *Isoniazid reagent*, place the flask in a water bath maintained at a temperature of 55 ± 2°, and allow to stand for 70 min. Cool, and dilute with chloroform to volume.

Sample stock solution: Nominally equivalent to 30 µg/mL of testolactone in chloroform prepared as follows. Weigh, and finely powder NLT 10 Tablets. Transfer a weighed portion of the powder, equivalent to 50 mg of testolactone, to a 100-mL volumetric flask. Add 85 mL of chloroform, and shake by mechanical means for 30 min. Add chloroform to volume, and centrifuge a portion of 10 mL of the mixture. Transfer 3.0 mL of the clear supernatant to a 50-mL volumetric flask, and dilute with chloroform to volume.

Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 25-mL volumetric flask. Add 10.0 mL of *Isoniazid reagent*, place the flask in a water bath maintained at a temperature of 55 ± 2°, and allow to stand for 70 min. Cool, and dilute with chloroform to volume.

Blank solution: Transfer 5.0 mL of chloroform to a 25-mL volumetric flask. Add 10.0 mL of *Isoniazid reagent*, place the

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flask in a water bath maintained at a temperature of 55 ± 2°, and allow to stand for 70 min. Cool, and dilute with chloroform to volume.

Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 415 nm
Cell: 1 cm
Blank: *Blank solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of testolactone ($C_{19}H_{24}O_3$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

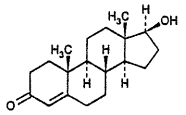
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Testolactone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of testolactone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 120 min
Analytical wavelength: UV maximum at about 247 nm
Standard solution: USP Testolactone RS in the same medium. [NOTE—An amount of methanol NMT 2% of the total volume of the *Standard solution* may be used to bring the testolactone standard into solution before dilution with water.]
Sample solution: Dilute filtered portions of the solution under test with water, if necessary, to a concentration similar to that of the *Standard solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of testolactone ($C_{19}H_{24}O_3$) dissolved by employing UV absorption.
Tolerances: NLT 80% (*Q*) of the labeled amount of testolactone ($C_{19}H_{24}O_3$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Testolactone RS

Testosterone



$C_{19}H_{28}O_2$ 288.42
Androst-4-en-3-one, 17-hydroxy-, (17β)-.
17β-Hydroxyandrost-4-en-3-one [58-22-0].

» Testosterone contains not less than 97.0 percent and not more than 103.0 percent of $C_{19}H_{28}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Testosterone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: methanol.
Melting range ⟨741⟩: between 153° and 157°.
Specific rotation ⟨781S⟩: between +101° and +105°.
Test solution: 10 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it in vacuum over phosphorus pentoxide for 4 hours: it loses not more than 1.0% of its weight.

Assay—
Standard preparation—Prepare as directed under *Single-Steroid Assay* ⟨511⟩, using USP Testosterone RS.
Assay preparation—Accurately weigh about 20 mg of Testosterone, previously dried; dissolve in a sufficient quantity of a mixture of equal volumes of alcohol and chloroform to make 10.0 mL; and mix.
Procedure—Proceed as directed for *Procedure* under *Single-Steroid Assay* ⟨511⟩, using a solvent system consisting of a mixture of benzene and ethyl acetate (1:1), through the fourth sentence of the second paragraph under *Procedure*. Then centrifuge the tubes for 5 minutes, and determine the absorbances of the supernatant in 1-cm cells at the wavelength of maximum absorbance at about 241 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{19}H_{28}O_2$ in the portion of Testosterone taken by the formula:

$$10C(A_U/A_S)$$

in which *C* is the concentration, in mg per mL, of USP Testosterone RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Testosterone Injectable Suspension

» Testosterone Injectable Suspension is a sterile suspension of Testosterone in an aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{28}O_2$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

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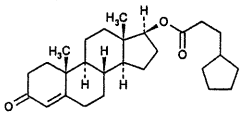
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USP Reference standards ⟨11⟩—
USP Testosterone RS
Identification—The testosterone obtained by filtration and washing, as directed in the *Assay*, and dried at 105° to constant weight, meets the requirements for *Identification* tests *A* and *B* under *Testosterone*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.5 USP Endotoxin Units per mg of testosterone.
Uniformity of dosage units ⟨905⟩: meets the requirements.
pH ⟨791⟩: between 4.0 and 7.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of previously well-mixed Injectable Suspension, equivalent to about 100 mg of testosterone, to a fine-porosity, sintered-glass filtering crucible, previously dried at 105° for 1 hour, and filter with suction. If the filtrate is not clear, again pass it through the same filter into a second receiver. Wash the residue in the filter with several 5-mL portions of water until 2 mL of the last washing, when evaporated on a steam bath, leaves a negligible residue. [NOTE—If the Injectable Suspension is passed through the filter twice, rinse the first receiver with the portions of water before passing them through the filter.] Dry the crucible and the collected testosterone at 105° for 1 hour. Completely dissolve the testosterone with five 25-mL portions of methanol, passing each portion through the crucible under gentle suction, and transfer the combined methanol solution to a 200-mL volumetric flask. Rinse the crucible and receiver with two 25-mL portions of methanol, add the rinsings to the main solution, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with methanol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Testosterone RS, in the same medium having a known concentration of about 10 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 241 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_{19}H_{28}O_2$ in each mL of the Injectable Suspension taken by the formula:

$$(10C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Testosterone RS in the Standard solution, V is the volume, in mL, of Injectable Suspension taken, and A_U and A_S are the absorbances of the solution from the Injectable Suspension and the Standard solution, respectively.

Testosterone Cypionate



$C_{27}H_{40}O_3$ 412.60
Androst-4-en-3-one, 17-(3-cyclopentyl-1-oxopropoxy)-, (17β)-.
Testosterone cyclopentanepropionate [58-20-8].

» Testosterone Cypionate contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{40}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Cholesteryl Caprylate RS
$C_{35}H_{60}O_2$ 512.86
USP Testosterone Cypionate RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
Melting range ⟨741⟩: between 98° and 104°.
Specific rotation ⟨781S⟩: between +85° and +92°.
Test solution: 20 mg per mL, in chloroform.
Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.
Free cyclopentanepropionic acid—Dissolve 500 mg in 10 mL of alcohol that previously has been neutralized to a faint blue color following the addition of 2 or 3 drops of bromothymol blue TS, and promptly titrate with 0.01 N sodium hydroxide VS: not more than 0.70 mL of 0.01 N sodium hydroxide is required (0.20% of cyclopentanepropionic acid).

Assay—
Internal standard solution—Dissolve 80 mg of USP Cholesteryl Caprylate RS in a mixture of methanol and chloroform (4:1) in a 100-mL volumetric flask, then add the same solvent mixture to volume.
Standard preparation—Weigh accurately about 10 mg of USP Testosterone Cypionate RS into a suitable vial, add by pipet 10 mL of *Internal standard solution*, and mix.
Assay preparation—Prepare as directed for *Standard preparation*, using an accurately weighed portion of about 10 mg of Testosterone Cypionate instead of the Reference Standard.
Procedure—Inject 1 µL of the *Assay preparation* and the *Standard preparation*, successively, into a suitable gas chromatograph fitted with a flame-ionization detector. Under typical conditions, the instrument contains a 3-mm × 1.2-m glass column packed with 1% (w/w) phase G6 on packing S1AB. The column temperature is maintained at 260° and the helium carrier gas flows at 50 mL per minute. In a suitable chromatogram, the resolution factor, R (see *Chromatography* ⟨621⟩), is not less than 3 between the internal standard and testosterone cypionate peaks, and five replicate injections of a single *Standard preparation* show a coefficient of variation of not more than 2% in the peak area ratio of testosterone cypionate to internal standard. Measure the areas under the peaks for testosterone cypionate and cholesteryl caprylate in each chromatogram. Calculate the ratio, R_U, of the area of the testosterone cypionate peak to the area of the internal standard peak in the chromatogram from the *Assay preparation*, and similarly calculate the ratio, R_S, in the chromatogram from the *Standard preparation*. Calculate the quantity, in mg, of $C_{27}H_{40}O_3$ in the portion of Testosterone Cypionate taken by the formula:

$$W(R_U/R_S)$$

in which W is the weight, in mg, of USP Testosterone Cypionate RS in the *Standard preparation*, and the other terms are as defined therein.

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Testosterone Cypionate Injection

» Testosterone Cypionate Injection is a sterile solution of Testosterone Cypionate in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{27}H_{40}O_3$. It may contain a suitable solubilizing agent.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Cholesteryl Caprylate RS
$C_{35}H_{60}O_2$ 512.86
USP Testosterone Cypionate RS

Identification—Dilute a suitable volume of Injection with chloroform to obtain a solution having a concentration of about 400 µg of testosterone cypionate per mL. Prepare a 20- × 20-cm thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic siliceous earth, by placing it in a developing chamber containing and equilibrated with a mixture of chloroform and corn oil (90:10), and allowing the solvent front to move about three-fourths of the length of the plate. Remove the plate, and allow the chloroform to evaporate. Apply 10 µL each of the solution under test and of a solution of USP Testosterone Cypionate RS in chloroform containing about 400 µg per mL on the plate, on a line about 2.5 cm from the bottom edge and about 1.5 cm apart. Place the plate in a developing chamber that contains and has been equilibrated with a mixture of methanol and water (90:10) previously saturated with corn oil. Develop the chromatogram until the solvent front has moved to about 10 cm above the line of application. Remove the plate, and heat in an oven at 105° for a few minutes. Spray the plate with a mixture of alcohol and sulfuric acid (3:1), and heat in an oven at 105° for 1 to 2 minutes. Observe the plate under long-wavelength UV light: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Internal standard solution and *Standard preparation*—Prepare as directed in the *Assay* under *Testosterone Cypionate.*

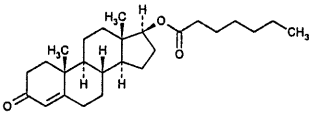
Assay preparation—Transfer 1 mL of Injection, accurately measured, into a glass-stoppered, 50-mL centrifuge tube. Add 30 mL of a mixture of methanol and water (9:1), insert the stopper, and shake for 15 minutes. Centrifuge, remove the dilute methanol layer without disturbing the oil, and transfer it to a 200-mL volumetric flask. Repeat the extraction with three additional 30-mL portions of the dilute methanol, collecting the combined extracts in the volumetric flask. Dilute the combined extracts with the dilute methanol to volume, mix, and chill the contents of the flask to –8°. Remove the flask from the freezer, and immediately filter a portion of the contents. Allow the filtrate to reach room temperature, transfer a portion of it, equivalent to about 3 mg of testosterone cypionate, to a suitable vial, and evaporate to dryness. Add by pipet 3 mL of *Internal standard solution*, and shake vigorously to dissolve the residue.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Testosterone Cypionate.* Calculate the quantity, in mg, of $C_{27}H_{40}O_3$ in the portion of Injection taken by the formula:

$$600(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Testosterone Cypionate RS in the *Standard preparation;* and V is the volume, in mL, of the filtrate used in the *Assay preparation.*

Testosterone Enanthate



$C_{26}H_{40}O_3$ 400.59
Androst-4-en-3-one, 17-(1-oxoheptyl)oxy-, (17β)-.
Testosterone heptanoate [315-37-7].

» Testosterone Enanthate contains not less than 97.0 percent and not more than 103.0 percent of $C_{26}H_{40}O_3$.

Packaging and storage—Preserve in well-closed containers, and store in a cool place.

USP Reference standards ⟨11⟩—
USP Testosterone Enanthate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: alcohol.
Absorptivities at 240 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

C: Reflux 25 mg with 2 mL of a 1 in 100 solution of potassium hydroxide in methanol for 1 hour. Cool the mixture, add 10 mL of water, filter, and wash the precipitate with water until the last washing is neutral to litmus. Dry the precipitate in vacuum at 60° for 3 hours: the testosterone so obtained melts between 151° and 157°.

Melting range ⟨741⟩: between 34° and 39°, the initial temperature of the bath not exceeding 20°.

Specific rotation ⟨781S⟩: between +77° and +82°.
Test solution: 20 mg per mL, in dioxane.

Water Determination, *Method I* ⟨921⟩: not more than 0.5%.

Free heptanoic acid—Dissolve 500 mg in 10 mL of alcohol that previously has been neutralized to a faint blue color following the addition of 2 or 3 drops of bromothymol blue TS, and promptly titrate with 0.01 N sodium hydroxide VS: not more than 0.6 mL of 0.01 N sodium hydroxide is required (0.16% of heptanoic acid).

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Eluant: a mixture of cyclohexane and ethyl acetate (2:1).
Visualization: 19.

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Limits—No individual impurity exceeds 1.0%, and the total of observed impurities does not exceed 2.0%.

Assay—Dissolve about 40 mg of Testosterone Enanthate, accurately weighed, in chloroform to make 100 mL, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, add chloroform to volume, and mix. Dissolve a suitable quantity of USP Testosterone Enanthate RS, accurately weighed, in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a Standard solution having a known concentration of about 40 µg per mL. Pipet 5 mL each of the solution of Testosterone Enanthate and the Standard solution into separate, glass-stoppered, 50-mL conical flasks, and place 5.0 mL of chloroform in a similar flask to provide a blank. Treat each flask as follows. Add 10.0 mL of a solution of 375 mg of isoniazid and 0.47 mL of hydrochloric acid in 500 mL of methanol, mix, and allow to stand for 45 minutes. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 380 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of $C_{26}H_{40}O_3$ in the Testosterone Enanthate taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Testosterone Enanthate RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Testosterone Enanthate and the Standard solution, respectively.

Testosterone Enanthate Injection

» Testosterone Enanthate Injection is a sterile solution of Testosterone Enanthate in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{26}H_{40}O_3$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—
USP Testosterone Enanthate RS

Identification—Dilute a suitable volume of Injection with chloroform to obtain a solution having a concentration of about 400 µg of testosterone enanthate per mL. Proceed as directed in the *Identification* test under *Testosterone Cypionate Injection*, beginning with "Prepare a 20- × 20-cm thin-layer chromatographic plate," but using USP Testosterone Enanthate RS. The R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Reference Standard solution.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Chromatographic solvent—Equilibrate, by shaking in a separator, 95 mL of alcohol, 5 mL of water, and 50 mL of chromatographic *n*-heptane. Allow the layers to separate.

Isoniazid reagent—Dissolve 375 mg of isoniazid and 0.47 mL of hydrochloric acid in 500 mL of methanol.

Standard preparation—Dissolve a suitable quantity of USP Testosterone Enanthate RS, accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 40 µg per mL.

Assay preparation—Transfer to a 10-mL volumetric flask an accurately measured volume of Injection, equivalent to about 100 mg of testosterone enanthate, add chromatographic *n*-heptane to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, add chromatographic *n*-heptane to volume, and mix.

Procedure—Mix in a beaker 3 g of silanized chromatographic siliceous earth and 3 mL of the upper layer of the *Chromatographic solvent*. Pack the mixture into a 250- × 25-mm chromatographic tube that contains a small pledget of glass wool above the stem constriction. Mix in a beaker 3 g of silanized chromatographic siliceous earth and 2.0 mL of *Assay preparation*, transfer the mixture to the tube, and pack. Dry-wash the beaker with 1 g of silanized chromatographic siliceous earth, and transfer to the tube. Place a small pad of glass wool above the column packing. Pass 35 mL of the lower layer of the *Chromatographic solvent* through the column, and collect the eluate in a 50-mL volumetric flask. Add alcohol to volume, and mix. Pipet 10 mL of the resulting solution into a glass-stoppered, 50-mL conical flask, and evaporate on a water bath to dryness. Pipet 5 mL of methanol into the flask, and swirl to dissolve the residue. Pipet 5 mL of *Standard preparation* into a similar flask. To each flask add 10.0 mL of *Isoniazid reagent*, mix, and allow to stand for about 45 minutes. Concomitantly determine the absorbances of both solutions at the wavelength of maximum absorbance at about 380 nm, with a suitable spectrophotometer, using as a blank 5 mL of methanol that has been treated similarly with *Isoniazid reagent*. Calculate the quantity, in mg, of $C_{26}H_{40}O_3$ in each mL of the Injection taken by the formula:

$$2.5(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Testosterone Enanthate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Testosterone Propionate

$C_{22}H_{32}O_3$ 344.49
Androst-4-en-3-one, 17-(1-oxopropoxy)-, (17β)-.
Testosterone propionate [57-85-2].

» Testosterone Propionate contains not less than 97.0 percent and not more than 103.0 percent of $C_{22}H_{32}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Testosterone Propionate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.

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Medium: alcohol.
Absorptivities at 241 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: It responds to *Identification* test *C* under *Testosterone Enanthate.*
Melting range ⟨741⟩: between 118° and 123°.
Specific rotation ⟨781S⟩: between +83° and +90°.
Test solution: 20 mg, previously dried, per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.5% of its weight.
Assay—Proceed with Testosterone Propionate as directed in the *Assay* under *Testosterone Enanthate,* except to use USP Testosterone Propionate RS and otherwise substitute Testosterone Propionate throughout. Calculate the quantity, in mg, of $C_{22}H_{32}O_3$ in the Testosterone Propionate taken by the formula given therein.

Testosterone Propionate Injection

» Testosterone Propionate Injection is a sterile solution of Testosterone Propionate in a suitable vegetable oil. It contains not less than 88.0 percent and not more than 112.0 percent of the labeled amount of $C_{22}H_{32}O_3$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Testosterone Propionate RS
Identification—Dilute a suitable volume of Injection with chloroform to obtain a solution having a concentration of about 400 µg of testosterone propionate per mL. Proceed as directed in the *Identification* test under *Testosterone Cypionate Injection,* beginning with "Prepare a 20- × 20-cm thin-layer chromatographic plate," except to use USP Testosterone Propionate RS. The R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Chromatographic solvent and *Isoniazid reagent*—Prepare as directed in the *Assay* under *Testosterone Enanthate Injection.*
Standard preparation—Prepare as directed in the *Assay* under *Testosterone Enanthate Injection,* using USP Testosterone Propionate RS.
Assay preparation—Transfer to a 10-mL volumetric flask an accurately measured volume of Injection, equivalent to about 100 mg of testosterone propionate, add chromatographic *n*-heptane to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, add chromatographic *n*-heptane to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Testosterone Enanthate Injection.* Calculate the quantity, in mg, of $C_{22}H_{32}O_3$ in each mL of the Injection taken by the formula:

$$2.5(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Testosterone Propionate RS in the *Standard preparation,* V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Add the following:

▲Testosterone Topical Solution

DEFINITION
Testosterone Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of testosterone ($C_{19}H_{28}O_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Solution A: Acetonitrile, water, and trifluoroacetic acid (10: 90: 0.05)
Solution B: Acetonitrile and trifluoroacetic acid (100: 0.05)
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
12	10	90
13	0	100
15	0	100
16	80	20
21	80	20

[NOTE—Post time of the gradient may be extended to more than 21 min to allow system to properly re-equilibrate.]
Diluent: Absolute alcohol and *Solution A* (50:50)
Standard stock solution: 0.8 mg/mL of USP Testosterone RS in absolute alcohol
Standard solution: 80 µg/mL of USP Testosterone RS from the *Standard stock solution* in *Diluent*
Sample solution: Nominally 80 µg/mL of testosterone from Topical Solution prepared as follows. Dispense a suitable amount of Topical Solution to a screw-cap glass container, seal the container, and sonicate briefly to disperse bubbles. [NOTE—Do not store sample in an open container.] Transfer a suitable volume of the solution to an appropriate volumetric flask and dilute with *Diluent* to volume. Sonicate the resulting solution. [NOTE—Sonication for 30–60 s may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 240 nm. For *Identification B,* use a diode array detector in the range of 210–400 nm.
Column: 3.9-mm × 15-cm; 5-µm packing L7. [NOTE—A guard column with a similar packing may be used.]
Flow rate: 0.7 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.3
Relative standard deviation: NMT 1.0%

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of testosterone ($C_{19}H_{28}O_2$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of testosterone from the *Sample solution*
r_S = peak response of testosterone from the *Standard solution*
C_S = concentration of USP Testosterone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of testosterone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Sensitivity solution: 0.11 µg/mL of USP Testosterone RS from the *Standard solution* in *Diluent*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 1.3 for the testosterone peak, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual degradation product in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*
r_T = peak response of testosterone from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified degradation product	—	0.3
Total degradation products	—	5.0

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL. The total combined molds and yeasts count does not exceed 10^1 cfu/mL. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

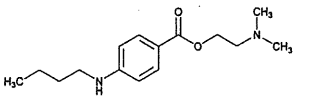
ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Testosterone RS▲ 2S (USP41)

Tetanus Immune Globulin

» Tetanus Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic solution of globulins derived from the blood plasma of adult human donors who have been immunized with tetanus toxoid. It has a potency of not less than 50 antitoxin units per mL based on the U.S. Standard Tetanus Antitoxin and the U.S. Control Tetanus Test Toxin, tested in guinea pigs. It contains not less than 10 g and not more than 18 g of protein per 100 mL, of which not less than 90 percent is gamma globulin. It contains 0.3 M glycine as a stabilizing agent, and it contains a suitable preservative.

Packaging and storage—Preserve at a temperature between 2° and 8°.
Expiration date—The expiration date for Tetanus Immune Globulin containing a 10% excess of potency is not later than 3 years after date of issue from manufacturer's cold storage (5°, 1 year).
Labeling—Label it to state that it is not for intravenous injection.

Tetracaine



$C_{15}H_{24}N_2O_2$ 264.36
Benzoic acid, 4-(butylamino)-, 2-(dimethylamino)ethyl ester; 2-(Dimethylamino)ethyl *p*-(butylamino)benzoate [94-24-6].

DEFINITION
Tetracaine contains NLT 98.0% and NMT 102.0% of tetracaine ($C_{15}H_{24}N_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Prepare the standard solutions and sample solutions immediately before use or store them at a temperature of 2°–8°, and protect from light.
Solution A: 1.36 g/L of monobasic potassium phosphate in water is prepared as follows. Dissolve 1.36 g of monobasic potassium phosphate in about 600 mL of water, and add 0.5 mL of phosphoric acid. Dilute with water to 1 L.
Solution B: Acetonitrile
Solution C: 1.0 mL/L of hydrochloric acid
Mobile phase: See *Table 1*.

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
3	80	20
18	40	60
23	40	60
23.1	80	20
28	80	20

Diluent: Acetonitrile and *Solution C* (20:80)
Standard stock solution: 0.46 mg/mL of USP Tetracaine Hydrochloride RS in *Diluent*. Sonicate for 5 min.
Standard solution: 0.046 mg/mL of USP Tetracaine Hydrochloride RS from *Standard stock solution* in *Diluent*
Sample stock solution: 0.4 mg/mL of Tetracaine in *Diluent*. Sonicate for 5 min.
Sample solution: 0.04 mg/mL of Tetracaine from *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tetracaine ($C_{15}H_{24}N_2O_2$) in the portion of Tetracaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Tetracaine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tetracaine, 264.36
M_{r2} = molecular weight of tetracaine hydrochloride, 300.82

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Organic Impurities**

Solution A, Solution B, Solution C, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 4.6 µg/mL of USP Tetracaine Hydrochloride RS and 4 µg/mL each of USP Tetracaine Related Compound B RS, USP Tetracaine Related Compound C RS, and USP Aminobenzoic Acid RS in *Diluent*
Sample solution: 1 mg/mL of Tetracaine in *Diluent*
System suitability
Sample: *Standard solution*
[Note—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between tetracaine and tetracaine related compound B
Relative standard deviation: NMT 5.0% for tetracaine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoic acid, tetracaine related compound B, and tetracaine related compound C in the portion of Tetracaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of corresponding tetracaine related compound from the *Sample solution*
r_S = peak response of corresponding tetracaine related compound from the *Standard solution*
C_S = concentration of corresponding tetracaine related compound in the *Standard solution* (mg/mL)
C_U = concentration of Tetracaine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Tetracaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Tetracaine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tetracaine, 264.36
M_{r2} = molecular weight of tetracaine hydrochloride, 300.82

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid[a]	0.3	0.4
Tetracaine hydrochloride	1	—
Tetracaine related compound B	1.7	0.4
Tetracaine related compound C	2.1	0.4
Individual unspecified impurity	—	0.4
Total impurities	—	0.8

[a] 4-Aminobenzoic acid.

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩

Analysis: Dry under vacuum over phosphorus pentoxide for 18 h.
Acceptance criteria: NMT 0.5%

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminobenzoic Acid RS
  USP Tetracaine RS
  USP Tetracaine Related Compound B RS
  4-(Butylamino)benzoic acid.
  $C_{11}H_{15}NO_2$ 193.24
  USP Tetracaine Related Compound C RS
  Methyl 4-(butylamino)benzoate.
  $C_{12}H_{17}NO_2$ 207.35
  USP Tetracaine Hydrochloride RS

Tetracaine Ointment

» Tetracaine Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{15}H_{24}N_2O_2$ in a suitable ointment base.

Packaging and storage—Preserve in collapsible ointment tubes.
USP Reference standards ⟨11⟩—
USP Tetracaine Hydrochloride RS

Identification—
A: The solution employed for measurement of absorbance in the *Assay* exhibits a maximum at 310 ± 2 nm.
B: Dissolve 5 g in 50 mL of ether, extract the ether solution with 5 mL of 3 N hydrochloric acid, and filter the acid extract. Add 2 mL of potassium thiocyanate solution (1 in 2) to the filtrate: a crystalline precipitate is formed, and when recrystallized from water and dried at 80° for 2 hours, it melts between 130° and 132° (see *Melting Range or Temperature* ⟨741⟩).
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Standard preparation—Transfer about 20 mg of USP Tetracaine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, dissolve in water, add water to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, add 5 mL of dilute hydrochloric acid (1 in 240) and 10 mL of *Buffer B.6* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers*) dilute with water to volume, and mix. The concentration of USP Tetracaine Hydrochloride RS in the *Standard preparation* is about 10 µg per mL.
Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 9 mg of tetracaine, to a separator, and dissolve in 15 mL of ether. Extract with one 20-mL portion and two 10-mL portions of dilute hydrochloric acid (1 in 240), collecting the acid extracts in a second separator. Render the aqueous solution alkaline by the addition of 5 mL of sodium carbonate TS, and extract immediately with two 50-mL portions of ether, collecting the ether extracts in another separator. Wash the ether solution with 20 mL of water, discard the washing, and extract the ether solution with two 20-mL portions and one 5-mL portion of dilute hydrochloric acid (1 in 240), collecting the acid extracts in a 50-mL volumetric flask. Dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 10 mL of *Buffer B.6* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers*) dilute with water to volume, and mix.
Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 310 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{15}H_{24}N_2O_2$ in the portion of Ointment taken by the formula:

$$(264.36/300.82)(C)(A_U/A_S)$$

in which 264.36 and 300.82 are the molecular weights of tetracaine and tetracaine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Tetracaine and Menthol Ointment

» Tetracaine and Menthol Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of tetracaine ($C_{15}H_{24}N_2O_2$) and menthol ($C_{10}H_{20}O$) in a suitable ointment base.

Packaging and storage—Preserve in collapsible ointment tubes.
USP Reference standards ⟨11⟩—
USP Menthol RS
USP Tetracaine Hydrochloride RS

Identification—
A: The solution employed for measurement of absorbance in the *Assay for tetracaine* exhibits a maximum at 310 ± 2 nm (*presence of tetracaine*).
B: Dissolve 5 g in 50 mL of ether, extract the ether solution with 5 mL of 3 N hydrochloric acid, and filter the acid extract. Add 2 mL of potassium thiocyanate solution (1 in 2) to the filtrate: a crystalline precipitate is formed, and when recrystallized from water and dried at 80° for 2 hours, it melts between 130° and 132° (see *Melting Range or Temperature* ⟨741⟩) (*presence of tetracaine*).
C: When chromatographed as directed in the *Assay for menthol*, the *Assay preparation* exhibits a major peak for menthol, the retention time of which corresponds to that exhibited by menthol in the *Standard preparation.*
Minimum fill ⟨755⟩: meets the requirements.

Assay for tetracaine—
Standard preparation—Prepare as directed in the *Assay* under *Tetracaine Ointment.*
Assay preparation—Using Ointment, proceed as directed in the *Assay* under *Tetracaine Ointment.*
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Tetracaine Ointment.*

Assay for menthol—
Internal standard solution—Dissolve decanol in *n*-hexane to obtain a solution having a concentration of about 1 mg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Menthol RS in *n*-hexane to obtain a solution having a known concentration of about 1 mg per mL. Transfer

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5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with ether to volume, and mix. Combine 2.0 mL of this solution and 2.0 mL of ether in a suitable container, and mix. This *Standard preparation* has a known concentration of about 0.05 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of Ointment, equivalent to about 5 mg of menthol, to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *n*-hexane to volume, mix, and sonicate. Using a suitable syringe attached firmly to a 25- × 12.5-mm chromatographic cartridge containing packing L4, force 2.0 mL of the solution through the cartridge at a rate of 1 mL per 12 seconds. Wash the cartridge at the same rate with two 5-mL portions of *n*-hexane, and discard the washings. Force two 2.0-mL portions of ether through the cartridge, combine the ether eluates in a suitable container, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and contains a 2-mm × 1.8-m column packed with 10% phase G16 on support S1AB. The column temperature is maintained isothermally at about 170°, the injection port temperature is maintained at about 260°, and the detector block temperature is maintained at about 240°. Dry helium is used as the carrier gas at a flow rate of about 50 mL per minute.

System suitability—Chromatograph three injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the retention time of menthol is about 0.7 relative to decanol; the resolution, *R*, between the 2 peaks is not less than 2.5; and the relative standard deviation of the ratio of the peak response obtained with menthol to that obtained with decanol is not more than 2%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Assay preparation* and the *Standard preparation* into the gas chromatograph, and measure the peak responses for menthol and decanol in each chromatogram. Calculate the quantity, in mg, of $C_{10}H_{20}O$ in the portion of Ointment taken by the formula:

$$100C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Menthol RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of menthol to decanol obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Tetracaine Hydrochloride

$C_{15}H_{24}N_2O_2 \cdot HCl$ 300.82

Benzoic acid, 4-(butylamino)-, 2-(dimethylamino)ethyl ester, monohydrochloride;
2-(Dimethylamino)ethyl *p*-(butylamino)benzoate monohydrochloride [136-47-0].

DEFINITION

Tetracaine Hydrochloride contains NLT 98.0% and NMT 102.0% of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL, *Chloride* ⟨191⟩**
  Sample solution: 20 mg/mL
  Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Prepare the standard solutions and sample solutions immediately before use, or store them at a temperature of 2°–8°. Protect them from light.

Buffer: 1.36 g/L of monobasic potassium phosphate in water prepared as follows. Dissolve 1.36 g of monobasic potassium phosphate in about 600 mL water, and add 0.5 mL of phosphoric acid. Dilute with water to 1 L.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	80	20
3	80	20
18	40	60
23	40	60
23.1	80	20
28	80	20

Diluent: Acetonitrile and water (20:80)
Standard stock solution: 0.4 mg/mL of USP Tetracaine Hydrochloride RS in *Diluent*. Sonicate for 5 min.
Standard solution: 0.04 mg/mL of USP Tetracaine Hydrochloride RS from the *Standard stock solution* in *Diluent*
Sample stock solution: 0.4 mg/mL of Tetracaine Hydrochloride in *Diluent*. Sonicate for 5 min.
Sample solution: 0.04 mg/mL of Tetracaine Hydrochloride from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Tetracaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (mg/mL)

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C_U = concentration of Tetracaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.004 mg/mL each of USP Tetracaine Hydrochloride RS, USP Tetracaine Related Compound B RS, USP Tetracaine Related Compound C RS, and USP Aminobenzoic Acid RS in *Diluent*

Sample solution: 1 mg/mL of Tetracaine Hydrochloride in *Diluent*

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between tetracaine and tetracaine related compound B

Relative standard deviation: NMT 5.0% for tetracaine hydrochloride

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid, tetracaine related compound B, and tetracaine related compound C in the portion of Tetracaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of corresponding tetracaine related compound from the *Sample solution*

r_S = peak response of corresponding tetracaine related compound from the *Standard solution*

C_S = concentration of corresponding tetracaine related compound in the *Standard solution* (mg/mL)

C_U = concentration of Tetracaine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Tetracaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Tetracaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid[a]	0.3	0.4
Tetracaine hydrochloride	1	—
Tetracaine hydrochloride related compound B	1.7	0.4
Tetracaine hydrochloride related compound C	2.1	0.4
Individual unspecified impurity	—	0.4
Total impurities	—	0.8

[a] 4-Aminobenzoic acid.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%
- **STERILITY TESTS** ⟨71⟩: Where the label states that Tetracaine Hydrochloride is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Tetracaine Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms, it contains NMT 0.7 USP Endotoxin Units/mg of tetracaine hydrochloride.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Aminobenzoic Acid RS
USP Tetracaine Hydrochloride RS
USP Tetracaine Related Compound B RS
4-(Butylamino)benzoic acid.
$C_{11}H_{15}NO_2$ 193.24
USP Tetracaine Related Compound C RS
Methyl 4-(butylamino)benzoate.
$C_{12}H_{17}NO_2$ 207.35

Tetracaine Hydrochloride Cream

» Tetracaine Hydrochloride Cream contains Tetracaine Hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of tetracaine ($C_{15}H_{24}N_2O_2$) in a suitable water-miscible base.

Packaging and storage—Preserve in collapsible, lined metal tubes.

USP Reference standards ⟨11⟩—
USP Tetracaine Hydrochloride RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* ***197U*** ▲ (CN 1-May-2020): *Assay preparation* compared to the *Standard preparation* from the *Assay*

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Minimum fill ⟨755⟩: meets the requirements.

USP Monographs

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pH ⟨791⟩: between 3.2 and 3.8.

Assay—

pH 6 Acetate buffer—Dissolve 250 g of sodium acetate in about 500 mL of water in a 1000-mL volumetric flask, add 5.0 mL of glacial acetic acid, dilute with water to volume, and mix.

Standard preparation—Transfer about 25 mg of USP Tetracaine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, dissolve in isopropyl alcohol, add isopropyl alcohol to volume, and mix. Transfer 2.0 mL of this solution to another 100-mL volumetric flask, add 2.0 mL of *pH 6 Acetate buffer*, dilute with isopropyl alcohol to volume, and mix. The concentration of USP Tetracaine Hydrochloride RS in the *Standard preparation* is about 5 µg per mL.

Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 4.5 mg of tetracaine, to a 50-mL beaker, add 25 mL of isopropyl alcohol, and warm on a steam bath to dissolve the specimen completely. Transfer the solution with the aid of isopropyl alcohol to a 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix. Transfer 10.0 mL of this solution to another 100-mL volumetric flask, add 2.0 mL of *pH 6 Acetate buffer*, dilute with isopropyl alcohol to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 310 nm, with a suitable spectrophotometer, using a 1 in 50 solution of *pH 6 Acetate buffer* in isopropyl alcohol as the blank. Calculate the quantity, in mg, of $C_{15}H_{24}N_2O_2$ in the portion of Cream taken by the formula:

$$(264.36/300.82)(C)(A_U/A_S)$$

in which 264.36 and 300.82 are the molecular weights of tetracaine and tetracaine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Tetracaine Hydrochloride Injection

DEFINITION

Tetracaine Hydrochloride Injection is a sterile solution of Tetracaine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

IDENTIFICATION

- **A.**

Sample solution: 100 mg in 10 mL of water

Analysis: To the *Sample solution* add 1 mL of potassium thiocyanate solution (1 in 4). A crystalline precipitate is formed. Recrystallize the precipitate from water, and dry at 80° for 2 h.

Acceptance criteria: The crystalline precipitate melts between 130° and 132°.

- **B.** The retention time of the major peak of tetracaine in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (20:20:60) containing 0.06% of sulfuric acid, 0.5% of sodium sulfate, and 0.02% of sodium 1-heptanesulfonate. The pH is about 2.6.

Diluent: Methanol and water (1:1)

Standard solution: 1 mg/mL of USP Tetracaine Hydrochloride RS in *Diluent*

System suitability solution: 4 mg/mL of salicylic acid and 1 mg/mL of tetracaine hydrochloride in *Standard solution*

Sample solution: Nominally 1 mg/mL of tetracaine hydrochloride in *Diluent* from Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 305 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for salicylic acid and tetracaine are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2 between salicylic acid and tetracaine peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tetracaine from the *Sample solution*

r_S = peak response of tetracaine from the *Standard solution*

C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tetracaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.7 USP Endotoxin Units/mg of tetracaine hydrochloride
- **pH** ⟨791⟩: 3.2–6.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, under refrigeration and protected from light. It may be packaged in 100-mL multiple-dose containers. Injection supplied as a component of spinal anesthesia trays may be stored at room temperature for 12 months.
- **LABELING:** Label it to indicate that the Injection is not to be used if it contains crystals, or if it is cloudy or discolored.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Tetracaine Hydrochloride RS

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Tetracaine Hydrochloride for Injection

DEFINITION
Tetracaine Hydrochloride for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: The *Sample solution* from the *Assay*.
Acceptance criteria: Meets the requirements
• **B.**
Sample solution: 100 mg in 10 mL of water
Analysis: To the *Sample solution* add 1 mL of potassium thiocyanate solution (1 in 4). A crystalline precipitate is formed. Recrystallize the precipitate from water, and dry at 80° for 2 h.
Acceptance criteria: The crystalline precipitate melts between 130° and 132°.

ASSAY
• **PROCEDURE**
Buffer: *Buffer B.6* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers, Table 12*)
Diluent: Dilute hydrochloric acid (1 in 200)
Standard stock solution: 0.2 mg/mL of USP Tetracaine Hydrochloride RS in water
Standard solution: 0.01 mg/mL of USP Tetracaine Hydrochloride RS prepared as follows. Pipet 5 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 5 mL of *Diluent* and 10 mL of *Buffer*, and dilute with water to volume.
Sample stock solution: Nominally 0.2 mg/mL of tetracaine hydrochloride in water prepared as follows. Transfer to a tared 20-mL beaker the contents of a sufficient number of containers of Tetracaine Hydrochloride for Injection to yield about 100 mg of tetracaine hydrochloride. Weigh immediately, and transfer with the aid of water to a 500-mL volumetric flask. Dilute with water to volume.
Sample solution: Nominally 0.01 mg/mL of tetracaine hydrochloride prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 5 mL of *Diluent* and 10 mL of *Buffer*, then dilute with water to volume.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 310 nm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of labeled amount of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Tetracaine Hydrochloride for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tetracaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Buffer: *Buffer B.6* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers, Table 12*)
Diluent: Dilute hydrochloric acid (1 in 200)
Standard stock solution: 0.2 mg/mL of USP Tetracaine Hydrochloride RS in water
Standard solution: 0.01 mg/mL of USP Tetracaine Hydrochloride RS prepared as follows. Transfer 5 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 5 mL of *Diluent* and 10 mL of *Buffer*, and dilute with water to volume.
Sample stock solution: Transfer the contents of one container, with the aid of water, to a 200-mL volumetric flask, and add water to volume.
Sample solution: Nominally 0.01 mg/mL of tetracaine hydrochloride from the *Sample stock solution* prepared as follows. Pipet a portion of the *Sample stock solution*, equivalent to 1 mg of tetracaine hydrochloride, to a 100-mL volumetric flask, add 5 mL of *Diluent* and 10 mL of *Buffer*, and dilute with water to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in each container taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tetracaine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: Meets the requirements

IMPURITIES
• **RESIDUE ON IGNITION**
Sample: 500 mg
Analysis: Transfer the *Sample* to a beaker, and dissolve in 10 mL of methanol. Filter through paper previously washed with methanol, collecting the filtrate in an ignited and tared crucible, and wash the beaker and the filter paper with 25–30 mL of methanol. Evaporate with the aid of heat and a current of air to dryness, and proceed as directed in *Residue on Ignition* ⟨281⟩, beginning with "Heat gently at a temperature as low as practicable".
Acceptance criteria: NMT 0.1%
• **ORGANIC IMPURITIES**
Standard solution: 0.2 mg/mL of 4-(butylamino) benzoic acid in methanol
Sample solution: 50 mg/mL of Tetracaine Hydrochloride for Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Chloroform, methanol, and isopropylamine (98:7:2)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter. Develop the plate in a suitable chromatographic chamber containing the *Developing solvent system* until the solvent front has

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moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry in a current of warm air. Examine the plate under short-wavelength UV light.

Acceptance criteria: Any spot from the *Sample solution*, other than the principal spot, is not more intense than the principal spot from the *Standard solution* (0.4%), and the sum of the intensities of any such spots is not greater than 0.8%.

SPECIFIC TESTS

- **pH** ⟨791⟩: 5.0–6.0 in a solution (1 in 100)
- **Water Determination** ⟨921⟩, *Method I*: NMT 2.0%
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 0.7 USP Endotoxin Units/mg of tetracaine hydrochloride
- **Completeness of Solution** ⟨641⟩
  Sample: Nominally 10 mg of tetracaine hydrochloride
  Acceptance criteria: The *Sample* dissolves in 1 mL of water in NMT 2 s to yield a colorless solution free from undissolved solid.
- **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products*, ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Other Requirements:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, preferably Type I glass.
- **USP Reference Standards** ⟨11⟩
  USP Tetracaine Hydrochloride RS

Tetracaine Hydrochloride Ophthalmic Solution

» Tetracaine Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Tetracaine Hydrochloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{15}H_{24}N_2O_2 \cdot HCl$. It may contain suitable antimicrobial and thickening agents.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate that the Ophthalmic Solution is not to be used if it contains crystals, or if it is cloudy or discolored.
USP Reference standards ⟨11⟩—
USP Tetracaine Hydrochloride RS
Identification—Add 5 mL of Ophthalmic Solution to 5 mL of water in a test tube, then add 1 mL of potassium thiocyanate solution (1 in 4): a crystalline precipitate is formed. Recrystallize the precipitate from water, and dry at 80° for 2 hours: the crystals so obtained melt between 130° and 132°.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 3.7 and 6.0.

Assay—
Mobile phase—Prepare 0.01 *M* of dibasic ammonium phosphate in water, and adjust with phosphoric acid to a pH of 3.0. Prepare a filtered and degassed mixture of this solution and acetonitrile (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Tetracaine Hydrochloride RS in water to obtain a solution having a known concentration of about 0.1 mg per mL.
Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of tetracaine hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column containing packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 500 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{15}H_{24}N_2O_2 \cdot HCl$ in each mL of the Ophthalmic Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and r_U and r_S are the tetracaine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Tetracaine Hydrochloride Topical Solution

» Tetracaine Hydrochloride Topical Solution is an aqueous solution of Tetracaine Hydrochloride. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{15}H_{24}N_2O_2 \cdot HCl$. It contains a suitable antimicrobial agent.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate that the Topical Solution is not to be used if it contains crystals, or if it is cloudy or discolored.
USP Reference standards ⟨11⟩—
USP Tetracaine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—*Solutions:* solutions of the Topical Solution employed for measurement of absorbance in the *Assay*.
B: It responds to the tests for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 4.5 and 6.0.

Assay—
Standard preparation—Prepare as directed in the *Assay* under *Tetracaine Hydrochloride in Dextrose Injection*.

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Assay preparation—Using an accurately measured volume of Topical Solution, prepare as directed in the *Assay* under *Tetracaine Hydrochloride in Dextrose Injection.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Tetracaine Hydrochloride in Dextrose Injection.* Calculate the quantity, in mg, of $C_{15}H_{24}N_2O_2 \cdot HCl$ in the volume of Topical Solution taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation*, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Tetracaine Hydrochloride in Dextrose Injection

DEFINITION

Tetracaine Hydrochloride in Dextrose Injection is a sterile solution of Tetracaine Hydrochloride and Dextrose in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amounts of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) and dextrose ($C_6H_{12}O_6$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL ⟨191⟩,** *Chloride*
  Sample solution: 100 mg in 5 mL of water
  Acceptance criteria: Meets the requirements
- **C.**
  Sample solution: Nominally 50 mg/mL of dextrose from Injection
  Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
  Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

ASSAY

- **TETRACAINE HYDROCHLORIDE**
  Buffer: *Buffer B.6* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers, Table 12*)
  Diluent: Dilute hydrochloric acid (1 in 200)
  Standard stock solution: 0.2 mg/mL of USP Tetracaine Hydrochloride RS in water
  Standard solution: 0.01 mg/mL of USP Tetracaine Hydrochloride RS prepared as follows. Pipet 5 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 5 mL of *Diluent* and 10 mL of *Buffer*, and dilute with water to volume.
  Sample stock solution: Nominally 0.2 mg/mL of tetracaine hydrochloride from Injection prepared as follows. Transfer a volume of Injection, equivalent to 10 mg of tetracaine hydrochloride, to a separator. Dilute with water to about 50 mL, and render alkaline by the addition of 5 mL of sodium carbonate TS. Extract immediately with two 50-mL portions of ether, collecting the extracts in a separator. Wash the ether extracts with 20 mL of water, discarding the wash solution, and extract the ether solution with two 20-mL portions and one 5-mL portion of *Diluent*, collecting the extracts in a 50-mL volumetric flask. Dilute with water to volume.
  Sample solution: Nominally 0.01 mg/mL of tetracaine hydrochloride from *Sample stock solution* prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 10 mL of *Buffer*, and dilute to volume with water.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: Maximum absorbance at about 310 nm
  Blank: Water
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*
  Calculate the percentage of labeled amount of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tetracaine hydrochloride in the *Sample solution* (mg/mL)

  Acceptance criteria: 95.0%–105.0%
- **DEXTROSE**
  Sample: Injection
  Analysis: Determine the angular rotation of the Injection in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩). Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times 100$$

a = observed angular rotation of the Injection (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample* (g/100 mL)

  Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

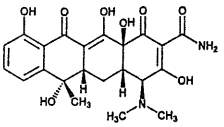
- **PARTICULATE MATTER IN INJECTIONS ⟨788⟩:** Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 1.0 USP Endotoxin Unit/mg of tetracaine hydrochloride.
- **pH ⟨791⟩:** 3.5–6.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, under refrigeration and protected from light. It may be packaged in 100-mL multiple-dose containers. Injection supplied as a component of spinal anesthesia trays may be stored at room temperature for 12 months.
- **LABELING:** Label it to indicate that the Injection is not to be used if it contains crystals, or if it is cloudy or discolored.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Tetracaine Hydrochloride RS

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Tetracycline



$C_{22}H_{24}N_2O_8$ 444.44
Trihydrate 498.49
2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, [4*S*-(4α,4aα,5aα,6β,12aα)]-;
(4*S*,4a*S*,5a*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide [60-54-8].
Trihydrate [6416-04-2].

DEFINITION
Tetracycline has a potency equivalent to NLT 975 µg/mg of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲** (CN 1-May-2020)
  Sample solution: 20 µg/mL in 0.25 N sodium hydroxide
  Analytical wavelength: 380 nm
  Analysis: Measure the absorptivity 6 min after preparation.
  Acceptance criteria: Absorptivity, calculated on the anhydrous basis and taking into account the potency of the Reference Standard, is between 104.5% and 111.95% of the absorptivity of USP Tetracycline Hydrochloride RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: Dilute 1 mL of phosphoric acid with water to 1 L.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
7.5	60	40
7.6	85	15
10	85	15

System suitability solution: 25 µg/mL each of USP Anhydrotetracycline Hydrochloride RS, USP Epitetracycline Hydrochloride RS, and USP 4-Epianhydrotetracycline Hydrochloride RS, and 100 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*
Standard solution: 100 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*
Sample solution: 90 µg/mL of Tetracycline in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 3-µm packing L60. [NOTE—L1 column is also suitable.]
Temperatures
Autosampler: 10°
Column: 50°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency equivalent, in µg/mg, of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Tetracycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of tetracycline from the *Sample solution*
r_S = peak response of tetracycline from the *Standard solution*
C_S = concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Tetracycline in the *Sample solution* (µg/mL)
P = potency of USP Tetracycline Hydrochloride RS (µg/mg)

Acceptance criteria: NLT 975 µg/mg on the anhydrous basis

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
  System suitability solution: 25 µg/mL each of USP Anhydrotetracycline Hydrochloride RS, USP Epitetracycline Hydrochloride RS, USP 4-Epianhydrotetracycline Hydrochloride RS, and USP Tetracycline Hydrochloride RS in *Solution A*
  Standard solution: 0.5 µg/mL of USP Anhydrotetracycline Hydrochloride RS, 3 µg/mL of USP Epitetracycline Hydrochloride RS, 2 µg/mL of USP 4-Epianhydrotetracycline Hydrochloride RS, and 0.1 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline, *System suitability solution*
  Relative standard deviation: NMT 2.8%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of anhydrotetracycline, epitetracycline, and 4-epianhydrotetracycline in the portion of Tetracycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of anhydrotetracycline, epitetracycline, or 4-epianhydrotetracycline from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
C_U = concentration of Tetracycline in the *Sample solution* (µg/mL)

Calculate the percentage of 2-acetyl analog or any unspecified impurity in the portion of Tetracycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of 2-acetyl analog or any unspecified impurity from the *Sample solution*
r_S = peak response of tetracycline from the *Standard solution*
C_S = concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Tetracycline in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of tetracycline, 444.44
M_{r2} = molecular weight of tetracycline hydrochloride, 480.90

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Epitetracycline[a]	0.9	3.0
Tetracycline	1.0	—
2-Acetyl analog[b]	1.3	2.0
4-Epianhydrotetracycline[c]	1.7	2.0
Anhydrotetracycline[d]	1.9	0.5
Any individual unspecified impurity	—	0.10

[a] (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.
[b] 2-Acetyl-2-decarbamoyltetracycline; (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-Acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione.
[c] (4*R*,4a*S*,12a*S*)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide.
[d] (4*S*,4a*S*,12a*S*)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide.

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 5 mg/mL in 0.1 N hydrochloric acid
  Acceptance criteria: −260° to −280° on the anhydrous basis
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩
  Sample solution: In an aqueous suspension (1 in 100)
  Acceptance criteria: 3.0–7.0
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 13.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only.

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Anhydrotetracycline Hydrochloride RS
  USP 4-Epianhydrotetracycline Hydrochloride RS
  USP Epitetracycline Hydrochloride RS
  USP Tetracycline Hydrochloride RS

Tetracycline Boluses

» Tetracycline Boluses contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

Packaging and storage—Preserve in tight containers.
Labeling—Label Boluses to indicate that they are intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
Identification—Shake a suitable quantity of finely powdered Boluses with methanol to obtain a solution containing the equivalent of 1 mg of tetracycline hydrochloride per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed under *Identification—Tetracyclines* ⟨193⟩.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation*.
Loss on drying ⟨731⟩—Dry about 100 mg of finely powdered Boluses, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight, or where the Boluses have a diameter of greater than 15 mm, it loses not more than 6.0% of its weight.
Assay—Transfer not less than 2 Boluses to a high-speed blender jar containing an accurately measured volume of 0.1 N hydrochloric acid, so that the solution so obtained contains not less than 150 µg of tetracycline hydrochloride per mL, and blend for about 3 to 5 minutes. Proceed as directed for tetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Tetracycline Oral Suspension

» Tetracycline Oral Suspension is Tetracycline with or without one or more suitable buffers, preservatives, stabilizers, and suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
USP 4-Epianhydrotetracycline Hydrochloride RS
Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for tetracycline, the retention time of which corresponds to that

USP Monographs

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exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Uniformity of dosage units ⟨905⟩—
F *OR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS* : meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 3.5 and 6.0.
Limit of 4-epianhydrotetracycline—Using the *Diluting solvent*, *Chromatographic system*, and *Procedure* set forth in the *Assay*, chromatograph a Standard solution prepared by dissolving an accurately weighed quantity of USP 4-Epianhydrotetracycline Hydrochloride RS in *Diluting solvent* to obtain a solution having a known concentration of about 10 µg per mL. Using the chromatogram so obtained and the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of 4-epianhydrotetracycline in the Oral Suspension taken by the formula:

$$(25C_E/T)(r_U/r_S)$$

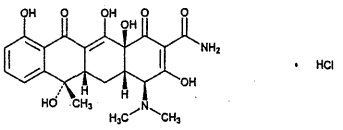
in which C_E is the concentration, in µg per mL, of USP 4-Epianhydrotetracycline Hydrochloride RS in the Standard solution, T is the quantity, in mg, of tetracycline hydrochloride equivalent in the portion of Oral Suspension taken, based on the labeled quantity, and r_U and r_S are the 4-epianhydrotetracycline peak responses obtained from the *Assay preparation* and the Standard solution, respectively: not more than 5.0% is found.

Assay—
Diluting solvent, *Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Tetracycline Hydrochloride*.
Assay preparation—Transfer an accurately measured volume of Oral Suspension, equivalent to about 125 mg of tetracycline hydrochloride, to a 250-mL volumetric flask, add 200 mL of *Diluting solvent*, and shake. Add *Diluting solvent* to volume, mix, and filter.
Procedure—Proceed as directed in the *Assay* under *Tetracycline Hydrochloride*. Calculate the quantity, in mg per mL, of $C_{22}H_{24}N_2O_8 \cdot HCl$ equivalent in the Oral Suspension taken by the formula:

$$(CP/4V)(r_U/r_S)$$

in which V is the volume, in mL, of Oral Suspension taken, and the other terms are as defined therein.

Tetracycline Hydrochloride



$C_{22}H_{24}N_2O_8 \cdot HCl$ 480.90
2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a, 5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, [4*S*-(4α,4aα,5aα, 6β,12aα)]-;
(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)- 1,4,4a,5,5a, 6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [64-75-5].

DEFINITION
Tetracycline Hydrochloride has a potency of NLT 900 µg/mg of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K.▲** (USP 1-May-2020) Do not dry the specimen.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
Sample solution: 1 mg/mL of Tetracycline Hydrochloride in methanol
▲Acceptance criteria: Meets the requirements▲ (USP 1-May-2020)

ASSAY
- **PROCEDURE**
Solution A: Dilute 1 mL of phosphoric acid with water to 1 L.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
7.5	60	40
7.6	85	15
10	85	15

System suitability solution: 25 µg/mL each of USP Anhydrotetracycline Hydrochloride RS, USP Epitetracycline Hydrochloride RS, USP 4-Epianhydrotetracycline Hydrochloride RS, and USP Tetracycline Hydrochloride RS in *Solution A*
Standard solution: 100 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*
Sample solution: 100 µg/mL of Tetracycline Hydrochloride in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 3-µm packing L60.
[NOTE—L1 column is also suitable.]
Temperatures
Autosampler: 10°
Column: 50°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency, in µg/mg, of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Tetracycline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Tetracycline Hydrochloride in the *Sample solution* (µg/mL)
P = potency of USP Tetracycline Hydrochloride RS (µg/mg)

Acceptance criteria: NLT 900 µg/mg

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.5 µg/mL of USP Anhydrotetracycline Hydrochloride RS, 3 µg/mL of USP Epitetracycline Hydrochloride RS, 2 µg/mL of USP 4-Epianhydrotetracycline Hydrochloride RS, and 0.1 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲anhydrotetracycline, epitetracycline, and 4-epianhydrotetracycline▲ (USP 1-May-2020) in the portion of Tetracycline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ▲anhydrotetracycline, epitetracycline, or 4-epianhydrotetracycline▲ (USP 1-May-2020) from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
C_U = concentration of Tetracycline Hydrochloride in the *Sample solution* (µg/mL)

▲Calculate the percentage of 2-acetyl analog or any unspecified impurity in the portion of Tetracycline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2-acetyl analog or any unspecified impurity from the *Sample solution*
r_S = peak response of tetracycline from the *Standard solution*
C_S = concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Tetracycline Hydrochloride in the *Sample solution* (µg/mL)▲ (USP 1-May-2020)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
▲4-▲ (USP 1-May-2020)Epitetracycline[a]	0.9	3.0
Tetracycline	1.0	—
▲2-Acetyl analog[b]	1.3	1.5▲ (USP 1-May-2020)
4-Epianhydrotetracycline[c]	1.7	2.0
Anhydrotetracycline[d]	1.9	0.5
▲Any individual unspecified impurity	—	0.10▲ (USP 1-May-2020)

[a] (4R,4aS,5aS,6S,12aS)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.
[b] 2-Acetyl-2-decarbamoyltetracycline; (4S,4aS,5aS,6S,12aS)-2-Acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4H,5H)-dione.
[c] (4R,4aS,12aS)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide.
[d] (4S,4aS,12aS)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 5 mg/mL in 0.1 N hydrochloric acid
Acceptance criteria: −240° to −255° on the dried basis
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩
Sample solution: In a solution (1 in 100)
Acceptance criteria: 1.8–2.8

Change to read:

- **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg
Analysis: Dry ▲the *Sample*▲ (USP 1-May-2020) in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 2.0%
- **STERILITY TESTS** ⟨71⟩: Where the label states that Tetracycline Hydrochloride is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Tetracycline Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.5 USP Endotoxin Units/mg of tetracycline hydrochloride. Where it is intended for use in preparing nonparenteral sterile dosage forms, it is exempt from the requirements for bacterial endotoxins.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

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- **LABELING:** Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Anhydrotetracycline Hydrochloride RS
  USP 4-Epianhydrotetracycline Hydrochloride RS
  USP Epitetracycline Hydrochloride RS
  USP Tetracycline Hydrochloride RS

Tetracycline Hydrochloride Capsules

DEFINITION

Tetracycline Hydrochloride Capsules contain NLT 90.0% and NMT 125.0% of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Dilute 1 mL of phosphoric acid with water to 1 L.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
7.5	60	40
7.6	85	15
10	85	15

System suitability solution: 25 µg/mL each of USP Anhydrotetracycline Hydrochloride RS, USP 4-Epianhydrotetracycline Hydrochloride RS, USP Epitetracycline Hydrochloride RS, and USP Tetracycline Hydrochloride RS in *Solution A*
Standard solution: 100 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*. ▲[NOTE—If needed, dilute the *Standard solution* concentration for *Identification B*.]▲ (USP 1-Dec-2019)
Sample solution: Nominally equivalent to 100 µg/mL of tetracycline hydrochloride in *Solution A* from NLT 20 Capsules prepared as follows. Transfer a portion of Capsule contents, equivalent to 25 mg of tetracycline hydrochloride, into a 250-mL volumetric flask. Dissolve with the aid of sonification, and dilute with *Solution A* to volume. Pass through a syringe filter of 0.22-µm pore size, discarding the first 2 mL of the filtrate. ▲[NOTE—If needed, dilute the *Sample solution* concentration for *Identification B*.]▲ (USP 1-Dec-2019)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. ▲For *Identification B*, use a diode array detector in the range of 220–400 nm.▲ (USP 1-Dec-2019)
Column: 4.6-mm × 15-cm; 3-µm packing L1
Temperatures
Autosampler: 4°
Column: 50°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for epitetracycline, tetracycline, 4-epianhydrotetracycline, and anhydrotetracycline are 0.9, 1.0, 1.6, and 1.8, respectively.]
Suitability requirements
Tailing factor: NMT 1.5, *Standard solution*
Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of tetracycline from the *Sample solution*
r_S = peak response of tetracycline from the *Standard solution*
C_S = concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tetracycline hydrochloride in the *Sample solution* (µg/mL)
P = potency of USP Tetracycline Hydrochloride RS (µg/mg)
F = correction factor, 0.001 mg/µg

Acceptance criteria: 90.0%–125.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: Water; 900 mL
Apparatus 2: 75 rpm. Maintain a distance of 45 ± 5 mm between the blade and the inside bottom of the vessel.
Times: 60 min ▲for 250-mg Capsules;▲ (USP 1-Dec-2019) 90 min for 500-mg Capsules
Detector: UV 276 nm
Standard solution: A known concentration of USP Tetracycline Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Analysis: Determine the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) dissolved by using UV absorption on portions of the *Sample solution* suitably diluted with *Medium*, if necessary, in comparison with the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) is dissolved.

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Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Water; 900 mL, deaerated
Apparatus 2: 75 rpm. Maintain a distance of 45 ± 5 mm between the blade and the inside bottom of the vessel.
Times: For Capsules labeled to contain 250 mg: 30 and 60 min; for Capsules labeled to contain 500 mg: 30, 60, and 90 min
Detector: UV 276 nm
Standard solution: A known concentration of USP Tetracycline Hydrochloride RS in *Medium*
Sample solution: At the times specified, withdraw a known volume of the solution under test, and pass it through a suitable filter. Dilute with *Medium*, if necessary. Replace the volume withdrawn with the same amount of *Medium*, preheated at 37.0 ± 0.5°.
Blank: *Medium*
▲**Analysis:** Determine the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) dissolved by using UV absorption on portions of the *Sample solution* suitably diluted with *Medium*, if necessary, in comparison with the *Standard solution*.▲ (USP 1-Dec-2019)
Tolerances: See *Table 2* and *Table 3*.

Table 2

For Capsules Labeled to Contain 250 mg	
Time (min)	Amount Dissolved (%; Q)
30	NLT 60
60	NLT 85

Table 3

For Capsules Labeled to Contain 500 mg	
Time (min)	Amount Dissolved (%; Q)
30	NLT 50
60	NLT 70
90	NLT 85

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 25 µg/mL each of USP Anhydrotetracycline Hydrochloride RS, USP 4-Epianhydrotetracycline Hydrochloride RS, USP Epitetracycline Hydrochloride RS, and USP Tetracycline Hydrochloride RS in *Solution A*
Standard solution: 0.5 µg/mL of USP Anhydrotetracycline Hydrochloride RS, 3 µg/mL each of USP 4-Epianhydrotetracycline Hydrochloride RS and USP Epitetracycline Hydrochloride RS, and 0.1 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each specified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*
r_S = peak response of corresponding Reference Standard from the *Standard solution*
C_S = concentration of corresponding Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of tetracycline hydrochloride in the *Sample solution* (µg/mL)
P = potency of the corresponding Reference Standard

Calculate the percentage of each unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of tetracycline from the *Standard solution*
C_S = concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tetracycline hydrochloride in the *Sample solution* (µg/mL)
P = potency of USP Tetracycline Hydrochloride RS (µg/mg)
F = correction factor, 0.001 mg/µg

Acceptance criteria: See *Table 4*. Disregard any peaks less than 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Epitetracycline[a]	0.9	3.0
Tetracycline	1.0	—
▲2-Acetyl analog[b, c]	1.3	—▲ (USP 1-Dec-2019)
4-Epianhydrotetracycline[d]	1.7	3.0
Anhydrotetracycline[e]	1.8	0.5
Any individual unspecified impurity	—	0.1

[a] (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.
[b] 2-Acetyl-2-decarbamoyltetracycline; (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione.
[c] Process impurities that are controlled in the drug substance are not to be reported. They are not to be included in total impurities. They are listed here for information only.
[d] (4*R*,4a*S*,12a*S*)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide.
[e] (4*S*,4a*S*,12a*S*)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide.

USP Monographs

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Anhydrotetracycline Hydrochloride RS
  USP 4-Epianhydrotetracycline Hydrochloride RS
  USP Epitetracycline Hydrochloride RS
  USP Tetracycline Hydrochloride RS

Tetracycline Hydrochloride for Injection

» Tetracycline Hydrochloride for Injection is sterile Tetracycline Hydrochloride or a sterile, dry mixture of sterile Tetracycline Hydrochloride, one form of which contains Magnesium Chloride or magnesium ascorbate and one or more suitable buffers, and may contain one or more suitable preservatives, solubilizers, stabilizers, and anesthetic agents, and the other form of which contains one or more suitable stabilizing agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{22}H_{24}N_2O_8 \cdot HCl$.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, protected from light.
Labeling—Label Tetracycline Hydrochloride for Injection that contains an anesthetic agent to indicate that it is intended for intramuscular administration only.
USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
USP 4-Epianhydrotetracycline Hydrochloride RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for tetracycline, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 USP Endotoxin Unit per mg of tetracycline hydrochloride.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined, Fluid D* being used instead of *Fluid A*.
pH ⟨791⟩: between 2.0 and 3.0, in a solution containing 10 mg per mL.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Limit of 4-epianhydrotetracycline—Using the *Diluting solvent*, *Chromatographic system*, and *Procedure* set forth in the *Assay*, chromatograph a Standard solution prepared by dissolving an accurately weighed quantity of USP 4-Epianhydrotetracycline Hydrochloride RS in *Diluting solvent* to obtain a solution having a known concentration of about 15 µg per mL. Using the chromatogram so obtained and the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of 4-epianhydrotetracycline hydrochloride in the Tetracycline Hydrochloride for Injection taken by the formula:

$$(10C_E/T)(r_U/r_S)$$

in which C_E is the concentration, in µg per mL, of USP 4-Epianhydrotetracycline Hydrochloride RS in the Standard solution; T is the quantity, in mg, of tetracycline hydrochloride in the portion of Tetracycline Hydrochloride for Injection taken to prepare the *Assay preparation*, based on the labeled quantity; and r_U and r_S are the 4-epianhydrotetracycline peak responses obtained from the *Assay preparation* and the Standard solution, respectively: not more than 3.0% is found.
Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—
Diluting solvent, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system* —Prepare as directed in the *Assay* under *Tetracycline Hydrochloride*.
Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Tetracycline Hydrochloride for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with *Diluting solvent* to obtain a solution containing about 0.5 mg of tetracycline hydrochloride per mL.
Assay preparation 2 (where the label states the quantity of tetracycline hydrochloride in a given volume of constituted solution)—Constitute Tetracycline Hydrochloride for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Diluting solvent* to obtain a solution containing about 0.5 mg of tetracycline hydrochloride per mL.
Procedure—Proceed as directed in the *Assay* under *Tetracycline Hydrochloride*. Calculate the quantity, in mg, of $C_{22}H_{24}N_2O_8.HCl$ withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(CP/1000)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of $C_{22}H_{24}N_2O_8.HCl$ in the container, or in the volume of constituted solution taken; D is the concentration, in mg of tetracycline hydrochloride per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein.

Tetracycline Hydrochloride Ointment

» Tetracycline Hydrochloride Ointment contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{22}H_{24}N_2O_8 \cdot HCl$.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
USP 4-Epianhydrotetracycline Hydrochloride RS

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Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for tetracycline, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay—
Diluting solvent , *Mobile phase*, *Resolution solution*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Tetracycline Hydrochloride*.
Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 300 mg of tetracycline hydrochloride, to a glass-stoppered conical flask, add 20 mL of cyclohexane, and shake. Add 35 mL of methanol, and sonicate for about 20 minutes. Filter this solution into a 100-mL volumetric flask, and rinse the sides of the conical flask with 40 mL of methanol, filtering the rinsing through the filter into the volumetric flask. Dilute with methanol to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Tetracycline Hydrochloride RS quantitatively in methanol to obtain a solution having a known concentration of about 1 mg per mL. Transfer 6.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix. This solution contains about 0.12 mg of USP Tetracycline Hydrochloride RS per mL.
Procedure—Proceed as directed in the *Assay* under *Tetracycline Hydrochloride*. Calculate the quantity, in mg, of $C_{22}H_{24}N_2O_8 \cdot HCl$ in the portion of Ointment taken by the formula:

$$2.5CP(r_U/r_S)$$

in which the terms are as defined therein.

Tetracycline Hydrochloride Ophthalmic Ointment

DEFINITION
Tetracycline Hydrochloride Ophthalmic Ointment contains NLT 90.0% and NMT 125.0% of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 0.1 M ammonium oxalate
Solution B: 0.2 M dibasic ammonium phosphate
Diluent: Dimethylformamide and *Solution A* (270:680)
Mobile phase: Dimethylformamide, *Solution A*, and *Solution B* (270:680:50). Adjust, if necessary, with 3 N ammonium hydroxide or 3 N phosphoric acid to a pH of 7.6–7.7.
System suitability solution: 100 µg/mL of tetracycline hydrochloride and 25 µg/mL of USP 4-Epianhydrotetracycline Hydrochloride RS in *Diluent*
Standard stock solution: 1 mg/mL of USP Tetracycline Hydrochloride RS in methanol
Standard solution: 0.12 mg/mL of USP Tetracycline Hydrochloride RS from *Standard stock solution* in *Diluent*
Sample stock solution: Nominally 3 mg/mL of tetracycline hydrochloride prepared as follows. Transfer a portion of Ophthalmic Ointment, containing nominally 300 mg of tetracycline hydrochloride, to a glass-stoppered conical flask. Add 20 mL of cyclohexane, and shake. Add 35 mL of methanol, and sonicate for 20 min. Filter this solution into a 100-mL volumetric flask, and rinse the sides of the conical flask with 40 mL of methanol, filtering the rinsing into the volumetric flask. Dilute with methanol to volume.
Sample solution: Nominally 0.12 mg/mL of tetracycline hydrochloride in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Columns
Guard: 4.6-mm × 3-cm; 10-µm packing L7
Analytical: 4.6-mm × 25-cm; 5–10-µm packing L7
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of 4-epianhydrotetracycline and tetracycline are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.2 between 4-epianhydrotetracycline and tetracycline, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of tetracycline from the *Sample solution*
r_S = peak response of tetracycline from the *Standard solution*
C_S = concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tetracycline hydrochloride in the *Sample solution* (mg/mL)
P = potency of USP Tetracycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–125.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP 4-Epianhydrotetracycline Hydrochloride RS
$C_{22}H_{22}N_2O_7 \cdot HCl$ 462.88
USP Tetracycline Hydrochloride RS

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Tetracycline Hydrochloride Soluble Powder

» Tetracycline Hydrochloride Soluble Powder contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{22}H_{24}N_2O_8 \cdot HCl$.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
Identification—Shake a suitable quantity of Powder with methanol to obtain a solution containing the equivalent of 1 mg of tetracycline hydrochloride per mL, and filter. Using the filtrate so obtained as the *Test Solution*, proceed as directed under *Identification—Tetracyclines* ⟨193⟩.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.
Assay—Transfer an accurately weighed quantity of Powder to a high-speed blender jar containing an accurately measured volume of 0.1 N hydrochloric acid, so that the solution so obtained contains not less than 150 µg of tetracycline hydrochloride per mL, and blend for about 3 to 5 minutes. Proceed as directed for tetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, diluting an accurately measured volume of this solution quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Tetracycline Hydrochloride for Topical Solution

» Tetracycline Hydrochloride for Topical Solution is a dry mixture of Tetracycline Hydrochloride and Epitetracycline Hydrochloride with Sodium Metabisulfite packaged in conjunction with a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of tetracycline hydrochloride, when constituted as directed.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
Identification—Dissolve a suitable quantity in methanol to obtain a solution containing 1 mg of tetracycline hydrochloride per mL, and filter, if necessary, to obtain a clear solution. Using the clear solution as the *Test Solution*, proceed as directed under *Identification—Tetracyclines* ⟨193⟩: the specified result is obtained.
pH ⟨791⟩: between 1.9 and 3.5, in the solution constituted as directed in the labeling.
Loss on drying ⟨731⟩—Dry the contents of 1 container, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

Content of epitetracycline hydrochloride—
Standard solution—Use the *Standard preparation* prepared as directed under the *Assay*.
Test solution—Open the column stopcock of the *Chromatographic column* remaining from the *Assay preparation* in the *Assay*, and collect the eluate in a low-actinic, 50-mL volumetric flask until the column runs dry. Use this as the *Test solution*.
Procedure—Proceed as directed for *Procedure* in the *Assay*. Calculate the quantity, in mg, of epitetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in each mL of the constituted Topical Solution taken by the formula:

$$0.002(WP/V)(A_U/A_S)$$

in which A_U is the absorbance of the solution from the *Test solution*, and the other terms are defined therein: the quantity of epitetracycline hydrochloride is between 115.0% and 140.0% of the quantity of tetracycline hydrochloride found as determined in the *Assay*.

Assay—
Edetate disodium solution—Dissolve 74.4 g of edetate disodium in about 1800 mL of water, adjust with ammonium hydroxide to a pH of 7.0, dilute with water to 2000 mL, and mix.
Stationary phase—Mix 95 mL of *Edetate disodium solution* and 5 mL of a mixture of glycerine and polyethylene glycol 400 (4:1).
Alkaline methanol solution—On the day of use, prepare a mixture of methanol and ammonium hydroxide (19:1).
Column support—Suspend 300 g of chromatographic siliceous earth in 2000 mL of 6 N hydrochloric acid in a suitable vessel, and mix for about 15 minutes. Filter, and wash the siliceous earth with water until the last washing is neutral to moistened litmus paper. Suspend the washed siliceous earth in 2000 mL of a mixture of ethyl acetate and methanol (1:1), and mix for about 15 minutes. Filter, and dry the siliceous earth in vacuum at 60° for about 16 hours. Weigh, add 0.5 mL of *Stationary phase* for each g of dried siliceous earth, and shake until the column packing is uniformly moist. Store in a tight container.
Chromatographic column—Prepare as directed under *Column Partition Chromatography* ⟨621⟩, using a 10- × 300-mm chromatographic tube equipped with a solvent reservoir on the top and a stopcock on the bottom and packed with 8 ± 0.1 g of *Column support*.
Standard preparation—Transfer about 22 mg of USP Tetracycline Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask, add 1 mL of methanol, and swirl to dissolve. Dilute with *Stationary phase* to volume, and mix. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, dilute with *Stationary phase* to volume, and mix. Pipet 2.0 mL of the resulting solution into the *Chromatographic column*, and allow it to permeate the *Column support*. Add 20 mL of benzene to the solvent reservoir, and collect the eluate at the rate of about 1 mL per minute, using a 50-mL graduated cylinder as a receiver. When the benzene level reaches the top of *Column support*, add 60 mL of chloroform to the solvent reservoir, and continue collecting the eluate until 30 mL has been collected. Discard this eluate, and continue collecting the eluate in a low-actinic, 50-mL volumetric flask. When the chloroform level reaches the top of the *Column support*, add 10 mL of a mixture of butyl alcohol and chloroform (1:1) to the solvent reservoir, and replace the low-actinic, 50-mL volumetric flask with a 10-mL graduated cylinder. Collect 8 mL of the eluate, close the column stopcock, and transfer the eluate to the low-actinic, 50-mL volumetric flask. Rinse the graduated cylinder with 2 mL of chloroform, and add the

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rinsing to the volumetric flask. The eluate in the 50-mL volumetric flask is the *Standard preparation.*

Assay preparation—Constitute the Topical Solution as directed in the labeling. Transfer an accurately measured volume of the constituted Topical Solution, equivalent to about 4.4 mg of tetracycline hydrochloride, to a 25-mL volumetric flask, dilute with *Stationary phase* to volume, and mix. Pipet 2.0 mL of this solution into the *Chromatographic column,* and allow it to penetrate the *Column support.* Add 20 mL of benzene to the solvent reservoir, and collect the eluate at the rate of about 1 mL per minute, using a 50-mL graduated cylinder as a receiver. When the benzene level reaches the top of the *Column support,* add 60 mL of chloroform to the solvent reservoir, and continue collecting the eluate until 30 mL has been collected. Discard this eluate, and continue collecting the eluate in a low-actinic, 50-mL volumetric flask. When the chloroform level reaches the top of the *Column support,* add 50 mL of a mixture of butyl alcohol and chloroform (1:1) to the solvent reservoir, and replace the low-actinic, 50-mL volumetric flask with a 10-mL graduated cylinder. Collect 8 mL of the eluate, close the column stopcock, and transfer the eluate to the low-actinic, 50-mL volumetric flask. Rinse the graduated cylinder with 2 mL of chloroform, and add the rinsing to the volumetric flask. The eluate in the 50-mL volumetric flask is the *Assay preparation.* Retain the column for the test for *Content of epitetracycline hydrochloride.*

Procedure—Add 2.0 mL of *Alkaline methanol solution* to the *Standard preparation,* and the *Assay preparation,* dilute each with chloroform to volume, and mix. Concomitantly, within 10 minutes of preparation, determine the absorbances of these solutions at the wavelength of maximum absorbance at about 366 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of tetracycline hydrochloride in each mL of the constituted Topical Solution taken by the formula:

$$0.0002(WP/V)(A_U/A_S)$$

in which W is the weight, in mg, of USP Tetracycline Hydrochloride RS taken, P is the potency, in µg per mg, of USP Tetracycline Hydrochloride RS, V is the volume, in mL, of constituted Topical Solution taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Tetracycline Hydrochloride Ophthalmic Suspension

» Tetracycline Hydrochloride Ophthalmic Suspension is a sterile suspension of sterile Tetracycline Hydrochloride in a suitable oil. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{22}H_{24}N_2O_8 \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers of glass or plastic, containing not more than 15 mL. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
USP 4-Epianhydrotetracycline Hydrochloride RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for tetracycline, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay.*

Sterility Tests ⟨71⟩ : meets the requirements.

Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay—

Diluting solvent, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Tetracycline Hydrochloride.*

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, equivalent to about 50 mg of tetracycline hydrochloride, with the aid of 30 mL of cyclohexane to a 125-mL separator, add 30 mL of *Diluting solvent,* insert the stopper, and shake. Allow to separate, and collect the lower layer in a 100-mL volumetric flask. Repeat the extraction with two additional 30-mL portions of *Diluting solvent,* combining the extracts in the 100-mL volumetric flask. Add *Diluting solvent* to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Tetracycline Hydrochloride.* Calculate the quantity, in mg per mL, of $C_{22}H_{24}N_2O_8 \cdot HCl$ equivalent in the Ophthalmic Suspension taken by the formula:

$$(CP/10V)(r_U/r_S)$$

in which V is the volume, in mL, of Ophthalmic Suspension taken, and the other terms are as defined therein.

Tetracycline Hydrochloride Compounded Oral Suspension

DEFINITION

Tetracycline Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

Prepare Tetracycline Hydrochloride Compounded Oral Suspension 25 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Tetracycline Hydrochloride	2.50 g
Cetylpyridinium Chloride	10 mg
Xanthan Gum	0.15 g
Dibasic Sodium Phosphate	60 mg
Monobasic Sodium Phosphate	0.65 g
Sodium Hydroxide	0.30 g
Purified Water	35 mL
Suspension Structured Vehicle or Sugar-Free Suspension Structured Vehicle, a sufficient quantity to make	100 mL

Dissolve *Dibasic Sodium Phosphate* and *Monobasic Sodium Phosphate* in 25 mL of *Purified Water.* Separately dissolve a quantity of *Cetylpyridinium Chloride* in *Purified Water* and dilute, if necessary, with *Purified Water* to obtain 5 mL of a solution containing 10 mg of *Cetylpyridinium Chloride.* Mix this solution with 5 mL of the aqueous phosphate solution and add the resulting solution, in divided portions, with

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mixing, to the *Tetracycline Hydrochloride* in a glass mortar to completely wet the powder, and make a smooth paste. Transfer the remaining 20 mL of the aqueous phosphate solution to a beaker. Using moderate heat, stir to form a vortex, and slowly sprinkle the *Xanthan Gum* into the vortex to produce a uniform dispersion. Add this dispersion to the paste in the glass mortar, and mix until smooth, then add 20 mL of the *Suspension Structured Vehicle* or *Sugar-Free Suspension Structured Vehicle* to the mixture. Dissolve the *Sodium Hydroxide* in 5 mL of *Purified Water*, and while mixing, slowly add this solution to the prepared mixture. Complete the suspension by adding a sufficient quantity of the *Suspension Structured Vehicle* or *Sugar-Free Suspension Structured Vehicle* to make a final volume of 100 mL, and pass this final dispersion through a hand homogenizer prior to transferring it to the dispensing container.

ASSAY

- **PROCEDURE**

Diluent: Dimethylformamide and ammonium oxalate (27:68)

Mobile phase: Dimethylformamide, 0.1 M ammonium oxalate, and 0.2 M dibasic ammonium phosphate (27:68:5). Adjust, if necessary, with 3 N ammonium hydroxide or 3 N phosphoric acid to a pH of 7.6–7.7.

System suitability solution: 100 µg/mL of tetracycline hydrochloride and 25 µg/mL of USP 4-Epianhydrotetracycline Hydrochloride RS

Standard solution: 0.5 mg/mL of USP Tetracycline Hydrochloride RS in *Diluent*

Sample solution: Equivalent to 125 mg of tetracycline hydrochloride, from Oral Suspension, to a 250-mL volumetric flask add 200 mL of *Diluent*, and shake. Add *Diluent* to volume and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Columns

Pre-injection guard: 4.6-mm × 3-cm; 10-µm packing L7

Analytical: 4.6-mm × 25-cm; 5–10-µm packing L7

Flow rate: 2 mL/min

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 4-epianhydrotetracycline and tetracycline are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.2 between the 4-epianhydrotetracycline and tetracycline peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Tetracycline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tetracycline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.5–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, and protect from freezing.
- **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded when stored at controlled room temperature
- **LABELING:** Label it to state the *Beyond-Use Date*. Label it to state that it should not be frozen and that it is to be well shaken before use.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP 4-Epianhydrotetracycline Hydrochloride RS

USP Tetracycline Hydrochloride RS

Tetracycline Hydrochloride Tablets

» Tetracycline Hydrochloride Tablets contain not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{22}H_{24}N_2O_8 \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Tetracycline Hydrochloride RS

USP 4-Epianhydrotetracycline Hydrochloride RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for tetracycline, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

Dissolution ⟨711⟩—

Medium: water; 900 mL.

Apparatus 2: 75 rpm. Maintain a distance of 45 ± 5 mm between the blade and the inside bottom of the vessel.

Time: 60 minutes.

Procedure—Determine the amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Tetracycline Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.

Limit of 4-epianhydrotetracycline—Using the *Diluting solvent*, *Chromatographic system*, and *Procedure* set forth in the *Assay*, chromatograph a Standard solution prepared by dissolving an accurately weighed quantity of USP 4-Epianhydrotetracycline Hydrochloride RS in *Diluting solvent* to obtain a solution having a known concentration of about 15 µg per mL. Using the chromatogram so obtained and the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of 4-epianhydrotetracycline hydrochloride in the Tablets taken by the formula:

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$$(10C_E/T)(r_U/r_S)$$

in which C_E is the concentration, in µg per mL, of USP 4-Epianhydrotetracycline Hydrochloride RS in the Standard solution; T is the quantity, in mg, of tetracycline hydrochloride in the portion of Tablets taken to prepare the *Assay preparation*, based on the labeled quantity; and r_U and r_S are the 4-epianhydrotetracycline peak responses obtained from the *Assay preparation* and the Standard solution, respectively: not more than 3.0% is found.

Assay—

Diluting solvent, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Tetracycline Hydrochloride.*

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of tetracycline hydrochloride, to a 100-mL volumetric flask, add 50 mL of *Diluting solvent*, mix, and sonicate for 5 minutes. Allow to cool, add *Diluting solvent* to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Tetracycline Hydrochloride.* Calculate the quantity, in mg, of $C_{22}H_{24}N_2O_8 \cdot HCl$ in the portion of Tablets taken by the formula:

$$(CP/10)(r_U/r_S)$$

in which the terms are as defined therein.

Tetracycline Hydrochloride and Novobiocin Sodium Tablets

» Tetracycline Hydrochloride and Novobiocin Sodium Tablets contain the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) and novobiocin ($C_{31}H_{36}N_2O_{11}$).

Packaging and storage—Preserve in tight containers.
Labeling—Label the Tablets to indicate that they are intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
USP Novobiocin RS
Identification—Shake a suitable quantity of finely powdered Tablets with methanol to obtain a solution containing 1 mg of tetracycline hydrochloride per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed under *Identification—Tetracyclines* ⟨193⟩.
Disintegration ⟨701⟩: 60 minutes, simulated gastric fluid TS being substituted for water in the test.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation* with respect to tetracycline hydrochloride and to novobiocin sodium.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, of finely powdered Tablets in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.
Limit of 4-epianhydrotetracycline ⟨226⟩—To an accurately weighed quantity of finely powdered Tablets, equivalent to about 250 mg of tetracycline hydrochloride, add 10 mL of 0.1 N hydrochloric acid, and adjust with 6 N ammonium hydroxide to a pH of 7.8. Transfer this solution with the aid of *EDTA Buffer* to a 50-mL volumetric flask, dilute with *EDTA Buffer* to volume, and mix. Use this solution, without delay, as the *Test Solution:* not more than 2.0% is found.
Assay for tetracycline hydrochloride—Proceed as directed for tetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, except to use *Escherichia coli* ATCC 10536 as the test organism instead of *Staphylococcus aureus* ATCC 29737 and an inoculum composition of about 0.2 mL of stock suspension in each 100 mL of *Medium 3*. Transfer not less than 5 Tablets to a high-speed blender jar containing an accurately measured volume of 0.1 N hydrochloric acid, so that, after blending for about 3 to 5 minutes, the solution so obtained contains not less than 150 µg of tetracycline hydrochloride per mL. Dilute an accurately measured volume of this solution quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration of tetracycline hydrochloride assumed to be equal to the median dose level of the Standard.
Assay for novobiocin—Proceed as directed for novobiocin under *Antibiotics—Microbial Assays* ⟨81⟩, blending not less than 5 Tablets for 3 to 5 minutes in a high-speed glass blender jar containing 1.0 mL of polysorbate 80 and a sufficient accurately measured volume of *Buffer B.3* to provide a stock solution of convenient concentration. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with *Buffer B.6* to obtain a *Test Dilution* having a concentration of novobiocin assumed to be equal to the median dose level of the Standard.

Tetracycline Hydrochloride, Novobiocin Sodium, and Prednisolone Tablets

» Tetracycline Hydrochloride, Novobiocin Sodium, and Prednisolone Tablets contain not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) and novobiocin ($C_{31}H_{36}N_2O_{11}$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone ($C_{21}H_{28}O_5$).

Packaging and storage—Preserve in tight containers.
Labeling—Label the Tablets to indicate that they are intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
USP Novobiocin RS
USP Prednisolone RS
Disintegration ⟨701⟩: 60 minutes, simulated gastric fluid TS being substituted for water in the test.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation* with respect to tetracycline hydrochloride and to novobiocin sodium and for *Content Uniformity* with respect to prednisolone.

Procedure for content uniformity for prednisolone—Mobile phase, Internal standard solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Prednisolone.*
Standard preparation—Prepare as directed in the *Assay for prednisolone.*
Test preparation—Transfer 1 Tablet to a suitable container, add 7 mL of diluted methanol (2 in 7) for each 1.5 mg of prednisolone in the Tablet, based on the labeled amount, and allow to stand for 90 minutes, occasionally agitating gently to

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ensure that the Tablet disintegrates. For each 1.5 mg of prednisolone, add 3.0 mL of *Internal standard solution*, 12 mL of water-saturated chloroform, and about 10 glass beads. Securely close the container, and shake by mechanical means for 30 minutes. Carefully open the container, add 0.5 mL of sodium carbonate solution (1 in 4), reclose the container, and shake by mechanical means for 5 minutes. Centrifuge, remove the upper layer by aspiration, discarding the aspirated liquid, and retain the clear chloroform layer (*Test preparation*). *Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Prednisolone*. Calculate the quantity, in mg, of prednisolone ($C_{21}H_{28}O_5$) in the Tablet taken by the formula:

$$0.015C(R_U/R_S)$$

in which the terms are as defined therein.

Limit of 4-epianhydrotetracycline ⟨226⟩—To an accurately weighed quantity of finely powdered Tablets, equivalent to about 250 mg of tetracycline hydrochloride, add 10 mL of 0.1 N hydrochloric acid, and adjust with 6 N ammonium hydroxide to a pH of 7.8. Transfer this solution with the aid of *EDTA Buffer* to a 50-mL volumetric flask, dilute with *EDTA Buffer* to volume, and mix. Use this solution, without delay, as the *Test Solution:* not more than 2.0% is found.

Other requirements—Tablets respond to the *Identification* test and meet the requirements of the test for *Loss on drying* under *Tetracycline Hydrochloride and Novobiocin Sodium Tablets*.

Assay for tetracycline hydrochloride and Assay for novobiocin—Using Tablets, proceed as directed in the *Assay for tetracycline hydrochloride* and the *Assay for novobiocin* under *Tetracycline Hydrochloride and Novobiocin Sodium Tablets*.

Assay for prednisolone—

Mobile phase, Internal standard solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Prednisolone*.

Standard preparation—Transfer about 10 mg of USP Prednisolone RS, accurately weighed, to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, and swirl to dissolve. Dilute with water-saturated chloroform to volume, and mix. Transfer 15.0 mL of this solution to a suitable container, add 7 mL of diluted methanol (2 in 7), securely close the container, and shake by mechanical means for 30 minutes. Carefully open the container, add 0.5 mL of sodium carbonate solution (1 in 4), reclose the container, and shake by mechanical means for 5 minutes. Centrifuge, remove the upper layer by aspiration, discarding the aspirated liquid, and retain the clear chloroform layer (*Standard preparation*).

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1.5 mg of prednisolone, to a suitable container containing about 10 glass beads. Add 3.0 mL of *Internal standard solution*, 12 mL of water-saturated chloroform, and 7 mL of diluted methanol (2 in 7), securely close the container, and shake by mechanical means for 30 minutes. Carefully open the container, add 0.5 mL of sodium carbonate solution (1 in 4), reclose the container, and shake by mechanical means for 5 minutes. Centrifuge, remove the upper layer by aspiration, discarding the aspirated liquid, and retain the clear chloroform layer (*Assay preparation*).

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Prednisolone*. Calculate the quantity, in mg, of prednisolone ($C_{21}H_{28}O_5$) in the portion of Tablets taken by the formula:

$$0.015C(R_U/R_S)$$

in which the terms are as defined therein.

Tetracycline Hydrochloride and Nystatin Capsules

» Tetracycline Hydrochloride and Nystatin Capsules contain not less than 90.0 percent and not more than 125.0 percent of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$), and not less than 90.0 percent and not more than 135.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Tetracycline Hydrochloride RS
USP Nystatin RS

Identification—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing about 1 mg of tetracycline hydrochloride per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed under *Identification—Tetracyclines* ⟨193⟩.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 75 rpm.
Time: 60 minutes.

Procedure—Determine the amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Tetracycline Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 70% (*Q*) of the labeled amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ is dissolved in 60 minutes.

Loss on drying ⟨731⟩—Dry about 100 mg of Capsule contents, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 4.0% of its weight.

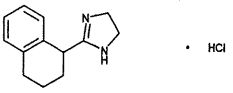
Limit of 4-epianhydrotetracycline ⟨226⟩—To a quantity of Capsule contents, equivalent to about 250 mg of tetracycline hydrochloride, add 10 mL of 0.1 N hydrochloric acid, and adjust with 6 N ammonium hydroxide to a pH of 7.8. Transfer this solution with the aid of *EDTA Buffer* to a 50-mL volumetric flask, dilute with *EDTA Buffer* to volume, and mix. Use this solution, without delay, as the *Test Solution:* not more than 3.0% is found.

Assay for tetracycline hydrochloride—Proceed with Capsules as directed in the *Assay* under *Tetracycline Hydrochloride Capsules*.

Assay for nystatin—Proceed as directed for Nystatin under *Antibiotics—Microbial Assays* ⟨81⟩, blending not less than 5 Capsules for 3 to 5 minutes in a high-speed blender with a sufficient, accurately measured, volume of dimethylformamide to obtain a solution of convenient concentration. Dilute an accurately measured portion of this solution quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute this stock solution quantitatively with *Buffer B.6* to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.

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Tetrahydrozoline Hydrochloride



$C_{13}H_{16}N_2 \cdot HCl$ 236.74

1*H*-Imidazole, 4,5-dihydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl)-, monohydrochloride;
2-(1,2,3,4-Tetrahydro-1-naphthyl)-2-imidazoline monohydrochloride [522-48-5].

DEFINITION

Tetrahydrozoline Hydrochloride contains NLT 98.0% and NMT 102.0% of tetrahydrozoline hydrochloride ($C_{13}H_{16}N_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩,** *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General ⟨191⟩,** *Chloride*
  Sample solution: 5 mg/mL
  Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**
  Buffer: 2.64 g/L of dibasic ammonium phosphate in water, adjusted with ammonium hydroxide to a pH of 9.0
  Mobile phase: Acetonitrile and *Buffer* (15:85)
  Standard solution: 0.025 mg/mL of USP Tetrahydrozoline Hydrochloride RS in water
  Sample solution: 0.025 mg/mL of Tetrahydrozoline Hydrochloride in water
  Chromatographic system
  (See *Chromatography ⟨621⟩, System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 15-cm; 3.5-µm packing L1
  Column temperature: 37°
  Flow rate: 1.2 mL/min
  Injection volume: 25 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of tetrahydrozoline hydrochloride ($C_{13}H_{16}N_2 \cdot HCl$) in the portion of Tetrahydrozoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Tetrahydrozoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Tetrahydrozoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Organic Impurities**
  Diluent: Methanol and 1 M sodium hydroxide (75:25)
  Standard solution: 0.1 mg/mL of USP Tetrahydrozoline Hydrochloride RS in *Diluent*
  Sample solution: 100 mg/mL of Tetrahydrozoline Hydrochloride in *Diluent*
  Chromatographic system
  (See *Chromatography ⟨621⟩, System Suitability*.)
  Mode: GC
  Detector: Flame ionization
  Column: 25-m × 0.32-mm; 1.0-µm coating of G1
  Temperatures
  Injection port: 220°
  Detector: 220°
  Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
160	0	160	8
160	20	220	4

Carrier gas: Helium
Flow rate: 2.5 mL/min
Injection volume: 1 µL
Injection type: Split, 40:1
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3%
Signal-to-noise ratio: NLT 50
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tetrahydrozoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of tetrahydrozoline from the *Standard solution*
C_S = concentration of USP Tetrahydrozoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Tetrahydrozoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
α-Cyanotetraline[a]	0.5	0.1
Tetrahydrozoline[b]	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	0.2

[a] 1,2,3,4-Tetrahydronaphthalene-1-carbonitrile.
[b] 2-(1,2,3,4-Tetrahydro-1-naphthyl)-2-imidazoline.

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SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tetrahydrozoline Hydrochloride RS

Tetrahydrozoline Hydrochloride Nasal Solution

» Tetrahydrozoline Hydrochloride Nasal Solution is a solution of Tetrahydrozoline Hydrochloride in water adjusted to a suitable tonicity. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{13}H_{16}N_2 \cdot HCl$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Tetrahydrozoline Hydrochloride RS
Identification—The UV absorption spectrum of the Nasal Solution, diluted with water, if necessary, to a concentration of about 1 in 4000, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Tetrahydrozoline Hydrochloride RS, concomitantly measured.
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
pH ⟨791⟩: between 5.3 and 6.5.

Assay—
Oxidized nitroprusside reagent—Dissolve 1.0 g of sodium nitroferricyanide in water to make 10.0 mL (*Solution A*). Dissolve 1.0 g of potassium ferricyanide in water to make 10.0 mL (*Solution B*). Transfer 1.0 mL each of *Solution A* and *Solution B* to a 100-mL volumetric flask, add 1 mL of sodium hydroxide solution (1 in 10), and allow to stand until the solution changes to a light yellow color (about 20 to 30 minutes). Dilute with water to volume, and mix. Store in a refrigerator or keep in an ice bath, and use within 4 hours.
Standard preparation—Dissolve a suitable quantity of USP Tetrahydrozoline Hydrochloride RS, accurately weighed, in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 100 µg per mL.
Assay preparation—Transfer an accurately measured volume of Nasal Solution, equivalent to about 10 mg of tetrahydrozoline hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Transfer 5.0 mL each of the *Standard preparation* and the *Assay preparation* to separate glass-stoppered test tubes. Pipet 5 mL of water into a third tube to provide a blank. To each tube add 4.0 mL of *Oxidized nitroprusside reagent*, mix, and allow to stand at 30° for 15 minutes. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of $C_{13}H_{16}N_2 \cdot HCl$ in each mL of the Nasal Solution taken by the formula:

$$0.1(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Tetrahydrozoline Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Nasal Solution taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Tetrahydrozoline Hydrochloride Ophthalmic Solution

DEFINITION
Tetrahydrozoline Hydrochloride Ophthalmic Solution is a sterile, isotonic solution of Tetrahydrozoline Hydrochloride in water. It contains NLT 90.0% and NMT 110.0% of the labeled amount of tetrahydrozoline hydrochloride ($C_{13}H_{16}N_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Diluent: Dilute hydrochloric acid (1 in 100)
  Sample solution: Dilute with *Diluent* (1 in 4000).
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of a similar solution of USP Tetrahydrozoline Hydrochloride RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 20 mM dibasic ammonium phosphate in water. Adjust with ammonium hydroxide to a pH of 9.0.
  Mobile phase: Acetonitrile and *Solution A* (15:85)
  Standard solution: 0.025 mg/mL of USP Tetrahydrozoline Hydrochloride RS in water
  Sample solution: Nominally 0.025 mg/mL of tetrahydrozoline hydrochloride prepared as follows. Transfer a suitable volume of Ophthalmic Solution to a suitable volumetric flask. Dilute with water to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 15-cm; 3.5-µm packing L1
  Column temperature: 37°
  Flow rate: 1.2 mL/min
  Injection volume: 25 µL
  Run time: NLT 2 times the retention time of tetrahydrozoline
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: 0.8–2.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of tetrahydrozoline hydrochloride ($C_{13}H_{16}N_2 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

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r_U = peak response of tetrahydrozoline from the *Sample solution*
r_S = peak response of tetrahydrozoline from the *Standard solution*
C_S = concentration of USP Tetrahydrozoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tetrahydrozoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 20 mM dibasic ammonium phosphate in water. Adjust with ammonium hydroxide to a pH of 9.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
11.0	85	15
12.0	60	40
26.0	60	40
27.0	30	70
41.0	30	70
42.0	85	15
50.0	85	15

Standard stock solution: 0.25 mg/mL of USP Tetrahydrozoline Hydrochloride RS in water
Standard solution: 5 µg/mL of USP Tetrahydrozoline Hydrochloride RS from the *Standard stock solution* in water
Sensitivity solution: 0.25 µg/mL of USP Tetrahydrozoline Hydrochloride RS from the *Standard solution* in water
Sample solution: Nominally 250 µg/mL of tetrahydrozoline hydrochloride in water prepared as follows. Transfer a suitable volume of Ophthalmic Solution to a suitable volumetric flask. Dilute with water to volume and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 37°
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified and any unspecified degradation product in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified and any unspecified degradation product from the *Sample solution*
r_S = peak response of tetrahydrozoline from the *Standard solution*
C_S = concentration of USP Tetrahydrozoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tetrahydrozoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tetrahydrozoline acid[a]	0.8	2.0
Tetrahydrozoline	1.0	—
Tetrahydrozoline amide[b]	1.8	2.0
Tetrahydrozoline related compound A[c]	4.4	2.0
Tetrahydrozoline methyl ester[d]	5.3	2.0
Any unspecified degradation product	—	1.0
Total degradation products	—	3.0

[a] 1,2,3,4-Tetrahydronaphthalene-1-carboxylic acid.
[b] *N*-(2-Aminoethyl)-1,2,3,4-tetrahydronaphthalene-1-carboxamide.
[c] 1,2,3,4-Tetrahydronaphthalene-1-carbonitrile.
[d] Methyl 1,2,3,4-tetrahydronaphthalene-1-carboxylate.

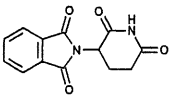
SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **pH** ⟨791⟩: 5.8–6.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tetrahydrozoline Hydrochloride RS

Thalidomide



$C_{13}H_{10}N_2O_4$ 258.23
1*H*-Isoindole-1,3(2*H*)-dione, 2-(2,6-dioxo-3-piperidinyl)-, (±)-.
(±)-*N*-(2,6-Dioxo-3-piperidyl)phthalimide.
α-(*N*-Phthalimido)glutarimide [50-35-1].

» Thalidomide contains not less than 98.0 percent and not more than 101.5 percent of $C_{13}H_{10}N_2O_4$, calculated on the anhydrous basis.

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Packaging and storage—Preserve in tight containers, protected from light, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Thalidomide RS

Change to read:

Identification, ***▲Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—The total aerobic microbial count using the *Plate Method* is not more than 1000 cfu per g, and the total combined molds and yeasts count is not more than 100 cfu per g.

Water Determination, *Method Ic* ⟨921⟩ **:** not more than 0.5%.

Solvent: anhydrous dimethyl sulfoxide.

Chromatographic purity—

Solution A—Prepare a filtered and degassed mixture of water, acetonitrile, and phosphoric acid (95: 5: 0.1).

Solution B—Prepare a filtered and degassed mixture of water, acetonitrile, and phosphoric acid (85: 15: 0.1).

Diluent—Prepare a mixture of water, acetonitrile, and phosphoric acid (50: 50: 0.1).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Phthalic acid stock solution—Transfer about 100 mg of phthalic acid to a 100-mL volumetric flask, dissolve in a mixture of acetonitrile and water (80:5), and dilute with acetonitrile to volume. Mix, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a concentration of about 0.1 mg per mL.

Standard stock solution—Dissolve, with the aid of sonication, an accurately weighed quantity of USP Thalidomide RS in acetonitrile to obtain a solution having a known concentration of about 1 mg per mL.

Standard solution—Pipet 2.0 mL of the *Standard stock solution* and 2.0 mL of the *Phthalic acid stock solution* into a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pipet 10.0 mL of this solution into a 100-mL volumetric flask, add 10.0 mL of phosphoric acid solution (1 in 100), dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.0002 mg of phthalic acid per mL.

Test solution—Transfer about 100 mg of Thalidomide, accurately weighed, to a 50-mL volumetric flask, and dissolve, with the aid of sonication, in 40 mL of a mixture of water, acetonitrile, and phosphoric acid (50: 50: 0.1). Dilute with *Diluent* to volume, and mix. Pipet 10.0 mL of this solution into a 100-mL volumetric flask, add 10.0 mL of phosphoric acid solution (1 in 100), dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 218-nm detector and a 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–15	100→50	0→50	linear gradient
15–20	50→100	50→0	linear gradient
20–30	100	0	isocratic

Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.35 for phthalic acid and about 1.0 for thalidomide; the tailing factor for the phthalic acid and thalidomide peaks is not more than 2.0; and the relative standard deviation determined from the phthalic acid peak for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 200 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the percentage of each impurity in Thalidomide by the formula:

$$50{,}000(C_P/W)(r_i/r_P)$$

in which C_P is the concentration, in mg per mL, of phthalic acid in the *Standard solution; W* is the amount, in mg, of Thalidomide taken to prepare the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_P is the phthalic acid peak response obtained from the *Standard solution:* not more than 0.1% of any individual impurity is found; and not more than 0.3% of total impurities is found.

Ordinary impurities ⟨466⟩—

Test solution—Dissolve an accurately weighed quantity of Thalidomide in acetonitrile to obtain a solution having a concentration of about 2 mg per mL.

Standard solution—Dissolve an accurately weighed quantity of glutamine in a mixture of acetonitrile and water (1:1) to obtain a solution having a concentration of about 0.1 mg per mL.

Eluant: a mixture of methylene chloride, methanol, and acetic acid (75: 25: 0.05).

Application volume: 2 µL (*Standard solution*) and 100 µL (*Test solution*).

Visualization: 4.

Limit: 0.1%.

Change to read:

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and phosphoric acid (85: 15: 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard preparation—Transfer about 150 mg of phenacetin, accurately weighed, to a 100-mL volumetric flask, dissolve in about 80 mL of acetonitrile, dilute with acetonitrile to volume, and mix.

Standard preparation—Dissolve, with the aid of sonication, an accurately weighed quantity of USP Thalidomide RS in acetonitrile to obtain a solution having a known concentration of about 1 mg per mL. Transfer 10.0 mL of this solution and 5.0 mL of *Internal standard preparation* to a 100-mL volumetric flask, add 10.0 mL of phosphoric acid solution (1 in 100), dilute with water to volume, and mix.

Assay preparation—Transfer about 100 mg of Thalidomide, accurately weighed, to a 100-mL volumetric flask, and dissolve, with the aid of sonication, in 80 mL of acetonitrile. Dilute with acetonitrile to volume, and mix. Pipet 10.0 mL of this solution and 5.0 mL of *Internal standard preparation* into a 100-mL volumetric flask, add 10.0 mL of phosphoric acid solution (1 in 100), dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 237-nm detector and a 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the

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peak responses as directed for *Procedure:* the resolution, *R*, between thalidomide and phenacetin is not less than 3.0; the column efficiency determined from the thalidomide and phenacetin peaks is not less than 7000 and 9000 theoretical plates, respectively; the tailing factor is not more than 2.0; and the relative standard deviation for ▲the response ratio of thalidomide to phenacetin▲ (ERR 1-May-2019) is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{10}N_2O_4$ in the portion of Thalidomide taken by the formula:

$$1000C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Thalidomide RS in the *Standard preparation;* and R_U and R_S are the peak area ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Thalidomide Capsules

» Thalidomide Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of thalidomide ($C_{13}H_{10}N_2O_4$).

Packaging and storage—Preserve in tight containers, protected from light, at controlled room temperature. Do not repackage.

USP Reference standards ⟨11⟩—
USP Thalidomide RS

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—*Test solution*—Prepare a solution of it in acetonitrile containing about 3000 µg of thalidomide per mL.
Application volume: 5 µL.
Developing solvent system: a mixture of normal butyl acetate, glacial acetic acid, and butyl alcohol (50:25:5).

B: The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—The total aerobic microbial count using the *Plate Method* is not more than 1000 cfu per g, and the total combined molds and yeasts count is not more than 100 cfu per g. It meets the requirements of the test for absence of *Escherichia coli.*

Dissolution ⟨711⟩—
Medium—Add 1.0 mL of polyoxyethylene (23) lauryl ether solution, prepared by dissolving 50 g in 100 mL of water, to 0.225 M hydrochloric acid; 4000 mL.
Apparatus 2: 75 rpm.
Time: 60 minutes.
Determine the amount of $C_{13}H_{10}N_2O_4$ dissolved by employing the following method.
Mobile phase—Prepare as directed in the *Assay* under *Thalidomide.*
Internal standard solution—Prepare a solution of phenacetin in acetonitrile containing about 375 µg per mL. Pipet 20.0 mL of this solution into a 100-mL volumetric flask, add 10.0 mL of phosphoric acid solution (1 in 100), dilute with water to volume, and mix.
Standard solution—Dissolve an accurately weighed quantity of USP Thalidomide RS in acetonitrile to obtain a solution having a known concentration of about 0.25 mg per mL. Pipet 10.0 mL of this solution into a 100-mL volumetric flask, add 10.0 mL of phosphoric acid solution (1 in 100), dilute with water to volume, and mix. Add 5.0 mL of *Internal standard solution* to 20.0 mL of this solution, and mix. This solution contains about 0.02 mg of USP Thalidomide RS per mL.
Test solution—Add 5.0 mL of *Internal standard solution* to each 20.0 mL of filtered solution under test, and mix.
Chromatographic system—Prepare as directed in the *Assay* under *Thalidomide.*
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{10}N_2O_4$ dissolved by the formula:

$$2500C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Thalidomide RS in the *Standard solution;* and R_U and R_S are the peak area ratios of thalidomide to the internal standard obtained from the *Test solution* and the *Standard solution,* respectively.
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{13}H_{10}N_2O_4$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase, Internal standard preparation, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Thalidomide.*
Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of thalidomide, to a 100-mL volumetric flask, add 80 mL of acetonitrile to dissolve, and sonicate for about 20 minutes. Dilute with acetonitrile to volume, and mix. Transfer 20.0 mL of this solution and 5.0 mL of *Internal standard preparation* to a 100-mL volumetric flask, add 10.0 mL of phosphoric acid solution (1 in 100), dilute with water to volume, and mix.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of thalidomide ($C_{13}H_{10}N_2O_4$) in the portion of Capsules taken by the formula:

$$1000C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Thalidomide RS in the *Standard preparation;* and R_U and R_S are the peak area ratios of thalidomide to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thallous Chloride Tl 201 Injection

» Thallous Chloride Tl 201 Injection is a sterile, isotonic, aqueous solution of radioactive thallium (^{201}Tl) in the form of thallous chloride suitable for

USP Monographs

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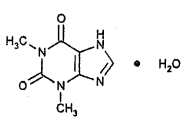
intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{201}Tl as chloride, expressed in megabecquerels (microcuries or millicuries) per mL, at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5.0 percent of the total radioactivity. It may contain a preservative or stabilizer.

Packaging and storage—Preserve in single-dose or multiple-dose containers.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{201}Tl as labeled thallous chloride expressed as total megabecquerels (microcuries or millicuries) and concentration as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{201}Tl is 73.1 hours.
Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{201}Tl of known purity that exhibits a major photopeak at an energy of 167 KeV and a minor photopeak of 135 KeV.
Bacterial Endotoxins Test ⟨85⟩—The limit of endotoxin content is not more than $175/V$ USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩: between 4.5 and 7.5.
Radiochemical purity —Soak a 2.5- × 15.0-cm cellulose polyacetate strip in 0.05 M edetate disodium for 45 to 60 minutes. Remove the strip with forceps, taking care to handle the outer edges only. Place the strip between two absorbent pads, and blot to remove excess solution. Apply not less than 5 µL of a previously mixed solution consisting of equal volumes of Injection and 0.05 M edetate disodium to the center of the blotted strip, and mark the point of application. Attach the strip to the support bridge of an electrophoresis chamber containing equal portions of 0.05 M edetate disodium in each side of the chamber. Ensure that each end of the strip is in contact with the 0.05 M edetate disodium. Attach the chamber cover, and perform the electrophoresis at 250 volts for 30 minutes. Remove the strip from the chamber, and allow to air-dry without blotting. Using a suitable scanner and counting assembly, determine the radioactivity. Not less than 95.0% of the radioactivity on the strip migrates toward the cathode as a single peak.
Radionuclidic purity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity of each radionuclidic impurity in the Injection by use of a calibrated system. Not less than 95.0% of the total radioactivity is present as thallium 201. In addition, not more than 2.0% of thallium 200 (half-life is 26.1 hours), not more than 0.3% of lead 203 (half-life is 52.02 hours), and not more than 2.7% of thallium 202 (half-life is 12.23 days) are present.

Content of thallium—
Standard thallium solution—Transfer 235 mg of thallous chloride, accurately weighed, to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with saline TS containing 0.9% benzyl alcohol to volume, and mix. This standard solution contains 2 µg of thallium per mL.
Procedure—Transfer 1.0-mL portions of the *Standard thallium solution* and the Injection to separate screw-cap test tubes. To each tube add 2 drops of a solution, prepared by carefully mixing 18 mL of nitric acid and 82 mL of hydrochloric acid, and mix. Then add to each tube 1.0 mL of sulfosalicylic acid solution (1 in 10), and mix. Add 2 drops of 12 N hydrochloric acid to each tube, and mix. To each tube add 4 drops of rhodamine B solution (50 mg of rhodamine B diluted with hydrochloric acid to 100.0 mL), and mix. Add 1.0 mL of diisopropyl ether. Screw the caps on tightly, shake the tubes by hand for 1 minute, accurately timed, releasing any pressure build-up by loosening the caps slightly. Recap the tubes and allow the phases to separate. Transfer 0.5 mL of the diisopropyl ether layer from each tube to clean tubes. Visually compare the ether layers: the color of the ether layer from the Injection is not darker than that from the *Standard thallium solution.*
Iron—Into separate cavities of a spot plate, place 0.1 mL of the Injection and 0.1 mL of *Standard Iron Solution* (see *Iron* ⟨241⟩) diluted with water to a concentration of 5 µg per mL. Add to each cavity 0.1 mL of hydroxylamine hydrochloride solution (1 in 10), 1 mL of sodium acetate solution (1 in 4), and 0.1 mL of 0.5% dipyridyl solution (0.5 g of 2,2′-dipyridyl dissolved in 100 mL of water containing 0.15 mL of hydrochloric acid), and mix. After 5 minutes, the color of the specimen of Injection is not darker than that of the *Standard Iron Solution.*

Copper—
Standard copper solution—Dissolve 0.982 g of $CuSO_4 \cdot 5H_2O$ in 1000 mL of 0.1 N hydrochloric acid. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix to obtain a Standard solution containing 5 µg of copper per mL.
Procedure—Into separate cavities of a spot plate, place 0.2 mL of the Injection and 0.2 mL of *Standard copper solution.* Add to each cavity 0.2 mL of water and 0.1 mL of iron thiocyanate solution (1.5 g ferric chloride and 2 g potassium thiocyanate dissolved in water and diluted with water to 100.0 mL). Mix, then add 0.1 mL of sodium thiosulfate solution (1 in 100), and again mix. The time required for the specimen of Thallous Chloride Tl 201 Injection to decolorize is equal to or longer than that observed for the *Standard copper solution.*
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility,* the latter test being started on the day of final manufacture, and except that it is not subject to the recommendations on *Container Content.*
Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity in MBq (µCi or mCi) per mL of Injection by use of a calibrated system.

Theophylline



$C_7H_8N_4O_2 \cdot H_2O$ 198.18
$C_7H_8N_4O_2$ 180.17
1*H*-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-, monohydrate;
Theophylline monohydrate [5967-84-0].
Anhydrous [58-55-9].

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DEFINITION

Theophylline contains one molecule of water of hydration or is anhydrous. It contains NLT 97.0% and NMT 102.0% of theophylline ($C_7H_8N_4O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in 80% flask volume of water. Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 0.2 mg/mL of USP Theophylline Related Compound F RS in water

System suitability solution: 1 mg/mL of USP Theophylline RS and 1 µg/mL of USP Theophylline Related Compound F RS, from *Impurity stock solution*, in water. Sonicate as needed to aid in the dissolution.

Standard solution: 0.2 mg/mL of USP Theophylline RS in water

Sample solution: 0.2 mg/mL of Theophylline in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 2.1-mm × 10-cm; 1.7-µm packing L1

Column temperature: 40°

Flow rate: 0.4 mL/min

Injection volume: 1 µL

System suitability

Samples: *System suitability solution* and *Standard solution*. [NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline ($C_7H_8N_4O_2$) in the portion of Theophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U = concentration of Theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.15%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Impurity stock solution, and **System suitability solution:** Proceed as directed in the *Assay*.

Standard stock solution: 50 µg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS

Standard solution: 1 µg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS, from *Standard stock solution*, in water

Sample solution: 1.0 mg/mL of Theophylline in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector

For unspecified impurities with absorption maxima near 220 nm: UV 220 nm

For specified impurities and unspecified impurities with absorption maxima near 270 nm: UV 270 nm

Column: 2.1-mm × 10-cm; 1.7-µm packing L1

Column temperature: 40°

Flow rate: 0.4 mL/min

Injection volume: 1 µL

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 3.0% for each peak present in the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

For impurities detected at 270 nm

Calculate the percentage of caffeine, theophylline related compound B, theophylline related compound C, and theophylline related compound D in the portion of Theophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of caffeine, theophylline related compound B, theophylline related compound C, or theophylline related compound D from the *Sample solution*

r_S = peak response of the corresponding Reference Standard from the *Standard solution*

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C_S = concentration of USP Caffeine RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, or USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)
C_U = concentration of Theophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity with absorption maxima near 270 in the portion of Theophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified impurity from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = concentration of Theophylline in the *Sample solution* (mg/mL)

For impurities detected at 220 nm
Calculate the percentage of any other individual unspecified impurity with absorption maxima near 220 in the portion of Theophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified impurity from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = concentration of Theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C	0.36	0.10
Theophylline related compound B	0.63	0.10
Theophylline related compound D	0.69	0.10
Theophylline	1.0	—
Theophylline related compound F[a]	1.09	—
Caffeine	1.20	0.10
Any other individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] Included for establishing system suitability only.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: The hydrous form loses 7.5%–9.5% of its weight, and the anhydrous form loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **Labeling:** Label it to indicate whether it is hydrous or anhydrous.
• **USP Reference Standards** ⟨11⟩
USP Caffeine RS
USP Theophylline RS
USP Theophylline Related Compound B RS
3-Methyl-1*H*-purine-2,6-dione.
$C_6H_6N_4O_2$ 166.14
USP Theophylline Related Compound C RS
N-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.
$C_7H_{10}N_4O_3$ 198.18
USP Theophylline Related Compound D RS
Theophyllidine;
N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide hydrochloride monohydrate.
$C_6H_{10}N_4O \cdot HCl \cdot H_2O$ 208.65
USP Theophylline Related Compound F RS
Etophylline;
7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.
$C_9H_{12}N_4O_3$ 224.22

Theophylline Capsules

» Theophylline Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Theophylline RS

Identification—
A: The contents of the Capsules respond to *Identification* tests *A* and *B* under *Theophylline Tablets*.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.
Procedure—Determine the amount of $C_7H_8N_4O_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 268 nm of filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, if necessary, in comparison with a Standard solution having a known concentration of USP Theophylline RS in the same medium.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_7H_8N_4O_2$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

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Assay—

Mobile phase—Prepare a solution containing a mixture of water, methanol, and glacial acetic acid (64:35:1).

Standard preparation—Dissolve an accurately weighed quantity of USP Theophylline RS in methanol to obtain a solution having a known concentration of about 400 µg per mL.

Assay preparation for hard Capsules—Remove, as completely as possible, the contents of not less than 20 Capsules, weigh, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of anhydrous theophylline, to a 250-mL volumetric flask, add about 150 mL of methanol, and shake to dissolve. Dilute with methanol to volume, mix, and filter, using a membrane filter.

Assay preparation for soft Capsules—Cut open 20 Capsules, and place them in a 200-mL volumetric flask. Add 50 mL of 6 N ammonium hydroxide, shake to dissolve the contents, add water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Transfer an accurately measured portion of the filtrate, equivalent to about 100 mg of anhydrous theophylline, to a 250-mL volumetric flask, add methanol to volume, mix, and filter through a membrane filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses. Calculate the quantity, in mg, of anhydrous theophylline in the portion of Capsule contents taken by the formula:

$$0.25C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Theophylline RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Theophylline Extended-Release Capsules

DEFINITION

Theophylline Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 10 mM ammonium acetate prepared as follows. Transfer 0.77 g of ammonium acetate to a 1-L volumetric flask, and dissolve in 80% of the flask volume of water. Adjust with glacial acetic acid to a pH of 5.4 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93.5	6.5
2.5	93.5	6.5
5.0	10	90
5.1	93.5	6.5
7.0	93.5	6.5

Diluent: Dilute 138 mL of ammonium hydroxide solution with water to 1 L.

Standard solution: 0.2 mg/mL of USP Theophylline RS

Sample stock solution: Nominally 4 mg/mL of theophylline prepared as follows. Transfer the contents of a suitable number of Capsules to a 500-mL volumetric flask. Add 150 mL of *Diluent* and heat on a hot plate to just boiling with occasional stirring. Remove from the hot plate and sonicate for 5 min while still hot. Cool to ambient temperature and dilute with water to volume.

Sample solution: Nominally 0.2 mg/mL of theophylline from *Sample stock solution*. Centrifuge and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm. For *Identification A*, use a photodiode array detector in the range of 210–400 nm.

Column: 2.1-mm × 10-cm; 1.7-µm packing L7

Column temperature: 40 ± 2°

Flow rate: 0.4 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

[NOTE—The following tests, which were assigned numbers chronologically, are placed in groups corresponding to product dosing intervals. Thus, individual tests do not necessarily appear in numerical order.]

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For products labeled for dosing every 12 h

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and 2, Delayed-Release Dosage Forms, Method B Procedure* except to use *Acceptance Table 2*.

Medium: pH 1.2 simulated gastric fluid (without pepsin) for the first hour; pH 6.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 50 rpm

Times: 1, 2, 4, 6, and 8 h

Standard solution: A known concentration of USP Theophylline RS in *Medium*

Sample solution: Filtered portions of the solution under test. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 271 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at each time point using the UV absorption.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	3–15
2	20–40
4	50–75
6	65–100
8	NLT 80

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Solution A: 6.8 g/L of monobasic potassium phosphate. Adjust with either 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 4.5 ± 0.05.

Medium: *Solution A*; 900 mL

Apparatus 2: 75 rpm

Times: 1, 2, 4, and 8 h

Analysis: Proceed as directed in *Test 1*.

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
1	10–30
2	30–55
4	55–80
8	NLT 80

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and 2, Delayed-Release Dosage Forms, Method B Procedure* except to use *Acceptance Table 2*.

Medium: pH 1.2 simulated gastric fluid (without pepsin) for 1 h; pH 7.5 simulated intestinal fluid (without enzyme); 900 mL

Apparatus 2: 50 rpm

Times: 1, 2, 3, 4, and 7 h

Analysis: Proceed as directed in *Test 1*.

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (%)
1	1–17
2	30–60
3	50–90
4	NLT 65
7	NLT 85

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and 2, Delayed-Release Dosage Forms, Method A Procedure* except to use *Acceptance Table 2*.

Medium: pH 3.0 phosphate buffer prepared by adjusting 0.05 M potassium phosphate buffer with phosphoric acid to a pH of 3.0 ± 0.05, for the first 3.5 h, followed by the addition of 5.3 M sodium hydroxide to adjust to a pH of 7.4 ± 0.05; 900 mL

Apparatus 2: 50 rpm

Times: 1, 2, 3.5, and 5 h

Analysis: Proceed as directed in *Test 1*.

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	13–38
2	25–50
3.5	37–65
5	85–115

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium, Apparatus 2, and **Analysis:** Proceed as directed in *Test 4*.

Times: 1, 3.5, 5, 7, and 10 h

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved (%)
1	10–30
3.5	30–60
5	50–80
7	NLT 65

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Table 6 *(continued)*

Time (h)	Amount Dissolved (%)
10	NLT 80

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Solution A: Dissolve 40.8 g of monobasic potassium phosphate in 6 L of water, add 667 mg of octoxynol 9, and adjust with dilute hydrochloric acid or sodium hydroxide to a pH of 4.5.

Medium: *Solution A*; 900 mL

Apparatus 2: 50 rpm

Times: 1, 2, 4, and 8 h

Analysis: Proceed as directed in *Test 1*.

Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved (%)
1	10–40
2	35–70
4	60–90
8	NLT 85

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: pH 7.5 simulated intestinal fluid (without enzyme); 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, 6, and 8 h

Analysis: Proceed as directed in *Test 1*.

Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved (%)
1	3–30
2	15–50
4	45–80
6	NLT 70
8	NLT 85

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

Medium 1: 0.1 N hydrochloric acid; 900 mL

Medium 2: Simulated intestinal fluid (without enzyme); 900 mL

Apparatus 1: 50 rpm

Times: 1, 2, 3, 4, and 6 h

Determine the percentage of the labeled amount of theophylline dissolved at the times specified, using *Medium 1* for the first h and *Medium 2* for the next 5 h.

Analysis: Proceed as directed in *Test 1*.

Tolerances: See *Table 9*.

Table 9

Time (h)	Amount Dissolved (%)
1	5–15
2	25–45
3	50–65
4	NLT 70
6	NLT 85

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 10: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*. Proceed as directed for *Test 3*.

Times: 1, 2, 4, and 8 h

Tolerances: See *Table 10*.

Table 10

Time (h)	Amount Dissolved (%)
1	6–27
2	25–50
4	65–85
8	NLT 80

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

For products labeled for dosing every 24 h

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: 0.05 M pH 6.6 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 1000 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, 5, and 8 h

Analysis: Proceed as directed in *Test 1*.

Tolerances: See *Table 11*.

Table 11

Time (h)	Amount Dissolved (%)
1	5–15
2	12–30
4	25–50
5	30–60
8	55–75

The percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved at the times given conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Standard solution: 12 µg/mL of USP Theophylline RS

Sample solution: Nominally 12 µg/mL of theophylline, prepared as follows. Using a mortar and pestle, triturate the contents of 1 Capsule with 20 mL of water. With the aid of water, transfer the mixture to a 100-mL volumetric flask. Add 25 mL of 6 N ammonium hydroxide, shake or

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sonicate for 45 min, and cool to room temperature. Dilute with water to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate. Dilute a portion of the filtrate with water to obtain the solution.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 270 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Concomitantly determine the absorbance of the *Sample solution* and *Standard solution*.

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in each Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Theophylline RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Sample stock solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.1 mg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS

System suitability solution: 1 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS from the *Standard stock solution*

Standard solution: 2.0 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS from the *Standard stock solution*

Sample solution: Nominally 1.0 mg/mL of theophylline from the *Sample stock solution*. Centrifuge and use the supernatant.

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 12* for relative retention times.]

Suitability requirements

Relative standard deviation: NMT 5.0% for the theophylline and theophylline related compound D RS peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline related compound D from the *Sample solution*

r_S = peak response of theophylline related compound D from the *Standard solution*

C_S = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 12*. Disregard peaks less than 0.1%.

Table 12

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound D	0.45	0.2
Theophylline	1.0	—
Any other individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **LABELING:** The labeling indicates whether the product is intended for dosing every 12 or 24 h, and states with which in vitro *Dissolution Test* the product complies.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Theophylline RS

USP Theophylline Related Compound D RS

Theophyllidine;

N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide hydrochloride monohydrate.

$C_6H_{10}N_4O \cdot HCl \cdot H_2O$ 208.65

Theophylline Oral Solution

DEFINITION

Theophylline Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of theophylline ($C_7H_8N_4O_2$).

IDENTIFICATION

• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 10 mM ammonium acetate prepared as follows. Transfer 771 mg/L of ammonium acetate to a suitable flask, and dissolve in water. Adjust with glacial acetic acid to a pH of 5.4 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93.5	6.5
2.5	93.5	6.5
5.0	10	90
5.1	93.5	6.5
7.0	93.5	6.5

Standard solution: 0.2 mg/mL of USP Theophylline RS in water
Sample solution: Nominally 0.2 mg/mL of theophylline from Oral Solution in water. Centrifuge and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm. For *Identification* test *A*, use a photodiode array detector in the range of 210–400 nm.
Column: 2.1-mm × 10-cm; 1.7-µm packing L7
Column temperature: 40 ± 2°
Flow rate: 0.4 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Impurity stock solution: 0.1 mg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water
System suitability solution: 10 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS from *Impurity stock solution*, and 10 µg/mL of USP Saccharin Sodium RS in water
Standard solution: 2.0 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS from *Impurity stock solution* in water
Sample solution: Nominally 1.0 mg/mL of theophylline from a portion of Oral Solution in water. Centrifuge and use the supernatant.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between theophyllidine and saccharin, *System suitability solution*
Relative standard deviation: NMT 3.0% for theophylline and theophylline related compound D peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of theophylline related compound D in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline related compound D from the *Sample solution*
r_S = peak response of theophylline related compound D from the *Standard solution*
C_S = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound D	0.45	0.2
Saccharin[a]	0.50	—
Theophylline	1.0	—
Any other individual unspecified degradation product	—	0.2
Total impurities	—	1.0

[a] Included as a potential excipient. Do not include in the calculation of total impurities.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*. The total aerobic microbial count does not exceed 1×10^2 cfu/mL, and the total combined molds and yeasts count does not exceed 5×10^1 cfu/mL.
• **PH** ⟨791⟩: 3.0–4.7
• **ALCOHOL DETERMINATION** ⟨611⟩, *Method II* (if present)
Analysis: Use acetone as the internal standard.

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Acceptance criteria: 90.0%–115.0% of the labeled amount of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** Label it to indicate the alcohol content (if present).
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alcohol Determination—Acetonitrile RS
  USP Alcohol Determination—Alcohol RS
  USP Saccharin Sodium RS
  USP Theophylline RS
  USP Theophylline Related Compound D RS
  Theophyllidine;
  N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide hydrochloride monohydrate.
  $C_6H_{10}N_4O \cdot HCl \cdot H_2O$ 208.65

Theophylline Compounded Oral Suspension

DEFINITION

Theophylline Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of theophylline ($C_7H_8N_4O_2$).

Prepare Theophylline Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Theophylline	500 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. Calculate the amount of *Theophylline* on the anhydrous basis. If using tablets, place the required number in a suitable mortar, and comminute to a fine powder, or use *Theophylline* powder. Add about 20 mL of the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container. Add enough of the liquid *Vehicle* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 2.500 g of sodium citrate and 1.633 g of citric acid (anhydrous) in 850 mL of ASTM Type I water, add 150 mL of methanol, and pass through 0.45-µm membrane filters.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Make adjustments, if necessary.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0.0
20.0	100	0.0
20.01	5.0	95.0
25.0	5.0	95.0

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
25.01	100	0.0

Diluent: *Solution A*

System suitability solution: 0.10 mg/mL of USP Theophylline RS and 0.20 mg/mL of USP Caffeine RS in *Solution A*

Standard solution: 0.10 mg/mL of USP Theophylline RS in *Solution A*

Sample solution: 0.10 mg/mL of theophylline prepared from Oral Suspension and *Solution A*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between the theophylline and caffeine peaks
Column efficiency: NLT 1000 theoretical plates for the theophylline peak
Tailing factor: NMT 1.5 for the theophylline peak
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature. Do not refrigerate.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **LABELING:** Label to state that it is to be well shaken before use, protected from light, not to be refrigerated, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Caffeine RS
  USP Theophylline RS

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Theophylline Tablets

» Theophylline Tablets contain not less than 94.0 percent and not more than 106.0 percent of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Theophylline RS

Identification—
A: Triturate a quantity of finely powdered Tablets, equivalent to about 500 mg of theophylline, with 10-mL and 5-mL portions of solvent hexane, and discard the solvent hexane. Triturate the residue with two 10-mL portions of a mixture of equal volumes of 6 N ammonium hydroxide and water, and filter each time. Evaporate the combined filtrates to about 5 mL, neutralize, if necessary, with 6 N acetic acid, using litmus, and then cool to about 15°, with stirring. Collect the precipitate on a filter, wash it with cold water, and dry at 105° for 2 hours: the theophylline so obtained melts between 270° and 274°, the procedure for *Class I* being used (see *Melting Range or Temperature* ⟨741⟩). Retain the remaining portion of the theophylline for use in *Identification* test *B*.
B: The IR absorption spectrum of a potassium bromide dispersion of the residue obtained in *Identification* test *A* exhibits maxima only at the same wavelengths as that of a potassium bromide dispersion of USP Theophylline RS.
C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_7H_8N_4O_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 272 nm of filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Theophylline RS in the same medium.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_7H_8N_4O_2$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase , *Internal standard solution*, and *Standard preparation*—Prepare as directed in the *Assay* under *Theophylline*.
Assay preparation—Place 10 Tablets in a 500-mL volumetric flask, add 50 mL of water, and when the Tablets have disintegrated add 50 mL of 6 N ammonium hydroxide. Shake until no more dissolves, dilute with water to volume, mix, and filter through a dry filter with the aid of suction, if necessary, into a dry flask, discarding the first 20 mL of the filtrate. Transfer an accurately measured aliquot portion of this concentrate, equivalent to about 10 mg of theophylline, to a 100-mL volumetric flask. Add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.
Chromatographic system—Proceed as directed in the *Assay* under *Theophylline*.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Theophylline*. Calculate the quantity, in mg, of $C_7H_8N_4O_2$ per Tablet taken by the formula:

$$5000(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Theophylline RS in the *Standard preparation*, V is the volume, in mL, of concentrate taken for the *Assay preparation*, and R_U and R_S are the response ratios of the theophylline peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Theophylline in Dextrose Injection

DEFINITION
Theophylline in Dextrose Injection is a sterile solution of Theophylline and Dextrose in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$) and NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$).

IDENTIFICATION
- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**

Sample solution: Theophylline in Dextrose Injection
Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A red-to-orange precipitate of cuprous oxide is formed.

ASSAY
- **THEOPHYLLINE**

Solution A: 10 mM ammonium acetate prepared as follows. Transfer 771 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in 80% of the flask volume of water. Adjust with glacial acetic acid to a pH of 4.8 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93.5	6.5
2.5	93.5	6.5
5.0	10	90
5.1	93.5	6.5
7.0	93.5	6.5

Standard solution: 0.1 mg/mL of USP Theophylline RS
Sample solution: Nominally 0.1 mg/mL of theophylline prepared as follows. Transfer 5 mg of theophylline from a volume of Injection to a 50-mL volumetric flask. Dissolve and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm. For *Identification A*, use a photodiode array detector in the range of 210–400 nm.
Column: 2.1-mm × 10-cm; 1.7-µm packing L7

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Column temperature: 40°
Flow rate: 0.4 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

• **DEXTROSE**
Sample solution: Nominally 2–5 g of dextrose per 100 mL, prepared as follows. Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.
Analysis
Sample: *Sample solution*
Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).
Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, and **Mobile phase:** Prepare as directed in the *Assay*.
System suitability solution: 0.4 µg/mL each of USP Theophylline Related Compound D RS and 5-hydroxymethylfurfural
Standard solution: 0.4 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS
Sample solution: Nominally 400 µg/mL of theophylline in water prepared as follows. Transfer 4 mg of theophylline from a volume of Injection to a 10-mL volumetric flask. Dissolve and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L7
Column temperature: 40°
Flow rate: 0.4 mL/min
Injection volume: 2.5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.1 between theophylline related compound D and 5-hydroxymethylfurfural, *System suitability solution*
Relative standard deviation: NMT 5.0% for theophylline and theophylline related compound D, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of theophylline related compound D in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline related compound D from the *Sample solution*
r_S = peak response of theophylline related compound D from the *Standard solution*
C_S = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (µg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound D	0.44	0.2
5-HMF[a]	0.47	—
Theophylline	1.0	—
Any other individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] 5-Hydroxymethylfurfural; the content of this impurity is controlled in the *Limit of 5-Hydroxymethylfurfural and Related Substances* test.

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• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Cation-exchange column: Proceed as directed in *Chromatography* ⟨621⟩ using a chromatographic tube capable of providing a 0.8- × 4-cm bed volume (or 2 mL) of 100- to 200-mesh, strongly acidic styrene-divinylbenzene cation-exchange resin. Condition the column by washing with 30 mL of water, discarding the eluate.

Sample solution: Pass a volume of Injection containing 100 mg of hydrous dextrose through the resin bed in the *Cation-exchange column*, allowing the sample to flow down the wall of the column so as not to disturb the resin bed, and collect the eluate in a 50-mL volumetric flask. Wash the column with 25 mL of water, and collect the eluate in the same 50-mL volumetric flask. Dilute the eluate with water to volume.

Blank solution: Pass 27 mL of water through a freshly conditioned *Cation-exchange column*, collecting the eluate in a 50-mL volumetric flask. Fill with water to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: *Blank solution*

Analysis

Samples: *Sample solution* and *Blank solution*

Determine the absorbance of the *Sample solution* and *Blank solution* with a suitable spectrophotometer.

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 1.0 USP Endotoxin Unit/mg of anhydrous theophylline

• **PH** ⟨791⟩

Sample solution: NMT 5% of dextrose from a portion of Injection in water, diluted with water if necessary

Acceptance criteria: 3.5–6.5

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass, or of a suitable plastic material. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Theophylline RS

USP Theophylline Related Compound D RS

Theophyllidine;

N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide hydrochloride monohydrate.

$C_6H_{10}N_4O \cdot HCl \cdot H_2O$ 208.65

Theophylline, Ephedrine Hydrochloride, and Phenobarbital Tablets

» Theophylline, Ephedrine Hydrochloride, and Phenobarbital Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of anhydrous theophylline ($C_7H_8N_4O_2$), ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$), and phenobarbital ($C_{12}H_{12}N_2O_3$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Theophylline RS

USP Ephedrine Sulfate RS

USP Phenobarbital RS

Identification—Place a quantity of finely powdered Tablets, equivalent to about 24 mg of ephedrine hydrochloride, in a 15-mL centrifuge tube, add 4.0 mL of a mixture of chloroform and methanol (4:1), mix by sonication for 10 minutes, and filter to obtain the test solution. Prepare separate Standard solutions containing known concentrations of about 36 mg of USP Theophylline RS per mL, about 9.6 mg of USP Ephedrine Sulfate RS per mL, and about 5 mg of USP Phenobarbital RS per mL, respectively, in the mixture of chloroform and methanol (4:1). Apply 2 µL of the test solution and of each of the three Standard solutions at equidistant points about 2.5 cm above one edge of a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a suitable chamber previously equilibrated with a solvent system consisting of a mixture of chloroform, acetone, methanol, and ammonium hydroxide (50:10:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, air-dry, and locate the spots on the plate by viewing under short-wavelength UV light: the R_F values of the spots obtained from the test solution correspond to those obtained from the Standard solutions.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Determine the amounts of theophylline, ephedrine hydrochloride, and phenobarbital dissolved using the following procedure.

Mobile phase—Dissolve accurately weighed quantities of monobasic potassium phosphate and sodium 1-hexanesulfonate in water, and dilute quantitatively with water to obtain a solution having concentrations of 0.953 mg per mL (0.007 M monobasic potassium phosphate) and 0.564 mg per mL (0.003 M sodium 1-hexanesulfonate), respectively. Adjust, if necessary, with 0.3 M phosphoric acid or 0.2 M monobasic potassium phosphate to a pH of 3.0 ± 0.05, to obtain a *Phosphate buffer*. The *Mobile phase* is a mixture of *Phosphate buffer* and methanol (75:25).

Standard preparation—Dissolve accurately weighed quantities of USP Theophylline RS, USP Ephedrine Sulfate RS, and USP Phenobarbital RS in water, and dilute quantitatively and stepwise with water to obtain a solution having known concentrations of about 145 µg of anhydrous theophylline per mL, 28 µg of ephedrine sulfate per mL, and 9 µg of phenobarbital per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 3.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the theophylline and phenobarbital peaks is not less than 4.0, the tailing factors for the ephedrine and phenobarbital peaks are not more than 3.0 and 2.0, respectively, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject an accurately measured volume (about 75 µL) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the responses for the major peaks. The elution order is theophylline, ephedrine, and phenobarbital (last). Calculate the quantity, in mg, of $C_7H_8N_4O_2$ dissolved by the formula:

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$$(0.9C)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Theophylline RS in the *Standard preparation*, and r_U and r_S are the peak responses for theophylline obtained from the solution under test and the similarly chromatographed *Standard preparation*, respectively. Calculate the quantity, in mg, of $C_{10}H_{15}NO \cdot HCl$ dissolved by the formula:

$$(201.69/214.27)(0.9C')(r'_U/r'_S)$$

in which 201.69 is the molecular weight of ephedrine hydrochloride, 214.27 is one-half the molecular weight of ephedrine sulfate, C' is the concentration, in µg per mL, of USP Ephedrine Sulfate RS in the *Standard preparation*, and r'_U and r'_S are the peak responses for ephedrine obtained from the solution under test and the similarly chromatographed *Standard preparation*, respectively. Calculate the quantity, in mg, of $C_{12}H_{12}N_2O_3$ dissolved by the formula:

$$(0.9C'')(r''_U/r''_S)$$

in which C'' is the concentration, in µg per mL, of USP Phenobarbital RS in the *Standard preparation*, and r''_U and r''_S are the peak responses for phenobarbital obtained from the solution under test and the similarly chromatographed *Standard preparation*, respectively.

Tolerances—Not less than 75% *(Q)* of the labeled amounts of $C_7H_8N_4O_2$, $C_{10}H_{15}NO \cdot HCl$, and $C_{12}H_{12}N_2O_3$ are dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—Mix 240 mL of acetonitrile with 760 mL of 0.01 M, pH 7.8 phosphate buffer (see *Buffer Solutions* under *Solutions* in the section *Reagents, Indicators, and Solutions*).

Internal standard solution—Dissolve 25 mg of butabarbital sodium in 50 mL of dibasic potassium phosphate solution (17 in 1000). Mix 40 mL of this solution with 10 mL of sodium metaperiodate solution (1 in 100).

Standard preparation—Transfer about 120 mg of USP Theophylline RS, about 8 mg of USP Phenobarbital RS, and about 25 mg of USP Ephedrine Sulfate RS, each accurately weighed, to a 200-mL volumetric flask. Add 10 mL of methanol and 100 mL of chloroform, mix to dissolve, add chloroform to volume, and mix. Pipet 15 mL of this solution into a 50-mL volumetric flask, add chloroform to volume, and mix. The concentrations of anhydrous theophylline, phenobarbital, and ephedrine sulfate in the *Standard preparation* are about 180 µg per mL, 12 µg per mL, and 37.5 µg per mL, respectively.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 120 mg of anhydrous theophylline, to a 200-mL volumetric flask, and shake by mechanical means for 20 minutes with a mixture of 10 mL of methanol and 100 mL of chloroform. Add chloroform to volume, mix, and filter. Pipet 15 mL of the clear filtrate into a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

Standard solution and *Assay solution*—Pipet 10-mL portions of the *Standard preparation* and the *Assay preparation* into separate glass-stoppered, 25-mL conical flasks, and treat each as follows: Evaporate in a warm-water bath with the aid of a current of air to dryness. Add by pipet 4 mL of *Internal standard solution*, insert the stopper in the flask, mix to dissolve the residue, and allow to stand at room temperature for 30 minutes. Pipet 1 mL of propylene glycol solution (1 in 100) into the flask, insert the stopper, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 241-nm detector and a 30-cm × 4-mm stainless steel column that contains packing L1. Set a flow rate of 1.0 mL per minute for the *Mobile phase*, and allow the system to equilibrate until a stable baseline is obtained on the recorder. Chromatograph a 10-µL portion of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the peaks are completely resolved, and the resolution factors between each two neighboring peaks are not less than 1.5. Five replicate injections of the *Standard preparation* show a relative standard deviation of not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Assay solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. In the order of increasing elution times, the five peaks correspond to the reagent (iodate), theophylline, phenobarbital, butabarbital, and benzaldehyde (from ephedrine). Designate the peak response ratio of each component to the internal standard in the *Standard solution* as R_S, and that of each component to the internal standard in the *Assay solution* as R_U.

Calculation for theophylline—Calculate the quantity, in mg, of anhydrous theophylline ($C_7H_8N_4O_2$) in the portion of Tablets taken by the formula:

$$0.667C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Theophylline RS in the *Standard preparation*.

Calculation for ephedrine hydrochloride—Calculate the quantity, in mg, of ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken by the formula:

$$(201.69/214.27)(0.667C)(R_U/R_S)$$

in which 201.69 is the molecular weight of ephedrine hydrochloride, 214.27 is one-half the molecular weight of ephedrine sulfate, and C is the concentration, in µg per mL, of USP Ephedrine Sulfate RS in the *Standard preparation*.

Calculation for phenobarbital—Calculate the quantity, in mg, of phenobarbital ($C_{12}H_{12}N_2O_3$) in the portion of Tablets taken by the formula:

$$0.667C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Phenobarbital RS in the *Standard preparation*.

Theophylline and Guaifenesin Capsules

» Theophylline and Guaifenesin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of anhydrous theophylline ($C_7H_8N_4O_2$) and guaifenesin ($C_{10}H_{14}O_4$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Guaifenesin RS
USP Theophylline RS

Identification—Transfer a quantity of the contents of the Capsules, equivalent to about 150 mg of theophylline, to a separator, and add 15 mL of water. To a second separator

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transfer 15 mL of an aqueous Standard solution containing USP Theophylline RS and USP Guaifenesin RS corresponding, proportionately, to the amounts of theophylline and guaifenesin in the Capsules and having a known concentration of about 10 mg of theophylline per mL. Treat the contents of each separator as follows. Add 25 mL of chloroform, and shake vigorously for 0.5 minute. Allow the layers to separate, filter the lower chloroform layer through glass wool, and evaporate the filtrate to dryness. Dissolve the residue in 10 mL of chloroform. Apply separately 10 µL of each solution so obtained to a thin-layer chromatographic cellulose sheet with fluorescent indicator (see *Chromatography* ⟨621⟩), allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methanol and water (95:5) until the solvent front has moved about 10 cm above the starting line. Remove the sheet from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the sheet to short-wavelength UV light: the R_F values of the spots obtained from the test preparation correspond to those obtained from the Standard solution.

Dissolution ⟨711⟩—
Medium: simulated gastric fluid; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amounts of theophylline ($C_7H_8N_4O_2$) and guaifenesin ($C_{10}H_{14}O_4$) dissolved in filtered portions of the solution under test and employing the procedure set forth in the *Assay*, making any necessary volumetric adjustments, in comparison with Standard solutions having known concentrations of USP Theophylline RS and USP Guaifenesin RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amounts of $C_7H_8N_4O_2$ and $C_{10}H_{14}O_4$ are dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
pH 6.5 buffer solution—Dissolve 1.36 g of monobasic potassium phosphate in water to make 1000 mL. Carefully adjust with 2.5 N sodium hydroxide to a pH of 6.5, and filter.
Mobile phase—Prepare a degassed solution of *pH 6.5 buffer solution* and methanol (70:30).
Internal standard solution—Dissolve about 400 mg of caffeine in 1000 mL of a solution of methanol and water (90:10), and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Theophylline RS in *pH 6.5 buffer solution*, and dilute quantitatively with *pH 6.5 buffer solution* to obtain a solution (*Solution T*) having a known concentration of about 900*J* µg per mL, in which *J* is the ratio of the labeled amount of theophylline to that of guaifenesin. Transfer about 90 mg of USP Guaifenesin RS, accurately weighed, to a 200-mL volumetric flask, add about 150 mL of *pH 6.5 buffer solution*, shake to dissolve, dilute with *pH 6.5 buffer solution* to volume, and mix. Pipet 10 mL of this solution, 10 mL of *Internal standard solution*, and 5 mL of *Solution T* into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 90 µg of guaifenesin and about 90*J* µg of theophylline per mL.
Assay preparation—Transfer a number of Capsules, equivalent to about 900 mg of guaifenesin, to a 200-mL volumetric flask, add about 160 mL of *pH 6.5 buffer solution*, heat to dissolve completely, cool to room temperature, dilute with *pH 6.5 buffer solution* to volume, and mix. Dilute 10.0 mL of this solution with *pH 6.5 buffer solution* to 100.0 mL. Pipet 10 mL of the diluted solution and 10 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph six replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation of the ratio of peak responses (peak response of ingredient/peak response of internal standard) is not more than 2.0% for theophylline and not more than 2.5% for guaifenesin. The resolution, *R*, between theophylline and caffeine is not less than 3.0.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for theophylline, 1.0 for caffeine, and 1.5 for guaifenesin. Calculate the quantities, in mg, of anhydrous theophylline ($C_7H_8N_4O_2$) and guaifenesin ($C_{10}H_{14}O_4$) in the portion of Capsules taken by the formula:

$$10C(R_U/R_S)$$

in which *C* is the concentration, in µg per mL, of the appropriate USP Reference Standard in the *Standard preparation*, and R_U and R_S are the ratios of the peak responses of the corresponding analyte to those of caffeine in the *Assay preparation* and the *Standard preparation*, respectively.

Theophylline and Guaifenesin Oral Solution

» Theophylline and Guaifenesin Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$) and not less than 86.7 percent and not more than 113.3 percent of the labeled amount of guaifenesin ($C_{10}H_{14}O_4$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Guaifenesin RS
USP Theophylline RS
Identification—Transfer a volume of Oral Solution, equivalent to about 150 mg of theophylline, to a separator, add 15 mL of water, and proceed as directed in the *Identification* test under *Theophylline and Guaifenesin Capsules*, beginning with "To a second separator."
Alcohol Determination, *Method II* ⟨611⟩ ***(if present):*** between 90.0% and 110.0% of the labeled amount of C_2H_5OH, determined by the gas-liquid chromatographic procedure, acetone being used as the internal standard.

Assay—
pH 6.5 buffer solution and *Mobile phase*—Prepare as directed in the *Assay* under *Theophylline and Guaifenesin Capsules*.
Caffeine solution—Dissolve about 400 mg of caffeine in 1000 mL of a mixture of methanol and water (90:10), and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Theophylline RS in *pH 6.5 buffer solution*, and dilute quantitatively with *pH 6.5 buffer solution* to obtain a solution (*Solution T*) having a known concentration of about 900*J* µg per mL, in which *J* is the ratio of the labeled amount

USP Monographs

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of theophylline to that of guaifenesin. Transfer about 90 mg of USP Guaifenesin RS, accurately weighed, to a 200-mL volumetric flask, add about 150 mL of *pH 6.5 buffer solution*, shake to dissolve, dilute with *pH 6.5 buffer solution* to volume, and mix. Pipet 10 mL of this solution and 5 mL of *Solution T* into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 90 µg of guaifenesin and about 90*J* µg of theophylline per mL.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 90 mg of guaifenesin, to a 200-mL volumetric flask, dilute with *pH 6.5 buffer solution* to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph a mixture of 4 mL of *Standard preparation* and 1 mL of *Caffeine solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the theophylline and caffeine peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0 for theophylline and not more than 2.5% for guaifenesin.

Procedure—Proceed as directed in the *Assay* under *Theophylline and Guaifenesin Capsules*. Calculate the quantities, in mg, of anhydrous theophylline ($C_7H_8N_4O_2$) and guaifenesin ($C_{10}H_{14}O_4$) per mL of the Oral Solution taken by the formula:

$$(C/V)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of the appropriate USP Reference Standard in the *Standard preparation*, *V* is the volume, in mL, of Oral Solution taken, and r_U and r_S are the peak responses of the corresponding analyte in the *Assay preparation* and the *Standard preparation*, respectively.

Theophylline Sodium Glycinate

Glycine, mixture with 3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione, monosodium salt.
Theophylline sodium mixture with glycine [8000-10-0].

» Theophylline Sodium Glycinate is an equilibrium mixture containing Theophylline Sodium ($C_7H_7N_4NaO_2$) and Glycine ($C_2H_5NO_2$) in approximately equimolecular proportions buffered with an additional mole of Glycine. Dried at 105° for 4 hours, it contains theophylline sodium glycinate equivalent to not less than 44.5 percent and not more than 47.3 percent of anhydrous theophylline ($C_7H_8N_4O_2$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Glycine RS
USP Theophylline RS

Identification—

A: Dissolve about 1 g in 20 mL of warm water, and neutralize the solution with 6 N acetic acid: a white, crystalline precipitate of theophylline is formed. Filter, wash the precipitate with small portions of cold water, and dry it at 105° for 1 hour: the theophylline so obtained melts between 270° and 274°, the procedure for *Class I* being used (see *Melting Range or Temperature* ⟨741⟩). Retain the remaining portion of the theophylline for use in *Identification* test *B*.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020)Residue obtained in *Identification* test *A*.

C: Ignite it: the residue colors a nonluminous flame intensely yellow and effervesces with acids.

pH ⟨791⟩: between 8.5 and 9.5, in a saturated solution.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 2.0% of its weight.

Glycine content—

Mobile phase—Prepare a solution containing 470 mg of sodium acetate trihydrate and 1 mL of glacial acetic acid in 2 liters of water. Adjust with 10 N sodium hydroxide to a pH of 4.3. Mix 3 volumes of the resulting solution with 7 volumes of acetonitrile. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Glycine RS in *Mobile phase*, and dilute quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 1.3 mg per mL.

Test preparation—Transfer about 153 mg of Theophylline Sodium Glycinate, accurately weighed, to a 50-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 3.9-mm × 30-cm column that contains packing L8. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 2000 theoretical plates, the tailing factor for the glycine peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the glycine peaks. Calculate the quantity, in mg, of $C_2H_5NO_2$ in the portion of Theophylline Sodium Glycinate taken by the formula:

$$50C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Glycine RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not less than 42.0 percent and not more than 48.0 percent, on the dried basis, is found.

Assay—

Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Theophylline*.

Assay preparation—Transfer about 550 mg of Theophylline Sodium Glycinate, previously dried and accurately weighed, to a 250-mL volumetric flask, add about 125 mL of *Mobile phase*, shake by mechanical means until solution is complete, dilute with *Mobile phase* to volume, and mix.

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Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Theophylline*. Calculate the quantity, in mg, of anhydrous theophylline ($C_7H_8N_4O_2$) in the portion of Theophylline Sodium Glycinate taken by the formula:

$$2500C(R_U/R_S)$$

in which the terms are as defined therein.

Theophylline Sodium Glycinate Oral Solution

» Theophylline Sodium Glycinate Oral Solution contains an amount of theophylline sodium glycinate equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Theophylline RS
Labeling—Label Oral Solution to state both the content of theophylline sodium glycinate and the content of anhydrous theophylline.
Identification—Mix a volume of Oral Solution, equivalent to about 500 mg of theophylline, with 10 mL of 6 N ammonium hydroxide, and evaporate on a steam bath to a volume of about 20 mL. Neutralize with 6 N acetic acid to litmus, and cool, with stirring, to about 15°. Collect the precipitate on a filter, wash with cold water, and dry at 105° for 4 hours: the theophylline so obtained melts between 270° and 274°, the procedure for *Class I* being used (see *Melting Range or Temperature* ⟨741⟩), and meets the requirements for *Identification* test *B* under *Theophylline Sodium Glycinate*.
pH ⟨791⟩: between 8.3 and 9.1.
Alcohol Determination, *Method II* ⟨611⟩: between 17.0% and 23.0% of C_2H_5OH.

Assay—

Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Theophylline*.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 220 mg of theophylline sodium glycinate, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Theophylline*. Calculate the quantity, in mg, of anhydrous theophylline ($C_7H_8N_4O_2$) in each mL of the Oral Solution taken by the formula:

$$(1000C/V)(R_U/R_S)$$

in which V is the volume, in mL, of Oral Solution taken, and the other terms are as defined therein.

Theophylline Sodium Glycinate Tablets

» Theophylline Sodium Glycinate Tablets contain an amount of theophylline sodium glycinate equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

Packaging and storage—Preserve in well-closed containers.
Labeling—Label the Tablets to state both the content of theophylline sodium glycinate and the content of anhydrous theophylline.
USP Reference standards ⟨11⟩—
USP Theophylline RS
Identification—Triturate a quantity of finely powdered Tablets, equivalent to about 500 mg of theophylline, with 10-mL and 15-mL portions of solvent hexane, and discard the solvent hexane. Triturate the residue with two 10-mL portions of a mixture of equal volumes of 6 N ammonium hydroxide and water, and filter each time. Evaporate the combined filtrates to about 5 mL, neutralize, if necessary, with 6 N acetic acid, using litmus, and cool to about 15°, with stirring. Collect the precipitate on a filter, wash it with cold water, and dry at 105° for 1 hour: the theophylline so obtained melts between 270° and 274°, the procedure for *Class I* being used (see *Melting Range or Temperature* ⟨741⟩), and responds to *Identification* test *B* under *Theophylline Sodium Glycinate*.

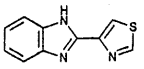
Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.

Procedure—Determine the amount of anhydrous theophylline ($C_7H_8N_4O_2$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 272 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Theophylline RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of anhydrous $C_7H_8N_4O_2$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Place 20 Tablets in a 200-mL volumetric flask, add 50 mL of water, and when the tablets have disintegrated add 50 mL of 6 N ammonium hydroxide. Shake until no more dissolves, then dilute with water to volume, mix, and filter through a dry filter into a dry flask, discarding the first 20 mL of the filtrate. Transfer an accurately measured aliquot of the subsequent filtrate, equivalent to about 250 mg of theophylline, to a 250-mL conical flask, add 20.0 mL of 0.1 N silver nitrate VS, and heat on a steam bath for 15 minutes. Filter through a filter crucible under reduced pressure, and wash the precipitate with three 10-mL portions of water. Acidify the combined filtrate and washings with nitric acid, and add an excess of 3 mL of the acid. Cool, add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

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Thiabendazole



$C_{10}H_7N_3S$ 201.25
1*H*-Benzimidazole, 2-(4-thiazolyl)-.
2-(4-Thiazolyl)benzimidazole [148-79-8].

» Thiabendazole contains not less than 98.0 percent and not more than 101.0 percent of $C_{10}H_7N_3S$, calculated on the dried basis. [NOTE—Thiabendazole labeled solely for veterinary use is exempt from the requirements of the tests for *Residue on ignition*, *Selenium*, and *Chromatographic purity*.]

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Thiabendazole RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—Do not dry specimens.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 5 µg per mL.
Medium: 0.1 N hydrochloric acid.

C: Dissolve about 5 mg in 5 mL of 0.1 N hydrochloric acid, add 3 mg of *p*-phenylenediamine dihydrochloride, and shake to dissolve. Add about 0.1 g of zinc dust, mix, and allow to stand for 2 minutes. Add 5 mL of a solution prepared by dissolving 20 g of ferric ammonium sulfate in 75 mL of water, adding 10 mL of 1 N sulfuric acid, and diluting with water to 100 mL: a blue or blue-violet color develops.

D: The R_f value of the principal spot in the chromatogram of the *Identification test preparation* corresponds to that of *Standard preparation A*, as obtained in the test for *Chromatographic purity*.

Loss on drying ⟨731⟩ —Dry it in vacuum at 100° for 2 hours: it loses not more than 0.5% of its weight.
Melting range ⟨741⟩: between 296° and 303°.
Residue on ignition ⟨281⟩: not more than 0.1%.
Selenium ⟨291⟩: 0.003%, a 200-mg test specimen being used.

Chromatographic purity—
Standard preparations—Dissolve USP Thiabendazole RS in glacial acetic acid, and mix to obtain a solution having a known concentration of 1.0 mg per mL. Dilute quantitatively with glacial acetic acid to obtain *Standard preparations A*, *B*, and *C* having the following compositions:

Standard preparation	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimen)
A	(1 in 4)	250	0.5
B	(3 in 20)	150	0.3
C	(1 in 20)	50	0.1

Test preparation—Dissolve an accurately weighed quantity of Thiabendazole in glacial acetic acid to obtain a solution containing 50 mg per mL.
Identification preparation—Dilute a portion of the *Test preparation* quantitatively with glacial acetic acid to obtain a solution containing 0.25 mg per mL.
Procedure—Apply separately 10 µL of the *Test preparation*, 10 µL of the *Identification preparation*, and 10 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of toluene, glacial acetic acid, acetone, and water (60:20:8:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations*. No secondary spot from the chromatogram of the *Test preparation* is larger or more intense than the principal spot obtained from *Standard preparation* (0.5%), and the sum of the intensities of all secondary spots obtained from the *Test preparation* corresponds to not more than 1.0%.
Assay—Dissolve about 160 mg of Thiabendazole, accurately weighed, in 10 mL of glacial acetic acid. Add 50 mL of acetic anhydride, 1 mL of mercuric acetate TS, and 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS (the color change at the endpoint is from blue to blue-green). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 20.13 mg of $C_{10}H_7N_3S$.

Thiabendazole Oral Suspension

» Thiabendazole Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of thiabendazole ($C_{10}H_7N_3S$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Thiabendazole RS

Identification—
A: Mix a portion of Oral Suspension, equivalent to about 0.5 g of thiabendazole, with about 20 mL of water, and filter. Wash the residue with 20 mL of water, discard the washing, dissolve the residue in 30 mL of 0.1 N hydrochloric acid, and filter. Collect the filtrate in a separator, render it alkaline with 1 N sodium hydroxide, and extract with 10 mL of carbon disulfide. Pass the carbon disulfide layer through a dry filter, collecting the filtrate in an evaporating dish. Evaporate the solvent with the aid of gentle heat and a stream of nitrogen. [CAUTION—Do not overheat the residue.] The residue so

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obtained responds to *Identification* test *A* under *Thiabendazole*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH ⟨791⟩: between 3.4 and 4.2.

Assay—

pH 3.1 Phosphate buffer—Dissolve 13.8 g of monobasic sodium phosphate in water to obtain 2000 mL of solution. Adjust this solution with phosphoric acid to a pH of 3.1 ± 0.05.

Mobile phase—Prepare a filtered and degassed mixture of *pH 3.1 Phosphate buffer* and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Thiabendazole RS in 0.1 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary, with 0.1 N hydrochloric acid to obtain a solution containing about 2 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.2 mg of USP Thiabendazole RS per mL.

Assay preparation—Transfer an accurately measured volume of Oral Suspension, equivalent to about 0.5 g of thiabendazole, to a 250-mL volumetric flask; dilute with 0.1 N hydrochloric acid to volume; and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 960 theoretical plates, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in g, of thiabendazole ($C_{10}H_7N_3S$) in each mL of the Oral Suspension taken by the formula:

$$2.5(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Thiabendazole RS in the *Standard preparation; V* is the volume, in mL, of Oral Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thiabendazole Chewable Tablets

Former Title: Thiabendazole Tablets

» Thiabendazole Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of thiabendazole ($C_{10}H_7N_3S$).

Packaging and storage—Preserve in tight containers.
Labeling—Label the Chewable Tablets to indicate that they are to be chewed before swallowing.
USP Reference standards ⟨11⟩—
USP Thiabendazole RS

Identification—

A: Triturate a quantity of powdered Chewable Tablets, equivalent to about 0.5 g of thiabendazole, with about 20 mL of water, and filter. Wash the residue with 20 mL of water, discard the washing, dissolve the residue in 30 mL of 0.1 N hydrochloric acid, and filter. Collect the filtrate in a separator, render it alkaline with 1 N sodium hydroxide, and extract with 10 mL of carbon disulfide. Pass the carbon disulfide layer through a dry filter, collecting the filtrate in an evaporating dish. Evaporate the solvent with the aid of gentle heat and a stream of nitrogen. *[Caution—Do not overheat the residue.]* The residue so obtained meets the requirements for *Identification* test *A* under *Thiabendazole*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Uniformity of dosage units ⟨905⟩: meet the requirements.
PROCEDURE FOR CONTENT UNIFORMITY—
Standard preparation—Dissolve an accurately weighed quantity of USP Thiabendazole RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 5 µg per mL.

Test preparation—Transfer 1 Chewable Tablet to a 1000-mL volumetric flask, add about 75 mL of 0.1 N hydrochloric acid, and heat on a steam bath for about 1 hour. Cool to room temperature, dilute with 0.1 N hydrochloric acid to volume, mix, and filter a portion of the solution, discarding the first 20 mL of the filtrate. Pipet 5 mL of the filtrate into a 500-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Test preparation* at the wavelength of maximum absorbance at about 302 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{10}H_7N_3S$ in the Chewable Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which T is the labeled quantity, in mg, of thiabendazole in the Chewable Tablet; C is the concentration, in µg per mL, of USP Thiabendazole RS in the *Standard preparation; D* is the concentration, in µg per mL, of thiabendazole in the *Test preparation,* based upon the labeled quantity per Chewable Tablet and the extent of dilution; and A_U and A_S are the absorbances of the *Test preparation* and the *Standard preparation,* respectively.

Assay—

Standard preparation and *Chromatographic system*—Prepare as directed in the *Assay* under *Thiabendazole Oral Suspension*.

pH 3.5 Phosphate buffer—Dissolve 13.8 g of monobasic sodium phosphate in water to obtain 2000 mL of solution. Adjust this solution with phosphoric acid to a pH of 3.5 ± 0.05.

USP Monographs

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Mobile phase—Prepare a filtered and degassed mixture of *pH 3.5 Phosphate buffer* and methanol (54:46). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

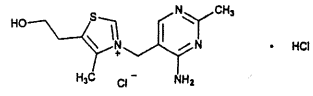
Assay preparation—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of thiabendazole, to a 1000-mL volumetric flask, add 100 mL of 0.1 N hydrochloric acid, mix, and warm the solution for a minimum of 30 minutes. Allow to cool to room temperature, dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Thiabendazole Oral Suspension*. Calculate the quantity, in mg, of thiabendazole ($C_{10}H_7N_3S$) in the portion of Chewable Tablets taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Thiabendazole RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thiamine Hydrochloride



$C_{12}H_{17}ClN_4OS \cdot HCl$ 337.27

Thiazolium, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methyl-, chloride, monohydrochloride; Thiamine monohydrochloride [67-03-8].

DEFINITION

Thiamine Hydrochloride contains NLT 98.0% and NMT 102.0% of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Analysis: Dry specimens at 105° for 2 h.
  Acceptance criteria: Meets the requirements
- **B. Identification Tests—General,** *Chloride* ⟨191⟩: A 20-mg/mL solution meets the requirements.

ASSAY

- **Procedure**
  Solution A: 0.005 M sodium 1-octanesulfonate in dilute glacial acetic acid (1 in 100)
  Solution B: Methanol and acetonitrile (3:2)
  Mobile phase: *Solution B* and *Solution A* (40:60)
  Internal standard solution: 2% (v/v) of methylbenzoate in methanol
  Standard solution: Prepare a 1-mg/mL solution of USP Thiamine Hydrochloride RS in *Mobile phase*. Transfer 20.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. The *Standard solution* contains 400 µg/mL of thiamine hydrochloride.
  Sample solution: Prepare a 2-mg/mL of Thiamine Hydrochloride in *Mobile phase*. Transfer 10.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  [Note—The flow rate may be adjusted as needed to obtain a retention time of about 12 min for thiamine hydrochloride.]
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 4.0 between the thiamine and methylbenzoate peaks
  Tailing factor: NMT 2.0 for the thiamine peak
  Column efficiency: NLT 1500 theoretical plates for the thiamine peak
  Relative standard deviation: NMT 2.0% for the ratios of thiamine peak areas to the internal standard peak area
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of Thiamine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak area of thiamine/peak area of the internal standard) from the *Sample solution*

R_S = internal standard ratio (peak area of thiamine/peak area of the internal standard) from the *Standard solution*

C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Thiamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.2%
- **Limit of Nitrate**
  Sample solution: 20 mg/mL of Thiamine Hydrochloride
  Analysis: To 2 mL of the *Sample solution* add 2 mL of sulfuric acid. Cool, and superimpose 2 mL of ferrous sulfate TS.
  Acceptance criteria: No brown ring is produced at the junction of the two layers.
- **Related Compounds**
  Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay*.
  Sample solution: 1.0 mg/mL of Thiamine Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.0-mm × 15-cm; packing L1
  Flow rate: 0.75 mL/min
  Injection size: 10 µL
  Analysis
  Sample: *Sample solution*
  Allow the *Sample solution* to elute for NLT three times the retention time of the main peak.
  Calculate the percentage of total secondary peaks in the portion of Thiamine Hydrochloride taken:

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$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the areas of all the peaks, except that of the thiamine peak
r_T = sum of the areas of all the peaks

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
- **pH** ⟨791⟩
  Sample solution: 10-mg/mL solution
  Acceptance criteria: 2.7–3.4
- **Water Determination,** *Method I* ⟨921⟩**:** NMT 5.0%
- **Absorbance of Solution**
  Sample solution: 100 mg/mL in water. Filter through a fine-porosity, sintered-glass funnel.
  Blank: Water
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV-Vis
  Analytical wavelength: 400 nm
  Cell: 1 cm
  Analysis
  Samples: *Sample solution* and *Blank*
  Determine the absorbance of the *Sample solution* against that of the *Blank*.
  Acceptance criteria: NMT 0.025

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Thiamine Hydrochloride RS

Thiamine Hydrochloride Injection

» Thiamine Hydrochloride Injection is a sterile solution of Thiamine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩**—**
USP Thiamine Hydrochloride RS

Identification—
A: It yields a white precipitate with mercuric chloride TS, and a red-brown precipitate with iodine TS. It also yields a precipitate with mercuric-potassium iodide TS, and with trinitrophenol TS.
B: Dilute a portion of Injection with water to a concentration of about 10 mg of thiamine hydrochloride per mL. To 0.5 mL of this solution add 5 mL of 0.5 N sodium hydroxide, then add 0.5 mL of potassium ferricyanide TS and 5 mL of isobutyl alcohol, shake the mixture vigorously for 2 minutes, and allow the liquid layers to separate: when illuminated from above by a vertical beam of UV light and viewed at a right angle to this beam, the air-liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified, but reappears when it is again made alkaline.
C: It responds to the tests for *Chloride* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.5 USP Endotoxin Units per mg of thiamine hydrochloride.
pH ⟨791⟩: between 2.5 and 4.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Thiamine Hydrochloride Oral Solution.*
Assay preparation—Quantitatively dilute an accurately measured volume of Injection with *Mobile phase* to obtain a solution containing about 500 µg of thiamine hydrochloride per mL. Pipet 10 mL of the resulting solution and 10 mL of *Internal standard solution* into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Thiamine Hydrochloride Oral Solution.* Calculate the quantity, in mg, of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in each mL of the Injection taken by the formula:

$$C(L/D)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Thiamine Hydrochloride RS in the *Standard preparation; L* is the labeled quantity, in mg per mL, of thiamine hydrochloride in the Injection; *D* is the concentration, in mg per mL, of thisamine hydrochloride in the *Assay preparation* on the basis of the labeled quantity and the extent of dilution; and R_U and R_S are the ratios of the peak responses of thiamine to methylparaben obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thiamine Hydrochloride Oral Solution

DEFINITION
Thiamine Hydrochloride Oral Solution contains NLT 95.0% and NMT 135.0% of the labeled quantity of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$).

IDENTIFICATION
- **A.**
  Sample solution: Dilute a portion of Oral Solution with water to a concentration of 10 mg/mL of thiamine hydrochloride.
  Analysis: To 0.5 mL of the *Sample solution* add 5 mL of 0.5 N sodium hydroxide, then add 0.5 mL of potassium ferricyanide TS and 5 mL of isobutyl alcohol. Shake the mixture vigorously for 2 min, and allow the liquid layers to separate. Illuminate from above by a vertical beam of UV light, and observe the air–liquid meniscus at a right angle to this beam.
  Acceptance criteria: The air–liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified, but reappears when it is again made alkaline.

ASSAY
- **Procedure**
  Mobile phase: Methanol and 0.04 M aqueous monobasic potassium phosphate (45:55)
  Internal standard solution: 100 µg/mL of methylparaben in *Mobile phase*
  Standard stock solution: 500 µg/mL of USP Thiamine Hydrochloride RS in *Mobile phase*
  Standard solution: Dilute a mixture of equal volumes of the *Standard stock solution* and *Internal standard solution* with

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Mobile phase to obtain a concentration of USP Thiamine Hydrochloride RS of about 50 µg/mL.
Sample stock solution: Equivalent to 500 µg/mL of thiamine hydrochloride in *Mobile phase* from an accurately measured volume of Oral Solution
Sample solution: Dilute a mixture of equal volumes of the *Sample stock solution* and *Internal standard solution* with *Mobile phase* to obtain a concentration of thiamine hydrochloride of about 50 µg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for thiamine and methylparaben are about 0.35 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between thiamine and methylparaben
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of thiamine to methylparaben from the *Sample solution*
R_S = peak area ratio of thiamine to methylparaben from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard stock solution* (µg/mL)
C_U = nominal concentration of thiamine hydrochloride in the *Sample stock solution* (µg/mL)

Acceptance criteria: 95.0%–135.0%

OTHER COMPONENTS
• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩**:** 90.0%–110.0% of the labeled quantity of C_2H_5OH, using acetone as the internal standard

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Thiamine Hydrochloride RS

Thiamine Hydrochloride Tablets

DEFINITION
Thiamine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$).

IDENTIFICATION
• **A.**
Sample solution: Triturate a quantity of powdered Tablets, equivalent to 10 mg of thiamine hydrochloride, with 10 mL of 0.5 N sodium hydroxide, and filter.
Analysis: To 5 mL of the *Sample solution* add 0.5 mL of potassium ferricyanide TS and 5 mL of isobutyl alcohol, shake the mixture vigorously for 2 min, and allow the liquid layers to separate. Illuminate from above by a vertical beam of UV light, and observe the air–liquid meniscus at a right angle to this beam.
Acceptance criteria: The air–liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified, but reappears when it is again made alkaline.
• **B.**
Sample solution: Triturate a quantity of powdered Tablets, equivalent to 10 mg of thiamine hydrochloride, with 10 mL of water, and filter.
Analysis 1: To 2 mL of the *Sample solution* add iodine TS.
Acceptance criteria 1: A red-brown precipitate is formed.
Analysis 2: To 2 mL of the *Sample solution* add mercuric chloride TS.
Acceptance criteria 2: A white precipitate is formed.
Analysis 3: *Identification Tests—General* ⟨191⟩, *Chloride*
Acceptance criteria 3: Meet the requirements
• **C.**
Sample solution: Use the remainder of the *Sample solution* from *IdentificationB.*
Analysis: Add 1 mL of lead acetate TS and 1 mL of 2.5 N sodium hydroxide.
Acceptance criteria: A yellow color is produced. Heat the mixture for several minutes on a steam bath: the color changes to brown, and, on standing, a precipitate of lead sulfide separates.

ASSAY
• **THIAMINE ASSAY** ⟨531⟩, *Chemical Methods, Procedure 1*
Sample solution: Place NLT 20 Tablets in a flask of suitable size, half fill the flask with 0.2 N hydrochloric acid, and heat on a steam bath, with frequent agitation, until the Tablets have dissolved or have disintegrated so that a uniform dispersion is obtained. Cool, transfer the contents of the flask to a volumetric flask, and dilute with 0.2 N hydrochloric acid to volume. If the mixture is not clear, either centrifuge it or filter it through paper known not to adsorb thiamine. Dilute a portion of the clear solution with 0.2 N hydrochloric acid to obtain a 0.2-µg/mL solution of thiamine hydrochloride.
Analysis: Proceed as directed in the chapter.
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms*
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: A known concentration of USP Thiamine Hydrochloride RS in *Medium*
Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary
Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 1.40 mg/mL of sodium 1-hexanesulfonate
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) dissolved:

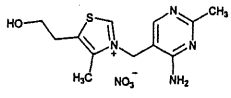
$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak area of thiamine from the *Sample solution*
r_S = peak area of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = labeled claim of thiamine hydrochloride (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Thiamine Hydrochloride RS

Thiamine Mononitrate



$C_{12}H_{17}N_5O_4S$ 327.36
Thiazolium, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methyl-, nitrate (salt);
Thiamine nitrate (salt) [532-43-4].

DEFINITION
Thiamine Mononitrate contains NLT 98.0% and NMT 102.0% of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$), calculated on the dried basis.

IDENTIFICATION
• **A.**
Sample solution: 20 mg/mL of Thiamine Mononitrate
Analysis: To 2 mL of the *Sample solution* add 2 mL of sulfuric acid. Cool, and superimpose 2 mL of ferrous sulfate TS.
Acceptance criteria: A brown ring is produced at the junction of the two liquids.
• **B.**
Sample: 5 mg
Analysis: Dissolve the *Sample* in a mixture of 1 mL of lead acetate TS and 1 mL of 2.5 N sodium hydroxide. Heat for several min on a steam bath.
Acceptance criteria: After dissolution of the *Sample*, a yellow color is produced. After heating the solution, the color changes to brown and on standing, a precipitate of lead sulfide separates.
• **C.** A solution of Thiamine Mononitrate yields a white precipitate with mercuric chloride TS, and a red-brown precipitate with iodine TS. It also yields a precipitate with mercuric–potassium iodide TS, and with trinitrophenol TS.
• **D.**
Sample solution: Dissolve 5 mg in 5 mL of 0.5 N sodium hydroxide.
Analysis: To the *Sample solution* add 0.5 mL of potassium ferricyanide TS and 5 mL of isobutyl alcohol. Shake the mixture vigorously for 2 min, and allow the liquid layers to separate.
Acceptance criteria: When illuminated from above by a vertical beam of UV light and viewed at a right angle to this beam, the air–liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified, but reappears when it is again made alkaline.

ASSAY
• **PROCEDURE**
Solution A: 0.005 M sodium 1-octanesulfonate in dilute glacial acetic acid (1 in 100)
Solution B: Methanol and acetonitrile (3:2)
Mobile phase: *Solution A* and *Solution B* (60:40)
Internal standard solution: 2.0% (v/v) of methylbenzoate in methanol
Standard solution: Prepare a 1-mg/mL solution of USP Thiamine Hydrochloride RS in *Mobile phase*. Transfer 20.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. The *Standard solution* contains 400 µg/mL of thiamine hydrochloride.
Sample solution: Prepare a 2-mg/mL solution of Thiamine Mononitrate in *Mobile phase*. Transfer 10.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 1 mL/min. [NOTE—The flow rate may be adjusted as needed to obtain a retention time of about 12 min for thiamine hydrochloride.]
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between the thiamine and methylbenzoate peaks
Tailing factor: NMT 2.0 for the thiamine peak
Column efficiency: NLT 1500 theoretical plates for the thiamine peak
Relative standard deviation: NMT 2.0% for the ratios of the thiamine peak area to the internal standard peak area
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of Thiamine Mononitrate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = internal standard ratio (peak area of thiamine/peak area of the internal standard) from the *Sample solution*
R_S = internal standard ratio (peak area of thiamine/peak area of the internal standard) from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Thiamine Mononitrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

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Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Standard: 0.40 mL of 0.020 N hydrochloric acid
  Sample: 500 mg of Thiamine Mononitrate
  Acceptance criteria: NMT 0.06%
- **RELATED COMPOUNDS**
  Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay*.
  Sample solution: 1.0 mg/mL of Thiamine Mononitrate in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.0-mm × 15-cm; packing L1
  Flow rate: 0.75 mL/min
  Injection size: 10 µL
  Analysis
  Sample: *Sample solution*
  Allow the *Sample solution* to elute for NLT three times the retention time of the main peak.
  Calculate the percentage of total secondary peaks in the portion of Thiamine Mononitrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the areas of all the peaks, except that of the thiamine peak
r_T = sum of the areas of all the peaks

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: 20-mg/mL solution
  Acceptance criteria: 6.0–7.5
- **LOSS ON DRYING** ⟨731⟩: Dry 500 mg at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Thiamine Hydrochloride RS

Thiamine Mononitrate Oral Solution

DEFINITION

Thiamine Mononitrate Oral Solution contains NLT 95.0% and NMT 115.0% of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$).

IDENTIFICATION

- **A.**
  Sample solution: Dilute a portion of Oral Solution with water to a concentration of 10 mg/mL of thiamine mononitrate.
  Analysis: To 0.5 mL of the *Sample solution* add 5 mL of 0.5 N sodium hydroxide, then add 0.5 mL of potassium ferricyanide TS and 5 mL of isobutyl alcohol. Shake the mixture vigorously for 2 min, and allow the liquid layers to separate. Illuminate from above by a vertical beam of UV light and observe the air–liquid meniscus at a right angle to this beam.
  Acceptance criteria: The air–liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified, but reappears when it is again made alkaline.
- **B.**
  Sample: 5 mL of Oral Solution
  Analysis: Add 2 mL of sulfuric acid to the *Sample*, cool, and superimpose 2 mL of ferrous sulfate TS.
  Acceptance criteria: A brown ring is produced at the junction of the two liquids.

ASSAY

- **PROCEDURE**
  Mobile phase: Methanol and 0.04 M aqueous monobasic potassium phosphate (45:55)
  Internal standard solution: 100 µg/mL of methylparaben in *Mobile phase*
  Standard stock solution: 500 µg/mL of USP Thiamine Hydrochloride RS in *Mobile phase*
  Standard solution: Dilute a mixture of equal volumes of the *Standard stock solution* and *Internal standard solution* with *Mobile phase* to obtain a concentration of USP Thiamine Hydrochloride RS of 50 µg/mL.
  Sample stock solution: Equivalent to 500 µg/mL of thiamine mononitrate in *Mobile phase* from an accurately measured volume of Oral Solution
  Sample solution: Dilute a mixture of equal volumes of the *Sample stock solution* and *Internal standard solution* with *Mobile phase* to obtain a concentration of thiamine mononitrate of 50 µg/mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  Injection size: 25 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for thiamine and methylparaben are about 0.35 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 6.0 between the thiamine and methylparaben
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak area ratio of thiamine to methylparaben from the *Sample solution*
R_S = peak area ratio of thiamine to methylparaben from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard stock solution* (µg/mL)
C_U = nominal concentration of thiamine mononitrate in the *Sample stock solution* (µg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

Acceptance criteria: 95.0%–115.0%

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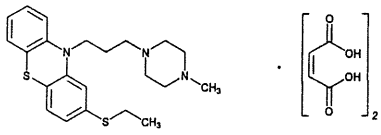
OTHER COMPONENTS

- **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩**:** 90.0%–110.0% of the labeled quantity of C_2H_5OH, using acetone as the internal standard

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Thiamine Hydrochloride RS

Thiethylperazine Maleate



$C_{22}H_{29}N_3S_2 \cdot 2C_4H_4O_4$ 631.76

10*H*-Phenothiazine, 2-(ethylthio)-10-[3-(4-methyl-1-piperazinyl)propyl]-, (*Z*)-2-butenedioate (1:2); 2-(Ethylthio)-10-[3-(4-methyl-1-piperazinyl)propyl] phenothiazine maleate (1:2) [1179-69-7].

DEFINITION

Thiethylperazine Maleate contains NLT 98.0% and NMT 101.5% of thiethylperazine maleate ($C_{22}H_{29}N_3S_2 \cdot 2C_4H_4O_4$), calculated on the dried basis.

[NOTE—Throughout the following procedures, protect test or assay samples, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample solution: Dissolve 250 mg of Thiethylperazine Maleate in 30 mL of glacial acetic acid. Warm on a steam bath to dissolve.

Analysis: To the *Sample solution* add 1 drop of crystal violet TS. Titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.59 mg of thiethylperazine maleate ($C_{22}H_{29}N_3S_2 \cdot 2C_4H_4O_4$).

Acceptance criteria: 98.0%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **SELENIUM** ⟨291⟩

Test solution: 100 mg of Thiethylperazine Maleate, mixed with 100 mg of magnesium oxide

Acceptance criteria: NMT 30 ppm; the absorbance of the solution from the *Test solution* is NMT one-half that from the *Standard Solution.*

- **ORGANIC IMPURITIES**

[NOTE—Conduct this test promptly without exposure to daylight and with minimum exposure to artificial light.]

Diluent: Methanol and ammonium hydroxide (49:1)

Sample solution: 20 mg/mL of Thiethylperazine Maleate in *Diluent*

Standard solution A: 20.0 mg/mL of USP Thiethylperazine Maleate RS in *Diluent*

Standard solutions B–F: Solutions containing 0.2, 0.1, 0.06, 0.04, and 0.02 mg/mL, respectively, from *Standard solution A* in *Diluent.* The final concentrations of *Standard solutions B–F* represent 1%, 0.5%, 0.3%, 0.2%, and 0.1% concentrations of *Standard solution A.*

Chromatographic system

Application volume: 5 µL

Developing solvent system: Ether, cyclohexane, methanol, and ammonium hydroxide (25:10:5:1)

Analysis: Apply equal spots of the *Sample solution* and *Standard solutions A–F* as directed in *Ordinary Impurities* ⟨466⟩. Examine the plate under short- and long-wavelength UV light, then spray the plate with Dragendorff's TS, dry the plate with a stream of nitrogen, and then spray with hydrogen peroxide TS.

Acceptance criteria: Any secondary spot in the *Sample solution* is NMT 0.5%, and the sum of all secondary spots is NMT 2.0%.

SPECIFIC TESTS

- **PH** ⟨791⟩

Sample solution: 1 mg/mL in water, warming, if necessary, to dissolve

Acceptance criteria: 2.8–3.8

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Thiethylperazine Maleate RS

Thiethylperazine Maleate Suppositories

DEFINITION

Thiethylperazine Maleate Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of thiethylperazine maleate ($C_{22}H_{29}N_3S_2 \cdot 2C_4H_4O_4$).

[NOTE—Throughout the following procedures, protect test or assay samples, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B.** The R_F value of the principal spot and its color, as visualized by the spray reagents in the chromatogram of the *Sample solution,* corresponds to that of *Standard solution A,* as obtained in *Organic Impurities.*

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and 10% ammonium carbonate (9:1)

Diluent: Methanol, chloroform, and ammonium hydroxide (55:45:1)

Standard solution: 0.1 mg/mL of USP Thiethylperazine Maleate RS in *Diluent*

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Sample solution: Weigh not fewer than 20 Suppositories, and freeze them at 0° for about 30 min. Grind the Suppositories into small particles, and transfer a portion of the mass, equivalent to about 10 mg of thiethylperazine maleate, to a 100-mL volumetric flask. Add about 30 mL of *Diluent*, and gently shake for about 10 min or until the mass dissolves. Dilute with *Diluent* to volume, and filter through about 2 g of anhydrous sodium sulfate, discarding the first portion of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 5-µm, base-deactivated packing L1
Column temperature: 45°
Flow rate: 2 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between the thiethylperazine peak and adjacent peaks
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thiethylperazine maleate ($C_{22}H_{29}N_3S_2 \cdot 2C_4H_4O_4$) in the portion of Suppositories taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Thiethylperazine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiethylperazine maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **Organic Impurities**
Diluent: Methanol, chloroform (stabilized with 0.75% alcohol), and ammonium hydroxide (55:45:1)
Standard stock solution: 10 mg/mL of USP Thiethylperazine Maleate RS in *Diluent*
Standard solutions: Dilute the *Standard stock solution* quantitatively with *Diluent* to obtain the *Standard solution* shown in *Table 1*.

Table 1

Standard solution	Dilution	Concentration (µg/mL)	Percentage (%, for comparison with Sample solution)
A	(1 in 20)	500	5
B	(1 in 33)	300	3
C	(1 in 50)	200	2
D	(1 in 100)	100	1
E	(1 in 200)	50	0.5

Sample solution: Transfer a number of Suppositories, equivalent to about 20 mg of thiethylperazine maleate, to a funnel having a fritted disk of a fine pore size. Add 50 mL of *n*-pentane, macerate, and mix with a glass stirring rod, collecting the filtrate in a filter flask under reduced pressure. Rinse the stirring rod and funnel with five 10-mL portions of *n*-pentane, and discard the filtrate. Transfer the funnel to a separate filter flask, add three 10-mL portions of *Diluent*, and collect the filtrate under reduced pressure. Evaporate the filtrate to dryness, add 2.0 mL of *Diluent*, and mix. Filter the resulting solution through a filter of 0.45-µm pore size, discarding the initial portion of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Absorbent: 0.25-mm layer of chromatographic silica gel mixture. Equilibrate for 3 h in the *Developing solvent system*. Remove and allow solvent to evaporate.
Application volume: 10 µL
Developing solvent system: Methylene chloride, isopropyl alcohol, methanol, and ammonium hydroxide (85:15:2:1)

Analysis
Samples: *Standard solutions A–E* and *Sample solution*
Develop the chromatogram in a separate lined chamber in the *Developing solvent system*, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to evaporate in a stream of nitrogen for about 10 min. Examine the plate under short- and long-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spots in the chromatograms of the *Standard solutions*. Spray the plate with Dragendorff's reagent, followed by a 9% solution of hydrogen peroxide in water, and cover with a glass plate for 5 min. Remove the glass plate, and observe under white light. Record the R_F values and estimate the concentration of the secondary spots observed in the *Sample solution*.

Acceptance criteria: No secondary spot from the chromatogram of the *Sample solution* observed, using the visualization methods above, is larger or more intense than the principal spot of *Standard solution E* (NMT 0.5%), and the sum of the intensities of the secondary spots of the *Sample solution* corresponds to NMT 5%.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers at temperatures below 25°. Do not expose unwrapped Suppositories to sunlight.
• **USP Reference Standards** ⟨11⟩
USP Thiethylperazine Maleate RS

Thiethylperazine Maleate Tablets

DEFINITION
Thiethylperazine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of thiethylperazine maleate ($C_{22}H_{29}N_3S_2 \cdot 2C_4H_4O_4$).
[Note—Throughout the following procedures, protect test or assay samples, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

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IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and 10% ammonium carbonate (9:1)

Diluent: Acetonitrile and water (9:1)

Standard solution: 0.2 mg/mL of USP Thiethylperazine Maleate RS in *Diluent*. Use sonication for about 5 min to dissolve the sample.

Sample solution: 0.2 mg/mL of thiethylperazine maleate, prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of thiethylperazine maleate, to a 250-mL volumetric flask. Add 150 mL of *Diluent*, and shake to disperse the powder. Sonicate the mixture for 10 min, then shake by mechanical means for 1 h. Dilute with *Diluent* to volume. Pass through a filter of 0.45-µm pore size, discarding the first portion of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm, base-deactivated packing L1

Column temperature: 45°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between the thiethylperazine peak and adjacent peaks

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of thiethylperazine maleate ($C_{22}H_{29}N_3S_2 \cdot 2C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Thiethylperazine Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of thiethylperazine maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 1000 mL

Apparatus 1: 120 rpm

Time: 30 min

Standard solution: USP Thiethylperazine Maleate RS at a known concentration in *Medium*

Sample solutions: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Wavelength of maximum absorption at about 263 nm

Tolerances: NLT 75% (*Q*) of the labeled amount of thiethylperazine maleate ($C_{22}H_{29}N_3S_2 \cdot 2C_4H_4O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

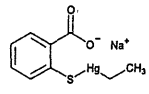
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Thiethylperazine Maleate RS

Thimerosal



$C_9H_9HgNaO_2S$ 404.81

Mercury, ethyl(2-mercaptobenzoato-*S*)-, sodium salt; Ethyl (sodium *o*-mercaptobenzoato)mercury [54-64-8].

DEFINITION

Thimerosal contains NLT 97.0% and NMT 102.0% of thimerosal ($C_9H_9HgNaO_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.**

Sample solution: 10 mg/mL

Analysis: To the *Sample solution* add a few drops of silver nitrate TS.

Acceptance criteria: A pale yellow precipitate is formed.

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 0.05% Trifluoroacetic acid, prepared by adding 1.0 mL of trifluoroacetic acid to 2 L of water

Mobile phase: Methanol and *Solution A* (60:40)

Standard stock solution: 250 µg/mL of USP Thimerosal RS in water

Impurity stock solution: 250 µg/mL of USP Thimerosal Related Compound A RS in methanol and water (90:10)

System suitability solution: 25 µg/mL each of USP Thimerosal RS and USP Thimerosal Related Compound A RS from *Standard stock solution* and *Impurity stock solution*, respectively, in water

Standard solution: 25 µg/mL of USP Thimerosal RS in water from *Standard stock solution*

Sample solution: 25 µg/mL of Thimerosal in water

[NOTE—Prepare both the *Standard solution* and *Sample solution* at a concentration of NMT ±10% of the specified concentration.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 222 nm

Column: 2.1-mm × 10-cm; 2-µm packing L1

Autosampler temperature: 4°

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Flow rate: 0.35 mL/min
Injection volume: 2.5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of thimerosal and thimerosal related compound A are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3.5 between the thimerosal and thimerosal related compound A peaks, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of thimerosal ($C_9H_9HgNaO_2S$) in the portion of Thimerosal taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Thimerosal RS in the *Standard solution* (µg/mL)
C_U = concentration of Thimerosal in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **MERCURY IONS**
Iodide reagent: 332 mg/mL of potassium iodide. Prepare fresh daily. Keep the stopper in the flask, and protect from light.
Standard solution: 95 µg/mL of mercuric chloride
Sample stock solution: 5 mg/mL of Thimerosal
Sample solution A: 1 mg/mL of Thimerosal from *Sample stock solution*
Sample solution B: 1 mg/mL of Thimerosal from *Sample stock solution* and 9.5 µg/mL of mercuric chloride from *Standard solution*
Instrumental conditions
Mode: UV
Analytical wavelength: Determine the wavelength of maximum absorbance for the tetraiodomercurate ion at about 323 nm using the solution prepared by mixing 1.0 mL of the *Standard solution* and 5.0 mL of the *Iodide reagent*, and diluting with water to 10.0 mL.
Cell: 1 cm
Blank: Water
Analysis
Protect all solutions from light before determining their absorbances.
Samples: Label five 10-mL volumetric flasks C, D, E, F, and R. Transfer 5.0 mL of *Sample solution A* to flasks C and D, 5.0 mL of *Sample solution B* to flasks E and F, and 5.0 mL of water to flask R. Dilute flasks C and E with water to volume. Dilute flasks D, F, and R with *Iodide reagent* to volume.
Determine the absorbances of the solutions in flasks C, D, E, F, and R as A_C, A_D, A_E, A_F, and A_R, respectively.
Calculate the percentage of mercury ions in the portion of Thimerosal taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (A_r/M_r) \times 100$$

A_U = absorbance of the *Sample solution* obtained by: $A_U = A_D - A_R - A_C$
A_S = absorbance of the *Standard solution* obtained by: $A_S = A_F - A_R - A_E - A_U$
C_S = concentration of mercuric chloride in *Sample solution B* (mg/mL)
C_U = concentration of Thimerosal in *Sample solution B* (mg/mL)
A_r = atomic weight of mercury, 200.59
M_r = molecular weight of mercuric chloride, 271.50

Acceptance criteria: NMT 0.70%

• **ORGANIC IMPURITIES**
Solution A: 0.05% Trifluoroacetic acid, prepared by adding 1.0 mL of trifluoroacetic acid to 2 L of water
Mobile phase: Methanol and *Solution A* (60:40)
Stock solution 1: 250 µg/mL of USP Thimerosal RS in water
Stock solution 2: 250 µg/mL of USP Thimerosal Related Compound A RS in methanol and water (90:10)
System suitability solution: 25 µg/mL each of USP Thimerosal RS and USP Thimerosal Related Compound A RS from *Stock solution 1* and *Stock solution 2*, respectively, in water
Standard solution: 0.25 µg/mL of USP Thimerosal Related Compound A RS from *Stock solution 2* in water
Sample solution: 250 µg/mL of Thimerosal in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm
Column: 2.1-mm × 10-cm; 2-µm packing L1
Autosampler temperature: 4°
Flow rate: 0.35 mL/min
Injection volume: 2.5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between the thimerosal and thimerosal related compound A peaks, *System suitability solution*
Relative standard deviation: NMT 3%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Thimerosal taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of thimerosal related compound A from the *Standard solution*
C_S = concentration of USP Thimerosal Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Thimerosal in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiosalicylic acid[a]	0.36	0.10
Thimerosal	1.0	—
Thimerosal related compound A	1.3	0.10

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Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] 2-Sulfanylbenzoic acid.

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩

Analysis: Dry to constant weight under vacuum over phosphorus pentoxide.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards** ⟨11⟩

USP Thimerosal RS

USP Thimerosal Related Compound A RS

2,2′-Disulfanediyldibenzoic acid.

$C_{14}H_{10}O_4S_2$ 306.35

Thimerosal Topical Aerosol

DEFINITION

Thimerosal Topical Aerosol is an alcoholic solution of Thimerosal mixed with suitable propellants in a pressurized container. It contains NLT 85.0% and NMT 115.0% of the labeled quantity of thimerosal ($C_9H_9HgNaO_2S$).

[Note—Thimerosal Topical Aerosol is sensitive to some metals.]

IDENTIFICATION

• **A.**

Sample: 10 mL of Topical Aerosol

Analysis: Spray the *Sample* into a suitable container, add 10 mL of water, and heat on a steam bath until the odor of alcohol is no longer perceptible. Cool, and pass hydrogen sulfide through the solution.

Acceptance criteria: No black discoloration or black precipitate is formed.

• **B.**

Sample: 15 mL of Topical Aerosol

Analysis: Spray the *Sample* into a suitable container, add 15 mL of water, heat on a steam bath until the odor of alcohol is no longer perceptible, and add 2 drops of bromine. Mix with 1.5 mL of 3 N hydrochloric acid, filter, evaporate the excess bromine with a current of air, and pass hydrogen sulfide through the filtrate.

Acceptance criteria: A black precipitate is formed.

ASSAY

• **Procedure**

The *Standard solutions* and *Sample solution* may be diluted with water, if necessary, to yield solutions of suitable concentration, adaptable to the linear or working range of the instrument.

Solution A: Dissolve 50 g of stannous chloride in 100 mL of hydrochloric acid on a steam bath, cool, dilute with water to 500 mL, and mix. Use within 3 months.

Standard stock solution A: 1.8 µg/mL of USP Thimerosal RS

Standard stock solution B: 2.0 µg/mL of USP Thimerosal RS

Standard stock solution C: 2.2 µg/mL of USP Thimerosal RS

Standard solution A: Pipet 20 mL of *Standard stock solution A* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Standard solution B: Pipet 20 mL of *Standard stock solution B* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Standard solution C: Pipet 20 mL of *Standard stock solution C* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Sample stock solution: Weigh a filled Topical Aerosol container, and record the weight. Place the container in a dry ice–alcohol bath, and cool for 60 min. Remove the container from the bath, and carefully remove the spray cap with wire cutters, taking precautions to save all pieces of the spray head and cap. With the aid of three 5-mL portions of water, transfer the contents of the container to a beaker previously chilled in the bath. Dry the rinsed empty container and all of its parts in an oven at 105° for 2 h, cool, and weigh. Calculate the weight of the container contents. Add a few boiling chips to the beaker, and carefully stir to help evaporate the propellant. After the bulk of the propellant has evaporated, place the beaker on a steam bath, evaporate the volatile solvents, and cool. Transfer the residual liquid with the aid of 35.0 mL of alcohol to a 50-mL volumetric flask, and dilute with water to volume. Dilute a portion of this solution quantitatively with water to obtain nominally 2 µg/mL of thimerosal.

Sample solution: Pipet 20 mL of *Sample stock solution* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Instrumental conditions

Mode: Flameless atomic absorption spectroscopy

Lamp: Mercury hollow-cathode

Blank solution: Pipet 20 mL of water into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Analysis

Samples: *Standard solutions A, B,* and *C, Sample solution,* and *Blank solution*

Proceed with each of the *Samples* as follows. Separately pipet 3 mL into the scrubbing chamber of a suitable system designed for determination of mercury. Dilute with water to 150 mL, and add hydroxylamine hydrochloride solution (1 in 10) to reduce the excess permanganate. Add 5 mL of *Solution A,* and immediately attach the scrubbing chamber to the system. Concomitantly determine the absorbance of the vapor from each solution at an integration time of 15 s. Use the absorbance of the *Blank solution* to correct the

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absorbances of *Standard solutions A, B,* and *C,* and the *Sample solution.* Plot the corrected absorbances of the standards versus the respective concentrations of the *Standard stock solutions,* in µg/mL, and from the curve so obtained determine the concentration, *C,* in µg/mL, of the *Sample solution.*

Calculate the percentage of thimerosal ($C_9H_9HgNaO_2S$) in the Topical Aerosol taken:

$$\text{Result} = (C/C_U) \times 100$$

C = measured concentration of thimerosal in the *Sample solution* (µg/mL)

C_U = nominal concentration of thimerosal in the *Sample solution* (µg/mL)

Acceptance criteria: 85.0%–115.0%

SPECIFIC TESTS

• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩

Analysis: Weigh, chill, and open 1 Topical Aerosol container, and remove the propellant as directed for the *Sample solution* in the *Assay,* continuing until the bulk of the propellant has evaporated. Determine the alcohol content of the sample thus prepared by the *Gas Chromatographic Method* (see *Method II* in *Alcohol Determination* ⟨611⟩), using methyl ethyl ketone as the internal standard in place of acetonitrile.

Acceptance criteria: 18.7%–25.3% (w/w) of C_2H_5OH

• **OTHER REQUIREMENTS:** Meets the requirements for *Topical Aerosols* ⟨603⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, pressurized containers, and avoid exposure to excessive heat.

Thimerosal Topical Solution

DEFINITION

Thimerosal Topical Solution contains, in each 100 mL, NLT 95 mg and NMT 105 mg of thimerosal ($C_9H_9HgNaO_2S$).

[NOTE—Thimerosal Topical Solution is sensitive to some metals.]

IDENTIFICATION

• **A.**

Sample: 50 mL of Topical Solution

Analysis 1: Pass hydrogen sulfide through the *Sample.*

Acceptance criteria 1: No black discoloration or black precipitate is formed.

Analysis 2: Add 3 or 4 drops of bromine to the *Sample,* mix, and warm on a steam bath to expel the excess bromine. Add 5 mL of 3 N hydrochloric acid, filter, and pass hydrogen sulfide through the filtrate.

Acceptance criteria 2: A black precipitate is formed.

• **B.**

Sample: 1 mL of Topical Solution

Analysis: To the *Sample* add 9 mL of water, mix, and add 1 mL of cupric sulfate TS.

Acceptance criteria: A green color is produced immediately and is followed by the gradual precipitation of flocculent, greenish brown particles.

ASSAY

• **PROCEDURE**

The *Standard solutions* and *Sample solution* may be diluted with water, if necessary, to yield solutions of suitable concentration, adaptable to the linear or working range of the instrument.

Solution A: Dissolve 50 g of stannous chloride in 100 mL of hydrochloric acid on a steam bath, cool, and dilute with water to 500 mL. Use within 3 months.

Standard stock solution A: 1.8 µg/mL of USP Thimerosal RS

Standard stock solution B: 2.0 µg/mL of USP Thimerosal RS

Standard stock solution C: 2.2 µg/mL of USP Thimerosal RS

Standard solution A: Pipet 20 mL of *Standard stock solution A* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Standard solution B: Pipet 20 mL of *Standard stock solution B* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Standard solution C: Pipet 20 mL of *Standard stock solution C* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Sample stock solution: Pipet 2 mL of Topical Solution into a 1000-mL volumetric flask, and dilute with water to volume.

Sample solution: Pipet 20 mL of *Sample stock solution* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Instrumental conditions

Mode: Flameless atomic absorption spectroscopy

Lamp: Mercury hollow-cathode

Blank solution: Pipet 20 mL of water into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.

Analysis

Samples: *Standard solutions A, B,* and *C, Sample solution,* and *Blank solution*

Proceed with each of the *Samples* as follows. Pipet 3 mL into the scrubbing chamber of a suitable system designed for determination of mercury. Dilute with water to 150 mL, and add hydroxylamine hydrochloride solution (1 in 10) to reduce the excess permanganate. Add 5 mL of *Solution A,* and immediately attach the scrubbing chamber to the system. Concomitantly determine the absorbance of each solution at an integration time of 15 s. Use the absorbance of the *Blank solution* to correct the absorbances of *Standard solutions A, B,* and *C,* and the *Sample solution.* Plot the corrected absorbances of the standards versus their respective concentrations of the *Standard stock solutions,* in µg/mL, and from the curve so obtained, determine the concentration, *C,* in µg/mL, of the *Sample solution.*

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Calculate the quantity, in mg, of thimerosal ($C_9H_9HgNaO_2S$) in each 100 mL of Topical Solution taken:

$$\text{Result} = C \times D \times V \times F$$

C	= measured concentration of the *Sample solution* (µg/mL)
D	= dilution of the *Sample stock solution*, 500
V	= volume of Topical Solution, 100 mL
F	= unit conversion, 1 mg/1000 µg

Acceptance criteria: 95–105 mg

SPECIFIC TESTS

- **pH** ⟨791⟩: 9.6–10.2

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **USP Reference Standards** ⟨11⟩
  USP Thimerosal RS

Thimerosal Tincture

DEFINITION

Thimerosal Tincture contains, in each 100 mL, NLT 90 mg and NMT 110 mg of thimerosal ($C_9H_9HgNaO_2S$).
[Note—Thimerosal Tincture is sensitive to some metals.]

IDENTIFICATION

- **A.**
  Sample: 25 mL of Tincture
  Analysis: Heat the *Sample* on a steam bath until the odors of alcohol and acetone are no longer perceptible. Cool and pass hydrogen sulfide through the solution.
  Acceptance criteria: No black discoloration or black precipitate is formed.
- **B.**
  Sample: 50 mL of Tincture
  Analysis: Evaporate the *Sample* on a steam bath to a volume of approximately 20 mL, cool, and add 3 or 4 drops of bromine. Add 5 mL of 3 N hydrochloric acid, filter, and pass hydrogen sulfide through the filtrate.
  Acceptance criteria: A black precipitate is formed.

ASSAY

- **Procedure**

The *Standard solutions* and *Sample solution* may be diluted with water, if necessary, to yield solutions of suitable concentration, adaptable to the linear or working range of the instrument.
Solution A: Dissolve 50 g of stannous chloride in 100 mL of hydrochloric acid on a steam bath, cool, dilute with water to 500 mL, and mix. Use within 3 months.
Standard stock solution A: 1.8 µg/mL of USP Thimerosal RS
Standard stock solution B: 2.0 µg/mL of USP Thimerosal RS
Standard stock solution C: 2.2 µg/mL of USP Thimerosal RS
Standard solution A: Pipet 20 mL of *Standard stock solution A* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.
Standard solution B: Pipet 20 mL of *Standard stock solution B* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.
Standard solution C: Pipet 20 mL of *Standard stock solution C* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.
Sample stock solution: Pipet 2 mL of Tincture into a 1000-mL volumetric flask, and dilute with water to volume.
Sample solution: Pipet 20 mL of *Sample stock solution* into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.
Instrumental conditions
Mode: Flameless atomic absorption spectroscopy
Lamp: Mercury hollow-cathode
Blank solution: Pipet 20 mL of water into a 100-mL volumetric flask. Add 5 mL of sulfuric acid, cool, add 3 mL of nitric acid, and mix. Add potassium permanganate crystals, while mixing, until the purple color persists for NLT 15 min. Add 200 mg of potassium persulfate, mix, and heat on a steam bath for 2 h. Cool, and dilute with water to volume.
Analysis
Samples: *Standard solutions A, B,* and *C, Sample solution,* and *Blank solution*
Proceed with each of the *Samples* as follows. Separately pipet 3 mL into the scrubbing chamber of a suitable system designed for determination of mercury. Dilute with water to 150 mL, and add hydroxylamine hydrochloride solution (1 in 10) to reduce the excess permanganate. Add 5 mL of *Solution A*, and immediately attach the scrubbing chamber to the system. Concomitantly determine the absorbance of the vapor from each solution at an integration time of 15 s. Use the absorbance of the *Blank solution* to correct the absorbances of *Standard solutions A, B,* and *C,* and the *Sample solution.* Plot the corrected absorbances of the standards versus the respective concentrations of the *Standard stock solutions,* in µg/mL, and from the curve so obtained, determine the concentration, *C,* in µg/mL, of the *Sample solution.*
Calculate the quantity, in mg, of thimerosal ($C_9H_9HgNaO_2S$) in each 100 mL of Tincture taken:

$$\text{Result} = C \times D \times V \times F$$

C	= measured concentration of the *Sample solution* (µg/mL)
D	= dilution of the *Sample stock solution*, 500
V	= volume of Tincture, 100 mL
F	= unit conversion, 1 mg/1000 µg

Acceptance criteria: 90–110 mg

SPECIFIC TESTS

- **Alcohol Determination,** *Method II* ⟨611⟩: 45.0%–55.0% of C_2H_5OH

ADDITIONAL REQUIREMENTS

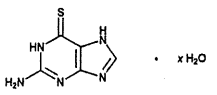
- **Packaging and Storage:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

USP Monographs

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Thimerosal RS

Thioguanine



$C_5H_5N_5S \cdot xH_2O$ (anhydrous) 167.19

$C_5H_5N_5S \cdot ½H_2O$ 176.20

6H-Purine-6-thione, 2-amino-1,7-dihydro-; 2-Aminopurine-6(1*H*)-thione [154-42-7]. Hemihydrate [5580-03-0].

DEFINITION
Thioguanine is anhydrous or contains one-half molecule of water of hydration. It contains NLT 96.0% and NMT 100.5% of thioguanine ($C_5H_5N_5S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: Transfer 100 mg of Thioguanine, previously dried, to a 100-mL volumetric flask. Dissolve in a mixture of 15 mL of water and 1.5 mL of 1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, add 1% hydrochloric acid to volume, and mix. Finally, transfer 5.0 mL of the last solution to a third 100-mL volumetric flask, then add 1% hydrochloric acid to volume, and mix.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Thioguanine RS.

ASSAY
• **PROCEDURE**
Phosphoric acid solution: Add 1 mL of phosphoric acid to 99 mL of water.
Mobile phase: 0.05 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Standard stock solution: 0.4 mg/mL of USP Thioguanine RS in 0.01 N sodium hydroxide
Standard solution: 0.04 mg/mL of USP Thioguanine RS in *Phosphoric acid solution* from *Standard stock solution*
Sample stock solution: 0.4 mg/mL of Thioguanine in 0.01 N sodium hydroxide
Sample solution: 0.04 mg/mL of Thioguanine in *Phosphoric acid solution* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 248 nm
Column: 4.6-mm × 5-cm; packing L1
Flow rate: 2.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of thioguanine ($C_5H_5N_5S$) in the portion of Thioguanine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Thioguanine RS in the *Standard solution* (mg/mL)
C_U = concentration of Thioguanine in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–100.5% on the dried basis

OTHER COMPONENTS
• **NITROGEN DETERMINATION,** *Method II* ⟨461⟩
Sample: 100 mg
Analysis: Each mL of 0.1 N sulfuric acid is equivalent to 1.401 mg of N.
Acceptance criteria: 40.2%–43.1% on the dried basis

IMPURITIES
• **SELENIUM** ⟨291⟩
Sample: 200 mg
Acceptance criteria: NMT 30 ppm
• **FREE SULFUR**
Sample solution: Dissolve 50 mg of Thioguanine in 5 mL of 1 N sodium hydroxide.
Acceptance criteria: The *Sample solution* is clear.
• **PHOSPHOROUS-CONTAINING SUBSTANCES**
Solution A: Dissolve 8.3 g of ammonium molybdate in 40 mL of water, add 33 mL of dilute sulfuric acid (2 in 7), and dilute with water to 100.0 mL. [NOTE—This solution is stable for about 2 weeks.]
Analysis: Transfer 50.0 mg of Thioguanine to a large test tube, add 1 mL of dilute sulfuric acid (2 in 7), and heat in a boiling water bath for 5 min. Cautiously add nitric acid, dropwise, continue heating until the mixture becomes colorless, and then heat for 1 min longer. Cool, dilute with water to about 10 mL, and transfer the solution to a 25-mL volumetric flask with the aid of a few mL of water. To the flask add 0.75 mL of *Solution A* and 1.0 mL of aminonaphtholsulfonic acid TS, dilute with water to volume, and mix. Determine the absorbance of this solution in a 1-cm cell, with a suitable spectrophotometer, at a wavelength of about 620 nm, using a reagent blank to set the instrument.
Acceptance criteria: NMT 0.03% as phosphate; the absorbance is not greater than that produced by 1.5 mL of a similar solution of monobasic potassium phosphate in water having a known concentration of 10 µg/mL of phosphate (PO_4), concomitantly measured.
• **ORGANIC IMPURITIES**
Mobile phase, Sample stock solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.04 mg/mL of USP Guanine RS in 0.01 N sodium hydroxide
Standard solution: 0.4 µg/mL of USP Guanine RS in *Mobile phase* from *Standard stock solution*
Sample solution: 0.04 mg/mL of Thioguanine in *Mobile phase* from *Sample stock solution*

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System suitability solution: Transfer 1.0 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with the *Sample solution* to volume.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for guanine and thioguanine are about 0.60 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between guanine and thioguanine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of guanine in the portion of Thioguanine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of guanine from the *Sample solution*
r_S = peak response of guanine from the *Standard solution*
C_S = concentration of guanine in the *Standard solution* (mg/mL)
C_U = concentration of Thioguanine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.5%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 105° for 5 h.
Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Label it to indicate its state of hydration.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Guanine RS
USP Thioguanine RS

Thioguanine Tablets

DEFINITION
Thioguanine Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of thioguanine ($C_5H_5N_5S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K.▲** (CN 1-May-2020) Record the spectra over the range from 3000 cm^{-1} to 650 cm^{-1}
Sample: Shake a portion of the powdered Tablets equivalent to 0.5 g of thioguanine with 10 mL of 1 M sodium hydroxide, and filter. Acidify the filtrate with hydrochloric acid, filter, and dissolve the precipitate in 25% ammonia water. Evaporate to dryness, and dry the residue at 105° at a pressure not exceeding 0.1 psi for 5 h.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: 6 g/L of anhydrous monobasic sodium phosphate in water adjusted with phosphoric acid to a pH of 3.0
Diluent: 0.01 M sodium hydroxide
Standard stock solution: 0.4 mg/mL of USP Thioguanine RS in *Diluent*
Standard solution: 0.04 mg/mL of USP Thioguanine RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: Nominally 0.4 mg/mL of thioguanine in *Diluent* prepared as follows. Transfer NLT 20 finely powdered Tablets, equivalent to 40 mg of thioguanine, to a 100-mL volumetric flask. Add 70 mL of *Diluent*, and shake for 15 min. Dilute with *Diluent* to volume.
Sample solution: 0.04 mg/mL of thioguanine in *Mobile phase* from the *Sample stock solution*. Pass a portion of this solution through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 248 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thioguanine ($C_5H_5N_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of thioguanine in the *Standard solution* (mg/mL)
C_U = nominal concentration of thioguanine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Sample solution: Pass a portion of the solution under test through a suitable filter. Transfer 2 mL of the filtrate and 2 mL of 1 M hydrochloric acid to a 20-mL volumetric flask, and dilute with water to volume.
Standard solution: USP Thioguanine RS in 0.1 M hydrochloric acid with a concentration similar to the *Sample solution*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 348 nm
Blank: 0.1 M hydrochloric acid
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thioguanine ($C_5H_5N_5S$) dissolved:

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$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
D = dilution factor for *Sample solution*, 10
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of thioguanine ($C_5H_5N_5S$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Diluent: 0.01 M sodium hydroxide

System suitability stock solution: 0.4 mg/mL each of USP Thioguanine RS and USP Guanine RS in 10% (v/v) phosphoric acid. Sonicate to dissolve.

System suitability solution: 0.04 mg/mL each of USP Thioguanine RS and USP Guanine RS in *Mobile phase* from the *System suitability stock solution*

Standard stock solution: 8 µg/mL of USP Thioguanine RS and 0.16 mg/mL of USP Guanine RS in *Diluent*

Standard solution: 0.8 µg/mL of USP Thioguanine RS and 0.016 mg/mL of USP Guanine RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Nominally 0.4 mg/mL of thioguanine prepared as follows. Transfer NLT 20 finely powdered Tablets, equivalent to 40 mg of thioguanine, to a 100-mL volumetric flask. Add 10 mL of *Diluent*, and sonicate for 5 min. Dilute with *Mobile phase* to volume. Pass a portion of this solution through a suitable filter.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between the thioguanine and guanine peaks, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of guanine in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Guanine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thioguanine in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of thioguanine from the *Standard solution*
C_S = concentration of USP Thioguanine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thioguanine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Guanine[a]	0.5	4
Thioguanine	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	4.5

[a] 2-Amino-1,7-dihydro-6*H*-purin-6-one.

ADDITIONAL REQUIREMENTS

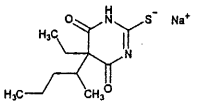
• **Packaging and Storage:** Preserve in tight containers. Store at 15°–25°.

• **USP Reference Standards** ⟨11⟩

USP Guanine RS
2-Amino-1,7-dihydro-6*H*-purin-6-one.
$C_5H_5N_5O$ 151.13

USP Thioguanine RS

Thiopental Sodium



$C_{11}H_{17}N_2NaO_2S$ 264.32

4,6(1*H*,5*H*)-Pyrimidinedione, 5-ethyldihydro-5-(1-methylbutyl)-2-thioxo-, monosodium salt, (±)-;
Sodium (±)-5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate [71-73-8].

DEFINITION

Thiopental Sodium contains NLT 97.0% and NMT 102.0% of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$), calculated on the dried basis.

IDENTIFICATION

• **A.**

Sample: 500 mg of Thiopental Sodium

Analysis: Dissolve the *Sample* in 10 mL of water in a separator, add 10 mL of 3 N hydrochloric acid, and extract the liberated thiopental with two 25-mL portions of chloroform. Evaporate the combined chloroform extracts to dryness. Add 10 mL of ether, evaporate again, and dry at 105° for 2 h.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Thiopental RS.

• **B. Identification Tests—General,** *Sodium* ⟨191⟩

Analysis: Ignite 500 mg.

Acceptance criteria: The residue meets the requirements.

• **C.**

Sample: 200 mg of Thiopental Sodium

Analysis 1: Dissolve the *Sample* in 5 mL of 1 N sodium hydroxide, and add 2 mL of lead acetate TS.

Acceptance criteria 1: A white precipitate is formed, and it gradually darkens when the mixture is boiled.

Analysis 2: Acidify the darkened mixture obtained from *Analysis 1* with hydrochloric acid.

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Acceptance criteria 2: Hydrogen sulfide is evolved, and it is recognizable by its darkening of moistened lead acetate test paper held in the vapor.

ASSAY

• **PROCEDURE**

Solution A: 4 g/L of sodium hydroxide

Standard solution: 5 µg/mL of USP Thiopental RS in *Solution A*

Sample solution: 5 µg/mL of Thiopental Sodium in *Solution A*

Instrumental conditions

Mode: UV

Analytical wavelength: 304 nm

Cell: 1 cm

Blank: *Solution A*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$) in the portion of Thiopental Sodium taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Thiopental RS in the *Standard solution* (µg/mL)

C_U = concentration of Thiopental Sodium in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of thiopental sodium, 264.32

M_{r2} = molecular weight of thiopental, 242.34

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **ORDINARY IMPURITIES** ⟨466⟩

Standard solution: 9.2 mg/mL of USP Thiopental RS in methanol

Sample solution: 10 mg/mL of Thiopental Sodium in methanol

Application volume: 40 µL

Eluant: Toluene and methanol (85:15)

Visualization: 1

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 80° for 4 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Thiopental RS

Thiopental Sodium for Injection

DEFINITION

Thiopental Sodium for Injection is a sterile mixture of Thiopental Sodium and anhydrous Sodium Carbonate as a buffer. It contains NLT 93.0% and NMT 107.0% of the labeled amount of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Nominally 500 mg of thiopental sodium from Thiopental Sodium for Injection

Analysis: Dissolve the *Sample* in 10 mL of water in a separator, add 10 mL of 3 N hydrochloric acid, and extract the liberated thiopental with two 25-mL portions of chloroform. Evaporate the combined chloroform extracts to dryness. Add 10 mL of ether, evaporate again, and dry at 105° for 2 h.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Thiopental RS.

• **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

Sample: Nominally 500 mg of thiopental sodium from Thiopental Sodium for Injection

Analysis: Ignite the *Sample*.

Acceptance criteria: The residue meets the requirements.

ASSAY

• **PROCEDURE**

Diluent: 4 g/L of sodium hydroxide in water

Standard solution: 5 µg/mL of USP Thiopental RS in *Diluent*

Sample stock solution: Nominally 50 mg/mL of thiopental sodium from the contents of 10 containers of Thiopental Sodium for Injection dissolved in a sufficient volume of water

Sample solution: Nominally 5 µg/mL of thiopental sodium in *Diluent* from the *Sample stock solution*

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 304 nm

Cell: 1 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$) in the portion of Thiopental Sodium for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (µg/mL)

C_U = nominal concentration of the *Sample solution* (µg/mL)

M_{r1} = molecular weight of thiopental sodium, 264.32

M_{r2} = molecular weight of thiopental, 242.34

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS

• **COMPLETENESS OF SOLUTION** ⟨641⟩

Sample solution: Mix 800 mg of Thiopental Sodium for Injection with 10 mL of carbon dioxide-free water.

Acceptance criteria: After 1 min, the solution is clear and free from undissolved solid.

USP Monographs

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- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 1.0 USP Endotoxin Unit/mg of thiopental sodium.
- **pH** ⟨791⟩

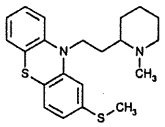
Sample solution: Use the *Sample solution* prepared in the test for *Completeness of Solution.*
Acceptance criteria: 10.2–11.2

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** Meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Thiopental RS

Thioridazine



$C_{21}H_{26}N_2S_2$ 370.57

10*H*-Phenothiazine, 10-[2-(1-methyl-2-piperidinyl) ethyl]-2-(methylthio)-;
10-[2-(1-Methyl-2-piperidyl)ethyl]-2-(methylthio) phenothiazine [50-52-2].

DEFINITION

Thioridazine contains NLT 99.0% and NMT 101.0% of $C_{21}H_{26}N_2S_2$, calculated on the dried basis.
[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and the solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample solution: 300 mg of Thioridazine in 60 mL of glacial acetic acid
Analysis: Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 37.06 mg of $C_{21}H_{26}N_2S_2$.
Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

[NOTE—Conduct this procedure without delay, under subdued light.]
Diluent: Methanol and ammonium hydroxide (49:1)
Standard solution A: 50 µg/mL of USP Thioridazine RS in *Diluent*
Standard solution B: 20 µg/mL of USP Thioridazine RS in *Diluent*
Sample solution: 10 mg/mL of Thioridazine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Chloroform, isopropyl alcohol, and ammonium hydroxide (74:25:1)
Analysis
Samples: *Standard solution A, Standard solution B,* and *Sample solution*
Immediately develop until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light.
Acceptance criteria: The chromatograms show principal spots at about the same R_F value; no secondary spot, if present from the *Sample solution,* is more intense than the principal spot from *Standard solution A* (0.5%); and the sum of the intensities of all secondary spots, if present in the *Sample solution,* is NMT 0.5%.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample in vacuum at 50° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Thioridazine RS

Thioridazine Hydrochloride

$C_{21}H_{26}N_2S_2 \cdot HCl$ 407.04

10*H*-Phenothiazine, 10-[2-(1-methyl-2-piperidinyl)ethyl]-2-(methylthio)-, monohydrochloride.
10-[2-(1-Methyl-2-piperidyl)ethyl]-2-(methylthio) phenothiazine monohydrochloride [130-61-0].

» Thioridazine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of $C_{21}H_{26}N_2S_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Thioridazine Hydrochloride RS
[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or by using low-actinic glassware.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

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B: A solution (1 in 100) in a mixture of water and alcohol (1:1) responds to test *B* in *Indentification Tests— General* ⟨191⟩ *Chemical Identification Tests, Chloride.*

Melting range ⟨741⟩: between 159° and 165°, but the range between beginning and end of melting does not exceed 3°.

pH ⟨791⟩: between 4.2 and 5.2, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.4% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Selenium ⟨291⟩: 0.003%, a 100-mg specimen being used, 100 mg of magnesium oxide being added to the *Test Solution.*

Chromatographic purity— [NOTE—Conduct this procedure in subdued lighting without delay.]

Diluting solution: a mixture of methanol and ammonium hydroxide (49:1).

Test solution—Transfer 100 mg of Thioridazine Hydrochloride, accurately weighed, to a 5-mL volumetric flask. Dissolve in and dilute with *Diluting solution* to volume, and mix.

Standard solutions—Dissolve an accurately weighed quantity of USP Thioridazine Hydrochloride RS in *Diluting solution,* and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of 20.0 mg per mL (*Standard solution A*). Dilute appropriate portions of this solution quantitatively with *Diluting solution* to obtain five *Standard solutions B* to *F* having known concentrations of 0.1, 0.067, 0.025, 0.02 and 0.01 mg per mL, respectively. The final concentrations of *Standard solutions B* to *F* represent 0.5%, 0.33%, 0.125%, 0.1% and 0.05% concentration of *Standard solution A,* respectively.

Application volume: 5 µL.

Developing solvent system: a mixture of chloroform, isopropyl alcohol, and ammonium hydroxide (74:25:1).

Procedure—Apply equal spots of the *Test solution* and *Standard solutions A* to *F* as directed under *Ordinary Impurities* ⟨466⟩. Examine the plate under short- and long-wavelength UV light, then spray the plate with Dragendorff's TS, dry the plate with a stream of nitrogen, and then spray with hydrogen peroxide TS: any secondary spot in the *Test solution* is not more than 0.5%, and the sum of all secondary spots is not more than 0.5%.

Assay—Dissolve about 350 mg of Thioridazine Hydrochloride, accurately weighed, in 80 mL of a solution of equal parts of glacial acetic acid and acetic anhydride, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 40.70 mg of $C_{21}H_{26}N_2S_2 \cdot HCl$.

Delete the following:

▲Thioridazine Hydrochloride Oral Solution

DEFINITION

Thioridazine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of thioridazine hydrochloride ($C_{21}H_{26}N_2S_2 \cdot HCl$).

[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and the solutions containing them by conducting the procedures without delay, under subdued light, or by using low-actinic glassware.]

IDENTIFICATION

• **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩

Sample solution: 2 mg/mL of thioridazine hydrochloride from a volume of Oral Solution in water. [NOTE—Use 2 mL of sodium bicarbonate solution (1 in 12) in place of the 2 mL of 1 N sodium hydroxide specified in the test.]

Acceptance criteria: Meets the requirements

ASSAY

[NOTE—Conduct this procedure with minimum exposure to light.]

• **PROCEDURE**

Diluent: Shake 125 mL of chloroform with 5 mL of ammonium hydroxide in a separator, slowly filter the bottom layer through filter paper containing anhydrous sodium sulfate, and discard the top layer.

Standard solution: 6 µg/mL of USP Thioridazine Hydrochloride RS in *Diluent*

Sample stock solution: Nominal concentration of 0.6 mg/mL in chloroform prepared as follows: Pipet a portion of Oral Solution, equivalent to 120 mg of thioridazine hydrochloride, into a separator containing 15 mL of water. Render alkaline with ammonium hydroxide, and extract with three 25-mL portions of *Diluent.* Filter the extracts through a pledget of glass wool into a 200-mL volumetric flask. Rinse the filter, and add chloroform to volume.

Sample solution: Nominally 6 µg/mL in *Diluent* from *Sample stock solution*

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 265 nm

Cell: 1 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution, Sample solution,* and *Blank*

Calculate the percentage of thioridazine hydrochloride ($C_{21}H_{26}N_2S_2 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Thioridazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of thioridazine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **ALCOHOL DETERMINATION** ⟨611⟩**:** NMT 4.75% of C_2H_5OH

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **LABELING:** Label it to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Thioridazine Hydrochloride RS▲ (USP 1-May-2020)

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Thioridazine Hydrochloride Tablets

DEFINITION

Thioridazine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of thioridazine hydrochloride ($C_{21}H_{26}N_2S_2 \cdot HCl$).

Throughout the following procedures, protect samples, the Reference Standard, and the solutions containing them by conducting the procedures without delay, under subdued light, or by using low-actinic glassware.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, water, and triethylamine (850:150:1)

System suitability solution: 0.1 mg/mL of USP Mesoridazine Besylate RS and 0.11 mg/mL of USP Thioridazine Hydrochloride RS in methanol

Standard solution: 125 µg/mL of USP Thioridazine Hydrochloride RS in methanol. Sonication may be used to aid dissolution.

Sample stock solution: Nominally 1.0 mg/mL prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a weighed portion of the powder, equivalent to 100 mg of thioridazine hydrochloride, to a 100-mL volumetric flask. Add 80 mL of methanol, and shake by mechanical means for 30 min. Dilute with methanol to volume, and sonicate for 45 min with intermittent shaking. Allow the undissolved solids to settle, and filter, discarding the first 20 mL of the filtrate.

Sample solution: 125 µg/mL in methanol from a portion of filtrate from the *Sample stock solution*. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mesoridazine and thioridazine are 0.44 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between mesoridazine and thioridazine, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of thioridazine hydrochloride ($C_{21}H_{26}N_2S_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thioridazine from the *Sample solution*

r_S = peak response of thioridazine from the *Standard solution*

C_S = concentration of USP Thioridazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of thioridazine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 1000 mL

Apparatus 2: 75 rpm

Time: 60 min

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Standard solution: USP Thioridazine Hydrochloride RS in *Medium* at a known concentration

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 262 nm

Blank: *Medium*

Tolerances: NLT 75% (*Q*) of the labeled amount of thioridazine hydrochloride ($C_{21}H_{26}N_2S_2 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

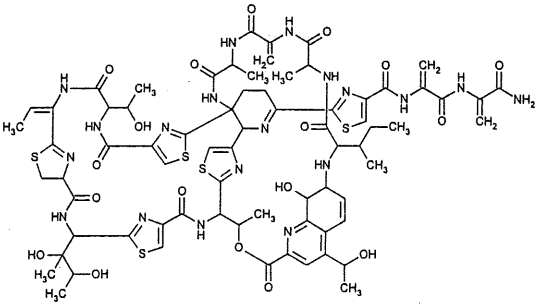
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Mesoridazine Besylate RS

USP Thioridazine Hydrochloride RS

Thiostrepton



$C_{72}H_{85}N_{19}O_{18}S_5$ 1664.89

[1393-48-2].

» Thiostrepton is an antibacterial substance produced by the growth of strains of *Streptomyces azureus* (Fam. Streptomycetaceae). It has a potency of not less than 900 USP Thiostrepton Units per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Thiostrepton RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)

USP Monographs

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Loss on drying ⟨731⟩—Dry about 1 g of sample in vacuum at 60° for 3 hours: it loses not more than 5.0% of its weight.
Residue on ignition ⟨281⟩: not more than 1.0%.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, preparing the *Test Dilution* as follows. Transfer about 60 mg of Thiostrepton, accurately weighed, to a beaker. Add about 125 mL of dimethyl sulfoxide, and stir with a magnetic stirrer until dissolved (about 5 minutes). Transfer this solution to a 200-mL volumetric flask, washing the beaker and the stirring bar with dimethyl sulfoxide. Add the washings to the volumetric flask, dilute with dimethyl sulfoxide to volume, and mix. Dilute this solution quantitatively with dimethyl sulfoxide to obtain a *Test Dilution* having a concentration of thiostrepton assumed to be equal to the median dose of the Standard.

Thiotepa



$C_6H_{12}N_3PS$ 189.22
Aziridine, 1,1′,1″-phosphinothioylidynetris-;
Tris(aziridin-1-yl)phosphine sulfide [52-24-4].

DEFINITION
Thiotepa contains NLT 97.0% and NMT 102.0% of thiotepa ($C_6H_{12}N_3PS$), calculated on the anhydrous basis.
[**CAUTION**—Great care should be taken to prevent inhaling particles of Thiotepa or exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 13.6 g/L of monobasic potassium phosphate in water. Adjust with 35 g/L of dibasic sodium phosphate to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (13:87)
System suitability solution: Transfer 10 mg of USP Thiotepa RS to a 4-mL vial, add 2 mL of methanol, and mix. Add 50 µL of 0.1% phosphoric acid solution. Place a cap on the vial, and heat at 65° for 50 s. Cool the solution, add 1 mL of methanol, and mix. [NOTE—The preparation generates methoxythiotepa.]
Standard solution: 1.5 mg/mL of USP Thiotepa RS in water
Sample solution: 1.5 mg/mL of Thiotepa in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for thiotepa and methoxythiotepa are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3 between methoxythiotepa and thiotepa, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of thiotepa ($C_6H_{12}N_3PS$) in the portion of Thiotepa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiotepa from the *Sample solution*
r_S = peak response of thiotepa from the *Standard solution*
C_S = concentration of USP Thiotepa RS in the *Standard solution* (mg/mL)
C_U = concentration of Thiotepa in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, and System suitability solution: Proceed as directed in the *Assay*.
Peak identification solution: Dissolve 15 mg of USP Thiotepa RS in 10 mL of water, add 1 g of sodium chloride, boil in a water bath for 10 min, and cool. [NOTE—The preparation generates thiotepa chloroethyl analog.]
Standard solution: 3.5 µg/mL of USP Thiotepa RS in water
Sample solution: 3.5 mg/mL of Thiotepa in water
Chromatographic system: Proceed as directed in the *Assay*, with a run time NLT 4 times the retention time of the thiotepa peak.
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for thiotepa and methoxythiotepa are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3 between methoxythiotepa and thiotepa, *System suitability solution*
Analysis
Samples: *Peak identification solution*, *Standard solution*, and *Sample solution*
Calculate the percentage of each impurity in the portion of Thiotepa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of thiotepa from the *Standard solution*
C_S = concentration of USP Thiotepa RS in the *Standard solution* (mg/mL)
C_U = concentration of Thiotepa in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiotepa	1.0	—

USP Monographs

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Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiotepa chloroethyl analog[a]	3.75	0.15
Any individual unspecified impurity	—	0.1
Total unspecified impurities	—	0.2

[a] *P,P*-Bis(aziridin-1-yl)-*N*-(2-chloroethyl)phosphinothioic amide.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Thiotepa RS

Thiotepa for Injection

DEFINITION

Thiotepa for Injection contains NLT 95.0% and NMT 110.0% of the labeled amount of thiotepa ($C_6H_{12}N_3PS$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 13.6 g/L of monobasic potassium phosphate in water. Adjust with 35 g/L of dibasic sodium phosphate in water to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (13:87)
System suitability solution: Transfer 10 mg of USP Thiotepa RS to a 4-mL vial, add 2 mL of methanol, and mix. Add 50 µL of 0.1% phosphoric acid solution. Place a cap on the vial, and heat at 65° for 50 s. Cool the solution, add 1 mL of methanol, and mix. [NOTE—The preparation generates methoxythiotepa.]
Standard solution: 1.5 mg/mL of USP Thiotepa RS in water
Sample solution: Nominally 1.5 mg/mL of thiotepa in water from Thiotepa for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for thiotepa and methoxythiotepa are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3 between methoxythiotepa and thiotepa, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thiotepa ($C_6H_{12}N_3PS$) in the portion of Thiotepa for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiotepa from the *Sample solution*
r_S = peak response of thiotepa from the *Standard solution*
C_S = concentration of USP Thiotepa RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiotepa in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, and **System suitability solution:** Proceed as directed in the *Assay*.
Peak identification solution: Dissolve 15 mg of USP Thiotepa RS in 10 mL of water, add 1 g of sodium chloride, boil in a water bath for 10 min, and cool. [NOTE—The preparation generates thiotepa chloroethyl analog.]
Standard solution: 3.75 µg/mL of USP Thiotepa RS in water
Sample solution: Nominally 3.75 mg/mL of thiotepa in water from Thiotepa for Injection. Pass through a suitable filter, and use the filtrate.
Chromatographic system: Proceed as directed in the *Assay*. In addition, the run time is NLT 4 times the retention time of the thiotepa peak.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3 between methoxythiotepa and thiotepa
Analysis
Samples: *Peak identification solution, Standard solution,* and *Sample solution*
Calculate the percentage of each impurity in the portion of thiotepa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of thiotepa from the *Standard solution*
C_S = concentration of USP Thiotepa RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiotepa in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiotepa	1.0	—
Thiotepa chloroethyl analog[a]	3.75	0.15
Any individual unspecified impurity	—	0.2

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Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities[b]	—	0.4

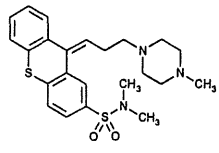
[a] *P,P*-Bis(aziridin-1-yl)-*N*-(2-chloroethyl)phosphinothioic amide.
[b] The impurity thiotepa chloroethyl analog is excluded.

PERFORMANCE TESTS
- **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

SPECIFIC TESTS
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 6.25 USP Endotoxin Units/mg of thiotepa
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL of thiotepa constituted as directed in the labeling
  Acceptance criteria: 5.5–7.5
- **Injections and Implanted Drug Products** ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions*: At the time of use, it meets the requirements.
- **Other Requirements:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* and store in a refrigerator, protected from light.
- **USP Reference Standards** ⟨11⟩
  USP Thiotepa RS

Thiothixene



$C_{23}H_{29}N_3O_2S_2$ 443.63

9*H*-Thioxanthene-2-sulfonamide, *N,N*-dimethyl-9-[3-(4-methyl-1-piperazinyl)propylidene]-, (*Z*)-.
N,N-Dimethyl-9-[3-(4-methyl-1-piperazinyl)propylidene] thioxanthene-2-sulfonamide [5591-45-7; 3313-26-6].

» Thiothixene contains not less than 96.0 percent and not more than 101.5 percent of $C_{23}H_{29}N_3O_2S_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Thiothixene RS
USP (*E*)-Thiothixene RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197S*▲ (CN 1-May-2020)—
Solution: 1 in 20.
Medium: chloroform.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: methanol.
Absorptivities at about 230 nm and 307 nm, calculated on the dried basis, do not differ by more than 4.0%.
Melting range, *Class I* ⟨741⟩: between 147° and 153.5°.
Loss on drying ⟨731⟩—Dry it in vacuum at 100° for 3 hours: it loses not more than 2.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.
Selenium ⟨291⟩: 0.003%.
Limit of (*E*)-thiothixene— [Note—Prepare all solutions in low-actinic glassware.]
Mobile phase—Transfer 6.9 g of monobasic sodium phosphate to a 1-liter volumetric flask, dissolve in and dilute with deionized water to volume, and mix. Filter through a suitable membrane filter. Mix 4 volumes of this solution with 6 volumes of methanol. The concentration of methanol may be adjusted to meet the system suitability requirements.
Standard preparations—
A—Using accurately weighed quantities of USP (*E*)-Thiothixene RS and USP Thiothixene RS, prepare a solution in methanol containing, in each mL, 0.4 mg and 1.2 mg, respectively.
B—Transfer 5.0 mL of *Standard preparation A* to a 100-mL volumetric flask, dilute with methanol to volume, and mix.
C—Transfer about 200 mg of Thiothixene, accurately weighed, to a 100-mL volumetric flask. Transfer 5.0 mL of *Standard preparation A* to the same flask, dissolve in and dilute with methanol to volume, and mix.
Test preparation—Transfer about 200 mg of Thiothixene, accurately weighed, to a 100-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix.
Procedure—Concomitantly introduce equal volumes (about 20 µL) of *Standard preparation C* and *Test preparation* into a high-pressure liquid chromatograph operated at room temperature and equipped with a suitable microsyringe or sampling valve, a column containing packing L9 (typically 25 cm × 4.6 mm), an UV detector capable of monitoring absorption at 254 nm, and a suitable recorder. The *Mobile phase* is maintained at a flow rate of about 1 to 1.5 mL per minute. In a suitable chromatographic system, three replicate injections of *Standard preparation B* show a resolution factor of not less than 2.2 between the thiothixene and (*E*)-thiothixene peaks, their retention times being 13 and 15 minutes, and between 16 and 18 minutes, respectively. Calculate the quantity, in mg, of (*E*)-thiothixene in the portion of Thiothixene taken by the formula:

$$5CH_U/(H_C - H_U)$$

in which *C* is the concentration of USP (*E*)-Thiothixene RS, in mg per mL, in *Standard preparation A;* and H_C and H_U are the peak responses of the (*E*)-thiothixene peaks corrected for the tailing of the main peak, obtained from *Standard preparation C* and the *Test preparation*, respectively: the limit of (*E*)-thiothixene is 1.0%.

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Assay— [NOTE—Perform the dilution operations in low-actinic glassware.]

Mobile phase—Mix 0.5 mL of ethanolamine with 3780 mL of methanol, mix 1400 mL of this solution with 200 mL of water, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Using an accurately weighed quantity of USP Thiothixene RS, prepare a solution in methanol having a known concentration of about 0.02 mg per mL.

Assay preparation—Transfer about 100 mg of Thiothixene, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 2 mL of the resulting solution into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)— The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L3. The flow rate is about 0.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 2000 theoretical plates, and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{29}N_3O_2S_2$ in the portion of Thiothixene taken by the formula:

$$5000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Thiothixene RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Thiothixene Capsules

» Thiothixene Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{23}H_{29}N_3O_2S_2$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Thiothixene RS

Identification—Dissolve a portion of the contents of Capsules in a solvent consisting of equal volumes of chloroform and methanol to obtain a solution containing 1 mg of thiothixene per mL. Shake by mechanical means for 10 minutes, clarify a portion of the mixture by centrifugation, filter, if necessary, and use the clear supernatant or filtrate for the test. Apply 10 µL of this test solution, 10 µL of a Standard solution containing 1 mg of USP Thiothixene RS per mL in the same medium, and 10 µL of a mixture of equal volumes of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, methanol, and diethylamine (65:35:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and locate the spots on the plate by viewing under short- and long-wavelength UV light. Spray the plate lightly with acidified iodoplatinate spray reagent (prepared by mixing 1 volume of hydrochloric acid with 50 volumes of potassium iodoplatinate TS): the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution and the mixed test-Standard solution.

Dissolution ⟨711⟩—

Medium—Dissolve 2.0 g of sodium chloride and 7 mL of hydrochloric acid in water to make 1000 mL, and mix; 900 mL.

Apparatus 1: 100 rpm.

Time: 20 minutes.

Buffer solution—On the day of use, prepare a mixture of 55 volumes of dibasic potassium phosphate solution (87 in 1000), 20 volumes of citric acid monohydrate solution (21 in 200), and 40 volumes of sodium hydroxide solution (1 in 25).

Methyl orange solution—Transfer 15.5 g of boric acid and 2.0 g of methyl orange to a glass-stoppered, 1000-mL flask. Add 500 mL of water, insert the stopper, and shake by mechanical means for not less than 3 hours. Filter through retentive filter paper, and wash the filtrate with two 100-mL portions of chloroform, discarding the chloroform washings. Store the *Methyl orange solution* over 50 mL of chloroform in a glass-stoppered bottle.

Procedure—Prepare a *Test preparation* by passing a 60-mL portion of the dissolution solution through a suitable filter, discarding the first 5 mL of the filtrate, and diluting the subsequent filtrate quantitatively, if necessary, to obtain a concentration of about 1 µg of thiothixene per mL. Prepare a *Standard preparation* of USP Thiothixene RS in *Dissolution Medium* having a known concentration of about 1 µg per mL. Transfer 40.0 mL each of the *Test preparation*, the *Standard preparation*, and *Dissolution Medium* to provide the blank, to individual separators, each containing 8.0 mL of *Buffer solution*, 10.0 mL of *Methyl orange solution*, and 50.0 mL of chloroform. Shake for 3 minutes, allow the layers to separate, transfer 40.0 mL of the chloroform layer, clarified by centrifugation, to a 60-mL separator containing 8.0 mL of dilute hydrochloric acid (1 in 120), shake for 1 minute, and allow the layers to separate. Concomitantly determine the absorbances of the aqueous layers in 1-cm cells, at the wavelength of maximum absorbance at about 508 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in µg per mL, of $C_{23}H_{29}N_3O_2S_2$ in the *Test preparation* taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Thiothixene RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively, and, from the known extent of dilution, determine the amount of it in the dissolution solution.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{23}H_{29}N_3O_2S_2$ is dissolved in 20 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay— [NOTE—Perform the dilution operations in low-actinic glassware.]

Mobile phase , *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Thiothixene.*

Assay preparation—Transfer, as completely as possible, the contents of not less than 20 Capsules to a tared beaker, and weigh. Mix, and transfer an accurately weighed portion of the powder, equivalent to about 10 mg of thiothixene, to a 500-mL volumetric flask. Add about 400 mL of methanol, shake by mechanical means for 10 minutes, place in an

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ultrasonic bath for 5 minutes, dilute with methanol to volume, and filter the suspension through a 5-µm polytef membrane filter.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Thiothixene.* Calculate the quantity, in mg, of $C_{23}H_{29}N_3O_2S_2$ in the portion of Capsules taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Thiothixene RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thiothixene Hydrochloride

$C_{23}H_{29}N_3O_2S_2 \cdot 2HCl \cdot 2H_2O$ 552.58
9*H*-Thioxanthene-2-sulfonamide, *N,N*-dimethyl-9-[3-(4-methyl-1-piperazinyl)propylidene]-, dihydrochloride, dihydrate, (*Z*)-.
N,N-Dimethyl-9-[3-(4-methyl-1-piperazinyl)propylidene] thioxanthene-2-sulfonamide dihydrochloride dihydrate [22189-31-7; 49746-09-0].
Anhydrous 516.56
[58513-59-0; 49746-04-5].

» Thiothixene Hydrochloride contains two molecules of water of hydration or is anhydrous. It contains not less than 97.0 percent and not more than 102.5 percent of $C_{23}H_{29}N_3O_2S_2 \cdot 2HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Thiothixene RS
USP (*E*)-Thiothixene RS

Identification—
A: It meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩, USP Thiothixene RS being used as the standard for comparison, chloroform being used instead of carbon disulfide, and a reagent blank being used in the matched reference cell.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: dilute methanolic hydrochloric acid (1 in 1200).
Absorptivities at 307 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

C: A solution (1 in 100) responds to the tests for *Chloride* ⟨191⟩.
Water Determination, *Method I* ⟨921⟩: between 6.2% and 7.5% for the dihydrate; not more than 1.0% for the anhydrous form.
Residue on ignition ⟨281⟩: not more than 0.2%.
Selenium ⟨291⟩: 0.003%.
Limit of (*E*)-thiothixene— [NOTE—Prepare all solutions in low-actinic glassware.]
Mobile phase—Prepare as directed in the test for *Limit of (E)-thiothixene* under *Thiothixene.*

Standard preparations and *Test preparation*—Prepare as directed in the test for *Limit of (E)-thiothixene* under *Thiothixene,* except to use 250 mg of Thiothixene Hydrochloride instead of 200 mg of Thiothixene.

Procedure—Proceed as directed for *Procedure* in the test for *Limit of (E)-thiothixene* under *Thiothixene.* Calculate the quantity, in mg, of (*E*)-thiothixene hydrochloride in the portion of Thiothixene Hydrochloride taken by the formula:

$$5CH_U(516.56/443.63)/(H_C - H_U)$$

in which 516.56 and 443.63 are the molecular weights of anhydrous thiothixene hydrochloride and thiothixene, respectively; and the other terms are as previously defined. The limit of (*E*)-thiothixene, as the hydrochloride, is 1.0%.
Assay— [NOTE—Perform the dilution operations in low-actinic glassware.]

Mobile phase, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay* under *Thiothixene.*

Assay preparation—Transfer about 116 mg of Thiothixene Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 2 mL of this solution into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Thiothixene.* Calculate the quantity, in mg, of $C_{23}H_{29}N_3O_2S_2 \cdot 2HCl$ in the portion of Thiothixene Hydrochloride taken by the formula:

$$(516.56/443.64)(5000C)(r_U/r_S)$$

in which 516.56 and 443.64 are the molecular weights of anhydrous thiothixene hydrochloride and thiothixene, respectively; C is the concentration, in mg per mL, of USP Thiothixene RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thiothixene Hydrochloride Injection

» Thiothixene Hydrochloride Injection is a sterile solution of Thiothixene Hydrochloride in Water for Injection. It contains an amount of thiothixene hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of thiothixene ($C_{23}H_{29}N_3O_2S_2$).

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Thiothixene RS
Identification—Transfer a volume of Injection, equivalent to about 20 mg of thiothixene hydrochloride, to a separator containing 20.0 mL of chloroform. Render the aqueous layer just basic with ammonium hydroxide, shake for 1 minute, and allow the layers to separate. Pass a portion of the chloroform layer through filter paper, previously washed with chloroform, and use the clear filtrate for the test. Proceed as directed in the *Identification* test under *Thiothixene Capsules,* beginning with "Apply 10 µL of this test solution."
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 88.0 USP Endotoxin Units per mg of thiothixene.
pH ⟨791⟩: between 2.5 and 3.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

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Assay— [NOTE—Perform the dilution operations in low-actinic glassware.]

Mobile phase, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay* under *Thiothixene.*

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 2 mg of thiothixene, to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Thiothixene.* Calculate the quantity, in mg, of $C_{23}H_{29}N_3O_2S_2$ in each mL of the Injection taken by the formula:

$$(100C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Thiothixene RS in the *Standard preparation,* V is the volume, in mL, of Injection taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thiothixene Hydrochloride for Injection

» Thiothixene Hydrochloride for Injection is a sterile, dry mixture of Thiothixene Hydrochloride and Mannitol. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{23}H_{29}N_3O_2S_2$.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution;* protect from light.

USP Reference standards ⟨11⟩—
USP Thiothixene RS

Identification—Constitute a vial of Thiothixene Hydrochloride for Injection with 2.2 mL of water. Further dilute one volume of the constituted solution with 4 volumes of methanol to obtain a test solution containing 1 mg of thiothixene per mL. Apply 10 µL of this test solution and 10 µL of a Standard solution containing 1 mg of USP Thiothixene RS per mL in the same medium to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of isopropyl alcohol and diethylamine (25:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, air-dry for 30 minutes, and then oven-dry at 110° for 30 minutes. Locate the spots on the plate by viewing under short-wavelength UV light. Spray the plate with alkaline permanganate spray reagent (prepared by dissolving 0.5 g of potassium permanganate and 2.0 g of sodium carbonate in 100 mL of water): the R_F value of the principal spot obtained from the test solution, under both detection conditions, corresponds to that obtained from the Standard solution.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 88.0 USP Endotoxin Units per mg of thiothixene.

pH ⟨791⟩: between 2.3 and 3.7, in the solution constituted as directed in the labeling.

Water Determination, *Method I* ⟨921⟩: not more than 4.0%.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay— [NOTE—Perform the dilution operations in low-actinic glassware.]

Mobile phase, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay* under *Thiothixene.*

Assay preparation—Constitute a vial of Thiothixene Hydrochloride for Injection with an accurately measured volume (V_L mL) of water as directed in the labeling. Transfer an accurately measured volume (V_F mL) of the constituted solution, equivalent to about 5 mg of thiothixene, to a 250-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Thiothixene.* Calculate the quantity, in mg, of $C_{23}H_{29}N_3O_2S_2$ in each mL of the constituted solution taken by the formula:

$$(250CV_L/V_P)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Thiothixene RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thiothixene Hydrochloride Oral Solution

» Thiothixene Hydrochloride Oral Solution contains an amount of thiothixene hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of thiothixene ($C_{23}H_{29}N_3O_2S_2$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Thiothixene RS

Identification—Transfer a portion of Oral Solution, equivalent to about 20 mg of thiothixene hydrochloride, to a separator containing 20.0 mL of chloroform. Render the aqueous layer just basic with ammonium hydroxide, shake for 1 minute, and allow the layers to separate. Pass a portion of the chloroform layer through filter paper, previously washed with chloroform; and use the clear filtrate for the test. Proceed as directed in the *Identification* test under *Thiothixene Capsules,* beginning with "Apply 10 µL of this test solution."

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH ⟨791⟩: between 2.0 and 3.0.

Alcohol Determination, *Method II* ⟨611⟩: if present, between 90.0% and 110.0% of the labeled amount, the labeled amount being not more than 7.0% of C_2H_5OH, determined by the gas-liquid chromatographic procedure, acetonitrile being used as the internal standard.

Assay— [NOTE—Perform the dilution operations in low-actinic glassware.]

Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Thiothixene.*

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 25 mg of thiothixene, to a 25-mL volumetric flask; dissolve in and dilute with methanol to volume; and mix. Pipet 2 mL of this solution

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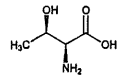
into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Thiothixene.* Calculate the quantity, in mg, of thiothixene ($C_{23}H_{29}N_3O_2S_2$) in each mL of the Oral Solution taken by the formula:

$$(1250C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Thiothixene RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Threonine



$C_4H_9NO_3$ 119.12

L-Threonine [72-19-5].

DEFINITION

Threonine contains NLT 98.5% and NMT 101.5% of L-threonine ($C_4H_9NO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample: 110 mg of Threonine

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant.* Perform the *Blank* determination.

Calculate the percentage of threonine ($C_4H_9NO_3$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 119.1 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.4%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of Threonine

Acceptance criteria: NMT 0.05%

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Threonine

Acceptance criteria: NMT 0.03%

- **IRON ⟨241⟩:** NMT 30 ppm
- **RELATED COMPOUNDS**

Diluent: Hydrochloric acid and water (1:99)

System suitability solution: 0.4 mg/mL each of USP L-Threonine RS and USP L-Proline RS in *Diluent*

Standard solution: 0.05 mg/mL of USP L-Threonine RS in *Diluent.* [NOTE—This solution has a concentration equivalent to about 0.5% of that of the *Sample solution.*]

Sample solution: 10 mg/mL Threonine in 2 N hydrochloric acid

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *System suitability solution, Standard solution,* and *Sample solution.*

After air-drying the plate, spray with *Spray reagent,* and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution.*

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 60 mg/mL in water

Acceptance criteria: –26.7° to –29.1°

- **pH ⟨791⟩**

Sample solution: 50 mg/mL solution

Acceptance criteria: 5.0–6.5

- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° for 3 h: it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP L-Proline RS

USP L-Threonine RS

Thyroid

DEFINITION

Thyroid is the cleaned, dried, and powdered thyroid gland previously deprived of connective tissue and fat. It is obtained from domesticated animals that are used for food by humans.

On hydrolysis it yields NLT 90.0% and NMT 110.0% each of the labeled amounts of levothyroxine ($C_{15}H_{11}I_4NO_4$) and liothyronine ($C_{15}H_{12}I_3NO_4$), calculated on the dried basis. It

USP Monographs

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is free from iodine in inorganic or any form of combination other than that peculiar to the thyroid gland. It may contain a suitable diluent such as lactose, sodium chloride, starch, sucrose, or dextrose.

IDENTIFICATION

• **A.** The retention times of the peaks for liothyronine and levothyroxine of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, water, and phosphoric acid (350:650:5), filtered and degassed

Reducing buffer solution: Freshly prepare 0.04 M tris(hydroxymethyl)aminomethane and 0.05 M methimazole in 0.11 M sodium chloride. Adjust, if necessary, with 6 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 8.4 ± 0.05.

Proteolytic enzyme: Freshly prepare a solution containing 3 mg/mL of bacterial protease in *Reducing buffer solution*.[1]

Enzyme deactivating solution: Phosphoric acid in acetonitrile (1:99)

Standard stock solution: Transfer accurately weighed quantities of about 9 mg of USP Liothyronine RS and about 38 mg of USP Levothyroxine RS to a 100-mL volumetric flask, add 50 mL of a mixture of acetonitrile, water, and ammonium hydroxide (500:500:1) and swirl to dissolve. Dilute with a mixture of acetonitrile and water (1:1) to volume, and mix. [NOTE—Protect solutions from light.]

Standard solution: Pipet 5 mL of the freshly prepared *Standard stock solution* into a 250-mL volumetric flask, dilute with *Reducing buffer solution* to volume, and mix to obtain a solution having known concentrations of about 1.8 µg/mL of liothyronine and 7.6 µg/mL of levothyroxine. Pipet 5 mL of this solution into a screw-capped 16- × 125-mm culture tube. Pipet 2 mL of *Enzyme deactivating solution* into the tube, place the cap on the tube, and shake the mixture vigorously. [NOTE—Prepare on the day of use.]

Sample solution: Transfer an accurately weighed portion of finely powdered Thyroid, equivalent to about 38 µg of levothyroxine, to a screw-capped 16- × 125-mm culture tube that has been flushed previously with nitrogen. Taking precautions to avoid unnecessary exposure to air, pipet 5 mL of *Proteolytic enzyme* into the tube. Allow nitrogen to flow gently over the mixture for 5 min. Place the cap on the tube, mix to disperse the contents, and place in a covered water bath maintained at a temperature of 37 ± 1° for 28 h. [NOTE—Protect the contents of the tubes from light.] Examine occasionally, and mix as necessary to ensure dispersion. At the end of the incubation period, pipet 2 mL of *Enzyme deactivating solution* into the tube, place the cap on the tube, mix vigorously, and centrifuge at about 2000 rpm for 5 min. Pass the supernatant through a filter of 0.45-µm pore size, discarding the first 1 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-cm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8 for liothyronine and levothyroxine peaks

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of levothyroxine ($C_{15}H_{11}I_4NO_4$) and liothyronine ($C_{15}H_{12}I_3NO_4$) in the portion of Thyroid taken:

$$\text{Result} = (V \times C) \times (r_U/r_S)$$

V = volume of *Sample solution*, 7 mL

C = concentration of the corresponding Reference Standard in the *Standard solution* (µg/mL)

r_U = peak response of the corresponding analytes from the *Sample solution*

r_S = peak response of the corresponding analytes from the *Standard solution*

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of levothyroxine ($C_{15}H_{11}I_4NO_4$) and liothyronine ($C_{15}H_{12}I_3NO_4$) on the dried basis

IMPURITIES

• **LIMIT OF INORGANIC IODIDES**

Extracting solution: Sulfuric acid and water (1:99)

Standard solution: Dissolve an accurately weighed quantity of potassium iodide in water to obtain a stock solution containing 0.131 mg, equivalent to 0.100 mg of iodide, per mL. Transfer 1.0 mL of this stock solution into a 100-mL volumetric flask, dilute with *Extracting solution* to volume, and mix. Each mL of the *Standard solution* contains 1.0 µg of iodide. [NOTE—Prepare this solution on the day of use.]

Sample solution: Transfer 1.00 g, or proportionately less if the dry basis levothyroxine content is greater than 38 µg per grain, of Thyroid to a beaker. Add 100.0 mL of *Extracting solution*, and sonicate for 5 min.

Electrode system: Use an iodide-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±1 mV (see *pH* ⟨791⟩).

Analysis: Transfer the *Standard solution* to a beaker containing a magnetic stirring bar. Rinse and dry the electrodes, insert in the solution, stir for 5 min or until the reading stabilizes, and read the potential in mV. Repeat this process using the *Sample solution*.

Acceptance criteria: The requirements of the test are met if the *Sample solution* has a higher potential, in mV, than the *Standard solution*, the limit is 0.01%.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Levothyroxine RS

USP Liothyronine RS

[1] A suitable grade is available as "Pronase" (Catalog number 53702) from Calbiochem-Behring, P.O. Box 12087, San Diego, CA 92112.

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Thyroid Tablets

DEFINITION

Thyroid Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of levothyroxine and liothyronine, the labeled amounts being 38 µg of levothyroxine and 9 µg of liothyronine for each 65 mg of the labeled content of thyroid.

IDENTIFICATION

• **A.** The retention times of the peaks for liothyronine and levothyroxine of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, water, and phosphoric acid (350:650:5), filtered and degassed

Reducing buffer solution: Freshly prepare 0.04 M tris(hydroxymethyl)aminomethane and 0.05 M methimazole in 0.11 M sodium chloride. Adjust, if necessary, with 6 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 8.4 ± 0.05.

Proteolytic enzyme: Freshly prepare a solution containing 3 mg/mL of bacterial protease[1] in *Reducing buffer solution*.

Enzyme deactivating solution: Phosphoric acid in acetonitrile (1:99)

Standard stock solution: Transfer accurately weighed quantities of about 9 mg of USP Liothyronine RS and about 38 mg of USP Levothyroxine RS to a 100-mL volumetric flask, add 50 mL of a mixture of acetonitrile, water, and ammonium hydroxide (500:500:1) and swirl to dissolve. Dilute with a mixture of acetonitrile and water (1:1) to volume, and mix. [NOTE—Protect solutions from light.]

Standard solution: Pipet 5 mL of the freshly prepared *Standard stock solution* into a 250-mL volumetric flask, dilute with *Reducing buffer solution* to volume, and mix to obtain a solution having known concentrations of about 1.8 µg/mL of liothyronine and 7.6 µg/mL of levothyroxine. Pipet 5 mL of this solution into a screw-capped 16- × 125-mm culture tube. Pipet 2 mL of *Enzyme deactivating solution* into the tube, place the cap on the tube, and shake the mixture vigorously. [NOTE—Prepare on the day of use.]

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of finely powdered Thyroid, equivalent to about 38 µg of levothyroxine, to a screw-capped 16- × 125-mm culture tube that has been flushed previously with nitrogen. Taking precautions to avoid unnecessary exposure to air, pipet 5 mL of *Proteolytic enzyme* into the tube. Allow nitrogen to flow gently over the mixture for 5 min. Place the cap on the tube, mix to disperse the contents, and place in a covered water bath maintained at a temperature of 37 ± 1° for 28 h. [NOTE—Protect the contents of the tubes from light.] Examine occasionally, and mix as necessary to ensure dispersion. At the end of the incubation period, pipet 2 mL of *Enzyme deactivating solution* into the tube, place the cap on the tube, mix vigorously, and centrifuge at about 2000 rpm for 5 min. Pass the supernatant through a filter of 0.45-µm pore size, discarding the first 1 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-cm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8 for liothyronine and levothyroxine

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of liothyronine ($C_{15}H_{12}I_3NO_4$) and levothyroxine ($C_{15}H_{11}I_4NO_4$) in the portion of Tablets taken:

$$\text{Result} = (V \times C) \times (r_U/r_S)$$

V = volume of *Sample solution*, 7 mL

C = concentration of the corresponding Reference Standards in the *Standard solution* (µg/mL)

r_U = peak response of the corresponding analytes from the *Sample solution*

r_S = peak response of the corresponding analytes from the *Standard solution*

Acceptance criteria: 90.0%–110.0% of the labeled amounts of liothyronine ($C_{15}H_{12}I_3NO_4$) and levothyroxine ($C_{15}H_{11}I_4NO_4$)

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Time: 15 min, with disks

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Standard stock solution: Accurately weigh 1.69 g of potassium iodate and transfer to a 1-L volumetric flask. Dissolve in about 200 mL of water, dilute with water to volume, and mix. This is a stock solution having a concentration of about 1 mg/mL with respect to iodine.

Standard solution: Pipet 8 mL of the *Standard stock solution* into a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer an appropriate aliquot, based on the dosage being analyzed (i.e., ¼ grain, 1 mL; ½ grain, 2 mL; 1 grain, 4 mL; 1½ grains, 6 mL; 2 grains, 8 mL; 2½ grains, 10 mL; 3 grains, 12 mL; 4 grains, 16 mL; 5 grains, 20 mL), to a 100-mL volumetric flask containing 8 g of anhydrous potassium carbonate dissolved in 70 mL of water. Add 1 mL of bromine TS, mix, add sufficient sodium sulfite (about 20 mg) until the solution becomes colorless, and dilute with water to volume, and mix.

Sample solution: Crush 1 Tablet in a porcelain crucible with a glass rod. Remove any sample adhering to the glass rod with a spatula, and add it to the crucible. Add 4 g of anhydrous potassium carbonate, mix carefully, and gently tap the crucible several times to compact the mixture. Overlay with 4 g more of anhydrous potassium carbonate, and again compact the material thoroughly by tapping. Place the crucible in a preheated muffle furnace, and ignite at 675°–700° for 25 min. Cool, add 30 mL of water, carefully heat on a hot plate to dissolve the residue, and pass through a funnel with a glass wool plug into a 100-mL volumetric flask. Repeat the heating and filtration with two additional 30-mL portions of water, and add these filtrates to the volumetric flask. Add 1 mL of bromine TS, mix, add sufficient sodium sulfite (about 20 mg) until the solution becomes colorless, and mix. Dilute with water to volume, and mix.

Blank solution: Add 8 g of anhydrous potassium carbonate into a 100-mL volumetric flask, and dissolve it in 70 mL of water. Add 1 mL of freshly prepared bromine TS, mix, add sufficient sodium sulfite (about 20 mg) until the solution

[1] A suitable grade is available as "Pronase" (Catalog number 53702) from Calbiochem-Behring, P.O. Box 12087, San Diego, CA 92112.

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becomes colorless, and dilute with water to volume, and mix.

Analysis: Transfer 10 mL of the *Sample solution* to a dry polarographic cell. Bubble nitrogen through the solution for 5 min, then direct the stream of nitrogen above the solution. Use a suitable differential pulse polarograph equipped with a saturated calomel reference electrode and a dropping mercury electrode with a 1-s drop time. Scan from –0.8 V to –1.5 V at the rate of 5 mV/s, and 50-mV pulses. Record the polarogram of the *Sample solution*, the *Standard solution*, and the *Blank solution*. At the peaks near –1.18 V in the polarograms from the *Standard solution* and the *Sample solution*, measure the heights from the baseline, as established by the *Blank solution*.

Calculate the amount of iodine, in µg, in the Tablet taken:

$$\text{Result} = (A_{r1}/A_{r2}) \times (r_U/r_S) \times (C \times V)$$

A_{r1} = atomic weight of iodine (I), 126.90
A_{r2} = atomic weight of potassium iodate (KIO_3), 214.00
r_U = peak height from the *Sample solution*
r_S = peak height from the *Standard solution*
C = concentration of the aliquot portion of potassium iodate solution used to prepare the *Standard solution*, 54.08 µg/mL
V = volume of the aliquot portion of potassium iodate solution used to prepare the *Standard solution* (mL)

Proceed as directed for *Uniformity of Dosage Units* ⟨905⟩, *Content Uniformity*, using the results obtained by this procedure to determine the total iodine content of individual Tablets, and use the *Sample solution* from the *Assay* to perform the composite determination for iodine.

Acceptance criteria: The amount of iodine in each Tablet is within 85.0%–115% of the composite assay for iodine, with a relative standard deviation of NMT 6.0%.

SPECIFIC TESTS

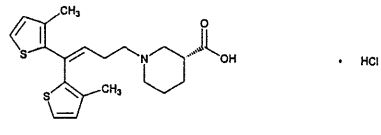
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** Meet the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets to indicate the amount of thyroid in either mg or grain, or both.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Levothyroxine RS
  USP Liothyronine RS

Tiagabine Hydrochloride

Change to read:



$C_{20}H_{25}NO_2S_2 \cdot HCl$ 412.00

3-Piperidinecarboxylic acid, 1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-, hydrochloride, (*R*)-;

(–)-(*R*)-1-[4,4-Bis(3-methyl-2-thienyl)-3-butenyl]nipecotic acid, hydrochloride [145821-59-6].

▲Free base

$C_{20}H_{25}NO_2S_2$ 375.55

[115103-54-3].

Monohydrate

$C_{20}H_{25}NO_2S_2 \cdot HCl \cdot H_2O$ 430.02

[145821-57-4▲ (USP 1-Aug-2019)].

DEFINITION

Tiagabine Hydrochloride contains NLT 97.5% and NMT 102.5% of tiagabine hydrochloride ($C_{20}H_{25}NO_2S_2 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Transfer about 5 mg of Tiagabine Hydrochloride to a test tube. Add 4 mL of 2-propanol, and sonicate ▲to dissolve,▲ (USP 1-Aug-2019) if necessary. Evaporate the solvent under inert atmosphere at 50°, using a nitrogen evaporator, for 2 h.

▲Analysis: The IR spectrum of the *Sample* corresponds to that of a similarly prepared USP Tiagabine Hydrochloride RS.▲ (USP 1-Aug-2019)

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Solution A: 1.38 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: Acetonitrile and *Solution A* (35:65)
Diluent: Methanol and water (50:50)
Internal standard solution: 0.4 mg/mL of butylparaben in *Diluent*
Standard stock solution: 1 mg/mL of USP Tiagabine Hydrochloride RS in *Diluent*
Standard solution: Transfer suitable volumes of the *Standard stock solution* and *Internal standard solution* into a suitable volumetric flask and dilute with *Diluent* to volume.
Sample stock solution: 1 mg/mL of Tiagabine Hydrochloride in *Diluent*
Sample solution: Transfer suitable volumes of the *Sample stock solution* and *Internal standard solution* into a suitable volumetric flask and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: ▲1 mL/min▲ (USP 1-Aug-2019)
Injection volume: 20 µL
▲Run time: NLT 3.5 times the retention time of tiagabine▲ (USP 1-Aug-2019)
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5.5 between tiagabine and butylparaben

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Relative standard deviation: NMT 1.5% for the peak response ratios

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tiagabine hydrochloride ($C_{20}H_{25}NO_2S_2 \cdot HCl$) in the portion of Tiagabine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times {}^{\blacktriangle}(M_{r1}/M_{r2}) \times {}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}}\ 100$$

R_U = peak area ratio of tiagabine hydrochloride to the internal standard from the *Sample solution*

R_S = peak area ratio of tiagabine hydrochloride to the internal standard from the *Standard solution*

C_S = concentration of USP Tiagabine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Tiagabine Hydrochloride in the *Sample solution* (mg/mL)

${}^{\blacktriangle}M_{r1}$ = molecular weight of tiagabine hydrochloride, 412.00

M_{r2} = molecular weight of tiagabine hydrochloride monohydrate, 430.02${}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}}$

Acceptance criteria: 97.5%–102.5% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Change to read:

• **LIMIT OF (*S*)-(+) ISOMER**

Mobile phase: Hexane, isopropyl alcohol, alcohol, and trifluoroacetic acid (80: 14: 6: 0.5). [NOTE—Increase or decrease the percentage of hexane or alcohol, but keep the percentage of isopropyl alcohol constant. Make other adjustments, if necessary (see *Chromatography* ⟨621⟩, *System Suitability*).]

Standard solution: 0.1 mg/mL of USP Racemic Tiagabine Hydrochloride Mixture RS prepared as follows. Transfer a suitable quantity of USP Racemic Tiagabine Hydrochloride Mixture RS into a suitable volumetric flask, dissolve in a few drops of methanol, and dilute with isopropyl alcohol to volume.

Sample solution: 2 mg/mL of Tiagabine Hydrochloride prepared as follows. Transfer a suitable quantity of Tiagabine Hydrochloride in a suitable volumetric flask, dissolve in a few drops of methanol, and dilute with isopropyl alcohol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 25-cm; ${}^{\blacktriangle}$10-µm${}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}}$ packing L40

Flow rate: 0.8 mL/min

Injection volume: 10 µL

${}^{\blacktriangle}$Run time: NLT 1.5 times the retention of the (*R*)-(–) isomer${}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}}$

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times of the (*S*)-(+) isomer and the (*R*)-(–) isomer are about 0.76 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the (*S*)-(+) and (*R*)-(–) isomers

Analysis

Sample: *Sample solution*

Calculate the percentage of the (*S*)-(+) isomer in the portion of Tiagabine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the (*S*)-(+) isomer from the *Sample solution*

r_T = sum of the peak responses of the (*S*)-(+) isomer and (*R*)-(–) isomer from the *Sample solution*

Acceptance criteria: NMT 0.5%

Change to read:

• **ORGANIC IMPURITIES**

Solution A: Water adjusted with phosphoric acid to a pH of 2.3

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
30	45	55
40	10	90
45	10	90

Standard stock solution: 1 mg/mL of USP Tiagabine Hydrochloride RS in water

Standard solution: 0.001 mg/mL of USP Tiagabine Hydrochloride RS in water from the *Standard stock solution*

${}^{\blacktriangle}$System suitability stock solution: 1 mg/mL of USP Tiagabine Related Compound A RS in water${}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}}$

System suitability solution: 0.1 mg/mL each of USP Tiagabine Hydrochloride RS and USP Tiagabine Related Compound A RS in water prepared from *Standard stock solution* ${}^{\blacktriangle}$and *System suitability stock solution*${}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}}$

Sample solution: 1 mg/mL of Tiagabine Hydrochloride in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: ${}^{\blacktriangle}$1 mL/min${}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}}$

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 9.0 between tiagabine hydrochloride and tiagabine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

${}^{\blacktriangle}{}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}}$

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tiagabine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times {}^{\blacktriangle}(1/F){}^{\blacktriangle}_{\text{(USP 1-Aug-2019)}} \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses, excluding the solvent peaks, from the *Sample solution*

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F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

▲Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tiagabine diol analog[a]	0.51	0.75	0.2
Tiagabine keto analog[b]	0.79	0.63	0.1
Desmethyl tiagabine[c]	0.93	1.00	0.1
Tiagabine	1.0	—	—
Tiagabine isomers[d]	1.13	1.00	0.6
Tiagabine methyl ester[e]	1.32	1.01	0.2
Tiagabine related compound A	1.39	1.04	0.2
Tiagabine alcohol analog[f]	1.98	0.97	0.2
Bisthiophenylmethanone[g]	2.27	0.39	0.1
Tiagabine alcohol mesylate[h]	2.33	0.96	0.1
Bisthiophenyl tetrahydrofuran[i]	2.54	0.94	0.1
Any unknown impurity	—	1.00	0.1
Total impurities	—	—	1.0

[a] (3*R*)-1-[3,4-Dihydroxy-4,4-bis(3-methylthiophen-2-yl)butyl]piperidine-3-carboxylic acid.
[b] (*R*)-1-[4,4-Bis(3-methylthiophen-2-yl)-3-oxobutyl]piperidine-3-carboxylic acid.
[c] (*R*,*ZE*)-1-[4-(3-Methylthiophen-2-yl)-4-(thiophen-2-yl)but-3-en-1-yl] piperidine-3-carboxylic acid.
[d] (*R*)-1-[4-(*x*-Methylthiophen-2-yl)-4-(*y*-methylthiophen-2-yl)but-3-en-1-yl] piperidine-3-carboxylic acid; where possible, *x*,*y* combinations include (3,4), (4,3), (4,4), (5,5), (4,5), (5,4), (3,5), and (5,3).
[e] Methyl (*R*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]piperidine-3-carboxylate.
[f] 4,4-Bis(3-methylthiophen-2-yl)but-3-en-1-ol.
[g] Bis(3-methylthiophen-2-yl)methanone.
[h] 4,4-Bis(3-methylthiophen-2-yl)but-3-en-1-yl methanesulfonate.
[i] 2,2-Bis(3-methylthiophen-2-yl)tetrahydrofuran. ▲(USP 1-Aug-2019)

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 6.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at a temperature not higher than 30°.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Racemic Tiagabine Hydrochloride Mixture RS
(*S*)-(+),(*R*)-(−)-1-[4,4-Bis(3-methyl-2-thienyl)-3 butenyl] nipecotic acid, hydrochloride.
$C_{20}H_{25}NO_2S_2 \cdot HCl$ 412.00
USP Tiagabine Hydrochloride RS
▲[NOTE—Available in monohydrate form.]▲ (USP 1-Aug-2019)
USP Tiagabine Related Compound A RS
(*R*)-Ethyl-1-[4,4-bis(3-methy-2-thienyl)-3-butenyl]-3-piperidinecarboxylate, hydrochloride.
$C_{22}H_{29}NO_2S_2 \cdot HCl$ 440.06

Tiagabine Hydrochloride Compounded Oral Suspension

DEFINITION

Tiagabine Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tiagabine hydrochloride ($C_{20}H_{25}NO_2S_2 \cdot HCl$). Prepare Tiagabine Hydrochloride Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Tiagabine Hydrochloride	100 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder or add *Tiagabine Hydrochloride* powder. Add the *Vehicle* in small portions and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tiagabine hydrochloride suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 5 mM octanesulfonic acid (50:50). Pass through a suitable filter of 0.45-µm pore size and degas.

Standard stock solution: 1.0 mg/mL of USP Tiagabine Hydrochloride RS in methanol

Standard solution: Transfer 0.2 mL of *Standard stock solution* to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing 20 µg/mL of tiagabine hydrochloride. Centrifuge, and pass through a suitable filter of 0.22-µm pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Centrifuge, and pass through a suitable filter of 0.22-µm pore size. Accurately pipet 0.2 mL of the Oral Suspension to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a nominal concentration of 20 µg/mL of tiagabine hydrochloride.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Flow rate: 0.4 mL/min
Column: 3.0-mm × 15-cm; 5-µm packing L10
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time of the tiagabine hydrochloride peak is 3.2 min.]
Suitability requirements
Relative standard deviation: NMT 1.7%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tiagabine hydrochloride ($C_{20}H_{25}NO_2S_2 \cdot HCl$) in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

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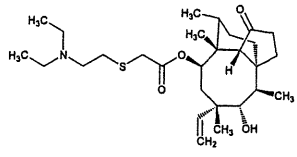
C_S = concentration of USP Tiagabine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tiagabine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–4.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator; NMT 60 days after the date it was compounded, when stored at controlled room temperature
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tiagabine Hydrochloride RS

Tiamulin



$C_{28}H_{47}NO_4S$ 493.74

Acetic acid, [[2-(diethylamino)ethyl]thio]-, 6-ethenyldecahydro-5-hydroxy-4,6,9,10-tetramethyl-1-oxo-3a,9-propano-3a*H*-cyclopentacycloocten-8-yl ester [3a*S*-(3aα,4β,5α,6α,8β,9α,9aβ,10*S**)]-;
[[2-(Diethylamino)ethyl]thio]acetic acid 8-ester with (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-octahydro-5,8-dihydroxy-4,6,9,10-tetramethyl-6-vinyl-3a,9-propano-3a*H*-cyclopentacycloocten-1(4*H*)-one [55297-95-5].

DEFINITION
Tiamulin contains NLT 96.5% and NMT 102.0% of tiamulin ($C_{28}H_{47}NO_4S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K.▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Dissolve 10.0 g of ammonium carbonate in water, add 22 mL of perchloric acid TS, and dilute with water to 1000 mL. Adjust with ammonium hydroxide to a pH of 10.0.
Mobile phase: Methanol, acetonitrile, and *Buffer* (490:210:300)
Diluent: Acetonitrile and *Buffer* (1:1)
Standard solution: 5.0 mg/mL of USP Tiamulin Fumarate RS in *Diluent*
Sample solution: 4.0 mg/mL of Tiamulin in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2 between tiamulin and its subsequent peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tiamulin ($C_{28}H_{47}NO_4S$) in the portion of Tiamulin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Tiamulin Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration Tiamulin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tiamulin, 493.74
M_{r2} = molecular weight of tiamulin fumarate, 609.82

Acceptance criteria: 96.5%–102.0% on the dried basis

IMPURITIES
- **LIMIT OF ALCOHOL AND TOLUENE**

Proceed as directed in *Residual Solvents* ⟨467⟩.
Acceptance criteria
Alcohol: NMT 1.0%
Toluene: NMT 0.08%
Sum of alcohol and toluene: NMT 1.0%
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Toluene solution: Transfer 0.1 mL of toluene to a 100-mL volumetric flask, and dilute with acetonitrile to volume. Transfer 0.1 mL of this solution to another 100-mL volumetric flask, and dilute with *Diluent* to volume.
Diluted sample solution: 0.04 mg/mL of Tiamulin from the *Sample solution* in *Diluent*
Analysis
Samples: *Standard solution, Sample solution, Toluene solution*, and *Diluted sample solution*
Calculate the area percentage of each impurity, relative to tiamulin, in the portion of Tiamulin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_T = peak area of tiamulin from the *Diluted sample solution*

Acceptance criteria: See *Table 1*. Disregard the toluene peak and any peak from the *Sample solution* less than 0.1 times the area of the principal peak from the *Diluted sample solution*.

USP Monographs

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Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mutilin	0.22	1.0
2-(Benzylsulfanyl)-*N*,*N*-diethylethanamine	0.50	1.0
2,2′-(Disulfane-1,2-diyl) bis(*N*,*N*-diethylethanamine)	0.66	1.0
Tiamulin	1.0	—
Hydroxy-11-oxotiamulin	1.1	1.0
1-Hydroxy-11-oxotiamulin	1.6	1.0
11-Oxotiamulin	2.4	1.0
Any individual unspecified impurity	—	0.2
Total impurities	—	3.0

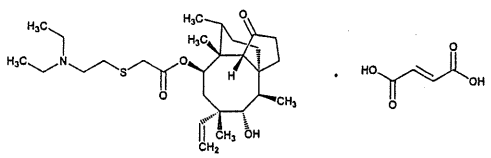
SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.4 USP Endotoxin Units/mg.
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 80° to constant weight.
  Acceptance criteria: NMT 1.0%
- **CLARITY AND COLOR OF SOLUTION**
  Sample: 2.5 g
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Analytical wavelength: 420 nm
  Analysis: Dissolve the *Sample* in methanol, and dilute with methanol to 50.0 mL.
  Acceptance criteria: The solution is clear, and its absorbance is NMT 0.050.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tiamulin RS
  USP Tiamulin Fumarate RS

Tiamulin Fumarate



$C_{28}H_{47}NO_4S \cdot C_4H_4O_4$ 609.82

Tiamulin hydrogen fumarate;
Acetic acid, [[2-(diethylamino)ethyl]thio]-, 6-ethenyldecahydro-5-hydroxy-4,6,9,10-tetramethyl-1-oxo-3a,9-propano-3a*H*-cyclopentacycloocten-8-yl ester [3a*S*-(3aα,4β,5α,6α,8β,9α,9aβ,10*S**)]-, (*E*)-2-butenedioate (1:1) (salt);
[[2-(Diethylamino)ethyl]thio]acetic acid 8-ester with (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-octahydro-5,8-dihydroxy-4,6,9,10-tetramethyl-6-vinyl-3a,9-propano-3a*H*-cyclopentacycloocten-1(4*H*)-one fumarate (1:1) (salt) [55297-96-6].

DEFINITION

Tiamulin Fumarate contains NLT 97.0% and NMT 102.0% of tiamulin fumarate ($C_{28}H_{47}NO_4S \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K [NOTE—When using 197K, intimately mix Tiamulin Fumarate with potassium bromide, but do not grind.▲ (CN 1-May-2020)]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Solution A: 6% perchloric acid
  Buffer: Transfer 10 g of ammonium carbonate to a 1000-mL volumetric flask, and dissolve in 800 mL of water. Add 24 mL of *Solution A*, and dilute with water to volume.
  Mobile phase: Methanol, acetonitrile, and *Buffer* (49:23:28)
  System suitability solution: 0.08 mg/mL of USP Tiamulin Fumarate RS and 0.08 mg/mL of USP Tiamulin Related Compound A RS in *Mobile phase*
  Standard solution: 4 mg/mL of USP Tiamulin Fumarate RS in *Mobile phase*
  Sample solution: 4 mg/mL of Tiamulin Fumarate in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 212 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 30 ± 3°
  Flow rate: 1.2 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  [NOTE—The tiamulin related compound A peak elutes before the tiamulin fumarate peak.]
  Resolution: NLT 2.0 between tiamulin related compound A and tiamulin fumarate, *System suitability solution*
  Tailing factor: NMT 2.0, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of tiamulin fumarate ($C_{28}H_{47}NO_4S \cdot C_4H_4O_4$) in the portion of Tiamulin Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tiamulin Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Tiamulin Fumarate in the *Sample solution* (mg/mL)

USP Monographs

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Acceptance criteria: 97.0%–102.0% on the dried basis

OTHER COMPONENTS

- **CONTENT OF FUMARATE**

Sample solution: 450 mg of Tiamulin Fumarate in 60 mL of a mixture of alcohol and water (1:1)

Analysis: Titrate the *Sample solution* with 0.1 N sodium hydroxide VS, using a glass–calomel electrode (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each milliliter of 0.1 N sodium hydroxide is equivalent to 5.8 mg of fumarate.

Acceptance criteria: 83.7–87.3 mg

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the area percentage of each impurity, relative to tiamulin fumarate, in the portion of Tiamulin Fumarate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = peak response of tiamulin fumarate from the *Sample solution*

Acceptance criteria: See *Table 1*. Disregard the peak due to fumarate at a relative retention time of 0.15.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pleuromutilin	0.25	1.0
Mutilin	0.3	1.0
14-Acetyl mutilin	0.5	1.0
11-Monoacetyl mutilin	0.6	1.0
Tiamulin related compound A	0.8	1.0
Tiamulin	1.0	—
11,14-Diacetyl mutilin	1.1	1.0
8-Dimethylderivative	1.3	1.0
Bisdimethylthioderivative	1.4	1.0
11-Ketoderivative	2.3	1.0
Any individual unspecified impurity	—	0.5
Total impurities	—	3.0

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 5.0 mg/mL in dioxane

Acceptance criteria: +24° to +28°, on the dried basis, measured at 20°

- **pH** ⟨791⟩

Sample solution: 10 mg/mL

Acceptance criteria: 3.1–4.1

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 105° for 3 h.

Acceptance criteria: NMT 0.5%

- **CLARITY AND COLOR OF SOLUTION**

Sample: 5 g

Analysis: Transfer the *Sample* to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.

Acceptance criteria: The solution is clear and colorless, and its absorbances determined in a 1-cm cell at 400 and 650 nm are NMT 0.150 and 0.030 absorbance units, respectively.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Tiamulin Fumarate RS

USP Tiamulin Related Compound A RS

Tosyl pleuromutilin.

Ticarcillin for Injection

DEFINITION

Ticarcillin for Injection contains an amount of Ticarcillin Disodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of ticarcillin ($C_{15}H_{16}N_2O_6S_2$).

ASSAY

- **PROCEDURE**

Buffer A: 13.8 g/L of monobasic sodium phosphate in water adjusted with phosphoric acid or 10 N sodium hydroxide to a pH of 4.3 ± 0.1 before final dilution

Buffer B: 6.9 g/L of monobasic sodium phosphate adjusted with 10 N sodium hydroxide to a pH of 6.4 ± 0.1 before final dilution

Mobile phase: Acetonitrile and *Buffer A* (5:95)

System suitability stock solution: 0.15 mg/mL of clavulanic acid from USP Clavulanate Lithium RS in *Buffer B*

System suitability solution: 30 µg/mL of clavulanic acid from *System suitability stock solution* and 1 mg/mL of USP Ticarcillin Monosodium Monohydrate RS in *Buffer B*. Use this solution on the day prepared.

Standard solution: 1 mg/mL of USP Ticarcillin Monosodium Monohydrate RS in *Buffer B*

Sample solution 1 (where the article is represented as being in a single-dose container): Nominally 0.9 mg/mL of ticarcillin prepared as follows. Constitute Ticarcillin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, and dilute with *Buffer B*.

Sample solution 2 (where the label states the quantity of ticarcillin in a given volume of constituted solution): Nominally 0.9 mg/mL of ticarcillin prepared as follows. Constitute Ticarcillin for Injection as directed in the labeling. Dilute a suitable aliquot of the constituted solution with *Buffer B*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4-mm × 30-cm; 3- to 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clavulanic acid and ticarcillin are 0.2 and 1.0, respectively.]

USP Monographs

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Suitability requirements
Resolution: NLT 5.0 between clavulanic acid and ticarcillin, *System suitability solution*
Column efficiency: NLT 1000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*
Calculate the percentage of the labeled amount of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) in the portion of Ticarcillin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of ticarcillin from *Sample solution 1* or *Sample solution 2*
r_S = peak response of ticarcillin from the *Standard solution*
C_S = concentration of USP Ticarcillin Monosodium Monohydrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ticarcillin in *Sample solution 1* or *Sample solution 2* (mg/mL)
P = potency of ticarcillin in USP Ticarcillin Monosodium Monohydrate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements

IMPURITIES
• **Dimethylaniline** ⟨223⟩**:** Meets the requirements

SPECIFIC TESTS
• **Content of Ticarcillin**
This test is to be performed on Ticarcillin for Injection that contains no added substances.
Solution A: 0.1 N methanolic hydrochloric acid prepared by diluting 4 mL of hydrochloric acid with methanol to 500 mL
Standard stock solution: 0.4 mg/mL of USP Ticarcillin Monosodium Monohydrate RS in water
Standard solution: 20 µg/mL of USP Ticarcillin Monosodium Monohydrate RS from *Standard stock solution* in *Solution A*
Sample stock solution: 0.4 mg/mL of ticarcillin from Ticarcillin for Injection in water
Sample solution: 20 µg/mL of ticarcillin from *Sample stock solution* in *Solution A*
Blank: Dilute 5.0 mL of water with *Solution A* to 100 mL.
Instrumental conditions
Analytical wavelength: 230 nm
Cell: 1 cm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) in the portion of Ticarcillin for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times P \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Ticarcillin Monosodium Monohydrate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ticarcillin in the *Sample solution* (µg/mL)
P = potency of ticarcillin in USP Ticarcillin Monosodium Monohydrate RS (µg/mg)

Acceptance criteria: 80.0%–94.0% on the anhydrous basis
• **Bacterial Endotoxins Test** ⟨85⟩**:** It contains NMT 0.05 USP Endotoxin Unit/mg of ticarcillin.
• **Sterility Tests** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
• **pH** ⟨791⟩
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: 6.0–8.0
• **Labeling** ⟨7⟩**,** *Labels and Labeling for Injectable Products***:** Meets the requirements
• **Water Determination,** *Method I* ⟨921⟩**:** NMT 6.0%
• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
This test is to be performed on Ticarcillin for Injection that contains no added substances.
Sample solution: 10 mg/mL of Ticarcillin for Injection in water
Acceptance criteria: +172° to +187°
• **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements for small-volume injections
• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
• **Other Requirements:** It meets the requirements of the *Identification* tests in *Ticarcillin Disodium*. It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
• **USP Reference Standards** ⟨11⟩
USP Clavulanate Lithium RS
USP Ticarcillin Monosodium Monohydrate RS

Ticarcillin and Clavulanic Acid Injection

DEFINITION
Ticarcillin and Clavulanic Acid Injection is a sterile isoosmotic solution of Ticarcillin Monosodium and Clavulanate Potassium in Water for Injection. It contains one or more suitable buffering agents and a tonicity-adjusting agent. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) and the equivalent of NLT 85.0% and NMT 120.0% of the labeled amount of clavulanic acid ($C_8H_9NO_5$).

IDENTIFICATION
• **A.** The retention time of the major peak for ticarcillin of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
• **Procedure**
Buffer A: 13.8 g/L of monobasic sodium phosphate in water adjusted with phosphoric acid or 10 N sodium hydroxide to a pH of 4.3 ± 0.1 before final dilution
Buffer B: 6.9 g/L of monobasic sodium phosphate in water adjusted with 10 N sodium hydroxide to a pH of 6.4 ± 0.1 before final dilution
Mobile phase: Acetonitrile and *Buffer A* (5:95)

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Standard stock solution: 0.6 mg/mL of USP Clavulanate Lithium RS in *Buffer B*
Standard solution: Transfer 100 mg of USP Ticarcillin Monosodium Monohydrate RS to a 100-mL volumetric flask. Add 150/*J* mL of *Standard stock solution*. Dilute with *Buffer B* to volume.

$$J = L_1/L_2$$

L_1 = labeled amount of ticarcillin (mg)
L_2 = labeled amount of clavulanic acid (mg)

Sample solution: Nominally 0.9 mg/mL of ticarcillin in *Buffer B* prepared as follows. Allow a container of Injection to thaw, and mix the solution. Dilute a suitable aliquot of Injection with *Buffer B*, stepwise if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 30-cm; 3- to 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clavulanic acid and ticarcillin are 0.2 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between ticarcillin and clavulanic acid
Column efficiency: NLT 1000 theoretical plates for ticarcillin and clavulanic acid
Tailing factor: NMT 2.0 for ticarcillin and clavulanic acid
Relative standard deviation: NMT 2.0% for ticarcillin and clavulanic acid
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of ticarcillin from the *Sample solution*
r_S = peak response of ticarcillin from the *Standard solution*
C_S = concentration of USP Ticarcillin Monosodium Monohydrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ticarcillin in the *Sample solution* (mg/mL)
P = potency of ticarcillin in USP Ticarcillin Monosodium Monohydrate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount of clavulanic acid ($C_8H_9NO_5$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of clavulanic acid from the *Sample solution*
r_S = peak response of clavulanic acid from the *Standard solution*
C_S = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clavulanic acid in the *Sample solution* (mg/mL)
P = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

Acceptance criteria: 90.0%–115.0% of the labeled amount of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) and 85.0%–120.0% of the labeled amount of clavulanic acid ($C_8H_9NO_5$)

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.07 USP Endotoxin Unit/mg of ticarcillin.
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PH** ⟨791⟩: 5.5–7.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
- **LABELING:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Clavulanate Lithium RS
USP Ticarcillin Monosodium Monohydrate RS

Ticarcillin and Clavulanic Acid for Injection

DEFINITION
Ticarcillin and Clavulanic Acid for Injection is a sterile, dry mixture of Ticarcillin Disodium and Clavulanate Potassium. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) and the equivalent of NLT 85.0% and NMT 120.0% of the labeled amount of clavulanic acid ($C_8H_9NO_5$). The labeled amounts represent proportions of ticarcillin to clavulanic acid of 15:1 or 30:1.

IDENTIFICATION
A. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Buffer A: 13.8 g/L of monobasic sodium phosphate in water adjusted with phosphoric acid or 10 N sodium hydroxide to a pH of 4.3 ± 0.1 before final dilution
Buffer B: 6.9 g/L of monobasic sodium phosphate in water adjusted with 10 N sodium hydroxide to a pH of 6.4 ± 0.1 before final dilution
Mobile phase: Acetonitrile and *Buffer A* (5:95)
Standard stock solution: 0.6 mg/mL of USP Clavulanate Lithium RS in *Buffer B*
Standard solution: Transfer 100 mg of USP Ticarcillin Monosodium Monohydrate RS to a 100-mL volumetric

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flask. Add 150/*J* mL of *Standard stock solution*. Dilute with *Buffer B* to volume.

$$J = L_1/L_2$$

L_1 = labeled amount of ticarcillin (mg)
L_2 = labeled amount of clavulanic acid (mg)

Sample solution: Nominally 0.9 mg/mL of ticarcillin prepared as follows. Dissolve the contents of one container of Ticarcillin and Clavulanic Acid for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Using a suitable hypodermic needle and syringe, withdraw all of the withdrawable contents from the container, and dilute stepwise with *Buffer B*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 30-cm; 3- to 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clavulanic acid and ticarcillin are 0.2 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between ticarcillin and clavulanic acid
Column efficiency: NLT 1000 theoretical plates for ticarcillin and clavulanic acid
Tailing factor: NMT 2.0 for ticarcillin and clavulanic acid
Relative standard deviation: NMT 2.0% for ticarcillin and clavulanic acid

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) in the portion of Ticarcillin and Clavulanic Acid for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of ticarcillin from the *Sample solution*
r_S = peak response of ticarcillin from the *Standard solution*
C_S = concentration of USP Ticarcillin Monosodium Monohydrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ticarcillin in the *Sample solution* (mg/mL)
P = potency of ticarcillin in USP Ticarcillin Monosodium Monohydrate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount of clavulanic acid ($C_8H_9NO_5$) in the portion of Ticarcillin and Clavulanic Acid for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of clavulanic acid from the *Sample solution*
r_S = peak response of clavulanic acid from the *Standard solution*
C_S = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clavulanic acid in the *Sample solution* (mg/mL)
P = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

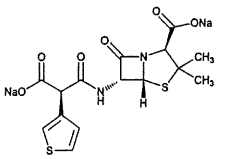
Acceptance criteria: 90.0%–115.0% of the labeled amount of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) and 85.0%–120.0% of the labeled amount of clavulanic acid ($C_8H_9NO_5$)

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.07 USP Endotoxin Unit/mg of ticarcillin.
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: 5.5–7.5
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 4.2%
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clavulanate Lithium RS
  USP Ticarcillin Monosodium Monohydrate RS

Ticarcillin Disodium



$C_{15}H_{14}N_2Na_2O_6S_2$ 428.38

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[(carboxy-3-thienylacetyl)amino]-3,3-dimethyl-7-oxo-, disodium salt, [2*S*-[2α,5α,6β(*S**)]]-;
N-(2-Carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-6-yl)-3-thiophenemalonamic acid disodium salt [4697-14-7].

DEFINITION
Ticarcillin Disodium has a potency equivalent to NLT 800 µg/mg of ticarcillin ($C_{15}H_{16}N_2O_6S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Solution A: Acetone and water (90:10)
  Standard: Dissolve 50 mg of USP Ticarcillin Monosodium Monohydrate RS in 1 mL of water, add 0.1 mL of

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hydrochloric acid, swirl, and allow to stand in iced water for 10 min. Pass through a filter. Wash the residue with 2 mL of water and dissolve in *Solution A*. Evaporate almost to dryness, then dry in an oven at 60° for 30 min.

Sample: Dissolve 50 mg of Ticarcillin Disodium in 1 mL of water, add 0.1 mL of hydrochloric acid, swirl, and allow to stand in iced water for 10 min. Pass through a filter. Wash the residue with 2 mL of water and dissolve in *Solution A*. Evaporate almost to dryness, then dry in an oven at 60° for 30 min.

Acceptance criteria: Meets the requirements

- **B.** Meets the requirements of the test for *Optical Rotation, Specific Rotation*
- **C.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **D. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 1.3 g/L of dibasic ammonium phosphate in water adjusted with phosphoric acid to a pH of 7.0

Mobile phase: Methanol and *Buffer* (20:80)

Standard solution: 0.1 mg/mL of USP Ticarcillin Monosodium Monohydrate RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Ticarcillin Disodium in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between the two ticarcillin peaks

Relative standard deviation: NMT 1.0% for the sum of the ticarcillin peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) in the portion of Ticarcillin Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = sum of the responses of the two ticarcillin peaks from the *Sample solution*

r_S = sum of the responses of the two ticarcillin peaks from the *Standard solution*

C_S = concentration of USP Ticarcillin Monosodium Monohydrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Ticarcillin Disodium in the *Sample solution* (mg/mL)

P = potency of ticarcillin in USP Ticarcillin Monosodium Monohydrate RS (µg/mg)

Acceptance criteria: NLT 800 µg/mg on the anhydrous basis

IMPURITIES

- **DIMETHYLANILINE** ⟨223⟩**:** Meets the requirements
- **ORGANIC IMPURITIES**

Solution A: 1.3 g/L of dibasic ammonium phosphate in water adjusted with phosphoric acid to a pH of 7.0

Solution B: Methanol and *Solution A* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	30	70
40	30	70

System suitability solution: 0.02 mg/mL of USP Ticarcillin Monosodium Monohydrate RS in *Solution A*

Sample solution: 1 mg/mL of Ticarcillin Disodium in *Solution A*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between the two ticarcillin peaks

Relative standard deviation: NMT 5.0% for the sum of the ticarcillin peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Ticarcillin Disodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the responses of the two ticarcillin peaks from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
	0.95	—
Ticarcillin	1.0	
Ticarcillin related compound A[a]	2.1	4
Any individual unspecified impurity	—	2.5

[a] Decarboxy ticarcillin; (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[[(thiophen-3-yl) acetyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL in water

Acceptance criteria: +172° to +187°

- **pH** ⟨791⟩

Sample solution: 10 mg/mL of ticarcillin from Ticarcillin Disodium in water

Acceptance criteria: 6.0–8.0

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 6.0%

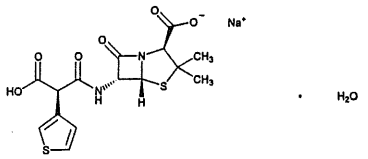
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- **STERILITY TESTS** ⟨71⟩: Where the label states that Ticarcillin Disodium is sterile, it meets the requirements in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Ticarcillin Disodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.05 USP Endotoxin Units/mg of ticarcillin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store in a refrigerator.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ticarcillin Monosodium Monohydrate RS
  $C_{15}H_{15}N_2NaO_6S_2 \cdot H_2O$ 424.42

Ticarcillin Monosodium



$C_{15}H_{15}N_2NaO_6S_2 \cdot H_2O$ 424.42

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[(carboxy-3-thienylacetyl)amino]-3,3-dimethyl-7-oxo, monosodium salt, [2*S*-[2α,5α,6β(*S**)]]-, monohydrate; (*R*)-*N*-[(2*S*,5*R*,6*R*)-2-Carboxy-3,4-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-6-yl]-3-thiophenemalonamic acid monosodium salt.

Anhydrous

$C_{15}H_{15}N_2NaO_6S_2$ 406.40

[74682-62-5].

DEFINITION

Ticarcillin Monosodium contains the equivalent of NLT 890 µg/mg of ticarcillin ($C_{15}H_{16}N_2O_6S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K.**▲ (CN 1-May-2020) Meets the requirements
- **B.** Meets the requirements of the test for *Optical Rotation, Specific Rotation.*
- **C.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay.*
- **D. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩
  Sample solution: 50 mg/mL
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Buffer: 1.3 g/L of dibasic ammonium phosphate in water adjusted with phosphoric acid to a pH of 7.0
  Mobile phase: Methanol and *Buffer* (20:80)
  Standard solution: 0.1 mg/mL of USP Ticarcillin Monosodium Monohydrate RS in *Mobile phase*
  Sample solution: 0.1 mg/mL of Ticarcillin Monosodium in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 220 nm
  Column: 4-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 1.5 between the two ticarcillin peaks
  Relative standard deviation: NMT 1.0% for the sum of the ticarcillin peaks
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the quantity, in µg/mg, of ticarcillin ($C_{15}H_{16}N_2O_6S_2$) in the portion of Ticarcillin Monosodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = sum of the responses of the two ticarcillin peaks from the *Sample solution*

r_S = sum of the responses of the two ticarcillin peaks from the *Standard solution*

C_S = concentration of USP Ticarcillin Monosodium Monohydrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Ticarcillin Monosodium in the *Sample solution* (mg/mL)

P = potency of ticarcillin in USP Ticarcillin Monosodium Monohydrate RS (µg/mg)

Acceptance criteria: NLT 890 µg/mg on the anhydrous basis

IMPURITIES

- **DIMETHYLANILINE** ⟨223⟩: Meets the requirements
- **ORGANIC IMPURITIES**
  Solution A: 1.3 g/L of dibasic ammonium phosphate in water adjusted with phosphoric acid to a pH of 7.0
  Solution B: Methanol and *Solution A* (50:50)
  Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	30	70
40	30	70

System suitability solution: 0.02 mg/mL of USP Ticarcillin Monosodium Monohydrate RS in *Solution A*
Sample solution: 1 mg/mL of Ticarcillin Monosodium in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between the two ticarcillin peaks

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Relative standard deviation: NMT 5.0% for the sum of the ticarcillin peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Ticarcillin Monosodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the responses of the two ticarcillin peaks from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ticarcillin	0.95 1.0	—
Ticarcillin related compound A[a]	2.1	4
Any individual unspecified impurity	—	2.5

[a] Decarboxy ticarcillin; (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[[(thiophen-3-yl) acetyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Buffer: 6.9 g/L of monobasic sodium phosphate adjusted with 10 N sodium hydroxide to a pH of 6.4 ± 0.1 before final dilution

Sample solution: 10 mg/mL in *Buffer*

Acceptance criteria: +181° to +197°

- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩

Sample solution: 10 mg/mL of ticarcillin from Ticarcillin Monosodium in water

Acceptance criteria: 2.5–4.0

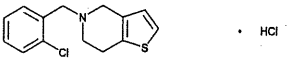
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 4.0%–6.0%
- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Ticarcillin Monosodium is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Ticarcillin Monosodium is sterile, or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.05 USP Endotoxin Units/mg of ticarcillin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store in a refrigerator.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ticarcillin Monosodium Monohydrate RS

Ticlopidine Hydrochloride



$C_{14}H_{14}ClNS \cdot HCl$ 300.25

Thieno[3,2-*c*]pyridine, 5-[(2-chlorophenyl)methyl]-4,5,6,7-tetrahydro-, hydrochloride;

5-(*o*-Chlorobenzyl)-4,5,6,7-tetrahydrothieno-[3,2-*c*]pyridine hydrochloride [53885-35-1].

DEFINITION

Ticlopidine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{14}H_{14}ClNS \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 1.1 g of monobasic sodium phosphate and 0.28 g of dibasic sodium phosphate in 1000 mL of water. The pH of solution is between 6.1 and 6.6. If necessary, adjust to the required pH using phosphoric acid or sodium hydroxide.

Mobile phase: Acetonitrile, methanol, and *Buffer* (6:7:7)

System suitability solution: 0.2 mg/mL of USP Ticlopidine Hydrochloride RS and 0.2 mg/mL of USP Sulconazole Nitrate RS in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]

Standard solution: 0.4 mg/mL of USP Ticlopidine Hydrochloride RS in *Mobile phase*

Sample solution: 0.4 mg/mL of Ticlopidine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 10-µm packing L7

Flow rate: 2 mL/min

Column temperature: 40°

Run time: 1.5 times the retention time of the ticlopidine peak

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.6 between ticlopidine hydrochloride and sulconazole nitrate, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{14}H_{14}ClNS \cdot HCl$ in the portion of Ticlopidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ticlopidine from the *Sample solution*

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r_S = peak response of ticlopidine from the *Standard solution*
C_S = concentration of ticlopidine in the *Standard solution*
C_U = concentration of ticlopidine in the *Sample solution*

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1% on a 1-g sample

ORGANIC IMPURITIES

• **Procedure 1**

Adsorbent: 0.25-mm thickness of silica

Developing solvent: Butanol, water, and glacial acetic acid (4:5:1). Shake well in a separatory funnel, allow it to settle, discard the lower aqueous layer, and use the upper organic layer.

Diluent: Methylene chloride and methanol (1:2)

Iodine–methanol reagent: Iodine TS and methanol (1:1)

Standard solution A: 15 mg/mL of USP Ticlopidine Hydrochloride RS in *Diluent*

Standard solution B: 2.5 mg/mL of each of USP Ticlopidine Related Compound A RS and USP Ticlopidine Related Compound B RS in *Diluent*

Sample solution: 15 mg/mL of Ticlopidine Hydrochloride in *Diluent*

Combined standard solution: Transfer 1.5 mL of *Standard solution B* and 250 µL of the *Sample solution* to a 25-mL volumetric flask and dilute to volume with *Diluent*.

Application size: 2, 5, and 10 µL of the *Combined standard solution* and 20 µL of the *Sample solution*

Analysis

Samples: *Sample solution* and *Combined standard solution*

Develop the plate to a distance of at least 15 cm from the origin, and remove the plate and air dry for at least 1 h. Analyze visually under UV light. Estimate the amounts of ticlopidine related compound A and ticlopidine related compound B. Spray the plate with the *Iodine–methanol reagent*, and estimate any other impurities by comparing to the ticlopidine hydrochloride spots in the *Combined standard solution*.

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Retardation Factor (R_f)	Acceptance Criteria, NMT (%)
Ticlopidine hydrochloride	1.00	—
Ticlopidine hydrochloride related compound A[a]	1.26	0.5
Ticlopidine hydrochloride related compound B[b]	1.41	0.5

[a] (4-Oxo-4,5,6,7-tetrahydrothieno-[3,2-*c*]pyridine.
[b] (5-(2-Chlorobenzyl)-4-oxo-4,5,6,7-tetrahydrothieno-[3,2-*c*]pyridine).

• **Procedure 2**

Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *N*-methyl ticlopidine in the portion of Ticlopidine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of *N*-methyl ticlopidine in the *Sample solution*
r_T = sum of all the peak responses from in the *Sample solution*

Calculate the percentage of any individual impurity in the portion of the Ticlopidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of any impurity in the *Sample solution*
r_S = peak response of ticlopidine in the *Standard solution*

Acceptance criteria

Individual impurities: See *Impurity Table 2*.

Total impurities: See *Impurity Table 2*.

Impurity Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ticlopidine hydrochloride	1	—
N-Methyl ticlopidine[a]	1.18	0.5
Any other individual impurity	—	0.10
Total impurities[b]	—	1.0

[a] 2-[*N*-Methyl-*N*-(2-chlorobenzyl)aminoethyl] thiophene hydrochloride.
[b] Total of *N*-methyl ticlopidine and sum of % individual impurities from *Procedure 1*.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩: Dry a 1.0-g sample at 80° for 5 h: it loses NMT 1.0% of its weight.

• **LIMIT OF FORMALDEHYDE**

Mobile phase: Acetonitrile, water, and hydrochloric acid (3: 2: 0.004)

2,4 Dintirophenyl hydrazine solution: 1.65 mg/mL of 2,4-dinitrophenylhydrazine in acetonitrile

Standard stock solution: Transfer a known amount of formaldehyde solution, equivalent to 37 mg of formaldehyde, into a 100-mL volumetric flask. Dilute with methanol to volume.

Standard solution: Dilute the *Standard stock solution* with methanol to prepare a 1.85-µg/mL solution.

Sample solution: 0.50 g of Ticlopidine Hydrochloride in 10 mL methanol (sonication may be necessary for complete dissolution)

Derivatized standard and **sample solutions:** Transfer 2.0 mL of *2,4-Dinitrophenyl hydrazine solution* to five different 10-mL volumetric flasks: 50 µL of 2 N hydrochloric acid and 150, 250, and 500 µL of the *Standard solution* to the first three flasks; 500 µL of the *Sample solution* to the fourth; and 500 µL of methanol to the fifth flask. Mix each solution well and allow the solutions to react for at least 30 min at ambient temperature. Dilute each flask with *Mobile phase* to volume and mix well. The solutions should be analyzed within 4 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

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System suitability
Sample: *Derivatized standard solution* prepared from 500 µL
Suitability requirements
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Derivatized standard solutions* and *Derivatized sample solution*
[NOTE—The approximate retention time for 2,4 dinitrophenylhydrazine is about 3.5 min and for the formaldehyde and 2,4 dinitrophenylhydrazine derivative is about 3.8 min.]
Calculate the formaldehyde concentration in ppm in the *Derivatized sample solution* as the concentration in the Ticlopidine Hydrochloride taken:

$$\text{Result} = (C \times D)/W$$

C = concentration of formaldehyde from the calibration curve generated from the peak areas of the derivatized methanol and the three *Derivatized standard solutions* (µg/mL)
D = dilution factor, 200
W = sample weight (g)

Acceptance criteria
Formaldehyde: NMT 20 ppm

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature below 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Sulconazole Nitrate RS
USP Ticlopidine Hydrochloride RS
USP Ticlopidine Hydrochloride Related Compound A RS
4-Oxo-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine.
USP Ticlopidine Hydrochloride Related Compound B RS
5-(2-Chlorobenzyl)-4-oxo-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine.

Ticlopidine Hydrochloride Tablets

DEFINITION
Ticlopidine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ticlopidine hydrochloride $C_{14}H_{14}ClNS \cdot HCl$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U.**▲ (CN 1-May-2020) Place a portion of powdered Tablets, equivalent to 100 mg of ticlopidine hydrochloride, in a suitable flask. Add 1 mL of water, and allow to stand for 15 min with occasional shaking. Add about 60 mL of *Mobile phase*, sonicate for 15 min, and shake by mechanical means for an additional 15 min. Dilute with *Mobile phaseMobile phase* to prepare 0.1 mg/mL of ticlopidine hydrochloride.
Acceptance criteria: The UV absorption spectra of the solution exhibits maxima at the same wavelength (232 nm) as that of a similar solution prepared from USP Ticlopidine Hydrochloride RS, concomitantly measured.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Buffer: 3.55 g/L of dibasic sodium phosphate
Mobile phase: Acetonitrile and *Buffer* (3:2). Adjust with phosphoric acid to a pH of 7.0 ± 0.1.
Standard solution: 0.1 mg/mL of USP Ticlopidine Hydrochloride RS in *Mobile phase*
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 250 mg of ticlopidine hydrochloride to a 250-mL volumetric flask, add 150 mL of *Mobile phase*, sonicate for about 5 min, and shake mechanically for 10 additional min. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 0.45-µm filter, and use the filtrate after discarding the first few mL. Transfer 5 mL of the filtrate into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 3.9-mm × 30-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 15 µL
Run time: Twice the retention time of the ticlopidine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{14}ClNS \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ticlopidine from the *Sample solution*
r_S = peak response of ticlopidine from the *Standard solution*
C_S = concentration of USP Ticlopidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ticlopidine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION**
Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 0.278 mg/mL of USP Ticlopidine Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Detection: UV 232 nm
Path length: 0.1 cm
Blank: *Medium*
Calculate the percentage of $C_{14}H_{14}ClNS \cdot HCl$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = Tablet label claim (mg)

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V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of $C_{14}H_{14}ClNS \cdot HCl$ is dissolved.

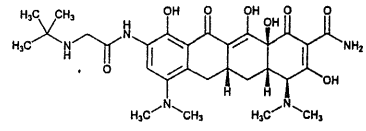
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers and stored at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ticlopidine Hydrochloride RS

Tigecycline



$C_{29}H_{39}N_5O_8$ 585.65

2-Naphthacenecarboxamide, 4,7-bis(dimethylamino)-9-[[[(1,1-dimethylethyl)amino]acetyl]amino]-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-, (4*S*,4a*S*,5a*R*,12a*S*)-;
(4*S*,4a*S*,5a*R*,12a*S*)-9-[2-(*tert*-Butylamino)acetamido]-4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide [220620-09-7].

DEFINITION

Tigecycline contains NLT 97.0% and NMT 102.0% of tigecycline ($C_{29}H_{39}N_5O_8$), calculated on the anhydrous and solvent-free basis.

[NOTE—Handle Standards and samples under moisture-controlled conditions to prevent degradation in quantitative applications where degradation could impact results.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Protect solutions containing tigecycline from light using low-actinic glassware. Store these solutions at 10° immediately after preparation and during analysis.

Buffer: 4.35 g/L of dibasic potassium phosphate and 0.93 g/L of edetate disodium, adjusted with phosphoric acid to a pH of 6.2

Mobile phase: Acetonitrile and *Buffer* (140:860)

Diluent: 4.35 g/L of dibasic potassium phosphate and 0.5 g/L of sodium bisulfite, adjusted with 1 N potassium hydroxide to a pH of 8.0

System suitability stock solution: Dissolve 10 mg of USP Tigecycline RS in 10.0 mL of water. Add 1 or 2 drops of trifluoroacetic acid, heat at about 65° for 15 min, and cool.

System suitability solution: Dilute 5.0 mL of the *System suitability stock solution* with water to 50.0 mL.

Standard solution: 0.1 mg/mL of USP Tigecycline RS in *Diluent*

Sample solution: 0.1 mg/mL of Tigecycline in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 248 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Temperatures

Column: 30°

Autosampler: 10°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for the tigecycline epimer and tigecycline are about 0.67 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the tigecycline epimer and tigecycline, *System suitability solution*

Tailing factor: 0.7–1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tigecycline ($C_{29}H_{39}N_5O_8$) in the portion of Tigecycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Tigecycline RS in the *Standard solution* (mg/mL)

C_U = concentration of Tigecycline in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Protect solutions containing tigecycline from light using low-actinic glassware. Store these solutions at 10° immediately after preparation and during analysis.

Solution A: Dissolve 4.35 g of dibasic potassium phosphate and 0.93 g of edetate disodium in 950 mL of water. Adjust with phosphoric acid to a pH of 6.4 ± 0.05, and add 50 mL of acetonitrile.

Solution B: Dissolve 4.35 g of dibasic potassium phosphate and 0.93 g of edetate disodium in 500 mL of water. Adjust with phosphoric acid to a pH of 6.4 ± 0.05, and add 500 mL of acetonitrile.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
40	57	43
55	0	100
58	0	100
59	85	15

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
66	85	15

Diluent: 4.35 g/L of dibasic potassium phosphate and 0.5 g/L of sodium bisulfite, adjusted with 1 N potassium hydroxide to a pH of 8.0

System suitability solution: 0.5 mg/mL of USP Tigecycline RS and 2.4 µg/mL of USP Tigecycline Related Compound B RS in *Diluent*

Standard solution: 0.005 mg/mL of USP Tigecycline RS in *Diluent*

Sensitivity solution: 0.25 µg/mL of USP Tigecycline RS in *Diluent*

Sample solution: 0.5 mg/mL of Tigecycline in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: 248 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Temperatures

Column: 30°

Autosampler: 10°

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between tigecycline related compound B and the tigecycline epimer, *System suitability solution*

Tailing factor: 0.7–1.5, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tigecycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of tigecycline from the *Standard solution*

C_S = concentration of USP Tigecycline RS in the *Standard solution* (mg/mL)

C_U = concentration of Tigecycline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Specified unidentified impurity	0.12	0.15
Specified unidentified impurity	0.23	0.15
Tigecycline open ring[a, b]	0.36	0.020
Specified unidentified impurity	0.50	0.15

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tigecycline 12-oxo-11-hydroxy[c]	0.55	0.15
Tigecycline related compound B[d]	0.64	0.25
Tigecycline epimer[e]	0.74	1.0
Specified unidentified impurity	0.76	0.15
Tigecycline	1.0	—
Tigecycline quinone analog[f]	1.3	0.15
Specified unidentified impurity	1.5	0.15
Minocycline	1.6	0.25
Tigecycline tricyclic analog[g]	1.7	0.15
Specified unidentified impurity	1.9	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	2.2

[a] To be reported if there is a peak with maximum absorbance at either 390 or 640 nm. The impurity is quantitated at 248 nm. The system may resolve two peaks. The limit is for the sum of the two peaks.

[b] (3*S*,4*R*)-7-[2-(*tert*-Butylamino)acetamido]-3-[4-carbamoyl-2-(dimethylamino)-3,5,6-trihydroxybenzyl]-5-(dimethylimino)-1,4,8-trihydroxy-3,4,5,8-tetrahydronaphthalene-2-carboxylate.

[c] (4*S*,4a*S*,12a*S*)-9-[2-(*tert*-Butylamino)acetamido]-4,7-bis(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydro-2-naphthacenecarboxamide.

[d] (4*S*,4a*S*,5a*R*,12a*S*)-9-Amino-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

[e] (4*R*,4a*S*,5a*R*,12a*S*)-9-[2-(*tert*-Butylamino)acetamido]-4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide.

[f] (*S*)-4-({6-[2-(*tert*-Butylamino)acetamido]-8-(dimethylamino)-5-hydroxy-4-oxo-1,2,3,4-tetrahydronaphthalen-2-yl}methyl)-2,5-dihydroxy-3,6-dioxocyclohexa-1,4-dienecarboxamide.

[g] (1*S*,4a*R*,4b*R*,10a*R*,11a*S*)-7-[2-(*tert*-Butylamino)acetamido]-9-(dimethylamino)-1,4,4a,6-tetrahydroxy-2,5,12-trioxo-1,2,4a,5,10,10a,11,11a-octahydro-1,4b-methanobenzo[*b*]fluorene-3-carboxamide.

SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: 10 mg/mL
  Acceptance criteria: 7.7–8.2
- **Water Determination,** *Method Ic* ⟨921⟩
  Sample solution: 50 mg/mL of Tigecycline in dimethyl sulfoxide
  Acceptance criteria: NMT 2.5%
- **Optical Rotation,** *Specific Rotation* ⟨781S⟩
  Sample solution: 1 mg/mL in water
  Acceptance criteria: −240° to −215° on the anhydrous and solvent-free basis
- **Bacterial Endotoxins Test** ⟨85⟩**:** Where the label states that Tigecycline is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 1 USP Endotoxin Unit/mg of tigecycline.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in air-tight, light-resistant containers under nitrogen, and store refrigerated.

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• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tigecycline RS
USP Tigecycline Related Compound B RS
(4*S*,4a*S*,5a*R*,12a*S*)-9-Amino-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.
$C_{23}H_{28}N_4O_7 \cdot HCl$ 508.95

Tigecycline for Injection

DEFINITION

Tigecycline for Injection contains NLT 96.0% and NMT 116.0% of the labeled amount of tigecycline ($C_{29}H_{39}N_5O_8$). It may contain suitable stabilizers and buffers.
[NOTE—Handle standards and samples under moisture-controlled conditions to prevent degradation in quantitative applications where degradation could impact results.]

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Protect solutions containing tigecycline from light using low-actinic glassware. Store these solutions at 10° immediately after preparation and during analysis.
Buffer: 4.35 g/L of dibasic potassium phosphate and 0.93 g/L of edetate disodium, adjusted with phosphoric acid to a pH of 6.2
Mobile phase: Acetonitrile and *Buffer* (140:860)
Diluent: 4.35 g/L of dibasic potassium phosphate and 0.5 g/L of sodium bisulfite, adjusted with 1 N potassium hydroxide to a pH of 8.0
System suitability stock solution: Dissolve 10 mg of USP Tigecycline RS in 10.0 mL of water. Add 1 or 2 drops of trifluoroacetic acid, heat at about 65° for 15 min, and cool.
System suitability solution: Dilute 5.0 mL of the *System suitability stock solution* with water to 50.0 mL.
Standard solution: 0.1 mg/mL of USP Tigecycline RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of tigecycline from Tigecycline for Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 248 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 10°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for the tigecycline epimer and tigecycline are about 0.67 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the tigecycline epimer and tigecycline, *System suitability solution*
Tailing factor: 0.7–1.5, *Standard solution*
Relative standard deviation: NMT 1.1%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tigecycline ($C_{29}H_{39}N_5O_8$) in the portion of Tigecycline for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tigecycline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tigecycline in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–116.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Protect solutions containing tigecycline from light using low-actinic glassware. Store these solutions at 10° immediately after preparation and during analysis.
Solution A: Dissolve 4.35 g of dibasic potassium phosphate and 0.93 g of edetate disodium in 950 mL of water. Adjust with phosphoric acid to a pH of 6.4 ± 0.05, and add 50 mL of acetonitrile.
Solution B: Dissolve 4.35 g of dibasic potassium phosphate and 0.93 g of edetate disodium in 500 mL of water. Adjust with phosphoric acid to a pH of 6.4 ± 0.05, and add 500 mL of acetonitrile.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
40	57	43
55	0	100
58	0	100
59	85	15
66	85	15

Diluent: 4.35 g/L of dibasic potassium phosphate and 0.5 g/L of sodium bisulfite, adjusted with 1 N potassium hydroxide to a pH of 8.0
System suitability solution: 0.5 mg/mL of USP Tigecycline RS and 2.4 µg/mL of USP Tigecycline Related Compound B RS in *Diluent*
Standard solution: 0.005 mg/mL of USP Tigecycline RS in *Diluent*
Sensitivity solution: 0.25 µg/mL of USP Tigecycline RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of tigecycline in *Diluent* from the combined contents of NLT 5 vials
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: 248 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Temperatures
Column: 30°
Autosampler: 10°
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between tigecycline related compound B and the tigecycline epimer, *System suitability solution*
Tailing factor: 0.7–1.5, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tigecycline for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of tigecycline from the *Standard solution*
C_S = concentration of USP Tigecycline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tigecycline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tigecycline open ring[a, b]	0.36	0.15
Tigecycline 12-oxo-11-hydroxy[c]	0.55	0.5
Tigecycline related compound B[d]	0.64	0.7
Tigecycline epimer[e]	0.74	2.0
Tigecycline	1.0	—
Tigecycline quinone analog[f]	1.3	0.3
Minocycline[g]	1.6	—
Tigecycline tricyclic analog[h]	1.7	0.5
Any individual unspecified degradation product	—	0.2
Total degradation products	—	6.0

[a] To be reported if there is a peak with maximum absorbance at either 390 or 640 nm. The impurity is quantitated at 248 nm. The system may resolve two peaks. The limit is for the sum of the two peaks.
[b] (3*S*,4*R*)-7-[2-(*tert*-Butylamino)acetamido]-3-[4-carbamoyl-2-(dimethylamino)-3,5,6-trihydroxybenzyl]-5-(dimethylimino)-1,4,8-trihydroxy-3,4,5,8-tetrahydronaphthalene-2-carboxylate.
[c] (4*S*,4a*S*,12a*S*)-9-[2-(*tert*-Butylamino)acetamido]-4,7-bis(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydro-2-naphthacenecarboxamide.
[d] (4*S*,4a*S*,5a*R*,12a*S*)-9-Amino-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.
[e] (4*R*,4a*S*,5a*R*,12a*S*)-9-[2-(*tert*-Butylamino)acetamido]-4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide.
[f] (*S*)-4-({6-[2-(*tert*-Butylamino)acetamido]-8-(dimethylamino)-5-hydroxy-4-oxo-1,2,3,4-tetrahydronaphthalen-2-yl}methyl)-2,5-dihydroxy-3,6-dioxocyclohexa-1,4-dienecarboxamide.
[g] Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.
[h] (1*S*,4a*R*,4b*R*,10a*R*,11a*S*)-7-[2-(*tert*-Butylamino)acetamido]-9-(dimethylamino)-1,4,4a,6-tetrahydroxy-2,5,12-trioxo-1,2,4a,5,10,10a,11,11a-octahydro-1,4b-methanobenzo[*b*]fluorene-3-carboxamide.

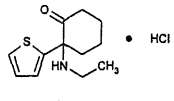
SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: Use the solution constituted as directed in the labeling.
  Acceptance criteria: 4.5–5.5
- **Particulate Matter in Injections** ⟨788⟩
  Sample solution: Use the solution constituted as directed in the labeling.
  Acceptance criteria: Meets the requirements
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 1.75 USP Endotoxin Units/mg
- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Tigecycline RS
  USP Tigecycline Related Compound B RS
  (4*S*,4a*S*,5a*R*,12a*S*)-9-Amino-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.
  $C_{23}H_{28}N_4O_7 \cdot HCl$ 508.95

Tiletamine Hydrochloride



$C_{12}H_{17}NOS \cdot HCl$ 259.80
Cyclohexanone, 2-(ethylamino)-2-(2-thienyl)-.

USP Monographs

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2-(Ethylamino)-2-(2-thienyl)cyclohexanone hydrochloride [14176-50-2].

» Tiletamine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{12}H_{17}NOS \cdot HCl$.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is for veterinary use only. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Tiletamine Hydrochloride RS
Clarity of solution—Dissolve 1.0 g of it in 10 mL of water: the solution is clear.

Change to read:

Identification—
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: ▲0.03▲ (ERR 1-Jun-2019) mg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 234 nm do not differ by more than 3.0%.
C: It meets the requirements of the tests for *Chloride* ⟨191⟩.
Melting range ⟨741⟩: between 190° and 195°, within a 2° range.
Bacterial Endotoxins Test ⟨85⟩ —Where the label states that Tiletamine Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.07 USP Endotoxin Unit per mg of tiletamine.
Sterility Tests ⟨71⟩ —Where the label states that Tiletamine Hydrochloride is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 3.0 and 5.0, in a solution (1 in 10).
Water Determination, *Method I* ⟨921⟩: not more than 1.0%.
Residue on ignition ⟨281⟩: not more than 0.5%.
Chromatographic purity—Prepare a test solution of Tiletamine Hydrochloride in methanol containing 50.0 mg per mL. Prepare a Standard solution in methanol containing 1.0 mg of USP Tiletamine Hydrochloride RS per mL. Prepare a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture as follows. Prewash the plate by developing it in a mixture of methanol and ether (8:2) to the top of the plate, and allow the plate to dry. Separately apply 5 µL of the test solution and the Standard solution to the plate, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, allow the plate to air-dry, and examine under short- and long-wavelength UV light: no individual secondary spot observed in the chromatogram obtained from the test solution is greater in size or intensity than the principal spot observed in the chromatogram obtained from the Standard solution, corresponding to 2%, and the total of any such spots observed does not exceed 3%.
Chloride content—Transfer about 250 mg of it, accurately weighed, to a conical flask, add 5 mL of water, 5 mL of glacial acetic acid, and 50 mL of methanol, and swirl to dissolve. Add 1 drop of eosin Y TS, and titrate with 0.1 N silver nitrate VS to the endpoint when the granular precipitate first turns to a permanent pink color. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride: between 13.24% and 14.06% is found.
Assay—Transfer about 300 mg of Tiletamine Hydrochloride, accurately weighed, to a conical flask, add 70 mL of glacial acetic acid and 10 mL of mercuric acetate TS, and swirl to dissolve. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 25.98 mg of $C_{12}H_{17}NOS \cdot HCl$.

Tiletamine and Zolazepam for Injection

» Tiletamine and Zolazepam for Injection is a sterile dry mixture of Tiletamine Hydrochloride and Zolazepam Hydrochloride. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of tiletamine ($C_{12}H_{17}NOS$) and zolazepam ($C_{15}H_{15}FN_4O$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Tiletamine Hydrochloride RS
USP Zolazepam Hydrochloride RS
Identification—Constitute a container of Tiletamine and Zolazepam for Injection with a volume of water sufficient to yield a test solution containing the equivalent of about 10 mg of tiletamine and 10 mg of zolazepam per mL. Prepare two Standard solutions containing in each mL 10 mg of USP Tiletamine Hydrochloride RS and 10 mg of USP Zolazepam Hydrochloride RS, respectively. Separately apply 2 µL of the test solution and the Standard solutions to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Place the plate in a saturated chamber containing ethyl acetate as the solvent system and lined with filter paper. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, allow the plate to air-dry, and examine under short-wavelength UV light: the R_F values of the principal spots obtained from the test solution correspond to those obtained from the Standard solutions.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.07 USP Endotoxin Unit per mg of combined tiletamine and zolazepam equivalents.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 2.0 and 3.5, when constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: not more than 20 mg in a container containing the equivalent of 250 mg of tiletamine and 250 mg of zolazepam.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩ and for *Uniformity of Dosage Units* ⟨905⟩.

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Assay—

Internal standard solution—Prepare a solution of tetraphenylethylene in chloroform containing 10 mg per mL.

Standard preparation—Transfer accurately weighed quantities of about 116 mg of USP Tiletamine Hydrochloride RS and 113 mg of USP Zolazepam Hydrochloride RS to a 250-mL flask, add 2 mL of water, and swirl to dissolve. Add 30 mL of *Alkaline borate buffer, pH 10.0* (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and swirl. Add 5.0 mL of *Internal standard solution* and 95.0 mL of chloroform, and shake by mechanical means for 30 minutes. Allow the phases to separate, and use the chloroform layer as the *Standard preparation.*

Assay preparation—Constitute a container of Tiletamine and Zolazepam for Injection with the volume of water specified in the labeling. Transfer an accurately measured volume of the resultant solution, equivalent to about 100 mg of tiletamine and 100 mg of zolazepam, to a 250-mL flask. Add 30.0 mL of *Alkaline borate buffer, pH 10.0* (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and swirl. Add 5.0 mL of *Internal standard solution* and 95.0 mL of chloroform, and shake by mechanical means for 30 minutes. Allow the phases to separate, and use the chloroform layer as the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.24-m column that contains 3% phase G2 on 100- to 120-mesh support S1AB. Helium is used as the carrier gas flowing at a rate of about 40 mL per minute. The column temperature is maintained at about 150° for 0.5 minute after injection and is programmed to rise to 230° at a rate of 10° per minute. The injector port is maintained at 160° and the detector at 250°. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for tiletamine, 0.8 for zolazepam, and 1.0 for tetraphenylethylene.

Procedure—Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of tiletamine ($C_{12}H_{17}NOS$) in each mL of the constituted solution taken by the formula:

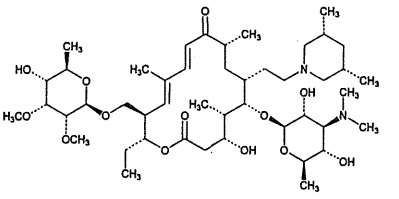
$$(223.33/259.79)(W/V)(R_U/R_S)$$

in which 223.33 and 259.79 are the molecular weights of tiletamine base and tiletamine hydrochloride, respectively; W is the weight, in mg, of USP Tiletamine Hydrochloride RS taken to prepare the *Standard preparation;* V is the volume, in mL, of the constituted solution taken to prepare the *Assay preparation;* and R_U and R_S are the peak area response ratios of the tiletamine peak to the tetraphenylethylene peak obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the quantity, in mg, of zolazepam ($C_{15}H_{15}FN_4O$) in each mL of the constituted solution taken by the formula:

$$(286.31/322.77)(W/V)(R_U/R_S)$$

in which 286.31 and 322.77 are the molecular weights of zolazepam base and zolazepam hydrochloride, respectively; W is the weight, in mg, of USP Zolazepam Hydrochloride RS taken to prepare the *Standard preparation;* V is the volume, in mL, of the constituted solution taken to prepare the *Assay preparation;* and R_U and R_S are the peak area response ratios of the zolazepam peak to the tetraphenylethylene peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Tilmicosin



$C_{46}H_{80}N_2O_{13}$ 869.13

Tylosin, 4^A-*O*-de(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-20-deoxo-20-(3,5-dimethyl-1-piperidinyl)-, 20(*cis*)-.

4^A-*O*-de(2,6-Dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-20-deoxo-20-(*cis*-3,5-dimethylpiperidino)-tylosin [108050-54-0].

» Tilmicosin contains not less than 85.0 percent of $C_{46}H_{80}N_2O_{13}$, calculated on the anhydrous basis. The content of tilmicosin *cis*-isomers is between 82.0 percent and 88.0 percent, and the content of tilmicosin *trans*-isomers is between 12.0 percent and 18.0 percent of total $C_{46}H_{80}N_2O_{13}$. [**CAUTION**—Tilmicosin is irritating to the eyes and may cause allergic reaction. Avoid contact.]

Packaging and storage—Preserve in well-closed, light-resistant containers. Avoid excessive heat.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Tilmicosin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits peaks for the tilmicosin *trans*-isomer and the tilmicosin *cis*-isomer, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay.*

Water Determination, *Method I* ⟨921⟩: not more than 5.0%, 20 mL of a mixture of methanol and pyridine (4:1) containing 10% of imidazole being used in place of methanol in the titration vessel.

Related compounds—

Dibutylammonium phosphate buffer and *Diluent*—Prepare as directed in the *Assay.*

Solution A—To 700 mL of water add 25 mL of *Dibutylammonium phosphate buffer,* dilute quantitatively with water to 1 L, and mix. Degas before use.

Solution B—Use degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Tilmicosin RS in acetonitrile to obtain a solution having a known concentration of about 0.25 mg per mL, sonicating if necessary to dissolve. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

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Test solution—Transfer about 200 mg of Tilmicosin, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile, and sonicate briefly to dissolve. Dilute with *Diluent* to volume, and mix. [NOTE—Use this solution within 24 hours.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1 and is programmed for gradient elution by delivering a mixture of *Solution A* and *Solution B* in a ratio of 82:18 initially, and by continuously varying the mixture linearly over a period of 30 minutes until the final ratio is 60:40. The flow rate is about 1.1 mL per minute. Chromatograph the *Standard solution,* and record the responses as directed for *Procedure:* the relative retention times are about 0.9 for the tilmicosin *trans*-isomers (two incompletely resolved peaks), 1.0 for the tilmicosin *cis*-isomer, and 1.1 for the tilmicosin *cis*-8-epimer.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of each related compound in the portion of Tilmicosin taken by the formula:

$$5(CP/W)(r_c/r_s)$$

in which C is the concentration, in mg per mL, of USP Tilmicosin RS in the *Standard solution; P* is the designated potency, in µg per mg, of tilmicosin in the USP Tilmicosin RS; *W* is the weight, in mg, of Tilmicosin taken to prepare the *Test solution;* r_c is the area response of the individual related compound peak, other than those obtained for tilmicosin *trans*-isomers, tilmicosin *cis*-isomer, and tilmicosin *cis*-8-epimer; and r_s is the sum of the peak area responses for the tilmicosin *trans*-isomers, the tilmicosin *cis*-isomer, and the tilmicosin *cis*-8-epimer obtained from the *Standard solution.* Not more than 3% of any individual related compound, calculated on the anhydrous basis, is found, and the sum of all the related compounds is not more than 10%, calculated on the anhydrous basis.

Assay—

Dibutylammonium phosphate buffer—Add, with stirring, 70 mL of dilute phosphoric acid (1 in 10) to 16.8 mL of dibutylamine. Allow to cool, and adjust with phosphoric acid to a pH of 2.5 ± 0.1. Dilute with water to 100 mL, and mix.

Mobile phase—To 700 mL of water, add 115 mL of acetonitrile, 55 mL of tetrahydrofuran, and 25 mL of *Dibutylammonium phosphate buffer.* Dilute with water to 1000 mL, and mix. Each component may be degassed before use, or the *Mobile phase* may be sparged with helium for 2 minutes before use. Store the *Mobile phase* in a sealed container when not in use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—Decreasing the proportion of acetonitrile or tetrahydrofuran increases resolution.]

Diluent—To 900 mL of water, add 5.71 g of phosphoric acid, adjust with 12.5 N sodium hydroxide to a pH of 2.5 ± 0.1, dilute with water to 1000 mL, and mix.

Standard preparation—Transfer about 25 mg of USP Tilmicosin RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile, and sonicate to dissolve. Dilute with *Diluent* to volume, and mix. [NOTE—Use this solution on the day prepared.]

Assay preparation—Transfer about 25 mg of Tilmicosin, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile, and sonicate to dissolve. Dilute with *Diluent* to volume, and mix. [NOTE—Use this solution on the day prepared.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the relative retention times are about 0.8 for the tilmicosin *trans*-isomers and 1.0 for the tilmicosin *cis*-isomers [NOTE—Tilmicosin *cis*-isomer and tilmicosin *cis*-8-epimer co-elute in this chromatographic system] ; the resolution, *R,* between the tilmicosin *trans*-isomers peak and the tilmicosin *cis*-isomers peak is not less than 1.25; the tailing factors for the peaks are not less than 0.7 and not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in µg, of tilmicosin *trans*- and *cis*-isomers in the portion of Tilmicosin taken by the formula:

$$50(CP/W)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of USP Tilmicosin RS in the *Standard preparation; P* is the designated potency, in µg per mg, of the relevant (*trans* or *cis*) tilmicosin isomers in the USP Tilmicosin RS; *W* is the weight, in mg, of Tilmicosin taken to prepare the *Assay preparation;* r_i is the peak area response for the relevant (*trans* or *cis*) tilmicosin isomers obtained from the *Assay preparation;* and r_s is the peak area response for the relevant (*trans* or *cis*) tilmicosin isomers obtained from the *Standard preparation.* Calculate the percentage of tilmicosin ($C_{46}H_{80}N_2O_{13}$) in the portion of Tilmicosin taken by the formula:

$$0.1(trans + cis)$$

in which *trans* and *cis* are the quantities, in µg per mg, of tilmicosin *trans*-isomers and tilmicosin *cis*-isomers in the Tilmicosin, as determined above. Calculate the percentages of tilmicosin *trans*-isomers and tilmicosin *cis*-isomers taken by the formula:

$$100\ isomer/(trans + cis)$$

in which *isomer* is the quantity, in µg per mg, of either the tilmicosin *trans*-isomers or the tilmicosin *cis*-isomers in the Tilmicosin, as determined above.

Tilmicosin Injection

» Tilmicosin Injection is a sterile solution of Tilmicosin in a mixture of Propylene Glycol and Water for Injection, and is solubilized with the aid of Phosphoric Acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of tilmicosin ($C_{46}H_{80}N_2O_{13}$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging;* protect from light. Store at or below 30°.

Labeling—Label the Injection to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Tilmicosin RS

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Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits major peaks for the tilmicosin *trans*-isomers and the tilmicosin *cis*-isomers, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.5 USP Endotoxin Unit per mg of tilmicosin.

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except that the test mixture is prepared as follows. Transfer aseptically 1 mL from each of 20 containers to a vessel containing 200 mL of a mixture containing 2 mL of polysorbate 20 in *Buffer B.16* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers*). After that solution has been filtered, wash the filter with three 100-mL portions of the same solution, instead of *Diluting Fluid A*.

pH ⟨791⟩: between 5.5 and 6.5.

Particulate Matter in Injections ⟨788⟩—Use the procedure under *Microscopic Particle Count Test:* not more than 50 particles per mL that are equal to or greater than 10 µm in effective spherical diameter, and not more than 5 particles per mL that are equal to or greater than 25 µm in effective spherical diameter are found.

Content of propylene glycol—

Internal standard solution—Prepare a solution of pentadecane in acetone containing about 0.5 mg per mL.

Standard solution—Transfer about 125 mg of propylene glycol, accurately weighed, to a 100-mL volumetric flask, dilute with acetone to volume, and mix. Mix equal, accurately measured volumes of this solution and the *Internal standard solution*. This solution contains about 0.625 mg of propylene glycol per mL.

Test solution—Transfer an accurately measured volume of Injection, equivalent to about 250 mg of propylene glycol, to a 200-mL volumetric flask, dilute with acetone to volume, and mix. Mix equal, accurately measured volumes of this solution and the *Internal standard solution*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 15-m fused silica column that has liquid phase G16 bonded to the inner surface at a thickness of 1 µm. The injection port and the detector block are maintained at about 250°, and the column is maintained at a temperature of about 100°. Helium is used as the carrier gas at a flow rate of about 15 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for pentadecane and 1.0 for propylene glycol, the resolution, *R*, between the pentadecane peak and the propylene glycol peak is not less than 7.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of propylene glycol in each mL of the Injection taken by the formula:

$$400(C/V)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of propylene glycol in the *Standard solution*, *V* is the volume, in mL, of Injection taken, and R_U and R_S are the ratios of the propylene glycol peak area response to the pentadecane peak area response obtained from the *Test solution* and the *Standard solution*, respectively. Between 80.0% and 120.0% of the labeled amount of propylene glycol is found.

Assay—

Dibutylammonium phosphate buffer—To 700 mL of water, add 168 mL of dibutylamine. Add phosphoric acid slowly until the dibutylamine is just dissolved, stirring vigorously during the addition. Allow to cool, and adjust with phosphoric acid to a pH of 2.55 ± 0.05. Dilute with water to 1000 mL, mix, and filter under vacuum.

Mobile phase—To 700 mL of water, add 115 mL of acetonitrile, 55 mL of tetrahydrofuran, and 25 mL of *Dibutylammonium phosphate buffer*. Dilute with water to 1000 mL, and mix. Each component may be filtered before mixing, or the *Mobile phase* may be filtered, minimizing solvent evaporation. Store the *Mobile phase* in a sealed container when not in use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—To 700 mL of water add 200 mL of acetonitrile and 25 mL of *Dibutylammonium phosphate buffer*, dilute with water to 1000 mL, and mix.

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Tilmicosin RS in acetonitrile to obtain a solution having a known concentration of about 2.5 mg per mL. Transfer 4.0 mL of this solution to a 20-mL volumetric flask, add 10 mL of water, and 0.5 mL of *Dibutylammonium phosphate buffer*, dilute with water to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 300 mg of tilmicosin, to a 30-mL volumetric flask, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the relative retention times are about 0.8 for the tilmicosin *trans*-isomers and 1.0 for the tilmicosin *cis*-isomers, the resolution, *R*, between the tilmicosin *trans*-isomers peak and the tilmicosin *cis*-isomers peak is not less than 1.25, the tailing factors for the peaks are not less than 0.7 and not more than 2, and the relative standard deviation for replicate injections is not more than 1.5%.

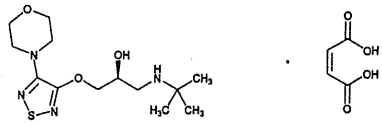
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of each of the tilmicosin isomers in each mL of the Injection taken by the formula:

$$0.6(CP/V)(r_I/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Tilmicosin RS in the *Standard preparation*, *P* is the potency, in µg per mg, of the relevant (*trans* or *cis*) tilmicosin isomers in the USP Tilmicosin RS, *V* is the volume of Injection taken to prepare the *Assay preparation*, r_I is the peak response of the relevant tilmicosin isomers obtained from the *Assay preparation*, and r_S is the peak area response for the relevant (*trans* or *cis*) tilmicosin isomers obtained from the *Standard preparation*. Calculate the quantity, in mg, of $C_{46}H_{80}N_2O_{13}$ in each mL of the Injection taken by adding the quantities, in mg per mL, of *cis*- and *trans*-isomers found.

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Timolol Maleate



$C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$ 432.49

2-Propanol, 1-[(1,1-dimethylethyl)amino]-3- [[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-, (*S*)-, (*Z*)-2-butenedioate (1:1) (salt);
(–)-1-(*tert*-Butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate (1:1) (salt) [26921-17-5].

DEFINITION

Timolol Maleate contains NLT 98.0% and NMT 102.0% of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197M or 197A▲ (CN 1-May-2020)
- **B.** The retention time of the timolol peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the *Enantiomeric Purity* test.

ASSAY

- **PROCEDURE**

Solution A: Dilute 0.5 mL of trifluoroacetic acid with water to 1 L.
Solution B: Dilute 0.5 mL of trifluoroacetic acid with acetonitrile to 1 L.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	84	16
2.4	84	16
8	20	80
8.1	84	16
11	84	16

Diluent: Methanol and water (60:40)
System suitability solution: 100 µg/mL of USP Timolol Maleate RS and 10 µg/mL of USP Timolol Related Compound D RS in *Diluent*
Standard solution: 100 µg/mL of USP Timolol Maleate RS in *Diluent*
Sample solution: 100 µg/mL of Timolol Maleate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 2.1-mm × 10-cm; 2.6-µm packing L1
Autosampler temperature: 4°
Flow rate: 0.4 mL/min
Injection volume: 2.5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between timolol and timolol related compound D, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of timolol from the *Sample solution*
r_S = peak response of timolol from the *Standard solution*
C_S = concentration of USP Timolol Maleate RS in the *Standard solution* (µg/mL)
C_U = concentration of Timolol Maleate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
▲Sensitivity solution: 0.5 µg/mL of USP Timolol Maleate RS in *Diluent*▲ (USP 1-Dec-2019)
System suitability solution: 100 µg/mL each of USP Timolol Maleate RS, USP Timolol Related Compound B RS, USP Timolol Related Compound C RS, USP Timolol Related Compound D RS, USP Timolol Related Compound E RS, and USP Timolol Related Compound F RS in *Diluent*. [NOTE—Prepare fresh and analyze immediately as USP Timolol Related Compound E RS degrades rapidly.]
Standard solution: 1 µg/mL of USP Timolol Maleate RS and 4 µg/mL each of USP Timolol Related Compound B RS, USP Timolol Related Compound C RS, USP Timolol Related Compound D RS, USP Timolol Related Compound E RS, and USP Timolol Related Compound F RS in *Diluent*. Sonicate if needed for 0.5 min.
Sample solution: 1 mg/mL of Timolol Maleate in *Diluent*
System suitability
Samples: ▲*Sensitivity solution*,▲ (USP 1-Dec-2019) *System suitability solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between timolol and timolol related compound D, *System suitability solution*
Relative standard deviation: NMT 4.0% for timolol, *Standard solution*
▲Signal-to-noise ratio: NLT 10 for timolol, *Sensitivity solution*▲ (USP 1-Dec-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of timolol maleate ester (free base of timolol related compound E) in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of timolol maleate ester from the *Sample solution*
r_S = peak response of timolol maleate ester from the *Standard solution*

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C_S = concentration of USP Timolol Related Compound E RS in the *Standard solution* (µg/mL)
C_U = concentration of Timolol Maleate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of timolol maleate ester, 414.48
M_{r2} = molecular weight of timolol related compound E, 530.55

Calculate the percentage of timolol bisthiadiazol analog (free base of timolol related compound C) in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of timolol bisthiadiazol from the *Sample solution*
r_S = peak response of timolol bisthiadiazol from the *Standard solution*
C_S = concentration of USP Timolol Related Compound C RS in the *Standard solution* (µg/mL)
C_U = concentration of Timolol Maleate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of timolol bisthiadiazol, 485.62
M_{r2} = molecular weight of timolol related compound C, 601.69 ▲(USP 1-Dec-2019)

Calculate the percentage of ▲timolol related compound B, D, or F▲(USP 1-Dec-2019) in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ▲timolol related compound B, D, or F▲(USP 1-Dec-2019) from the *Sample solution*
r_S = peak response of ▲timolol related compound B, D, or F▲(USP 1-Dec-2019) from the *Standard solution*
C_S = concentration of ▲USP Timolol Related Compound B RS, USP Timolol Related Compound D RS, or USP Timolol Related Compound F RS▲(USP 1-Dec-2019) in the *Standard solution* (µg/mL)
C_U = concentration of Timolol Maleate in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of timolol from the *Standard solution*
C_S = concentration of USP Timolol Maleate RS in the *Standard solution* (µg/mL)
C_U = concentration of Timolol Maleate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. ▲The reporting threshold is 0.05%.▲(USP 1-Dec-2019)

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Timolol related compound B	0.5	0.4
Timolol related compound D	0.8	0.4
Timolol maleate	1.0	—
▲Timolol maleate ester[a]▲(USP 1-Dec-2019)	1.4	0.4
▲Timolol bisthiadiazol analog[b]▲(USP 1-Dec-2019)	1.8	0.4
Timolol related compound F	2.0	0.4
Any individual unspecified impurity	—	0.10
▲Total impurities▲(USP 1-Dec-2019)	—	1.0

▲[a] (*S*)-3-(*tert*-Butylamino)-1-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-2-yl hydrogen maleate.
[b] *N*-(*tert*-Butyl)-2,3-bis(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-1-amine.▲(USP 1-Dec-2019)

Change to read:

- **ENANTIOMERIC PURITY**

Prepare all solutions in low-actinic glassware protected from light.
Mobile phase: Diethylamine, 2-propanol, and hexane (2:40:960)
Diluent: Methylene chloride and 2-propanol (25:75)
System suitability solution: 0.03 mg/mL each of USP Timolol Maleate RS and USP Timolol Related Compound A RS in *Diluent*
Standard solution: 0.03 mg/mL of USP Timolol Related Compound A RS in *Diluent*
Sample solution: 3 mg/mL of Timolol Maleate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 297 nm
Column: 4.6-mm × 25-cm; 5-µm packing L40
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* ▲and *Standard solution*▲(USP 1-Dec-2019)
Suitability requirements
Resolution: NLT 4.0 between the timolol related compound A and timolol peaks, *System suitability solution*
Relative standard deviation: NMT 1.5% for ▲the timolol related compound A peak, *Standard solution*▲(USP 1-Dec-2019)
Signal-to-noise ratio: NLT 10 for the timolol related compound A peak, ▲*Standard solution*▲(USP 1-Dec-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of timolol related compound A in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of timolol related compound A from the *Sample solution*
r_S = peak response of timolol related compound A from the *Standard solution*
C_S = concentration of USP Timolol Related Compound A RS in the *Standard solution* (mg/mL)

USP Monographs

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C_U = concentration of Timolol Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: 20 mg/mL of Timolol Maleate in water
  Acceptance criteria: 3.8–4.3
- **Loss on Drying** ⟨731⟩
  Analysis: Dry under vacuum at 100° to constant weight.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in well-closed containers, ▲protected from light.▲ (USP 1-Dec-2019)
- **USP Reference Standards** ⟨11⟩
  USP Timolol Maleate RS
  USP Timolol Related Compound A RS
  (*R*)-1-(*tert*-Butylamino)-3-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-2-ol maleate.
  $C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$ 432.49
  USP Timolol Related Compound B RS
  3-(*tert*-Butylamino)-2-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-1-ol.
  $C_{13}H_{24}N_4O_3S$ 316.42
  USP Timolol Related Compound C RS
  N-(*tert*-Butyl)-2,3-bis(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-1-amine maleate.
  $C_{19}H_{31}N_7O_4S_2 \cdot C_4H_4O_4$ 601.69
  USP Timolol Related Compound D RS
  4-Morpholino-1,2,5-thiadiazol-3-ol.
  $C_6H_9N_3O_2S$
  187.22
  USP Timolol Related Compound E RS
  (*S*, *Z*)-4-({1-(*tert*-Butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]propan-2-yl}oxy)-4-oxobut-2-enoic acid maleate salt (1:1).
  $C_{17}H_{26}N_4O_6S \cdot C_4H_4O_4$ 530.55
  USP Timolol Related Compound F RS
  3-Chloro-4-morpholino-1,2,5-thiadiazol.
  $C_6H_8ClN_3OS$ 205.67

Timolol Maleate Ophthalmic Solution

» Timolol Maleate Ophthalmic Solution is a sterile, aqueous solution of Timolol Maleate. It contains an amount of $C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$ equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of timolol ($C_{13}H_{24}N_4O_3S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Timolol Maleate RS

Identification—Dilute a suitable quantity of Ophthalmic Solution with water to obtain a solution having a concentration of about 20 µg of timolol per mL: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar preparation of USP Timolol Maleate RS, concomitantly measured.

Sterility Tests ⟨71⟩ **:** meets the requirements.

pH ⟨791⟩: between 6.5 and 7.5.

Assay—

pH 2.8 phosphate buffer—Dissolve 11.1 g of monobasic sodium phosphate in 1000 mL of water, adjust with phosphoric acid to a pH of 2.8 ± 0.05, filter, and degas.

Diluent—Prepare a mixture of acetonitrile and *pH 2.8 phosphate buffer* (2:1).

Mobile phase—Prepare a mixture of *pH 2.8 phosphate buffer* and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [Note—Minimize the time the Reference Standard, the Ophthalmic Solution, the standard stock solution, the *Standard preparation,* and the *Assay preparation* are exposed to direct light.]

Standard preparation—Transfer about 34 mg of USP Timolol Maleate RS, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this stock solution to a 50-mL volumetric flask, add 15 mL of *Diluent*, dilute with water to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 5 mg of timolol, to a 50-mL volumetric flask, add 15 mL of *Diluent*, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 295-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The column temperature is maintained at 40°, and the flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0, the column efficiency is not less than 3600 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak area responses for the major peaks. Calculate the quantity, in mg, of timolol ($C_{13}H_{24}N_4O_3S$) in each mL of Ophthalmic Solution taken by the formula:

$$(316.43/432.49)(50C/V)(r_U/r_S)$$

in which 316.43 and 432.49 are the molecular weights of timolol and timolol maleate, respectively, C is the concentration, in mg per mL, of USP Timolol Maleate RS in the *Standard preparation*, V is the volume, in mL, of Ophthalmic Solution taken, and r_U and r_S are the peak area responses of the timolol peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Timolol Maleate Tablets

DEFINITION

Timolol Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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• **B.** The UV (or UV-Vis) spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Transfer 0.5 mL of trifluoroacetic acid to a 1-L volumetric flask and dilute with water to volume.

Solution B: Transfer 0.5 mL of trifluoroacetic acid to a 1-L volumetric flask and dilute with acetonitrile to volume.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	84	16
2.4	84	16
8	20	80
8.1	84	16
11	84	16

Buffer: Transfer 11.04 g of monobasic sodium phosphate to a 1-L volumetric flask and dilute with water to volume. Adjust with phosphoric acid to a pH of 2.8 ± 0.05.

Diluent: Methanol and *Buffer* (20:80)

System suitability solution: 0.1 mg/mL of USP Timolol Maleate RS and 10.0 µg/mL of USP Timolol Related Compound D RS in *Diluent* with sonication if necessary

Standard solution: 0.1 mg/mL of USP Timolol Maleate RS in *Diluent* with sonication if necessary

Sample solution: 0.1 mg/mL of timolol maleate from NLT 20 finely ground Tablets (ground with a mortar and pestle) in *Diluent* with sonication if necessary, and filtration with 0.2-µm syringe filters, discarding the first 2 mL

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 295 nm. For *Identification* test *B*, use a diode array detector in the range of 210–400 nm.

Column: 2.1-mm × 10-cm; 2.6-µm packing L1

Flow rate: 0.4 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 2.5 between timolol and timolol related compound D, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Timolol Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of timolol maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 1: 100 rpm

Time: 20 min

Standard solution: USP Timolol Maleate RS in *Medium*

Sample solution: Sample per the chapter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Analysis: Determine the amount of timolol maleate in solution in filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the procedure in the *Assay*.

Tolerances: NLT 80% (*Q*) of the labeled amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Buffer, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.1 mg/mL of USP Timolol Maleate RS and 10 µg/mL of USP Timolol Related Compound D RS in *Diluent* with sonication if necessary

Standard solution: 2.0 µg/mL each of USP Timolol Maleate RS, USP Timolol Related Compound B RS, and USP Timolol Related Compound D RS in *Diluent*

Sample solution: 1.0 mg/mL of timolol maleate from NLT 20 finely ground Tablets (ground with a mortar and pestle) in *Diluent* with sonication if necessary, and filtration with 0.2-µm syringe filters, discarding the first 2 mL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 3 between timolol and timolol related compound D, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of timolol maleate related compound B and timolol maleate related compound D the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of each impurity from the *Standard solution*

C_S = concentration of each impurity in the *Standard solution* (mg/mL)

C_U = nominal concentration of timolol maleate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*

r_S = peak response of timolol from the *Standard solution*

USP Monographs

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C_S = concentration of USP Timolol Maleate RS in the *Standard solution*
C_U = nominal concentration of timolol maleate in the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Timolol related compound B	0.5	0.4
Timolol related compound D	0.8	0.4
Timolol	1.0	—
Any individual impurity	—	0.2
Total impurities	—	1.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Timolol Maleate RS
  USP Timolol Related Compound B RS
  3-(*tert*-Butylamino)-2-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-1-ol.
  $C_{13}H_{24}N_4O_3S$ 316.42
  USP Timolol Related Compound D RS
  4-Morpholino-1,2,5-thiadiazol-3-ol.
  $C_6H_9N_3O_2S$ 187.22

Timolol Maleate and Hydrochlorothiazide Tablets

DEFINITION

Timolol Maleate and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 10 mg/mL of USP Timolol Maleate RS in methanol
Standard solution B: 10 mg/mL of USP Hydrochlorothiazide RS in methanol
Sample solution: Norminally equivalent to 4 mg/mL of timolol maleate in methanol prepared as follows. Transfer a portion of powdered Tablets, equivalent to about 20 mg of timolol maleate, to a suitable centrifuge tube containing about 5 mL of methanol.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 3 µL
Developing solvent system: Chloroform, methanol, and ammonium hydroxide (80:20:1)
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Separately apply *Standard solution A*, *Standard solution B*, and the *Sample solution* to a thin-layer chromatographic plate, and develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Air-dry, and examine under short-wavelength UV light.
Acceptance criteria: The R_F values of the principal spots of the *Sample solution* correspond to the principal spots of *Standard solution A* and *Standard solution B*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 13.6 g of monobasic potassium phosphate in 100 mL of water. Adjust with phosphoric acid to a pH of 3.0 ± 0.05, and filter.
Mobile phase: Acetonitrile, methanol, *Buffer*, and water (160:40:40:760)
Standard stock solution: 0.1 mg/mL of USP Timolol Maleate RS and (0.1*J*) mg/mL of USP Hydrochlorothiazide RS, *J* being the ratio of the labeled amount, in mg, of hydrochlorothiazide to the labeled amount, in mg, of timolol maleate per Tablet prepared as follows. To a suitable amount of each reference material in a suitable volumetric flask add 0.05 M monobasic sodium phosphate to 10% of the final volume, followed by 25% of the final volume of acetonitrile. Sonicate for 4 min, and dilute with water to volume.
Standard solution: 0.02 mg/mL of USP Timolol Maleate RS and (0.02*J*) mg/mL of USP Hydrochlorothiazide RS, *J* being the ratio of the labeled amount, in mg, of hydrochlorothiazide to the labeled amount, in mg, of timolol maleate per Tablet in acetonitrile solution (1 in 10), from the *Standard stock solution*
Sample solution: Nominally equivalent to 0.02 mg/mL of timolol maleate from NLT 20 finely powdered Tablets prepared as follows. Transfer a portion of the powder, equivalent to about 20 mg of timolol maleate, to a 1-L volumetric flask, add about 100 mL of 0.05 M monobasic sodium phosphate, 125 mL of acetonitrile, and 100 mL of water, and mix by mechanical means. Allow to stand for 16 h, dilute with water to volume, mix, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzothiadiazine related compound A, hydrochlorothiazide, and timolol maleate are about 0.5, 0.6, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between hydrochlorothiazide and timolol maleate
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

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Calculate the percentage of the labeled amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of Timolol from the *Sample solution*
r_S = peak response of Timolol from the *Standard solution*
C_S = concentration of USP Timolol Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of timolol maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of timolol maleate and hydrochlorothiazide

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 20 min
Standard solution A: 11 µg/mL of USP Timolol Maleate RS in 0.1 N hydrochloric acid
Standard solution B: A suitable concentration of USP Hydrochlorothiazide RS in ethyl acetate
Sample solution: Filter a portion of the solution under test.
Blank: 0.1 N hydrochloric acid
Detector: UV 293 nm for timolol maleate; 270 nm for hydrochlorothiazide
Analysis
Samples: *Standard solution A*, *Sample solution*, and *Blank*
Transfer 10.0 mL each of *Blank*, *Standard solution A*, and *Sample solution* to three separators, respectively, and treat each of the solutions as follows. Add 20.0 mL of ethyl acetate, mix for 1 min, allow the phases to separate, filter the aqueous layer into a suitable vessel for the timolol maleate analysis, and retain the ethyl acetate layer for the hydrochlorothiazide determination. Determine the amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) by comparison of the UV absorbances of the aqueous layer from the *Sample solution* with that of the aqueous layer from *Standard solution A*. Filter the ethyl acetate layer through filter paper. Determine the amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) by comparison of the UV absobances of the ethyl acetate layer from the *Sample solution* with that of *Standard solution B*.
Tolerances: NLT 80% (*Q*) of each of the labeled amounts of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Content Uniformity* for timolol maleate and hydrochlorothiazide

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.5 mg/mL of USP Benzothiadiazine Related Compound A RS in methanol
Standard solution: 0.5 µg/mL of USP Benzothiadiazine Related Compound A RS in *Mobile phase* from the *Standard stock solution*
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between hydrochlorothiazide and timolol
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A from the *Standard solution*
C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (µg/mL)

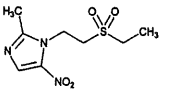
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonade.
$C_6H_8ClN_3O_4S_2$ 285.73
USP Hydrochlorothiazide RS
USP Timolol Maleate RS

Tinidazole



$C_8H_{13}N_3O_4S$ 247.27
1*H*-Imidazole, 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-; 1-[2-(Ethylsulfonyl)ethyl]-2-methyl-5-nitroimidazole [19387-91-8].

DEFINITION
Tinidazole contains NLT 98.0% and NMT 102.0% of tinidazole ($C_8H_{13}N_3O_4S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Acetonitrile, methanol, and water (10:20:70)
Standard solution: 0.1 mg/mL of USP Tinidazole RS prepared as follows. Transfer a suitable amount of USP Tinidazole RS to a suitable volumetric flask and add methanol to 10% of the final volume of the flask. Dilute with *Mobile phase* to volume.
Sample solution: 0.1 mg/mL of Tinidazole prepared as follows. Transfer a suitable amount of Tinidazole to a suitable volumetric flask and add methanol to 10% of the

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final volume of the flask. Dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 320 nm
Column: 3.0-mm × 25-cm; 5-µm packing L7
Flow rate: 0.5 mL/min
Injection volume: 20 µL
Run time: 1.5 times the retention time of tinidazole

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tinidazole ($C_8H_{13}N_3O_4S$) in the portion of Tinidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole from the *Sample solution*
r_S = peak response of tinidazole from the *Standard solution*
C_S = concentration of USP Tinidazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Tinidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

• **Organic Impurities**
Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution 1: Use the *Standard solution* from the *Assay*.
Standard stock solution 2: 0.05 mg/mL each of USP Tinidazole Related Compound A RS and USP Tinidazole Related Compound B RS prepared as follows. Transfer suitable amounts of USP Tinidazole Related Compound A RS and USP Tinidazole Related Compound B RS to a suitable volumetric flask and add methanol to 10% of the final volume of the flask. Dilute with *Mobile phase* to volume.
Standard solution: 0.1 µg/mL of USP Tinidazole RS and 0.2 µg/mL each of USP Tinidazole Related Compound A RS and USP Tinidazole Related Compound B RS in *Mobile phase* from *Standard stock solution 1* and *Standard stock solution 2*
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between tinidazole related compound A and tinidazole related compound B
Relative standard deviation: NMT 5.0% for each peak
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentages of tinidazole related compound A and tinidazole related compound B in the portion of Tinidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A or tinidazole related compound B from the *Sample solution*
r_S = peak response of tinidazole related compound A or tinidazole related compound B from the *Standard solution*
C_S = concentration of USP Tinidazole Related Compound A RS or USP Tinidazole Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Tinidazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Tinidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of tinidazole from the *Standard solution*
C_S = concentration of USP Tinidazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Tinidazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.6	0.2
Tinidazole related compound B	0.7	0.2
Tinidazole	1.0	—
Any unspecified impurity	—	0.10
Total impurities	—	0.4

SPECIFIC TESTS

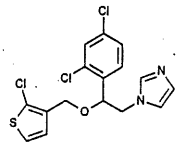
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 100°–105° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, protected from light, at controlled room temperature.

• **USP Reference Standards** ⟨11⟩
USP Tinidazole RS
USP Tinidazole Related Compound A RS
2-Methyl-5-nitroimidazole.
$C_4H_5N_3O_2$ 127.10
USP Tinidazole Related Compound B RS
1-(2-Ethyl-sulfonylethyl)-2-methyl-4-nitroimidazole.
$C_8H_{13}N_3O_4S$ 247.28

Tioconazole



$C_{16}H_{13}Cl_3N_2OS$ 387.71

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1*H*-Imidazole, 1-[2-[(2-chloro-3-thienyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-;
1-[2,4-Dichloro-[β-(2-chloro-3-thenyl)-oxy]phen ethyl] imidazole [65899-73-2].

DEFINITION

Tioconazole contains NLT 97.0% and NMT 103.0% of $C_{16}H_{13}Cl_3N_2OS$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B. PROCEDURE**

Standard solution: 50 mg/mL of USP Tioconazole RS in methanol
Sample solution: 50 mg/mL of Tioconazole in methanol
Developing solvent system: Chloroform, methanol, and glacial acetic acid (40:5:1)
Visualizing solution: Dissolve 0.85 g of bismuth subnitrate in 10 mL of glacial acetic acid, and dilute with water to 50 mL. Mix 10 mL of this solution, 50 mL of potassium iodide solution (2 in 25), and 20 mL of glacial acetic acid, and dilute to 100 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber and locate the spots on the plate by viewing under short- and long-wavelength UV light after drying the plate at 80° for 5 min. Spray the plate with *Visualizing solution*, air-dry for 2 min, and overspray with sodium nitrite solution (1 in 20). Air-dry the plate for 5 min, and examine it for brown spots on a pale yellow background.
Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.

- **C.** The retention time of the major peak for tioconazole of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (44:40:28). Degas the solution, and add 2.0 mL of ammonium hydroxide to 1120 mL of *Mobile phase*. [NOTE—Prepare the *Mobile phase* fresh daily.]
Standard solution: 200 µg/mL of USP Tioconazole RS in methanol
Sample solution: 200 µg/mL of Tioconazole in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 219 nm
Column: 5-mm × 25-cm; packing L1 with a precolumn containing packing L4 installed between the pump and the injector. [NOTE—Replace the precolumn daily.]
Flow rate: Adjust to obtain a retention time of between 12 and 17 min for tioconazole.
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates, determined from the analyte peak
Tailing factor: NMT 2.0 for the analyte peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{13}Cl_3N_2OS$ in the Tioconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tioconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0%

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.2%
- **Chloride and Sulfate,** *Chloride* ⟨221⟩: A 0.7-g portion dissolved in methanol shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.05%).

ORGANIC IMPURITIES

- **Procedure**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: Dissolve 1 mg each of USP Tioconazole Related Compound A RS, USP Tioconazole Related Compound B RS, and USP Tioconazole Related Compound C RS in 15.0 mL of methanol, and shake until the contents are completely dissolved.
Sample solution: Dissolve 100 mg of Tioconazole in 15.0 mL of methanol, and shake until the substance is completely dissolved.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate, in turn, the percentages of 1-[2,4-dichloro-β-[(3-thenyl)-oxy]phenethyl]imidazole hydrochloride (tioconazole related compound A), 1-[2,4-dichloro-β-[(2,5-dichloro-3-thenyl)-oxy] phenethyl]imidazole hydrochloride (tioconazole related compound B), and 1-[2,4-dichloro-β-[(5-bromo-2-chloro-3-thenyl)-oxy]phenethyl]imidazole hydrochloride (tioconazole related compound C) in the portion of Tioconazole taken:

$$\text{Result} = (r_U/r_S) \times (W_I/W_U) \times 100$$

r_U = peak response of the related compound from the *Sample solution*
r_S = peak response of the related compound from the *Standard solution*
W_I = weight of the respective USP Reference Standard taken to prepare the *Standard solution* (mg)
W_U = weight of Tioconazole taken to prepare the *Sample solution* (mg)

Acceptance criteria: The limit of each related compound is NMT 1.0%.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Tioconazole RS

USP Tioconazole Related Compound A RS
1-[2,4-Dichloro-β-[(3-thenyl)-oxy]phenethyl]imidazole hydrochloride.
$C_{16}H_{14}Cl_2N_2OS \cdot HCl$ 389.73

USP Tioconazole Related Compound B RS
1-[2,4-Dichloro-β-[(2,5-dichloro-3-thenyl)oxy]phenethyl] imidazole hydrochloride.
$C_{16}H_{12}Cl_4N_2OS \cdot HCl$ 458.62

USP Tioconazole Related Compound C RS
1-[2,4-Dichloro-β-[(5-bromo-2-chloro-3-thenyl)-oxy]-phenethyl]imidazole hydrochloride.
$C_{16}H_{13}BrCl_2N_2OS \cdot HCl$ 468.63

Tissue Human Amnion Chorion Membrane Dehydrated

DEFINITION

Tissue Human Amnion Chorion Membrane Dehydrated is a dried bi-layer allograft containing amnion and chorion, derived from donated human placental tissues obtained from scheduled Caesarian sections. Using sterile solutions and aseptic techniques, the amniotic membrane is dissected from the placenta, the amnion and chorion layers are separated and then washed, layered, and dried. The product is provided in a sterile sheet form. Placentas used to produce Tissue Human Amnion Chorion Membrane Dehydrated are obtained from donors that have passed all applicable donor eligibility requirements and relevant communicable disease testing.

SPECIFIC TESTS

- **HISTOLOGICAL EVALUATION**

Hydration solution: 0.9% (w/v) sodium chloride (NaCl)

Cryopreservation matrix: Optimum cutting temperature (OCT) formulation of water-soluble glycols and resins[1]

Tissue preparation: Cut a sample of Tissue Human Amnion Chorion Membrane Dehydrated NLT 0.5 cm × 0.5 cm and NMT 1.0 cm × 1.0 cm. Hydrate the sample in 5 mL of *Hydration solution* for 10–30 min. Add a thin layer of the *Cryopreservation matrix* into the bottom of a suitable embedding mold,[2] and freeze on dry ice for 5–10 min until the *Cryopreservation matrix* appears white. Add more *Cryopreservation matrix* on top of the frozen layer, and place the tissue sample into the *Cryopreservation matrix*. Use forceps to arrange the tissue in the *Cryopreservation matrix* in the desired orientation for sectioning. Place the tissue sample and embedding mold onto dry ice for 10–20 min until the *Cryopreservation matrix* is completely solid and appears white. Immediately place the frozen block in a –80° freezer until ready for sectioning.

Cryosectioning: Add a layer of liquid *Cryopreservation matrix* onto the cryostat sample holder of a suitable cryostat,[3] and then place the frozen tissue block onto the holder in the desired orientation for sectioning. Freeze the tissue block onto the holder in the cryostat sectioning chamber before trimming the frozen block. Using the cryostat, trim the frozen block as necessary in 50–100 µm sections until the tissue sample is accessed. Subsequently, cut 5-µm section(s) of the tissue sample. Press each section onto the positively charged side of a microscope slide,[4] allowing the section to freeze onto the slide. Store the sections at –80° until use.

Staining preparation: When ready to stain, thaw the frozen slides at room temperature for NLT 30 min. Fix the tissue slides in ice cold acetone for 10 min. Allow the slides to dry at room temperature for NLT 30 min.

- **VERHOEFF STAINING**

Verhoeff staining solution: Prepare fresh Verhoeff stain containing 60 mL of 5% alcoholic hematoxylin, 24 mL of 10% aqueous ferric chloride, and 24 mL of Lugol's iodine working solution.[5]

Analysis: Place the slides in 100 mL of deionized water twice for 5 min each. Submerge the slides in *Verhoeff staining solution* for 15 min. Place the slides in five changes of deionized water until the water appears clear, then perform two additional changes in 100 mL of deionized water for 5 min each. Dip the slides in 100 mL of 2% aqueous ferric chloride 10 times, and then allow the stain to differentiate for 60 s until the amnion begins to appear gray. Place the slides in two changes of 100 mL of deionized water for 5 min each. Submerge the slides in 100 mL of 5% aqueous sodium thiosulfate for 1 min, then submerge the slides in two exchanges of 100 mL of deionized water for 5 min each.

Add 3–4 drops of a mounting solution[6] to each slide and incubate for 5 min. Carefully place a coverslip over the tissue section, taking care not to trap any bubbles under the coverslip. Seal the edges of the coverslip with clear nail polish, and store the slides at 4° protected from light.

Image the tissue sections using a suitable microscope at 40× magnification with brightfield.

Acceptance criteria: The product must show the presence of both the amnion layer and the chorion layer. The amnion layer contains a thin, dark staining layer followed by light staining throughout the rest of the amnion. The chorion layer must show a thin layer of light staining followed by dark staining throughout the rest of the chorion.

- **HUMAN TYPE IV COLLAGEN IMMUNOSTAINING**

Phosphate buffered saline (PBS): 0.01 M phosphate buffer, 0.0027 M potassium chloride (KCl), and 0.137 M sodium chloride (NaCl), pH 7.4

1% Bovine serum albumin (BSA): 500 mg of BSA in 50 mL of *PBS*

Blocking buffer diluent: Add 50 µL of *t*-octylphenoxypolyethoxyethanol to 48.95 mL of *1% BSA*.

Blocking buffer: 2% (v/v) normal goat serum in *Blocking buffer diluent*

Human type IV collagen antibody solution: Dilute a suitable mouse anti-human type IV collagen antibody[7] (1:100) in *1% BSA*.

Secondary antibody solution: Dilute a suitable fluorescent labeled, goat anti-mouse antibody[8] 1:100 in *PBS*.

Sample: A sample of Tissue Human Amnion Chorion Membrane Dehydrated cut to NLT 0.5 cm × 0.5 cm and

[1] A suitable cryopreservation embedding medium can be obtained from Fisher Scientific, product #23-730-571 or a suitable equivalent.

[2] A suitable embedding mold is Thermo Scientific Shandon, product #22–19 or an equivalent.

[3] A suitable cryostat can be obtained from Thermo Scientific, product Microm HM 525 or a suitable equivalent.

[4] A suitable microscope slide can be obtained from Fisherbrand, product #12-550-15; Superfrost or a suitable equivalent.

[5] A suitable reagent can be obtained from Poly Scientific, product #K059-16OZ or a suitable equivalent.

[6] A suitable reagent can be obtained from Electron Microscopy Sciences/ Diatome, product #17985-10 or a suitable equivalent.

[7] A suitable antibody can be obtained from Abcam, product #ab6311 Mouse Monoclonal Antibody to Human Type IV Collagen or a suitable equivalent.

[8] A suitable reagent can be obtained from Life Technologies, product #A-21054 or a suitable equivalent.

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NMT 1.0 cm × 1.0 cm and prepared as directed in *Histological Evaluation* (see *Tissue preparation, Cryosectioning,* and *Staining preparation*).

Analysis: Place the slides in two exchanges of 100 mL of *PBS* for 5 min each. Circle each tissue section with a hydrophobic pen to make a hydrophobic ring surrounding the *Sample*. Place the slides into a humidity chamber for the following incubation steps. Add 50–100 µL of *Blocking buffer* to completely cover each tissue section within the hydrophobic ring, and incubate for 20 min. Blot off the excess *Blocking buffer*, but do not rinse the slides. Add 50–100 µL of *Human type IV collagen antibody solution* to completely cover each tissue section and incubate overnight for 12–24 h at 4°.

In parallel, prepare a negative control by incubating a *Sample* with 50–100 µL of *1% BSA* alone without primary antibody for the same amount of time followed by the same steps described below. Blot off the primary antibody or *1% BSA*, and rinse the slides by immersing in two exchanges of 100 mL of *PBS* for 5 min each. Add 50–100 µL of *Secondary antibody solution* to completely cover each tissue section, and incubate for 30 min. Blot off the *Secondary antibody solution* and place the slides in two exchanges of 100 mL of *PBS* for 5 min each, followed by 100 mL of deionized water for 5 min.

Add 3–4 drops of a mounting solution[9] to each slide and incubate for 5 min. Carefully place a coverslip over the tissue on each microscope slide, taking care not to trap any bubbles under the coverslip. Seal the edges of the coverslip with clear nail polish, and store the slides at 4° protected from light.

Image the tissue sections using a suitable fluorescent microscope at 40× magnification with an excitation wavelength of 628 nm and an emission wavelength of 692 nm. Other optical filters can also be used to image tissue autofluorescence relative to that seen with the specific fluorescence of the collagen type IV stain.

Acceptance criteria: Tissue Human Amnion Chorion Membrane Dehydrated must show positive staining for human type IV collagen in the amnion layer and throughout the chorion layer.

• **BALL-BURST TESTING**

Normal saline: 0.9% (w/v) sodium chloride (NaCl)

Sample: Tissue Human Amnion Chorion Membrane Dehydrated with dimensions of NLT 25 mm × 25 mm. [NOTE—At least five *Samples* should be tested for each lot.] Hydrate the *Samples* in *Normal saline* for NLT 30 min. [NOTE—Complete the *Analysis* rapidly so the *Sample* does not dry before testing.]

Analysis: Center the *Sample* between the ball-burst specimen clamp plates. Ensure that the test article is flat over the hole of the ball-burst fixture. Trim the *Sample* if it extends beyond the fixation screws so that the *Sample* rests flat between the screws. Tighten the screws on the ball-burst fixture on either side of the *Sample* to secure the *Sample* in place. Place the ball-burst attachment in the hole of the ball-burst clamp, with the ball of the attachment resting on the *Sample*. The polished steel ball is 6.35 mm in diameter, and the diameter of the test area is 10.7 mm. Place the ball-burst specimen clamp with the *Sample* and the ball-burst attachment approximately centered on the lower compression plate of a suitable materials testing system.[10] Test the *Sample* by lowering the upper compression piston until the *Sample* fails, at a rate of 305 ± 13 mm/min. Record the load, in Newtons (N), at failure.

Acceptance criteria: NLT 0.25 N per *Sample*

• **WATER DETERMINATION** ⟨921⟩

Sample: Weigh a sample of Tissue Human Amnion Chorion Membrane Dehydrated. The test sample should be NLT 40 and NMT 100 mg. [NOTE—Multiple grafts may be used when needed to create a sample size of 40 mg.]

Acceptance criteria: NMT 15%

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Tissue Human Amnion Chorion Membrane Dehydrated is packaged and supplied sterile in a double pouch configuration. Tissue Human Amnion Chorion Membrane Dehydrated can be stored between 0° and 38°.

• **LABELING:** The label shows the tissue identification number, expiration date, and size of Tissue Human Amnion Chorion Membrane Dehydrated contained in the pouch.

Titanium Dioxide

TiO_2 79.87

Titanium oxide (TiO_2) [13463-67-7].

DEFINITION

Titanium Dioxide contains NLT 99.0% and NMT 100.5% of titanium dioxide (TiO_2), calculated on the dried basis. If labeled as attenuation grade, then Titanium Dioxide contains NLT 99.0% and NMT 100.5% of TiO_2, calculated on the ignited basis. Attenuation grade material may contain suitable coatings, stabilizers, and treatments to assist formulation.

[NOTE—If labeled as attenuation grade, then all tests and assays are conducted on uncoated, untreated material. For UV attenuation grade, the test for *Loss on Drying* does not apply. The FDA requires the content of lead to be NMT 10 ppm (µg/g), that of antimony to be NMT 2 ppm (µg/g), and that of mercury to be NMT 1 ppm (µg/g) (21 CFR 73.1575).]

IDENTIFICATION

• **A.**

Sample: 500 mg

Analysis: Add 5 mL of sulfuric acid to the *Sample*, and heat gently. After fumes of sulfur trioxide appear, continue heating for a minimum of 10 s. Cool the suspension, and cautiously dilute with water to 100 mL. Filter, and to 5 mL of the clear filtrate, add a few drops of hydrogen peroxide TS.

Acceptance criteria: A yellow-red to orange-red color develops immediately.

ASSAY

• **PROCEDURE**

Sample solution: Transfer 300 mg of Titanium Dioxide to a 250-mL beaker. Add 20 mL of sulfuric acid and 7–8 g of ammonium sulfate. Mix, heat on a hot plate until fumes of sulfur trioxide appear, and continue heating over a strong flame until the solution is complete, or until it is apparent that the undissolved residue is siliceous matter. Cool, cautiously dilute with 100 mL of water, stir, heat carefully to boiling while stirring, and allow the insoluble matter to settle. Filter, transfer the entire residue to the filter, and wash thoroughly with cold 2 N sulfuric acid. Dilute the filtrate with water to 200 mL, and cautiously add about 10 mL of ammonium hydroxide.

Blank: 200 mL of 2 N sulfuric acid

[9] A suitable reagent can be obtained from Electron Microscopy Sciences/ Diatome, product #17985-10 or a suitable equivalent.

[10] A suitable materials system is the INSTRON Series 5500 Load Frames with a Series 2525 Load Cell or a suitable equivalent.

USP Monographs

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Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N potassium permanganate VS
Endpoint detection: Visual
Analysis: Prepare a zinc amalgam column in a 25-cm Jones reductor tube, placing a pledget of glass wool in the bottom of the tube, and filling the constricted portion of the tube with zinc amalgam prepared as follows. Add 20- to 30-mesh zinc to mercuric–chloride solution (1 in 50), using about 100 mL of the solution for each 100 g of zinc, and after about 10 min, decant the solution from the zinc, then wash the zinc by decantation. Wash the zinc amalgam column with 100-mL portions of 2 N sulfuric acid until 100 mL of the washing does not decolorize 1 drop of 0.1 N potassium permanganate.
Place 50 mL of ferric ammonium sulfate TS in a 1000-mL suction flask, and add 0.1 N potassium permanganate until a faint pink color persists for 5 min. Attach the Jones reductor tube to the neck of the flask, and pass 50 mL of 2 N sulfuric acid through the reductor at a rate of about 30 mL/min. Pass the *Sample solution* through the reductor at the same rate, and follow with 100 mL each of 2 N sulfuric acid and of water. During these operations, keep the reductor filled with a solution or water above the upper level of the amalgam. Taking precautions against the admission of atmospheric oxygen, gradually release the suction, wash down the outlet tube of the reductor and the sides of the receiver, and titrate immediately with *Titrant*. Perform a blank determination, and make any necessary correction.
Calculate the percentage of titanium dioxide (TiO_2) in the sample taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 79.88 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 99.0%–100.5% on the dried basis; if labeled as attenuation grade, 99.0%–100.5% on the ignited basis

IMPURITIES

• **ARSENIC,** *Method I* ⟨211⟩
Test preparation: Add 3.0 g of Titanium Dioxide to a 250-mL conical flask fitted with a thermometer and a vapor outlet. Add 50 mL of water, 500 mg of hydrazine sulfate, 500 mg of potassium bromide, 20 g of sodium chloride, and 25 mL of sulfuric acid. Arrange to collect the evolved vapors in 52 mL of water contained in the arsine generator flask, heat the test specimen to 90°, and maintain the temperature at 90°–100° for 15 min. Add 3 mL of hydrochloric acid to the solution in the generator flask.
Analysis: Follow the *Procedure* in the chapter, omitting the addition of 20 mL of 7 N sulfuric acid.
Acceptance criteria: Any red color produced by the *Test preparation* does not exceed that produced by the *Standard Preparation* (NMT 1 µg/g).

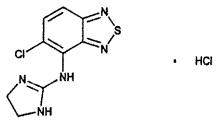
SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h
Acceptance criteria: NMT 0.5%
• **LOSS ON IGNITION** ⟨733⟩
Sample: 2 g (previously dried sample). If labeled as attenuation grade, 4 g
Analysis: Ignite the *Sample* at 800 ± 25° to constant weight.
Acceptance criteria: NMT 0.5%; if labeled as attenuation grade, NMT 13%
• **WATER-SOLUBLE SUBSTANCES**
Sample suspension: Suspend 4.0 g in 50 mL of water, mix, and allow to stand overnight.
Analysis: Transfer the *Sample suspension* to a 200-mL volumetric flask, add 2 mL of ammonium chloride TS, and mix. If the Titanium Dioxide does not settle, add another 2-mL portion of ammonium chloride TS. Allow the suspension to settle, dilute with water to volume, mix, and pass through a double thickness of fine-porosity filter paper, discarding the first 10 mL of the filtrate. Collect 100 mL of the clear filtrate, transfer to a tared platinum dish, evaporate on a hot plate to dryness, and ignite at a dull red heat to constant weight.
Acceptance criteria: The residue weighs NMT 5 mg (NMT 0.25%).
• **ACID-SOLUBLE SUBSTANCES**
Sample suspension: Suspend 5.0 g in 100 mL of 0.5 N hydrochloric acid, and heat on a steam bath for 30 min, with occasional stirring.
Analysis: Pass the *Sample suspension* through an appropriate filter medium until clear. Wash with three 10-mL portions of 0.5 N hydrochloric acid. Evaporate the combined filtrate and washings to dryness, and ignite at a dull red heat to constant weight.
Acceptance criteria: The residue weighs NMT 25 mg (NMT 0.5%).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** If intended for UV-attenuation, the material is labeled as attenuation grade. If intended for UV-attenuation, and any added coatings, stabilizers, or treatments are used, label it to indicate the name and amount of the additives.

Tizanidine Hydrochloride



$C_9H_8ClN_5S \cdot HCl$ 290.17
2,1,3-Benzothiadiazol-4-amine, 5-chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-, monohydrochloride;
5-Chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiadiazole monohydrochloride [64461-82-1].

DEFINITION

Tizanidine Hydrochloride contains NLT 98.0% and NMT 102.0% of tizanidine hydrochloride ($C_9H_8ClN_5S \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

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• **C. Identification Tests—General** ⟨191⟩, *Chloride*
Sample solution: 10 mg/mL
Acceptance criteria: Meets the requirements of test *A*

ASSAY

• **Procedure**
Buffer: 6.8 g/L of monobasic potassium phosphate. Adjust with 5.3 N potassium hydroxide to a pH of 7.5 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (20:80)
System suitability solution: 46 µg/mL of USP Tizanidine Hydrochloride RS and 0.12 µg/mL of USP Tizanidine Related Compound C RS in *Mobile phase*
Standard solution: 0.046 mg/mL of USP Tizanidine Hydrochloride RS in *Mobile phase*
Sample solution: 0.046 mg/mL of Tizanidine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; packing L7
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for tizanidine related compound C and tizanidine are 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6 between tizanidine and tizanidine related compound C, *System suitability solution*
Tailing factor: NMT 2.0 for tizanidine, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tizanidine hydrochloride ($C_9H_8ClN_5S \cdot HCl$) in the portion of Tizanidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of tizanidine from the *Sample solution*
r_S = peak area of tizanidine from the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Organic Impurities**
Solution A: Water and phosphoric acid (88:12)
Buffer: 3.5 g/L of sodium 1-pentanesulfonate. Adjust with *Solution A* or 1 N sodium hydroxide to a pH of 3.0 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (20:80)
System suitability stock solution: 0.1 mg/mL each of USP Tizanidine Related Compound A RS, USP Tizanidine Related Compound B RS, and USP Tizanidine Related Compound C RS in methanol
System suitability solution: 0.23 mg/mL of USP Tizanidine Hydrochloride RS, and 0.01 mg/mL each of USP Tizanidine Related Compound A RS, USP Tizanidine Related Compound B RS, and USP Tizanidine Related Compound C RS from *System suitability stock solution* in *Mobile phase*
Standard solution: 0.046 mg/mL of USP Tizanidine Hydrochloride RS in *Mobile phase*
Sample solution: 1.14 mg/mL of Tizanidine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times are listed in *Table 1.*]
Suitability requirements
Resolution: NLT 4.0 between tizanidine and tizanidine related compound C, *System suitability solution;* NLT 4.0 between tizanidine and tizanidine related compound B, *System suitability solution*
Column efficiency: NLT 5000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tizanidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak area for each impurity in the *Sample solution*
r_S = peak area of tizanidine in the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Tizanidine Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17
F = relative response factor in *Table 1*

Acceptance criteria: See *Table 1.*

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tizanidine related compound C	0.8	1.0	0.1
Tizanidine	1.0	—	—
Tizanidine related compound B	1.4	1.1	0.1
Tizanidine related compound A	10.2	1.1	0.1
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.3

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 4.3–5.3

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• **Loss on Drying** ⟨731⟩
Sample: 0.5 g
Analysis: Dry the *Sample* at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, and store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Tizanidine Hydrochloride RS
USP Tizanidine Related Compound A RS
4-Amino-5-chloro-2,1,3-benzothiadiazole.
$C_6H_4ClN_3S$ 185.63
USP Tizanidine Related Compound B RS
N-Acetyltizanidine.
$C_{11}H_{10}ClN_5OS$ 295.75
USP Tizanidine Related Compound C RS
1-Acetylimidazolidine-2-thione.
$C_5H_8N_2OS$ 144.20

Tizanidine Tablets

DEFINITION
Tizanidine Tablets contain Tizanidine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of tizanidine ($C_9H_8ClN_5S$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Solution A: Water and phosphoric acid (44:6)
Buffer: 3.5 g/L of sodium 1-pentanesulfonate. Adjust with *Solution A* or 1 N sodium hydroxide to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (20:80)
Tizanidine related compound A solution: 0.1 mg/mL of USP Tizanidine Related Compound A RS in methanol
Tizanidine related compound B solution: 0.1 mg/mL of USP Tizanidine Related Compound B RS in methanol
Tizanidine related compound C solution: 0.1 mg/mL of USP Tizanidine Related Compound C RS in methanol
System suitability solution: Transfer 23 mg of USP Tizanidine Hydrochloride RS to a 100-mL volumetric flask, and add 20 mL of *Mobile phase* and 10 mL each of *Tizanidine related compound A solution*, *Tizanidine related compound B solution*, and *Tizanidine related compound C solution*. Sonicate to dissolve the USP Tizanidine Hydrochloride RS, and dilute with *Mobile phase* to volume.
Standard solution: 0.046 mg/mL of USP Tizanidine Hydrochloride RS in *Mobile phase*
Sample solution: Transfer a weighed portion of finely powdered Tablets (NLT 20), equivalent to 20 mg of tizanidine, to a 500-mL volumetric flask. Add 250 mL of *Buffer* solution, sonicate for 15 min with occasional shaking, and shake by mechanical means for 15 min. Add 100 mL of acetonitrile, and mix. Allow to cool, and dilute with *Buffer* solution to volume. Centrifuge a portion of this solution at 2000 rpm or higher for 10 min. Pass a portion of this solution through a filter with a 0.45-µm or finer pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times are listed in *Table 1*.]
Suitability requirements
Resolution: NLT 4.0 between tizanidine and tizanidine related compound C; NLT 4.0 between tizanidine and tizanidine related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tizanidine ($C_9H_8ClN_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tizanidine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 1: 100 rpm
Time: 15 min
Solution A, Buffer, and **Mobile phase:** Prepare as directed in the *Assay*.
Standard solution: (*L*/500) mg/mL of USP Tizanidine Hydrochloride RS in *Medium*, where *L* is the label claim, in mg
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of tizanidine ($C_9H_8ClN_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of tizanidine from the *Sample solution*
r_S = peak response of tizanidine from the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of dissolution *Medium*, 500 mL
L = label claim, in mg
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17

Tolerances: NLT 80% (Q) of the labeled amount of tizanidine ($C_9H_8ClN_5S$) is dissolved.

Test 2

[NOTE—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.]

Medium: 0.1 N hydrochloric acid; 500 mL, deaerated
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: (L/500) mg/mL of USP Tizanidine Hydrochloride RS in *Medium*, where L is the label claim, in mg
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 228 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tizanidine ($C_9H_8ClN_5S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of dissolution *Medium*, 500 mL
L = label claim, in mg
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17

Tolerances: NLT 80% (Q) of the labeled amount of tizanidine ($C_9H_8ClN_5S$) is dissolved.

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Buffer, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: Transfer a weighed portion of finely powdered Tablets (NLT 20), equivalent to 20 mg of tizanidine, to a 100-mL volumetric flask. Add about 50 mL of *Buffer* solution, sonicate for about 15 min with occasional shaking, and shake by mechanical means for 15 min. Add 20 mL of acetonitrile, and mix. Allow to cool, dilute with *Buffer* solution to volume, and mix. Centrifuge a portion of this solution at 2000 rpm or higher for 10 min. Pass a portion of this solution through a filter with a 0.45-µm or finer pore size, and use the filtrate.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of tizanidine from the *Standard solution*
C_S = concentration of USP Tizanidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tizanidine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tizanidine, 253.71
M_{r2} = molecular weight of tizanidine hydrochloride, 290.17
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tizanidine related compound C[a]	0.8	—	—
Tizanidine	1.0	—	—
Tizanidine related compound B[a]	1.4	—	—
Tizanidine related compound A	10.2	1.1	0.2
Any unspecified degradation product	—	1.0	0.2
Total impurities	—	—	0.5

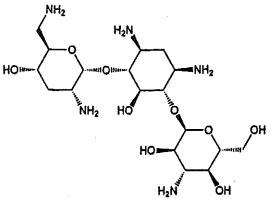
[a] Process impurity included for peak identification purposes only. Controlled in the drug substance.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tizanidine Hydrochloride RS
USP Tizanidine Related Compound A RS
4-Amino-5-chloro-2,1,3-benzothiadiazole.
$C_6H_4ClN_3S$ 185.63
USP Tizanidine Related Compound B RS
N-Acetyltizanidine.
$C_{11}H_{10}ClN_5OS$ 295.75
USP Tizanidine Related Compound C RS
1-Acetylimidazolidine-2-thione.
$C_5H_8N_2OS$ 144.19

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Tobramycin



$C_{18}H_{37}N_5O_9$ 467.51

D-Streptamine, *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→4)]-2-deoxy-;
O-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→6)]-2-deoxy-L-streptamine [32986-56-4].

DEFINITION

Tobramycin has a potency of NLT 900 µg/mg of tobramycin ($C_{18}H_{37}N_5O_9$), calculated on the anhydrous basis.

IDENTIFICATION

• **A.**

Standard solution: 6 mg/mL of USP Tobramycin RS in water

Sample solution: 6 mg/mL of Tobramycin in water

Solution A: *Standard solution* and *Sample solution* (1:1)

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 3 µL

Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)

Diluent: Butyl alcohol and pyridine (100:1)

Spray reagent: 10 mg/mL of ninhydrin in *Diluent*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Solution A*

Apply the *Samples* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying with *Spray reagent*.

Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots from the *Sample solution* and from *Solution A*, respectively, correspond to those obtained from the *Standard solution*.

• **B.** The retention time of the major peak of the *Derivatized sample solution* corresponds to that of the *Derivatized standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.

Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.

Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.

Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide with mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg of USP Tobramycin RS prepared as follows. Transfer 55 mg of USP Tobramycin RS to a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.

Standard solution: 0.22 mg/mL of USP Tobramycin RS from *Standard stock solution* in water

Sample stock solution: 1.1 mg of Tobramycin prepared as follows. Transfer 55 mg of Tobramycin to a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.

Sample solution: 0.22 mg/mL of Tobramycin from *Sample stock solution* in water

Derivatized standard solution, Derivatized sample solution, and Blank solution: Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.

To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 4.0 mL of the *Sample solution*, and 4.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.

System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile

System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Derivatized standard solution* and *System suitability solution*

[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*

Relative standard deviation: NMT 2.0% for tobramycin, *Derivatized standard solution*

Analysis

Samples: *Derivatized standard solution*, *Derivatized sample solution*, and *Blank solution*

Use the *Blank solution* to identify the solvent and reagent peaks.

USP Monographs

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Calculate the quantity, in µg/mg, of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Tobramycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of tobramycin from the *Derivatized sample solution*
r_S = peak area of tobramycin from the *Derivatized standard solution*
C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)
C_U = concentration of Tobramycin in the *Sample solution* (mg/mL)
P = potency of tobramycin in USP Tobramycin RS (µg/mg)

Acceptance criteria: NLT 900 µg/mg of tobramycin ($C_{18}H_{37}N_5O_9$) on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Analysis: Moisten the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.
Acceptance criteria: NMT 1.0%

• **ORGANIC IMPURITIES**
Solution A: Dilute 20 mL of sodium hypochlorite solution with water to 100 mL.
Solution B: Dissolve 1.1 g of potassium iodide in 60 mL of water, boil for 15 min, and slowly add a suspension of 1.5 g of soluble starch in 10 mL of water. Add 25 mL of water, and boil for 10 min. Allow to cool, and dilute with water to 100 mL.
Solution C: 29.2 g of sodium chloride in 100 mL of water
Sample solution: Transfer 50 mg of Tobramycin to a 10-mL volumetric flask. Dissolve in 7 mL of water, and adjust with 1 N sulfuric acid to a pH of 5.5 ± 0.4. Dilute with water to volume.
Standard solution: 0.05 mg/mL of Tobramycin from *Sample solution* in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 1 µL
Developing solvent system: Alcohol, *Solution C*, and water (30:50:20)
Analysis
Samples: *Sample solution* and *Standard solution*
Apply the *Samples* to the plate. Develop the chromatogram in a saturated chromatographic chamber containing the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, evaporate the solvent in a current of hot air, then heat the plate at 110° for 10 min. Lightly spray the hot plate with *Solution A*. Dry the plate in a current of cold air until a sprayed area of the plate below the origin gives at most a faint blue color with a drop of *Solution B*. Then spray the plate with *Solution B*.
Acceptance criteria: Bluish-purple spots are immediately visible. Other than the principal tobramycin spot, no spot observed in the *Sample solution* is more intense than the principal spot from the *Standard solution* (1.0%).

SPECIFIC TESTS

• **PH** ⟨791⟩
Sample solution: 100 mg/mL
Acceptance criteria: 9.0–11.0

• **WATER DETERMINATION** ⟨921⟩, *Method I*
Analysis: Use a mixture of formamide and methanol (1:3) as the solvent.
Acceptance criteria: NMT 8.0%

• **STERILITY TESTS** ⟨71⟩**:** Where the label states that Tobramycin is sterile, it meets the requirements.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Tobramycin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 2.00 USP Endotoxin Units/mg of tobramycin. Where it is intended for use in preparing ophthalmic dosage forms, it is exempt from the requirements for *Bacterial Endotoxins Test.*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where it is intended for use in preparing injectable or ophthalmic dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or ophthalmic dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tobramycin RS

Tobramycin Injection

DEFINITION

Tobramycin Injection is a sterile solution of Tobramycin Sulfate in Water for Injection, or of Tobramycin in Water for Injection prepared with the aid of Sulfuric Acid. It contains NLT 90.0% and NMT 120.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**
Diluent: Butyl alcohol and pyridine (100:1)
Standard solution: 6 mg/mL of USP Tobramycin RS in water
Sample solution: 6 mg/mL in water, from Injection diluted with water
Solution A: *Standard solution* and *Sample solution* (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 3 µL
Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)
Spray reagent: 10 mg/mL of ninhydrin in *Diluent*
Analysis
Samples: *Standard solution, Sample solution,* and *Solution A*
Apply the *Standard solution*, the *Sample solution*, and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent.*
Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots of the *Sample solution* and of *Solution A*, respectively, correspond to those of the *Standard solution.*

• **B.** The retention time of the major peak of the *Derivatized sample solution* corresponds to that of the *Derivatized standard solution*, obtained as directed in the *Assay.*

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ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.

Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.

Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.

Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg/mL of USP Tobramycin RS prepared as follows. Transfer 55 mg of USP Tobramycin RS to a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.

Standard solution: 0.22 mg/mL of USP Tobramycin RS from *Standard stock solution* in water

Sample solution: Nominally 0.2 mg/mL of tobramycin in water, from Injection in water

Derivatized standard solution, Derivatized sample solution, and Blank solution: Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.

To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 4.0 mL of the *Sample solution*, and 4.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.

System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.

System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with the *Derivatized standard solution* to volume, and use promptly.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Derivatized standard solution* and *System suitability solution*

[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis

Samples: *Derivatized standard solution*, *Derivatized sample solution*, and *Blank solution*

Use the *Blank solution* to identify the solvent and reagent peaks.

Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of tobramycin from the *Derivatized sample solution*

r_S = peak area of tobramycin from the *Derivatized standard solution*

C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)

P = potency of tobramycin in USP Tobramycin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 2.00 USP Endotoxin Units/mg of tobramycin.

• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **pH** ⟨791⟩**:** 3.0–6.5

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose glass or plastic containers. Glass containers are preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Tobramycin RS

Tobramycin for Injection

DEFINITION

Tobramycin for Injection contains an amount of Tobramycin Sulfate equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Butyl alcohol and pyridine (100:1)

Standard solution: 6 mg/mL of USP Tobramycin RS

Sample solution: 6 mg/mL in water, from Tobramycin for Injection diluted with water

Solution A: *Standard solution* and *Sample solution* (1:1)

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 3 µL

Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)

Spray reagent: 10 mg/mL of ninhydrin in *Diluent*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Solution A*

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Apply the *Standard solution*, the *Sample solution*, and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent*.

Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots of the *Sample solution* and of *Solution A*, respectively, correspond to those of the *Standard solution*.

- **B.** The retention time of the major peak of *Derivatized sample solution 1* or *Derivatized sample solution 2* corresponds to that of the *Derivatized standard solution*, obtained as directed in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.

Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.

Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.

Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg/mL of USP Tobramycin RS prepared as follows. Transfer 55 mg of USP Tobramycin RS to a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.

Standard solution: 0.22 mg/mL of USP Tobramycin RS from *Standard stock solution* in water

Sample solution 1 (where it is represented as being in a single-dose container)**:** Nominally 0.2 mg/mL of tobramycin prepared as follows. Constitute a container of Tobramycin for Injection in a volume of water corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents using a suitable hypodermic needle and syringe, and dilute with water.

Sample solution 2 (where the label states the quantity of tobramycin in a given volume of constituted solution)**:** Nominally 0.2 mg/mL of tobramycin prepared as follows. Constitute a container of Tobramycin for Injection in a volume of water equivalent to the volume of diluent specified in the labeling. Dilute a suitable aliquot of the constituted solution with water to an appropriate volume.

Derivatized standard solution, Derivatized sample solution 1 or **Derivatized sample solution 2**, and **Blank solution:** Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.

To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 4.0 mL of *Sample solution 1* or *Sample solution 2*, and 4.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, *Derivatized sample solution 1* or *Derivatized sample solution 2*, and the *Blank solution*, respectively.

System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.

System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with the *Derivatized standard solution* to volume, and use promptly.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Derivatized standard solution* and *System suitability solution*

[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis

Samples: *Derivatized standard solution*, *Derivatized sample solution 1* or *Derivatized sample solution 2*, and *Blank solution*

Use the *Blank solution* to identify the solvent and reagent peaks.

Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Tobramycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of tobramycin from *Derivatized sample solution 1* or *Derivatized sample solution 2*

r_S = peak area of tobramycin from the *Derivatized standard solution*

C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tobramycin in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of tobramycin in USP Tobramycin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩

Analysis: Moisten the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

Acceptance criteria: NMT 1.0%

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SPECIFIC TESTS

- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*: At the time of use, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 2.00 USP Endotoxin Units/mg of tobramycin.
- **STERILITY TESTS** ⟨71⟩: It meets the requirements in *Test for Sterility of the Product to Be Examined, Membrane Filtration*, 6 g being used if it is not packaged for dispensing.
- **pH** ⟨791⟩

  Sample solution: 40 mg/mL or where packaged for dispensing, use the solution constituted as directed in the labeling.

  Acceptance criteria: 6.0–8.0
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
- **USP REFERENCE STANDARDS** ⟨11⟩

  USP Tobramycin RS

Tobramycin Ophthalmic Ointment

DEFINITION

Tobramycin Ophthalmic Ointment contains NLT 90.0% and NMT 120.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

  Diluent: Butyl alcohol and pyridine (100:1)

  Standard solution: 6 mg/mL of USP Tobramycin RS in water

  Sample solution: Vigorously shake by mechanical means a quantity of Ophthalmic Ointment, containing nominally 3 mg of tobramycin with 2 mL of chloroform. Add 1 mL of water, shake vigorously by mechanical means for 1 min, and centrifuge for 15 min. Use the clear upper, aqueous layer.

  Solution A: *Standard solution* and *Sample solution* (1:1)

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

  Application volume: 3 µL

  Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)

  Spray reagent: 10 mg/mL of ninhydrin in *Diluent*

  Analysis

  Samples: *Standard solution, Sample solution,* and *Solution A*

  Apply the *Standard solution*, the *Sample solution*, and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent.*

  Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots of the *Sample solution* and of *Solution A*, respectively, correspond to those of the *Standard solution.*

ASSAY

- **PROCEDURE**

  Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.

  Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.

  Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.

  Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

  Standard stock solution: 1.1 mg of USP Tobramycin RS prepared as follows. Transfer 55 mg of USP Tobramycin RS into a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.

  Standard solution: 0.22 mg/mL of USP Tobramycin RS from *Standard stock solution* in water

  Sample solution: Nominally 0.045 mg/mL of tobramycin from Ophthalmic Ointment in water prepared as follows. Transfer a portion of Ophthalmic Ointment, containing nominally 4.5 mg of tobramycin, to a separator. Add 50 mL of ether, and extract with four 20- to 25-mL portions of water. Combine the water extracts in a 100-mL volumetric flask, and dilute with water to volume.

  Derivatized standard solution, Derivatized sample solution, and **Blank solution:** Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.

  To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 15.0 mL of the *Sample solution*, and 4.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.

  System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.

  System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *System Suitability.*)

  Mode: LC

  Detector: UV 365 nm

  Column: 3.9-mm × 30-cm; packing L1

  Flow rate: 1.2 mL/min

  Injection volume: 20 µL

  System suitability

  Samples: *Derivatized standard solution* and *System suitability solution*

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[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis

Samples: *Derivatized standard solution*, *Derivatized sample solution*, and *Blank solution*

Use the *Blank solution* to identify the solvent and reagent peaks.

Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of tobramycin from the *Derivatized sample solution*

r_S = peak area of tobramycin from the *Derivatized standard solution*

C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)

P = potency of tobramycin in USP Tobramycin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tobramycin RS

Tobramycin Inhalation Solution

DEFINITION

Tobramycin Inhalation Solution is a sterile, nonpyrogenic, preservative-free solution of Tobramycin in Water for Injection containing Sodium Chloride. It is prepared with the aid of Sulfuric Acid or Sodium Hydroxide and contains, in each mL, NLT 90.0% and NMT 110.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Derivatized sample solution* corresponds to that of the *Derivatized standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in about 800 mL of water. To this solution add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Allow to cool, and pass through a filter of 0.2-µm or finer pore size.

Blank: Water

2,4-Dinitrofluorobenzene reagent: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.

Tris(hydroxymethyl)aminomethane stock reagent: 15 mg/mL of tris(hydroxymethyl)aminomethane in water. This reagent may be used for 1 month if refrigerated when not in use.

Tris(hydroxymethyl)aminomethane reagent: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Tris(hydroxymethyl)aminomethane stock reagent* to a 200-mL volumetric flask, add dimethyl sulfoxide with mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg/mL of USP Tobramycin RS prepared as follows. Weigh a suitable amount of USP Tobramycin RS into a suitable volumetric flask. Add 1 N sulfuric acid, using 2% of the final volume, and enough water to dissolve the tobramycin, and dilute with water to volume.

Standard solution: 0.22 mg/mL of USP Tobramycin RS from the *Standard stock solution* in water

Sample solution: Nominally 0.192 mg/mL of tobramycin from the Inhalation Solution in water

Derivatization procedure: Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.

To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 4.0 mL of the *Sample solution*, and 4.0 mL of the *Blank*. To each flask add 10 mL of *2,4-Dinitrofluorobenzene reagent* and 10 mL of *Tris(hydroxymethyl)aminomethane reagent*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, *Derivatized sample solution*, and *Derivatized blank solution*, respectively.

System suitability solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Transfer 2 mL of this solution to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Derivatized standard solution*, *Derivatized blank solution*, and *System suitability solution*

[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]

Identify the solvent and reagent peaks using the *Derivatized blank solution*.

Suitability requirements

Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

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Analysis
Samples: *Derivatized standard solution* and *Derivatized sample solution*
Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Derivatized sample solution*
r_S = peak response from the *Derivatized standard solution*
C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)
P = potency of tobramycin in USP Tobramycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF SODIUM CHLORIDE**
Solution A: 2 g of gelatin and 50 mL of nitric acid in 1000 mL of water
Sample solution: Pipet 25 mL of Inhalation Solution into a suitable container. Add 70–100 mL of water and 10 mL of *Solution A*.
Analysis: Titrate potentiometrically with 0.1 N silver nitrate VS using a suitable silver electrode.
Acceptance criteria: 90.0%–110.0% of the labeled amount of sodium chloride

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: Acetonitrile, water, and phosphoric acid (5: 95: 0.08)
Solution B: Acetonitrile, water, and phosphoric acid (75: 25: 0.08)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	79	21
14	66	34
25	30	70
35	30	70
40	20	80
50	5	95

Blank: Water
2,4-Dinitrofluorobenzene reagent and **Tris(hydroxymethyl)aminomethane reagent:** Prepare as directed in the *Assay*.
System suitability stock solution: Dissolve USP Tobramycin RS in water, and adjust with 1 N sulfuric acid to a pH of 6.0. Dilute with water to obtain a solution having a known concentration of 1.1 mg/mL.
System suitability solution 1: 0.22 mg/mL of tobramycin from the *System suitability stock solution* in water
System suitability solution 2: Heat a portion of the *System suitability stock solution* in a suitable sealed glass container at 100° for 8–9 h. Cool to room temperature, and dilute with water to obtain a solution containing nominally 0.22 mg/mL of tobramycin.
Standard stock solution: Prepare as directed in the *Assay*.
Standard solution: 1.10 µg/mL of tobramycin from the *Standard stock solution* in water
Sample solution: Prepare as directed in the *Assay*.
Derivatization procedure: Heat all solutions at the same temperature and for the same duration as indicated. Move all flasks to and from the 60° constant-temperature bath at the same time.
To separate 50-mL flasks transfer 15.0 mL of *System suitability solution 1*, 15.0 mL of *System suitability solution 2*, 15.0 mL of *Standard solution*, 15.0 mL of *Sample solution*, and 15.0 mL of *Blank*. To each flask add 10 mL of *2,4-Dinitrofluorobenzene reagent* and 10 mL of *Tris(hydroxymethyl)aminomethane reagent*, shake, and insert the stopper. Place the flasks in a constant-temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, and dilute with acetonitrile to volume. Allow the solutions to stand for 16 h. The solutions thus obtained are *Derivatized system suitability solution 1*, *Derivatized system suitability solution 2*, *Derivatized standard solution*, *Derivatized sample solution*, and *Derivatized blank solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 4.6-mm × 25-cm; packing L11
Flow rate: 1.2 mL/min
Injection volume: 45 µL
System suitability
Samples: *Derivatized system suitability solution 1*, *Derivatized system suitability solution 2*, and *Derivatized standard solution*
[NOTE—See *Table 2* for relative retention times.]
Compare *Derivatized system suitability solutions 1* and *2* chromatograms to identify degradation peaks. Deoxystreptamine kanosaminide and nebramine will increase in response in *Derivatized system suitability solution 2*.
Suitability requirements
Capacity factor (*k'*): NLT 15.5 for tobramycin, *Derivatized system suitability solution 2*
Resolution: NLT 1.0 between the nebramine and kanamycin peaks, *Derivatized system suitability solution 2*
Relative standard deviation: NMT 2.0%, *Derivatized standard solution*
Analysis
Samples: *Derivatized system suitability solution 1*, *Derivatized standard solution*, *Derivatized sample solution*, and *Derivatized blank solution*
Disregard any peak corresponding to those from the *Derivatized blank solution*, and subtract the quantities of any such peaks found at the relative retention times of 0.36, 0.66, and 0.94 from those found in the *Derivatized sample solution*. For unknown peak determinations, disregard any peaks found in the chromatogram of the *Derivatized sample solution* that correspond to those in the chromatogram of *Derivatized system suitability solution 1*.
Calculate the percentage of each impurity in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak area of each impurity from the *Derivatized sample solution*
r_S = peak area of tobramycin from the *Derivatized standard solution*
C_S = concentration of USP Tobramycin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tobramycin in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Specified unidentified impurity	0.36	0.25
Deoxystreptamine kanosaminide	0.66	0.3
Nebramine	0.94	0.4
Kanamycin B	0.96	—
Tobramycin	1.0	—
Any individual unspecified impurity	—	0.1
Total unspecified impurities	—	0.2
Total impurities	—	1.0

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 60 USP Endotoxin Units/mL.
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **ABSORBANCE**
  Sample: Inhalation Solution
  Analysis: Determine the absorbance of the *Sample* at 410 nm in a 1-cm cell.
  Acceptance criteria: NMT 0.30
- **pH** ⟨791⟩: 4.5–6.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OSMOLALITY AND OSMOLARITY** ⟨785⟩: The osmolality is 135–285 mOsmol/kg.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in low-density, polyethylene, single-use ampules stored in light-resistant foil over-wrapped packaging, in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tobramycin RS

Tobramycin Ophthalmic Solution

DEFINITION

Tobramycin Ophthalmic Solution contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$). It may contain one or more suitable buffers, dispersants, preservatives, and tonicity agents.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**
  Diluent: Butyl alcohol and pyridine (100:1)
  Standard solution: 3 mg/mL of USP Tobramycin RS in water
  Sample solution: Ophthalmic Solution
  Solution A: *Standard solution* and *Sample solution* (1:1)
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 6 µL
  Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)
  Spray reagent: 10 mg/mL of ninhydrin in *Diluent*
  Analysis
  Samples: *Standard solution, Sample solution,* and *Solution A*
  Apply the *Standard solution,* the *Sample solution,* and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent.*
  Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots of the *Sample solution* and of *Solution A,* respectively, correspond to those of the *Standard solution.*
- **B.** The retention time of the major peak of the *Derivatized sample solution* corresponds to that of the *Derivatized standard solution* obtained as directed in the *Assay.*

ASSAY

- **PROCEDURE**
  Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.
  Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.
  Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.
  Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.
  Standard stock solution: 0.66 mg of USP Tobramycin RS prepared as follows. Transfer 33 mg of USP Tobramycin RS into a 50-mL volumetric flask. Add 20 mL of water, 1 mL of 1 N sulfuric acid, and mix. Dilute with water to volume.
  Standard solution: 0.132 mg/mL of USP Tobramycin RS from *Standard stock solution* in water
  Sample solution: Nominally 0.12 mg/mL of tobramycin from Ophthalmic Solution in water
  Derivatized standard solution, Derivatized sample solution, and **Blank solution:** Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.
  To separate 50-mL volumetric flasks transfer 5.0 mL of the *Standard solution,* 5.0 mL of the *Sample solution,* and 5.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C,* shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The

USP Monographs

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solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.

System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.

System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 4-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 20 µL

System suitability
Samples: *Derivatized standard solution* and *System suitability solution*
[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis
Samples: *Derivatized standard solution*, *Derivatized sample solution*, and *Blank solution*
Use the *Blank solution* to identify the solvent and reagent peaks.
Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of tobramycin from the *Derivatized sample solution*
r_S = peak area of tobramycin from the *Derivatized standard solution*
C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)
P = potency of tobramycin in USP Tobramycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PH** ⟨791⟩**:** 7.0–8.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Tobramycin RS

Tobramycin and Dexamethasone Ophthalmic Ointment

DEFINITION

Tobramycin and Dexamethasone Ophthalmic Ointment contains NLT 90.0% and NMT 120.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$), and NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Solution A: 100 mg/mL of sodium sulfate in water
Diluent: Butyl alcohol and pyridine (100:1)
Standard solution: 6 mg/mL of USP Tobramycin RS in water
Sample solution: To 1 g of Ophthalmic Ointment in a test tube add 2 mL of chloroform, and shake to dissolve. Add 0.5 mL of *Solution A*, shake vigorously, and centrifuge. Use the clear supernatant aqueous liquid. If, after centrifuging, an oily film remains on top of the supernatant aqueous liquid, transfer the supernatant aqueous liquid to a second test tube, and wash it with 2 mL of chloroform.
Solution B: *Standard solution* and *Sample solution* (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 3 µL
Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)
Spray reagent: 10 mg/mL of ninhydrin in *Diluent*

Analysis
Samples: *Standard solution*, *Sample solution*, and *Solution B*
Apply the *Standard solution*, the *Sample solution*, and *Solution B* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent*.
Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots of the *Sample solution* and of *Solution B*, respectively, correspond to those of the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Dexamethasone*.

ASSAY

- **TOBRAMYCIN**

Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.
Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.
Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.
Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while mixing, and dilute with dimethyl sulfoxide to volume. Use

USP Monographs

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this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg of USP Tobramycin RS prepared as follows. Transfer 55 mg of USP Tobramycin RS into a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.

Standard solution: 0.22 mg/mL of USP Tobramycin RS from *Standard stock solution* in water

Sample solution: Nominally 0.045 mg/mL of tobramycin from Ophthalmic Ointment in water prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 4.5 mg of tobramycin to a separator. Add 50 mL of ether, and extract with four 20- to 25-mL portions of water. Combine the water extracts in a 100-mL volumetric flask, and dilute with water to volume.

Derivatized standard solution, Derivatized sample solution, and **Blank solution:** Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time. To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 15.0 mL of the *Sample solution*, and 4.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.

System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.

System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL

System suitability
Samples: *Derivatized standard solution* and *System suitability solution*
[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis
Samples: *Derivatized standard solution, Derivatized sample solution,* and *Blank solution*
Use the *Blank solution* to identify the solvent and reagent peaks.
Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of tobramycin from the *Derivatized sample solution*
r_S = peak area of tobramycin from the *Derivatized standard solution*
C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)
P = potency of tobramycin in USP Tobramycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

• **DEXAMETHASONE**
Mobile phase: Methanol and water (55:45)
Diluent: Methanol and water (75:25)
System suitability stock solution: 1 mg/mL of anhydrous chlorobutanol and 0.2 mg/mL of USP Dexamethasone RS in *Diluent*
System suitability solution: 0.3 mg/mL of anhydrous chlorobutanol and 0.06 mg/mL of USP Dexamethasone RS in *Diluent* prepared as follows. Transfer 15.0 mL of the *System suitability stock solution* to a separator containing about 50 mL of *n*-hexane, and shake. Allow the layers to separate, and drain the lower phase into a 50-mL volumetric flask. Repeat the extraction with two 15-mL portions of *Diluent*, combining the lower phase from each extraction in the same 50-mL volumetric flask. Dilute with *Diluent* to volume.
Standard stock solution: 0.2 mg/mL of USP Dexamethasone RS in *Diluent*
Standard solution: 0.06 mg/mL of USP Dexamethasone RS in *Diluent* prepared as follows. Transfer 15.0 mL of the *Standard stock solution* to a separator containing about 50 mL of *n*-hexane, and shake. Allow the layers to separate, and drain the lower phase into a 50-mL volumetric flask. Repeat the extraction with two 15-mL portions of *Diluent*, combining the lower phase from each extraction in the same 50-mL volumetric flask. Dilute with *Diluent* to volume.
Sample solution: Transfer a portion of Ophthalmic Ointment containing nominally 3 mg of dexamethasone to a separator containing 50 mL of *n*-hexane, and shake. Add 15 mL of *Diluent*, and shake. Allow the layers to separate, and drain the lower phase into a 50-mL volumetric flask. Repeat the extraction with two 15-mL portions of *Diluent*, combining the lower phase from each extraction in the same 50-mL volumetric flask. Dilute with *Diluent* to volume, mix, and centrifuge. Use the clear solution.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 206 nm
Column: 8.0-mm × 10-cm; packing L1
Flow rate: 3 mL/min
Injection volume: 100 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for chlorobutanol and dexamethasone are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.8 between chlorobutanol and dexamethasone, *System suitability solution*
Tailing factor: NMT 2, *Standard solution*
Column efficiency: NLT 350 theoretical plates, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dexamethasone in the *Sample solution* (mg/mL)
P = potency of dexamethasone in USP Dexamethasone RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dexamethasone RS
  USP Tobramycin RS

Tobramycin and Dexamethasone Ophthalmic Suspension

DEFINITION
Tobramycin and Dexamethasone Ophthalmic Suspension is a sterile aqueous suspension containing Tobramycin and Dexamethasone. It contains NLT 90.0% and NMT 120.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$), and NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHY**
  Diluent: Butyl alcohol and pyridine (100:1)
  Standard solution: 6 mg/mL of USP Tobramycin RS in water
  Sample solution: To 1 mL of Ophthalmic Suspension in a test tube add 100 mg of sodium sulfate, disperse by shaking, and centrifuge. Use the clear supernatant.
  Solution A: *Standard solution* and *Sample solution* (1:1)
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 3 µL
  Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)
  Spray reagent: 10 mg/mL of ninhydrin in *Diluent*
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Solution A*
  Apply the *Standard solution*, the *Sample solution*, and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent*.
  Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots of the *Sample solution* and of *Solution A*, respectively, correspond to those of the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Dexamethasone*.

ASSAY
- **TOBRAMYCIN**
  Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.
  Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.
  Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.
  Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.
  Standard stock solution: 1.1 mg of USP Tobramycin RS prepared as follows. Transfer 55 mg of USP Tobramycin RS into a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.
  Standard solution: 0.22 mg/mL of USP Tobramycin RS from *Standard stock solution* in water
  Sample solution: Nominally 0.09 mg/mL of tobramycin from Ophthalmic Suspension in water
  Derivatized standard solution, Derivatized sample solution, and **Blank solution:** Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.
  To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 10.0 mL of the *Sample solution*, and 4.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.
  System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.
  System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 365 nm
  Column: 3.9-mm × 30-cm; packing L1

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Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *Derivatized standard solution* and *System suitability solution*
[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Derivatized standard solution*
Analysis
Samples: *Derivatized standard solution*, *Derivatized sample solution*, and *Blank solution*
Use the *Blank solution* to identify the solvent and reagent peaks.
Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of tobramycin from the *Derivatized sample solution*
r_S = peak area of tobramycin from the *Derivatized standard solution*
C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)
P = potency of tobramycin in USP Tobramycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

• **DEXAMETHASONE**
Mobile phase: Acetonitrile and water (45:55)
Standard solution: 0.04 mg/mL of USP Dexamethasone RS in methanol
Sample solution: Nominally 0.04 mg/mL of dexamethasone from Ophthalmic Suspension, freshly mixed and free of air bubbles, in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1400 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dexamethasone in the *Sample solution* (mg/mL)
P = potency of dexamethasone in USP Dexamethasone RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩: It meets the requirements in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
• **pH** ⟨791⟩: 5.0–6.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dexamethasone RS
USP Tobramycin RS

Tobramycin and Fluorometholone Acetate Ophthalmic Suspension

DEFINITION
Tobramycin and Fluorometholone Acetate Ophthalmic Suspension is a sterile aqueous suspension of Tobramycin and Fluorometholone Acetate. It contains NLT 90.0% and NMT 120.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) and NLT 90.0% and NMT 115.0% of the labeled amount of fluorometholone acetate ($C_{24}H_{31}FO_5$). It may contain suitable buffers, dispersants, tonicity-adjusting agents, and preservatives.

IDENTIFICATION
• **A.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Fluorometholone Acetate*.
• **B. THIN-LAYER CHROMATOGRAPHY**
Diluent: Butyl alcohol and pyridine (100:1)
Standard solution: 6 mg/mL of USP Tobramycin RS in water
Sample solution: Allow the Ophthalmic Suspension to settle, and decant 1 mL of the supernatant into a test tube. Add 0.1 g of sodium sulfate and centrifuge. Use the clear supernatant.
Solution A: *Standard solution* and *Sample solution* (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 3 µL
Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)
Spray reagent: 10 mg/mL of ninhydrin in *Diluent*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Solution A*
Apply the *Standard solution*, the *Sample solution*, and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent*.
Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots of the *Sample solution* and of

USP Monographs

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Solution A, respectively, correspond to those of the *Standard solution*.

ASSAY

• **TOBRAMYCIN**

Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.

Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.

Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.

Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg of USP Tobramycin RS prepared as follows. Transfer 55 mg of USP Tobramycin RS into a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.

Standard solution: 0.22 mg/mL of USP Tobramycin RS from *Standard stock solution* in water

Sample solution: Nominally 0.09 mg/mL of tobramycin from Ophthalmic Suspension in water

Derivatized standard solution, Derivatized sample solution, and **Blank solution:** Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.

To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 10.0 mL of the *Sample solution*, and 4.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.

System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.

System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Derivatized standard solution* and *System suitability solution*

[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis

Samples: *Derivatized standard solution, Derivatized sample solution,* and *Blank solution*

Use the *Blank solution* to identify the solvent and reagent peaks.

Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of tobramycin from the *Derivatized sample solution*

r_S = peak area of tobramycin from the *Derivatized standard solution*

C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)

P = potency of tobramycin in USP Tobramycin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

• **FLUOROMETHOLONE ACETATE**

Mobile phase: Acetonitrile and water (50:50)

System suitability solution: 0.04 mg/mL each of USP Fluorometholone RS and USP Fluorometholone Acetate RS in acetonitrile

Standard solution: 0.04 mg/mL of USP Fluorometholone Acetate RS in acetonitrile

Sample stock solution: Nominally 0.1 mg/mL of fluorometholone acetate in acetonitrile, prepared as follows. Transfer Ophthalmic Suspension, freshly mixed and free from air bubbles and containing nominally 2.5 mg of fluorometholone acetate, to a 25-mL volumetric flask. Dilute with acetonitrile to volume.

Sample solution: Nominally 0.04 mg/mL of fluorometholone acetate in acetonitrile, prepared as follows. Transfer 4.0 mL of *Sample stock solution* to a 10-mL volumetric flask, and dilute with acetonitrile to volume. Transfer a portion of this solution to a test tube, and centrifuge for 15 min. Use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for fluorometholone and fluorometholone acetate are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between fluorometholone and fluorometholone acetate, *System suitability solution*

Capacity factor, k′: 1.0–5.0, fluorometholone acetate peak, *Standard solution*

Column efficiency: NLT 1000 theoretical plates, *Standard solution*

Tailing factor: NMT 1.35, *Standard solution*

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of fluorometholone acetate ($C_{24}H_{31}FO_5$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak area of fluorometholone acetate from the *Sample solution*
r_S = peak area of fluorometholone acetate from the *Standard solution*
C_S = concentration of USP Fluorometholone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluorometholone in the *Sample solution* (mg/mL)
P = potency of fluorometholone acetate in USP Fluorometholone Acetate RS (mg/mg)

Acceptance criteria: 90.0%–115.0%

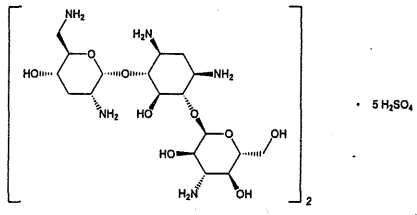
SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩: It meets the requirements in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩: 6.0–7.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluorometholone RS
  USP Fluorometholone Acetate RS
  USP Tobramycin RS

Tobramycin Sulfate



$(C_{18}H_{37}N_5O_9)_2 \cdot 5H_2SO_4$ 1425.42

D-Streptamine, *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[2,6-diamino-2,3,6-tridoexy-α-D-*ribo*-hexopyranosyl-(1→4)]-2-deoxy-, sulfate (2:5) (salt);
O-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→6)]-2-deoxy-L-streptamine, sulfate (2:5) (salt) [79645-27-5].

DEFINITION

Tobramycin Sulfate has a potency of NLT 634 µg/mg and NMT 739 µg/mg of tobramycin ($C_{18}H_{37}N_5O_9$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Butyl alcohol and pyridine (100:1)
Standard solution: 6 mg/mL of USP Tobramycin RS in water
Sample solution: 6 mg/mL of Tobramycin in water
Solution A: *Standard solution* and *Sample solution* (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 3 µL
Developing solvent system: Methanol, chloroform, and ammonium hydroxide (60:25:30)
Spray reagent: 10 mg/mL of ninhydrin in *Diluent*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Solution A*
Apply the *Standard solution*, the *Sample solution*, and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent.*
Acceptance criteria: Tobramycin appears as a pink spot, and the R_F values of the spots of the *Sample solution* and of *Solution A*, respectively, correspond to those of the *Standard solution.*

- **B.** The retention time of the major peak of the *Derivatized sample solution* corresponds to that of the *Derivatized standard solution* obtained as directed in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.
Solution A: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.
Solution B: 15 mg/mL of tris(hydroxymethyl) aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.
Solution C: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.
Standard stock solution: 1.1 mg of USP Tobramycin RS prepared as follows. Transfer 55 mg of USP Tobramycin RS into a 50-mL volumetric flask. Add 1 mL of 1 N sulfuric acid and enough water to dissolve it, and dilute with water to volume.
Standard solution: 0.22 mg/mL of USP Tobramycin RS from *Standard stock solution* in water
Sample solution: Nominally 0.2 mg/mL of tobramycin from Tobramycin Sulfate in water
Derivatized standard solution, Derivatized sample solution, and **Blank solution:** Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.
To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 4.0 mL of the *Sample solution*, and 4.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.
System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.

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System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL

System suitability
Samples: *Derivatized standard solution* and *System suitability solution*
[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis
Samples: *Derivatized standard solution*, *Derivatized sample solution*, and *Blank solution*
Use the *Blank solution* to identify the solvent and reagent peaks.
Calculate the quantity, in µg/mg, of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Tobramycin Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of tobramycin from the *Derivatized sample solution*
r_S = peak area of tobramycin from the *Derivatized standard solution*
C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)
C_U = concentration of Tobramycin Sulfate in the *Sample solution* (mg/mL)
P = potency of tobramycin in USP Tobramycin RS (µg/mg)

Acceptance criteria: 634–739 µg/mg

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Analysis: Moisten the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.
Acceptance criteria: NMT 1.0%

• **ORGANIC IMPURITIES**
Solution A: Dilute 20 mL of sodium hypochlorite solution with water to 100 mL.
Solution B: Dissolve 1.1 g of potassium iodide in 60 mL of water, boil for 15 min, and slowly add a suspension of 1.5 g of soluble starch in 10 mL of water. Add 25 mL of water, and boil for 10 min. Allow to cool, and dilute with water to 100 mL.
Solution C: 29.2 g of sodium chloride in 100 mL of water
Sample solution: Transfer 50 mg of Tobramycin Sulfate to a 10-mL volumetric flask, add 7 mL of water to dissolve it, and adjust with 1 N sulfuric acid to a pH of 5.5 ± 0.4. Dilute with water to volume.
Standard solution: 0.05 mg/mL of tobramycin from *Sample solution* in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 1 µL
Developing solvent system: Alcohol, *Solution C*, and water (30:50:20)
Analysis
Samples: *Sample solution* and *Standard solution*
Apply the *Sample solution* and the *Standard solution* to the plate. Develop the chromatogram in a saturated chromatographic chamber containing the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, evaporate the solvent in a current of hot air, then heat the plate at 110° for 10 min. Lightly spray the hot plate with *Solution A*. Dry the plate in a current of cold air until a sprayed area of the plate below the origin gives at most a faint blue color with a drop of *Solution B*. Then spray the plate with *Solution B*.
Acceptance criteria: Bluish-purple spots are immediately visible. Other than the principal tobramycin spot, no spot observed in the *Sample solution* is more intense than the principal spot from the *Standard solution* (1.0%).

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 40 mg/mL
Acceptance criteria: 6.0–8.0

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%

• **STERILITY TESTS** ⟨71⟩**:** Where the label states that Tobramycin Sulfate is sterile, it meets the requirements in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Tobramycin Sulfate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 2.00 USP Endotoxin Units/mg of tobramycin.

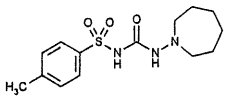
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where Tobramycin Sulfate is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tobramycin RS

Tolazamide



$C_{14}H_{21}N_3O_3S$ 311.40
Benzenesulfonamide, *N*-[[(hexahydro-1*H*-azepin-1-yl)amino] carbonyl]-4-methyl-.
1-(Hexahydro-1*H*-azepin-1-yl)-3-(*p*-tolylsulfonyl)urea [1156-19-0].

» Tolazamide contains not less than 97.5 percent and not more than 102.5 percent of $C_{14}H_{21}N_3O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

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USP Reference standards ⟨11⟩—
USP Tolazamide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The relative retention time of the major peak for tolazamide in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Loss on drying ⟨731⟩—Dry it at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Selenium ⟨291⟩: 0.003%, a 200-mg specimen being used.

Limit of N-aminohexamethyleneimine—

Trisodium pentacyanoaminoferroate solution—Mix 1.0 g of sodium nitroferricyanide and 3.2 mL of ammonium hydroxide in a glass-stoppered flask, insert the stopper in the flask, and refrigerate the mixture overnight. Pour the solution into 10 mL of dehydrated alcohol, and collect the yellow precipitate that is formed on coarse filter paper in a Buchner-type funnel by filtration under reduced pressure. Wash the residue on the filter with anhydrous ether, and store the dry solid in a desiccator. Dissolve a portion of the dry solid in water to obtain a solution containing 1.0 mg per mL, store in a refrigerator, and use within 7 days.

Buffer solution—Dissolve 0.96 g of anhydrous citric acid and 2.92 g of dibasic sodium phosphate in 200 mL of water. Adjust by adding phosphoric acid or 1 N sodium hydroxide, if necessary, to a pH of 5.4 ± 0.1.

Standard solution—Transfer, with the aid of a syringe, 100 mg of N-aminohexamethyleneimine to a 200-mL volumetric flask, dilute with acetone to volume, and mix. Dilute the resulting solution quantitatively with acetone to obtain a solution containing 12.5 µg per mL. Pipet 2 mL of this solution into a 25-mL glass-stoppered flask, add 8.0 mL of *Buffer solution*, shake the mixture, allow to stand for 15 minutes, and filter. Collect the filtrate in a suitable glass-stoppered tube, and use the filtrate as the *Standard solution*.

Test solution—Transfer 0.50 g of Tolazamide to a glass-stoppered, 25-mL flask, add 2.0 mL of acetone, insert the stopper in the flask, and shake the mixture vigorously for 15 minutes. Add 8.0 mL of *Buffer solution*, shake the mixture, allow to stand for 15 minutes, and filter. Collect the filtrate in a suitable glass-stoppered tube, and use the filtrate as the *Test solution*.

Procedure—Add 1.0 mL of *Trisodium pentacyanoaminoferroate solution* to the *Standard solution* and to the *Test solution*, and mix both solutions: the intensity of any pink color that may develop in the *Test solution* within 30 minutes does not exceed that produced in the *Standard solution* within 30 minutes (0.005%).

Chromatographic purity—

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (100:100:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve an accurately weighed quantity of USP Tolazamide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.014 mg per mL.

Test solution— [NOTE—Make solution fresh before each injection.] Transfer about 140 mg of Tolazamide, accurately weighed, to a 100-mL volumetric flask, dissolve in *Mobile phase*, sonicating if necessary, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 4000 theoretical plates; the tailing factor is not more than 3.0; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Inject a volume (about 50 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Tolazamide taken by the formula:

$$100(1/F)(r_i/r_s)$$

in which F is the relative response factor, which is equal to 0.52 for the *p*-toluenesulfonic acid peak eluting at a relative retention time of 0.23 and equal to 1.0 for all other peaks; r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 0.5% of any individual impurity is found; and not more than 1.5% of total impurities is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of hexane, water-saturated hexane, tetrahydrofuran, alcohol, and glacial acetic acid (475:475:20:15:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a suitable quantity of Tolbutamide in alcohol-free chloroform to obtain a solution having a known concentration of about 1.5 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Tolazamide RS in *Internal standard solution* to obtain a solution having a known concentration of about 3 mg per mL.

Assay preparation—Transfer about 30 mg of Tolazamide, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains 10-µm packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation for four replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for the internal standard and 1.0 for tolazamide. Calculate the quantity, in mg, of $C_{14}H_{21}N_3O_3S$ in the portion of Tolazamide taken by the formula:

$$10C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Tolazamide RS in the *Standard preparation*; and R_U and R_S are the ratios of the analyte peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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Tolazamide Tablets

» Tolazamide Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{14}H_{21}N_3O_3S$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Tolazamide RS
Identification—Triturate a quantity of Tablets, equivalent to about 250 mg of tolazamide, with 50 mL of chloroform, and filter. Evaporate the filtrate to dryness, and dry in vacuum at 60° for 3 hours: the residue so obtained responds to *Identification* test *A* under *Tolazamide*.

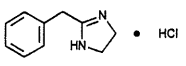
Dissolution ⟨711⟩—
Medium: 0.05 M Tris(hydroxymethyl)aminomethane, pH 7.6, adjusted, if necessary, with hydrochloric acid to a pH of 7.6; 900 mL.
Apparatus 2: 75 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{14}H_{21}N_3O_3S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 224 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Tolazamide RS in the same medium. [NOTE—Sonicate the Standard solution until the Reference Standard is dissolved.]
Tolerances—Not less than 70% (*Q*) of the labeled amount of $C_{14}H_{21}N_3O_3S$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Internal standard preparation , *Mobile phase*, and *Standard preparation*—Prepare as directed in the *Assay* under *Tolazamide*.
Assay preparation—Weigh and finely powder not less than 10 Tablets. Weigh accurately a portion of the powder, equivalent to about 300 mg of tolazamide, and transfer to a suitable container. Add 100.0 mL of *Internal standard solution* and about 20 glass beads. Securely close the container, and shake vigorously for approximately 30 minutes. Centrifuge, and use the clear liquid as the *Assay preparation*.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Tolazamide*. Calculate the quantity, in mg, of $C_{14}H_{21}N_3O_3S$ in the portion of Tablets taken by the formula:

$$100C(R_U/R_S)$$

in which the terms are as defined therein.

Tolazoline Hydrochloride



$C_{10}H_{12}N_2 \cdot HCl$ 196.68
1*H*-Imidazole, 4,5-dihydro-2-(phenylmethyl)-, monohydrochloride.
2-Benzyl-2-imidazoline monohydrochloride [59-97-2].

» Tolazoline Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of $C_{10}H_{12}N_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Tolazoline Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).
B: The R_F value of the principal spot in the chromatogram of the *Identification* corresponds to that of *Standard solution A*, as obtained in the test for *Chromatographic purity*.
Melting range ⟨741⟩: between 172.0° and 176.0°.
Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.2% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Standard solutions—Dissolve USP Tolazoline Hydrochloride RS in methanol, and mix to obtain *Standard solution A* having a known concentration of 100 µg per mL. Quantitatively dilute with methanol to obtain *Standard solutions*, designated below by letter, having the following compositions:

Standard solution	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimen)
A	undiluted	100	0.5
B	4 in 5	80	0.4
C	3 in 5	60	0.3
D	2 in 5	40	0.2
E	1 in 5	20	0.1

Test solution—Dissolve an accurately weighed quantity of Tolazoline Hydrochloride in methanol to obtain a solution containing 20 mg per mL.
Identification solution—Quantitatively dilute a portion of the *Test solution* with methanol to obtain a solution containing 100 µg per mL.
Detection reagent—Prepare (1) a solution of 0.5 g of potassium iodide in 50 mL of water, and (2) a solution of 1.5 g of soluble starch in 50 mL of boiling water. Just prior to use, mix 10 mL of each solution with 3 mL of alcohol.
Procedure—Apply separately 5 µL of the *Test solution*, 5 µL of the *Identification solution*, and 5 µL of each *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol and ammonium hydroxide (95:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to dry under a current of warm air for at least 30 minutes. Expose the plate to chlorine gas for not more than 5 minutes, and air-dry until the chlorine has dissipated (about 15 minutes). Spray the plate with *Detection reagent*,

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and immediately compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions:* the sum of the intensities of all secondary spots obtained from the *Test solution* corresponds to not more than 1.0%.

Assay—Dissolve about 300 mg of Tolazoline Hydrochloride, accurately weighed, in 100 mL of glacial acetic acid; add 25 mL of mercuric acetate TS; and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩), using a calomel–glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 19.67 mg of $C_{10}H_{12}N_2 \cdot HCl$.

Tolazoline Hydrochloride Injection

» Tolazoline Hydrochloride Injection is a sterile solution of Tolazoline Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{10}H_{12}N_2 \cdot HCl$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩**—**
USP Tolazoline Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—Obtain the test specimen as follows. Steam-distill a volume of Injection, equivalent to about 250 mg of tolazoline hydrochloride, for 5 to 10 minutes, and discard the distillate. Transfer the remaining solution to a separator, add about 2 mL of 1 N sodium hydroxide, and extract with 20 mL of ether. Filter the ether extract through cotton into a beaker, evaporate to dryness, and dry the residue in vacuum over silica gel for 4 hours.

B: It responds to *Identification* test *B* under *Tolazoline Hydrochloride.*

C: To 1 mL of Injection add 1 mL of ammonium reineckate TS: a pink precipitate is formed.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.8 USP Endotoxin Unit per mg of tolazoline hydrochloride.
pH ⟨791⟩: between 3.0 and 4.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Standard preparation—Transfer about 30 mg of USP Tolazoline Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

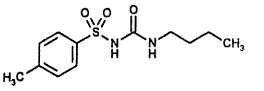
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 150 mg of tolazoline hydrochloride, to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 20.0 mL of this solution to a second 100-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Transfer 3.0 mL each of the *Standard preparation*, the *Assay preparation*, and methanol to provide the blank, to separate 25-mL volumetric flasks. To each flask add 1 mL of 0.5 N sodium hydroxide and 1 mL of dilute sodium nitroferricyanide TS (1 in 2), mix, and allow to stand for 10 minutes. Add 3 mL of sodium bicarbonate solution (1 in 12) to each flask, dilute with water to volume, mix, and allow to stand for 10 minutes. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 565 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{10}H_{12}N_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(0.5C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Tolazoline Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Tolbutamide



$C_{12}H_{18}N_2O_3S$ 270.35
Benzenesulfonamide, *N*-[(butylamino)carbonyl]-4-methyl-.
1-Butyl-3-(*p*-tolylsulfonyl)urea [64-77-7].

» Tolbutamide contains not less than 97.0 percent and not more than 103.0 percent of $C_{12}H_{18}N_2O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩**—**
USP Tolbutamide RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).
Melting range ⟨741⟩: between 126° and 130°.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.
Selenium ⟨291⟩: 0.003%, a 100-mg specimen, mixed with 100 mg of magnesium oxide, being used.
Limit of non-sulfonyl urea—Dissolve 500 mg in 10 mL of 0.5 N ammonium hydroxide: not more than a faint opalescence occurs.
Other requirements—Where the label states that Tolbutamide is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and for *Bacterial endotoxins* under *Tolbutamide for Injection.* Where the label states that Tolbutamide must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Tolbutamide for Injection.*

Assay—

Mobile phase—Prepare a filtered and degassed mixture of hexane, water-saturated-hexane, tetrahydrofuran, alcohol,

USP Monographs

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and glacial acetic acid (475:475:20:15:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a suitable quantity of tolazamide in alcohol-free chloroform to obtain a solution containing about 3 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Tolbutamide RS in *Internal standard solution* to obtain a solution having a known concentration of about 1.5 mg per mL.

Assay preparation—Transfer about 15 mg of Tolbutamide, accurately weighed, to a 10-mL volumetric flask. Dissolve in and dilute with *Internal standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%, and the resolution, *R*, between tolbutamide and tolazamide is not less than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for tolbutamide and 1.0 for tolazamide. Calculate the quantity, in mg, of $C_{12}H_{18}N_2O_3S$ in the portion of Tolbutamide taken by the formula:

$$10C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Tolbutamide RS in the *Standard preparation*, and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Tolbutamide for Injection

» Tolbutamide for Injection is prepared from Tolbutamide with the aid of Sodium Hydroxide. It contains an amount of tolbutamide sodium equivalent to not less than 95.0 percent and not more than 105.0 percent of the labeled amount of tolbutamide ($C_{12}H_{18}N_2O_3S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

USP Reference standards ⟨11⟩—
USP Tolbutamide RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Change to read:

▲ ***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197M***▲ (CN 1-May-2020)—Obtain the test specimen as follows. Place about 200 mg in a suitable container, dissolve in about 20 mL of water, add 2 mL of 2 N sulfuric acid, and extract with 10 mL of chloroform. Filter, and evaporate an aliquot of the chloroform layer. Dry the residue at 105° for 3 hours. Use USP Tolbutamide RS

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.35 USP Endotoxin Unit per mg of tolbutamide sodium.

pH ⟨791⟩: between 8.0 and 9.8, in a solution containing 50 mg per mL.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

Other requirements—It meets the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩, and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay* under *Tolbutamide.*

Internal standard solution—Prepare a solution of tolazamide in alcohol-free chloroform containing about 15 mg per mL.

Diluting solution—Prepare an alcohol-free chloroform solution containing 3% (v/v) of glacial acetic acid.

Standard preparation—Dissolve an accurately weighed quantity of USP Tolbutamide RS in *Internal standard solution* to obtain a known concentration of about 7.5 mg per mL. Add *Diluting solution* to obtain a *Standard preparation* having a final known concentration of about 1.5 mg of tolbutamide per mL.

Assay preparation—Add about 15 mL of water to 1 container of Tolbutamide for Injection, and shake vigorously to dissolve the contents. Transfer the contents, using adequate rinsing with water, to a 50-mL volumetric flask. Dilute with water to volume, and mix. Transfer an accurately measured portion of this solution, equivalent to about 75 mg of tolbutamide, to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, and dilute with *Diluting solution* to volume. Shake vigorously for about 15 minutes, and centrifuge or allow to stand for about 15 minutes. Use the lower, clear layer, as the *Assay preparation.*

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for tolbutamide and 1.0 for tolazamide. Calculate the quantity, in mg, of $C_{12}H_{18}N_2O_3S$ in the portion of solution taken for the *Assay preparation* by the formula:

$$50C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Tolbutamide RS in the *Standard preparation*, and R_U and R_S are the peak response ratios of the tolbutamide and internal standard peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Tolbutamide Tablets

» Tolbutamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{12}H_{18}N_2O_3S$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Tolbutamide RS

Identification—Triturate a quantity of finely powdered Tablets, equivalent to about 500 mg of tolbutamide, with 50 mL of chloroform, and filter. Evaporate the clear filtrate on a steam bath to dryness: the residue so obtained responds to *Identification* test *A* under *Tolbutamide.*

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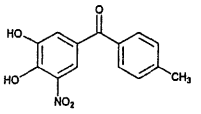
Dissolution ⟨711⟩—
Medium: pH 7.4 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.
Apparatus 2: 75 rpm.
Time: 30 minutes.
Procedure—Measure the amount in solution in filtered portions of the *Dissolution Medium*, suitably diluted with water, if necessary, at the wavelength of maximum absorbance at about 226 nm, with a suitable spectrophotometer, in comparison with a solution having a known concentration of USP Tolbutamide RS. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Dissolution Medium.*
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{12}H_{18}N_2O_3S$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase , Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Tolbutamide.*
Assay preparation—Weigh and finely powder not less than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 150 mg of tolbutamide, to a suitable container. Add 100.0 mL of *Internal standard solution* and about 20 glass beads. Securely close the container, and shake vigorously by mechanical means for approximately 30 minutes. Centrifuge and use the clear supernatant.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Tolbutamide.* Calculate the quantity, in mg, of $C_{12}H_{18}N_2O_3S$ in the portion of Tablets taken by the formula:

$$100C(R_U/R_S)$$

in which the terms are as defined therein.

Tolcapone



$C_{14}H_{11}NO_5$ 273.24
Methanone, (3,4-dihydroxy-5-nitrophenyl)(4-methylphenyl)-;
3,4-Dihydroxy-4′-methyl-5-nitrobenzophenone [134308-13-7].

DEFINITION
Tolcapone contains NLT 98.5% and NMT 101.5% of tolcapone ($C_{14}H_{11}NO_5$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Diluent: Methanol and acetonitrile (24:15)
Buffer: Transfer 6.8 g of monobasic potassium phosphate into a 1-L volumetric flask. Dissolve in about 980 mL of water. Adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume.
Mobile phase: Acetonitrile, methanol, and *Buffer* (25:40:35)
System suitability stock solution: 5 µg/mL of USP Tolcapone Related Compound A RS, 5 µg/mL of USP Tolcapone RS, and 10 µg/mL of USP Tolcapone Related Compound B RS in *Diluent*
System suitability solution: 0.1 µg/mL of USP Tolcapone Related Compound A RS, 0.1 µg/mL of USP Tolcapone RS, and 0.2 µg/mL of USP Tolcapone Related Compound B RS from *System suitability stock solution* prepared as follows. Transfer a suitable volume of *System suitability stock solution* to a suitable volumetric flask. Add 63% of the flask volume of *Diluent* and dilute with water to volume.
Standard stock solution: 1.0 mg/mL of USP Tolcapone RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Tolcapone RS from *Standard stock solution* prepared as follows. Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask. Add 55% of the flask volume of *Diluent* and dilute with water to volume.
Sample stock solution: 1.0 mg/mL of Tolcapone in *Diluent*
Sample solution: 0.1 mg/mL of Tolcapone from *Sample stock solution* prepared as follows. Transfer a suitable volume of *Sample stock solution* to a suitable volumetric flask. Add 55% of the flask volume of *Diluent* and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4.0-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times are given in *Table 1.*]
Suitability requirements
Resolution: NLT 4.0 between tolcapone related compound B and tolcapone, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tolcapone ($C_{14}H_{11}NO_5$) in the portion of Tolcapone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Tolcapone RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the anhydrous and solvent-free basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Diluent, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tolcapone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of any impurity from the *Sample solution*
r_S = peak area of tolcapone from the *Standard solution*
C_S = concentration of USP Tolcapone RS in the *Standard solution* (mg/mL)
C_U = concentration of Tolcapone in the *Sample solution* (mg/mL)
F = relative response factor of the impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tolcapone related compound A	0.6	0.87	0.1
Tolcapone	1.0	—	—
Tolcapone related compound B	1.4	1.0	0.2
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.1%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tolcapone RS
USP Tolcapone Related Compound A RS
4′-Methyl-3,4-dihydroxybenzophenone.
$C_{14}H_{12}O_3$ 228.24
USP Tolcapone Related Compound B RS
4-Hydroxy-3-methoxy-4′-methyl-5-nitrobenzophenone.
$C_{15}H_{13}NO_5$ 287.27

Tolcapone Tablets

DEFINITION
Tolcapone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of tolcapone ($C_{14}H_{11}NO_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197D**▲ (CN 1-May-2020)
Wavelength range: Between 2200 and 1090 cm^{-1}
Sample: Grind 10 Tablets to a fine powder.
Analysis: Transfer an amount of powder equivalent to 3 mg of tolcapone into a polystyrene vial containing 2 mixing beads. Add 300 mg of IR-grade potassium bromide, and disperse the material in the matrix by agitating the capped vial in a grinding mill for 2 min. Transfer a portion of the material to a sample cup. Record the diffuse reflectance IR spectrum.
Acceptance criteria: The spectrum thus obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Tolcapone RS, concomitantly measured.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Diluent 1: Methanol and acetonitrile (24:15)
Diluent 2: Acetonitrile, methanol, and water (25:40:35)
Buffer: Transfer 6.8 g of monobasic potassium phosphate into a 1-L volumetric flask. Dissolve in about 980 mL of water. Adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume.
Mobile phase: Acetonitrile, methanol, and *Buffer* (25:40:35)
System suitability solution: 100 µg/mL of USP Tolcapone RS, 10 µg/mL of USP Tolcapone Related Compound A RS, and 10 µg/mL of USP Tolcapone Related Compound B RS in *Diluent 2*
Standard stock solution: 1.0 mg/mL of USP Tolcapone RS in *Diluent 1*
Standard solution: 0.1 mg/mL of USP Tolcapone RS from *Standard stock solution* prepared as follows. Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask. Add 55% of the flask volume of *Diluent 1* and dilute with water to volume.
Sample stock solution: Nominally 1.0 mg/mL of tolcapone from NLT 20 Tablets prepared as follows. Finely powder the Tablets and transfer a portion of the powder equivalent to NLT 100 mg of tolcapone to a suitable volumetric flask, add 10% of the flask volume of water, and sonicate for 10 min. Add 65% of the flask volume of *Diluent 1*, and sonicate for 15 min. Allow the sample to settle. If the material is still undispersed, sonicate for an additional 5 min. Dilute with water to volume, and mix. Centrifuge a portion of this solution.
Sample solution: Nominally 0.1 mg/mL of tolcapone from the centrifuged *Sample stock solution* prepared as follows. Transfer a suitable volume of the *Sample stock solution* to a suitable volumetric flask. Dilute with *Diluent 2* to volume. Pass a portion of the solution through a filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.0-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times are given in *Table 1*.]
Suitability requirements
Resolution: NLT 4.0 between tolcapone related compound B and tolcapone, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tolcapone ($C_{14}H_{11}NO_5$) in the portion of Tablets taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tolcapone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tolcapone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: pH 6.8 phosphate buffer containing 1% of sodium lauryl sulfate; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Standard stock solution: 1 mg/mL of USP Tolcapone RS prepared as follows. Transfer a suitable quantity of USP Tolcapone RS to a suitable volumetric flask. Add 10% of the flask volume of methanol. Dissolve and dilute with *Medium* to volume.
Standard solution: (L/1000) mg/mL of USP Tolcapone RS from a suitable volume of *Standard stock solution* in *Medium*, where L is the label claim, in mg/Tablet
Sample solution: Filter portions of the solution under test, suitably diluted with *Medium* if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 271 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tolcapone ($C_{14}H_{11}NO_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tolcapone RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor if dilution was necessary
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of tolcapone ($C_{14}H_{11}NO_5$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Diluent 1, Diluent 2, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of any degradation product in the *Sample solution*
r_S = peak area of tolcapone in the *Standard solution*
C_S = concentration of USP Tolcapone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

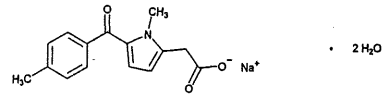
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tolcapone related compound A[a]	0.6	—
Tolcapone	1.0	—
Tolcapone related compound B[a]	1.4	—
Any individual unspecified degradation product	—	0.1
Total degradation products	—	0.5

[a] Process impurity, controlled in the drug substance, and included for information only. Not to be included in total degradation products.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Tolcapone RS
USP Tolcapone Related Compound A RS
4′-Methyl-3,4-dihydroxybenzophenone.
$C_{14}H_{12}O_3$ 228.24
USP Tolcapone Related Compound B RS
4-Hydroxy-3-methoxy-4′-methyl-5-nitrobenzophenone.
$C_{15}H_{13}NO_5$ 287.27

Tolmetin Sodium



$C_{15}H_{14}NNaO_3 \cdot 2H_2O$ 315.30
$C_{15}H_{14}NNaO_3$ 279.27
1*H*-Pyrrole-2-acetic acid, 1-methyl-5-(4-methylbenzoyl)-, sodium salt, dihydrate;
Sodium 1-methyl-5-*p*-toluoylpyrrole-2-acetate dihydrate [64490-92-2].
Anhydrous [35711-34-3].

DEFINITION

Tolmetin Sodium contains NLT 98.0% and NMT 102.0% of tolmetin sodium ($C_{15}H_{14}NNaO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 10 µg/mL in pH 7 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

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Medium: pH 7 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
Acceptance criteria: Meets the requirements
• **C. Identification Tests—General,** *Sodium* ⟨191⟩
Sample solution: 50 mg/mL
Acceptance criteria: Meets the requirements of the test for sodium

ASSAY
• **Procedure**
Sample: 300 mg
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis: Dissolve the *Sample* by warming in 150 mL of glacial acetic acid. Cool to room temperature, and titrate with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 27.93 mg of tolmetin sodium ($C_{15}H_{14}NNaO_3$).
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **Organic Impurities**
Standard stock solution: 12.5 mg/mL of USP Tolmetin Sodium RS in methanol
Standard solution: 62.5 µg/mL of USP Tolmetin Sodium RS in methanol from *Standard stock solution*
Sample solution: 12.5 mg/mL of Tolmetin Sodium in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Absorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Chloroform and glacial acetic acid (95:5)
Analysis
Samples: *Standard stock solution*, *Standard solution*, and *Sample solution*
Develop the chromatogram in the solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, air-dry, and view under short-wavelength UV light.
Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard stock solution*. Any other spot of the *Sample solution* does not exceed in size or intensity of the principal spot of the *Standard solution* (0.5%), and the sum of the total impurities, based on a comparison of the intensities of all such other spots with the *Standard solution*, does not exceed 2.0%.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample under vacuum at 60° for 4 h.
Acceptance criteria: 10.4%–12.4%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Tolmetin Sodium RS

Tolmetin Sodium Capsules

DEFINITION
Tolmetin Sodium Capsules contain an amount of tolmetin sodium equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of tolmetin ($C_{15}H_{15}NO_3$).

IDENTIFICATION
• **A. Ultraviolet Absorption**
Standard stock solution: 0.1 mg/mL of USP Tolmetin Sodium RS in methanol
Standard solution: 0.01 mg/mL of USP Tolmetin Sodium RS from the *Standard stock solution* diluted with 0.1 N sodium hydroxide
Sample solution: Transfer a quantity of Capsule contents, equivalent to 10 mg of tolmetin, to a 100-mL volumetric flask. Add 50 mL of methanol, shake for 2 min, and dilute with methanol to volume. Filter a portion of this solution, transfer 10.0 mL of the filtrate to a second 100-mL volumetric flask, and dilute with 0.1 N sodium hydroxide to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Concomitantly measure the UV absorption.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: 1.7 g/L of tetrabutylammonium phosphate. Adjust with phosphoric acid to a pH of 2.7 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (36:64)
Diluent: Acetonitrile and 0.01 N sodium hydroxide (40:60)
System suitability solution: 200 µg/mL of *p*-toluic acid and 500 µg/mL of USP Tolmetin Sodium RS in *Diluent*
Standard solution: 0.65 mg/mL of anhydrous tolmetin sodium from USP Tolmetin Sodium RS in *Diluent*
Sample solution: Transfer the equivalent of 60 mg of tolmetin from the powdered contents of NLT 20 Capsules to a 100-mL volumetric flask. Add 75 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and pass through a filter of 0.45-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40 ± 1.0°
Flow rate: 3 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between the *p*-toluic acid and tolmetin sodium peaks, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tolmetin ($C_{15}H_{15}NO_3$) in the portion of Capsule contents taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

USP Monographs

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r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the USP Tolmetin Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tolmetin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tolmetin, 257.29
M_{r2} = molecular weight of anhydrous tolmetin sodium, 279.27

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Solution A: Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid, and add water to 1000 mL.
Solution B: Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, and add 190 mL of 0.2 N sodium hydroxide and 400 mL of water. Adjust with 0.2 N sodium hydroxide to a pH of 7.4–7.6. Dilute with water to 1000 mL.
Medium: *Solution A* and *Solution B* (336:664). Adjust the solution with small amounts of either solution to a pH of 4.5; 900 mL.
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: USP Tolmetin Sodium RS in *Medium* at a known concentration
Sample solutions: Sample per *Dissolution* ⟨711⟩. Dilute with 0.1 N sodium hydroxide, as needed, and filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 322 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the quantity of tolmetin ($C_{15}H_{15}NO_3$) dissolved using filtered portions of the solution under test, suitably diluted with 0.1 N sodium hydroxide, in comparison with the *Standard solution*.
Tolerances: NLT 85% (*Q*) of the labeled amount of tolmetin ($C_{15}H_{15}NO_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tolmetin Sodium RS

Tolmetin Sodium Tablets

DEFINITION

Tolmetin Sodium Tablets contain an amount of tolmetin sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of tolmetin ($C_{15}H_{15}NO_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲ (CN 1-May-2020)
Standard stock solution: 0.1 mg/mL of USP Tolmetin Sodium RS in methanol
Standard solution: 0.01 mg/mL of USP Tolmetin Sodium RS from the *Standard stock solution* diluted with 0.1 N sodium hydroxide
Sample solution: Transfer a quantity of finely powdered Tablets, equivalent to 10 mg of tolmetin, to a 100-mL volumetric flask. Add 50 mL of methanol, shake for 2 min, and dilute with methanol to volume. Filter a portion of this solution, transfer 10 mL of the filtrate to a second 100-mL volumetric flask, and dilute with 0.1 N sodium hydroxide to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 1.7 g/L of tetrabutylammonium phosphate. Adjust with phosphoric acid to a pH of 2.7 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (36:64)
Diluent: Acetonitrile and 0.01 N sodium hydroxide (40:60)
System suitability solution: 200 µg/mL of *p*-toluic acid and 500 µg/mL of USP Tolmetin Sodium RS in *Diluent*
Standard solution: 0.65 mg/mL of anhydrous tolmetin sodium from USP Tolmetin Sodium RS in *Diluent*
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the powder, nominally equivalent to 60 mg of tolmetin, to a 100-mL volumetric flask. Add about 75 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and pass through a filter of 0.45-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40 ± 1.0°
Flow rate: 3 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between the *p*-toluic acid and tolmetin sodium peaks, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tolmetin ($C_{15}H_{15}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of tolmetin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of tolmetin, 257.29
M_{r2} = molecular weight of anhydrous tolmetin sodium, 279.27

Acceptance criteria: 90.0%–110.0%

USP Monographs

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PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Solution A: Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid, and add water to 1000 mL.

Solution B: Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, and add 190 mL of 0.2 N sodium hydroxide and 400 mL of water. Adjust the solution with 0.2 N sodium hydroxide to a pH of 7.4–7.6. Dilute with water to 1000 mL.

Medium: *Solution A* and *Solution B* (336:664). Adjust the solution with small amounts of either solution to a pH of 4.5; 900 mL.

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Tolmetin Sodium RS in *Medium* at a known concentration

Sample solutions: Sample per *Dissolution* ⟨711⟩. Dilute with 0.1 N sodium hydroxide, as needed, and filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 322 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tolmetin ($C_{15}H_{15}NO_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tolmetin Sodium RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*

Tolerances: NLT 75% (Q) of the labeled amount of tolmetin ($C_{15}H_{15}NO_3$) is dissolved.

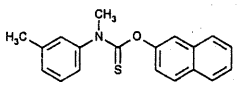
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tolmetin Sodium RS

Tolnaftate



$C_{19}H_{17}NOS$ 307.41

Carbamothioic acid, methyl(3-methylphenyl)-, *O*-2-naphthalenyl ester.

O-2-Naphthyl *m*,*N*-dimethylthiocarbanilate [2398-96-1].

» Tolnaftate contains not less than 98.0 percent and not more than 102.0 percent of $C_{19}H_{17}NOS$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Tolnaftate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The UV absorption spectrum of the solution employed for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Tolnaftate RS, concomitantly measured.

C: Prepare a test solution by dissolving 10 mg in 10 mL of alcohol. Apply 10 µL of this test solution and 10 µL of a Standard solution of USP Tolnaftate RS in alcohol having a concentration of 1.0 mg per mL to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram, using toluene as the solvent system, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, allow the solvent to evaporate, and view under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Melting range ⟨741⟩: between 110° and 113°.

Loss on drying ⟨731⟩—Dry it in vacuum at 65° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Assay—Dissolve about 50 mg of Tolnaftate, accurately weighed, in methanol, and dilute the solution quantitatively and stepwise with methanol to obtain a concentration of about 10 µg per mL. Dissolve an accurately weighed quantity of USP Tolnaftate RS in methanol, and dilute quantitatively and stepwise with methanol to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_{19}H_{17}NOS$ in the portion of Tolnaftate taken by the formula:

$$5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Tolnaftate RS in the Standard solution, and A_U and A_S are the absorbances of the solution of Tolnaftate and the Standard solution, respectively.

Tolnaftate Topical Aerosol

» Tolnaftate Topical Aerosol is a suspension of powder in suitable propellants in a pressurized container. The powder contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of tolnaftate ($C_{19}H_{17}NOS$).

Packaging and storage—Preserve in tight, pressurized containers. Store at controlled room temperature, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩—
USP Tolnaftate RS

Identification—It meets the requirements of the *Identification* test under *Tolnaftate Topical Powder*.

Other requirements—It meets the requirements for *Pressure Test*, *Minimum Fill*, and *Leakage Test* under *Aerosols*,

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Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers ⟨601⟩.

Assay—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Tolnaftate Topical Powder.*

Assay preparation—Remove the actuator button, and replace it with an actuator button that has a small-diameter, stiff polyethylene tube about 15 cm in length fitted tightly into the orifice. Deliver the entire contents of the Topical Aerosol into a conical flask, and heat the flask gently to expel the liquid phase. Cool, mix, and transfer an accurately weighed portion of the powder, equivalent to about 5 mg of tolnaftate, to a screw-capped, 50-mL centrifuge tube. Proceed as directed in the *Assay* under *Tolnaftate Topical Powder,* beginning with "Add 25.0 mL of methanol."

Procedure—Proceed as directed in the *Assay* under *Tolnaftate Topical Powder.*

Tolnaftate Cream

» Tolnaftate Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{17}NOS$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Tolnaftate RS
Identification—Evaporate 10 mL of the next-to-final chloroform solution prepared in the *Assay* on a steam bath just to dryness, and dissolve the residue in 1 mL of alcohol. Using this as the test solution, proceed as directed in *Identification* test *C* under *Tolnaftate:* the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.
Assay—Transfer a portion of Cream, equivalent to about 10 mg of tolnaftate and accurately weighed, to a 250-mL separator containing about 75 mL of chloroform. Wash the chloroform solution successively with two 25-mL portions of 0.1 N sodium hydroxide, two 25-mL portions of 0.1 N hydrochloric acid, and 25 mL of water. Filter the chloroform layer through a chloroform-washed cotton pledget into a 100-mL volumetric flask. Add chloroform to volume, and mix. [NOTE—Reserve a 10-mL portion of this solution for the *Identification* test.] Dilute 5.0 mL of the solution with chloroform to 50.0 mL, and mix. Dissolve an accurately weighed quantity of USP Tolnaftate RS in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of $C_{19}H_{17}NOS$ in the portion of Cream taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Tolnaftate RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Cream and the Standard solution, respectively.

Tolnaftate Gel

» Tolnaftate Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{17}NOS$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Tolnaftate RS
Identification—It responds to the *Identification* test under *Tolnaftate Cream.*
Minimum fill ⟨755⟩: meets the requirements.
Assay—Proceed with Gel as directed in the *Assay* under *Tolnaftate Cream,* except to omit the filtration of the chloroform layer through a chloroform-washed cotton pledget.

Tolnaftate Topical Powder

» Tolnaftate Topical Powder contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{17}NOS$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Tolnaftate RS
Identification—Evaporate the 5-mL portion of the methanol solution, reserved from the *Assay preparation,* on a steam bath just to dryness, and dissolve the residue in 1 mL of alcohol. Using this as the test solution, proceed as directed in *Identification* test *C* under *Tolnaftate:* the specified result is observed.
Minimum fill ⟨755⟩: meets the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve progesterone in methanol to obtain a solution containing about 1 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Tolnaftate RS in methanol to obtain a solution having a known concentration of about 0.22 mg per mL. Transfer 20.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution,* dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.088 mg of USP Tolnaftate RS per mL.

Assay preparation—Transfer an accurately weighed quantity of Topical Powder, equivalent to about 5 mg of tolnaftate, to a screw-capped, 50-mL centrifuge tube. Add 25.0 mL of methanol, place the cap on the tube, rotate on a rotating device for 10 minutes, and centrifuge at about 2000 rpm for 5 minutes. Pass the supernatant through a 0.45-µm porosity filter, and transfer 20.0 mL of the filtrate to a 50-mL volumetric flask, retaining the remaining portion of the filtrate (about 5 mL) for the *Identification* test. Add 5.0 mL of *Internal standard solution* to the volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,*

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between the analyte and internal standard peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for progesterone and 1.0 for tolnaftate. Calculate the quantity, in mg, of $C_{19}H_{17}NOS$ in the portion of Powder taken by the formula:

$$62.5C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Tolnaftate RS in the *Standard preparation*, and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Tolnaftate Topical Solution

» Tolnaftate Topical Solution contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{19}H_{17}NOS$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Tolnaftate RS

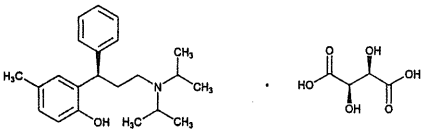
Identification—Evaporate 25 mL of the next-to-final chloroform solution prepared in the *Assay* on a steam bath just to dryness, and dissolve the residue in 1 mL of alcohol. Using this as the test solution, proceed as directed in *Identification* test C under *Tolnaftate:* the specified result is observed.

Assay—Pipet into a separator a volume of Topical Solution, equivalent to about 10 mg of tolnaftate, add 50 mL of chloroform, and extract with 50 mL of 0.1 N sodium hydroxide. Filter the chloroform layer through a chloroform-washed cotton pledget into a 250-mL volumetric flask, and extract the aqueous layer with two 45-mL portions of chloroform, filtering each portion into the flask. Add chloroform to volume, and mix. [NOTE—Reserve a 25-mL portion of this solution for the *Identification* test.] Dilute 25.0 mL of the solution with chloroform to 100.0 mL, and mix. Dissolve an accurately weighed quantity of USP Tolnaftate RS in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of $C_{19}H_{17}NOS$ in each mL of Topical Solution taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Tolnaftate RS in the Standard solution, V is the volume, in mL, of Topical Solution taken, and A_U and A_S are the absorbances of the solution from the Topical Solution and the Standard solution, respectively.

Tolterodine Tartrate



$C_{22}H_{31}NO \cdot C_4H_6O_6$ 475.57

(*R*)-2-[3-[Bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1) (salt);
(+)-(*R*)-2-[α-[2-(Diisopropylamino)ethyl]benzyl]-*p*-cresol L-tartrate (1:1) (salt);
(*R*)-2-[3-(Diisopropylamino)-1-phenylpropyl]-4-methylphenol tartrate [124937-52-6].

DEFINITION

Tolterodine Tartrate contains NLT 97.0% and NMT 103.0% of tolterodine tartrate ($C_{22}H_{31}NO \cdot C_4H_6O_6$), calculated on the as-is basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, water, and phosphoric acid (330:670:1)
Standard solution: 0.35 mg/mL of USP Tolterodine Tartrate RS in *Mobile phase*
Sample solution: 0.35 mg/mL of Tolterodine Tartrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0% from six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tolterodine tartrate ($C_{22}H_{31}NO \cdot C_4H_6O_6$) in the portion of Tolterodine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tolterodine Tartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Tolterodine Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the as-is basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

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• **ORGANIC IMPURITIES**

Solution A: Acetonitrile, water, and perchloric acid (100: 900: 1.5)

Solution B: Acetonitrile, water, and perchloric acid (500: 500: 1.5)

Solution C: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	75	25	0
5	75	25	0
22	0	100	0
47	0	0	100
57	0	0	100

Diluent: Acetonitrile and water (50:50)

System suitability solution: 10 mg/mL of USP Tolterodine System Suitability Mixture RS in *Diluent*. See *Table 2* for relative retention times of the main components of the mixture.

Table 2

Component of USP Tolterodine System Suitability Mixture RS	Relative Retention Time
p-Cresol	0.75
trans-Cinnamic acid	0.81
Monoisopropyl tolterodine	0.88
Tolterodine	1.0
Diol impurity	1.18
Tolterodine dimer[a]	1.44
6-Methyl-4-phenylchroman-2-ol[a]	1.48
Diol acetate impurity	1.54
6-Methyl-4-phenylchroman-2-one	1.59

[a] Undefined stereochemistry.

Standard solution: 0.01 mg/mL of USP Tolterodine Tartrate RS in *Diluent*

Sensitivity solution: 0.005 mg/mL of USP Tolterodine Tartrate RS in *Diluent* from the *Standard solution*

Sample solution: 10 mg/mL of Tolterodine Tartrate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 65°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between diol acetate impurity and 6-methyl-4-phenylchroman-2-one, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tolterodine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of tolterodine from the *Standard solution*

C_S = concentration of USP Tolterodine Tartrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Tolterodine Tartrate in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any peak below 0.05% and any peak eluting at retention times of less than 4 min.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Monoisopropyl tolterodine	0.88	1.6	0.25
Tolterodine	1.0	—	—
6-Methyl-4-phenylchroman-2-ol	1.48	1.9	0.25
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5

• **ENANTIOMERIC PURITY**

Buffer: Prepare pH 7.1 buffer as follows. Transfer 21.0 mL of 1 M solution of monobasic sodium phosphate and 53.3 mL of 0.5 M solution of dibasic sodium phosphate dihydrate to a 1000-mL volumetric flask, and dilute with water to volume. Dilute 100.0 mL of this solution with water to 1000.0 mL.

Mobile phase: Add 0.97 g of tetrabutylammonium bromide to a mixture of 930 mL of *Buffer* and 70 mL of isobutyl alcohol.

System suitability solution: 0.02 mg/mL each of USP Tolterodine Tartrate RS and USP Tolterodine S-Enantiomer RS in *Mobile phase*

Standard solution: 0.0004 mg/mL of USP Tolterodine S-Enantiomer RS in *Mobile phase*

Sample solution: 0.04 mg/mL of Tolterodine Tartrate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 2-mm × 10-cm; 5-µm packing L41

Flow rate: 0.2 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for tolterodine S-enantiomer and tolterodine are 0.9 and 1.0, respectively.]

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Suitability requirements
Resolution: NLT 1.4 between tolterodine *S*-enantiomer and tolterodine
Column efficiency: NLT 1500 theoretical plates for tolterodine
Relative standard deviation: NMT 3% for each of tolterodine *S*-enantiomer and tolterodine

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tolterodine *S*-enantiomer in the portion of Tolterodine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tolterodine *S*-enantiomer from the *Sample solution*
r_S = peak response of tolterodine *S*-enantiomer from the *Standard solution*
C_S = concentration of USP Tolterodine S-Enantiomer RS in the *Standard solution* (mg/mL)
C_U = concentration of Tolterodine Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum at 100° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Tolterodine S-Enantiomer RS
(*S*)-2-[3-(Diisopropylamino)-1-phenylpropyl]-4-methylphenol tartrate.
$C_{22}H_{31}NO \cdot C_4H_6O_6$ 475.57
USP Tolterodine System Suitability Mixture RS
The mixture contains tolterodine tartrate and the following impurities (other impurities may also be present):
p-Cresol.
C_7H_8O 108.14
trans-Cinnamic acid.
$C_9H_8O_2$ 148.16
Monoisopropyl tolterodine;
(*R*)-2-[3-(Isopropylamino)-1-phenylpropyl]-4-methylphenol.
$C_{19}H_{25}NO$ 283.41
Diol impurity;
2-(3-Hydroxy-1-phenylpropyl)-4-methylphenol.
$C_{16}H_{18}O_2$ 242.32
Tolterodine dimer;
N,*N*-Bis[3-(2-hydroxy-5-methylphenyl)-3-phenylpropyl]-*N*-isopropylamine.
$C_{35}H_{41}NO_2$ 507.72
6-Methyl-4-phenylchroman-2-ol.
$C_{16}H_{16}O_2$ 240.30
Diol acetate impurity;
3-(2-Hydroxy-5-methylphenyl)-3-phenylpropyl acetate.
$C_{18}H_{20}O_3$ 284.35
6-Methyl-4-phenylchroman-2-one.
$C_{16}H_{14}O_2$ 238.29
USP Tolterodine Tartrate RS

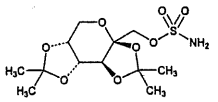
Tolu Balsam

DEFINITION
Tolu Balsam is a balsam obtained from *Myroxylon balsamum* (L.) Harms (Fam. Leguminosae).

SPECIFIC TESTS
• **Rosin, Rosin Oil, and Copaiba**
Sample: 1 g, powdered or crushed, if necessary
Analysis: Place the *Sample* in a small mortar, and add 10 mL of petroleum benzin. Triturate well for 1–2 min, filter into a test tube, and to the filtrate so obtained, add 10 mL of freshly prepared cupric acetate solution (5 mg/mL). Shake, and allow the phases to separate.
Acceptance criteria: The petroleum benzin layer does not show a green color.
• **Fats and Fixed Oils,** *Acid Value* ⟨401⟩
Sample solution: 1 g in 50 mL of neutralized alcohol
Analysis: Add phenolphthalein TS, and titrate with 0.5 N alcoholic potassium hydroxide VS.
Acceptance criteria: 112–168
• **Fats and Fixed Oils,** *Saponification Value* ⟨401⟩
Sample solution: Add sufficient 0.5 N alcoholic potassium hydroxide VS to the neutralized liquid obtained in the test for *Acid Value* to make the total volume of the alkali solution added 20.0 mL, heat the liquid on a steam bath for 30 min under a reflux condenser, and cool. Add 200 mL of water, or more if necessary.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.5 N alcoholic potassium hydroxide VS
Back-titrant: 0.5 N hydrochloric acid VS
Endpoint detection: Visual
Analysis: Titrate the excess potassium hydroxide in the *Sample solution* with *Back-titrant*. Perform a blank determination.
Acceptance criteria: The total volume of *Titrant* consumed, including that required to neutralize the free acid in the determination of the *Acid Value*, is equivalent to a saponification value between 154 and 220.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, and avoid exposure to excessive heat.

Topiramate



$C_{12}H_{21}NO_8S$ 339.36
β-D-Fructopyranose, 2,3:4,5-bis-*O*-(1-methylethylidene)-, sulfamate;
2,3:4,5-Di-*O*-isopropylidene-β-D-fructopyranose sulfamate [97240-79-4].

DEFINITION
Topiramate contains NLT 98.0% and NMT 102.0% of topiramate ($C_{12}H_{21}NO_8S$), calculated on the anhydrous basis.
[**Caution**—Great care must be exercised in handling topiramate because it is a suspected teratogen.]

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IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (50:50)
Standard solution: 2 mg/mL of USP Topiramate RS in *Mobile phase*
Sample solution: 2 mg/mL of Topiramate in *Mobile phase*
System suitability solution: 0.02 mg/mL each of USP Fructose RS and USP Topiramate Related Compound A RS in the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 50°
Detector: 55°
Flow rate: 0.6 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of topiramate
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 1* for the relative retention times for fructose, topiramate related compound A, and topiramate.]
Suitability requirements
Resolution: NLT 1.5 between topiramate related compound A and topiramate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of topiramate ($C_{12}H_{21}NO_8S$) in the portion of Topiramate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
C_U = concentration of Topiramate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **LIMIT OF SULFAMATE AND SULFATE**

[NOTE—Use water with resistivity of NLT 18 megohm-cm for preparation of the *Mobile phase*, *Standard solution*, and *Sample solution*.]
Buffer: 0.8 g/L of *p*-hydroxybenzoic acid in water
Mobile phase: Methanol and *Buffer* (2.5: 97.5). Adjust with sodium hydroxide solution to a pH of 9.4 ± 0.5.
Standard solution: 0.0045 mg/mL of sodium sulfate and 0.0030 mg/mL of sulfamic acid in *Mobile phase*
Sample solution: 6.0 mg/mL of Topiramate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4.6-mm × 15-cm; 5-µm packing L47
Detector temperature: 30°
Flow rate: 1.5 mL/min
[NOTE—A suitable background suppression unit may be used.]
Injection volume: 70 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention time of sulfamate is 0.44 relative to sulfate.]
Suitability requirements
Relative standard deviation: NMT 15.0% for sulfamate and sulfate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfate ions in the portion of Topiramate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the sulfate ion from the *Sample solution*
r_S = peak response of the sulfate ion from the *Standard solution*
C_S = concentration of sodium sulfate in the *Standard solution* (mg/mL)
C_U = concentration of Topiramate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of the sulfate anion, 96.04
M_{r2} = molecular weight of anhydrous sodium sulfate, 142.04

Calculate the percentage of sulfamate ions in the portion of Topiramate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the sulfamate ion from the *Sample solution*
r_S = peak response of the sulfamate ion from the *Standard solution*
C_S = concentration of sulfamic acid in the *Standard solution* (mg/mL)
C_U = concentration of Topiramate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sulfamate anion, 96.09
M_{r2} = molecular weight of sulfamic acid, 97.09

Acceptance criteria: NMT 0.1% of sulfate; NMT 0.1% of sulfamate
[NOTE— If *N*-methyltopiramate is a potential impurity, *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 3* are recommended; on the basis of synthetic route, perform either *Organic Impurities, Procedure 2* or *Organic Impurities, Procedure 3*.]

- **ORGANIC IMPURITIES, PROCEDURE 1**

Identification solution: 0.2 mg/mL of USP Topiramate Related Compound A RS in methanol
Standard solution A: 40 mg/mL of USP Topiramate RS in methanol
Standard solution B: 0.08 mg/mL of Topiramate from *Standard solution A* and methanol
Standard solution C: 0.04 mg/mL of Topiramate from *Standard solution A* and methanol
Sample solution: 40 mg/mL of Topiramate in methanol

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Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 0.20-mm layer of chromatographic silica gel mixture, prewashed with methanol and air-dried
Application volume: 20 µL
Developing solvent system: Acetonitrile, methanol, and 0.5 M sodium chlorideTS (35:15:50)
Spray reagent: 30-mg/mL solution of phenol in alcohol and concentrated sulfuric acid (95:5)
Analysis
Samples: *Standard solution B*, *Standard solution C*, and *Sample solution*
Proceed as directed in the chapter. After elution, air-dry the plate, spray the plate with the *Spray reagent*, and let the plate air-dry. Then dry the plate for 10 min in an oven at 125°. [NOTE—The approximate R_F values for topiramate and topiramate related compound A are 0.65 and 0.70, respectively. Disregard any spots at the origins of the chromatograms. Disregard any spot corresponding to topiramate related compound A because this impurity should be quantified using *Procedure 3*.] Examine the plate using visible light, and estimate the percentage of all secondary spots in the chromatogram of the *Sample solution* by comparing each spot with the principal spot from the chromatograms of each *Standard solution*.
Acceptance criteria: Any single spot is not greater in size and intensity than the spot for *Standard solution C*; NMT 0.1% of any individual impurity and NMT 0.5% of total impurities is found by TLC.

• **ORGANIC IMPURITIES, PROCEDURE 2**
Mobile phase: Prepare as directed in the *Assay*.
[NOTE—Prepare all solutions fresh before use.]
Sample solution: 40 mg/mL of Topiramate in *Mobile phase*.
[NOTE—Sonication may be used to aid dissolution.]
System suitability solution: 0.3 mg/mL each of USP Fructose RS and USP Topiramate Related Compound A RS in the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 50°
Detector: 55°
Flow rate: 0.6 mL/min
Injection volume: 50 µL
Run time: NLT 5 times the retention time of topiramate
System suitability
Sample: *System suitability solution*
[NOTE— See *Table 1* for the relative retention times for fructose, topiramate related compound A, and topiramate.]
Suitability requirements
Resolution: NLT 1.0 between topiramate related compound A and topiramate
Relative standard deviation: NMT 2.0% for topiramate
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Topiramate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of each impurity
r_T = sum of the peak areas of all the impurities and topiramate
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fructose	0.45	1.3	0.3
Topiramate related compound A	0.9	1.0	0.3
Topiramate	1.0	1.0	—
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

• **ORGANIC IMPURITIES, PROCEDURE 3**
Mobile phase: Methanol and water(32:68)
Standard solution: 10 mg/mL of USP Topiramate RS and 0.04 mg/mL of USP Topiramate Related Compound A RS in *Mobile phase*
Sample solution: 10 mg/mL of Topiramate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 15-cm; 5-µm packing L15
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between topiramate related compound A and topiramate
Relative standard deviation: NMT 2.0% for topiramate from six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Topiramate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of topiramate from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Response Factor	Acceptance Criteria, NMT (%)
Topiramate related compound A	1.1	0.3
Any individual unspecified impurity	1.0	0.10
Total impurities	1.0	0.5

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SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 4–10 mg/mL in methanol
  Acceptance criteria: –28.6° to –35.0°, measured at 20°
- **WATER DETERMINATION** ⟨921⟩, *Method I:* NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** If an *Organic Impurities* procedure other than *Procedure 2* is used, then the labeling states the test with which the article complies. The label also states that it is a suspected teratogen.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fructose RS
  USP Topiramate RS
  USP Topiramate Related Compound A RS
  2,3:4,5-Bis-*O*-(1-methylethylidene)-β-D-fructopyranose.
  $C_{12}H_{20}O_6$ 260.28

Topiramate Capsules

DEFINITION
Topiramate Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of topiramate ($C_{12}H_{21}NO_8S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
  Wavenumber range: 4000 to 650 cm^{-1}
  Standard solution: 20 mg/mL of USP Topiramate RS in acetone
  Sample solution: Open an appropriate number of Capsules to prepare a 20-mg/mL topiramate solution in acetone. Shake the solution for 30 min, and centrifuge for 10 min. Then pass an aliquot of the clear supernatant through a suitable nylon filter of 0.2-µm pore size, and use the filtrate for analysis.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Apply 50 µL to an NaCl plate, allow the solution to dry, then obtain the IR spectrum.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: 1.5 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.0.
  Diluent: Acetonitrile and water (20:80)
  Mobile phase: Methanol and *Buffer* (20:80)
  Standard solution: 6 mg/mL of USP Topiramate RS in *Diluent*
  Sample solution: Nominally 6 mg/mL of topiramate in *Diluent* from NLT 20 Capsules. [NOTE—Shake vigorously for at least 60 min, and pass a portion through a PTFE chemical-resistant filter of 0.45-µm pore size.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Refractive index
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 100 µL
  Temperatures
  Column: 35°
  Detector: 35°
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of topiramate ($C_{12}H_{21}NO_8S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of topiramate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm. Use an appropriate sinker as necessary.
  Time: 45 min
  Standard stock solution: 0.56 mg/mL of USP Topiramate RS in *Medium* prepared as follows. Transfer a suitable amount to a suitable volumetric flask. Add 2% of the flask volume of acetone to dissolve the solid. Dilute with *Medium* to volume.
  Standard solution: (*L*/900) mg/mL of USP Topiramate RS in *Medium*, where *L* is the label claim, in mg of topiramate per Capsule from the *Standard stock solution*
  Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size.
  Mobile phase: Methanol and water (32:68)
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Refractive index
  Column: 4.6-mm × 15-cm; 5-µm packing L7
  Flow rate: 2 mL/min
  Injection volume: 200 µL
  Temperatures
  Column: 35°
  Detector: 35°
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0% for Capsules labeled to contain more than 15 mg of topiramate; NMT 3.0% for Capsules labeled to contain less than or equal to 15 mg of topiramate
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of topiramate ($C_{12}H_{21}NO_8S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

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C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of topiramate is dissolved.

Test 2

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm. Use an appropriate sinker as necessary.

Time: 60 min

Standard solution: (L/900) mg/mL of USP Topiramate RS in *Medium*, where L is the label claim, in mg of topiramate per Capsule

Sample solution: Pass a portion of the solution under test through a suitable filter.

Mobile phase: Methanol and water (40:60)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 100 µL

Temperatures

Column: 35°

Detector: 35°

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of topiramate ($C_{12}H_{21}NO_8S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amount of topiramate ($C_{12}H_{21}NO_8S$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Limit of Sulfamate and Sulfate:** Use water with a resistivity of NLT 18 megohm-cm for the preparation of the *Mobile phase*, *Standard solution*, and *Sample solution*.

Buffer: 0.8 g/L of *p*-hydroxybenzoic acid in water

Mobile phase: Methanol and *Buffer* (2.5: 97.5). Adjust with sodium hydroxide solution to a pH of 9.4 ± 0.5.

Standard solution: 0.015 mg/mL each of sodium sulfate and sulfamic acid in *Mobile phase* from anhydrous sodium sulfate and sulfamic acid, respectively

Sample solution: Grind the contents of NLT 20 Capsules. Transfer an amount of powder equivalent to 300 mg of topiramate to a 50-mL volumetric flask. Add about 40 mL of *Mobile phase*, and stir for 30 min. Sonicate for 10 min, and dilute with *Mobile phase* to volume. Centrifuge, and pass through a polyethersulfone membrane filter of 0.45-µm pore size, discarding the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Conductivity

Column: 4.6-mm × 15-cm; 5-µm packing L47

Flow rate: 1.5 mL/min. [Note—A suitable background suppression unit may be used.]

Detector temperature: 30°

Injection volume: 70 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention time of the sulfamate peak is 0.44 relative to the sulfate peak.]

Suitability requirements

Relative standard deviation: NMT 15.0% for the sulfamate and sulfate peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sulfate in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sulfate from the *Sample solution*
r_S = peak response of sulfate from the *Standard solution*
C_S = concentration of sodium sulfate in the *Standard solution* (mg/mL)
C_U = nominal concentration of topiramate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sulfate anion, 96.04
M_{r2} = molecular weight of anhydrous sodium sulfate, 142.04

Calculate the percentage of sulfamate in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sulfamate from the *Sample solution*
r_S = peak response of sulfamate from the *Standard solution*
C_S = concentration of sulfamic acid in the *Standard solution* (mg/mL)
C_U = nominal concentration of topiramate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sulfamate anion, 96.09
M_{r2} = molecular weight of sulfamic acid, 97.09

Acceptance criteria: NMT 0.25% (w/w) of sulfate and NMT 0.25% (w/w) of sulfamate

• **Organic Impurities**

Diluent, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 1.2 mg/mL of USP Topiramate RS and 0.6 mg/mL of USP Topiramate Related Compound A RS in *Diluent*

System suitability

Sample: *Standard solution*

[Note—Identify the peaks due to topiramate related compound A and topiramate using the relative retention times given in *Table 1*.]

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Sample solution* and *Standard solution*

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Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*
r_S = peak response of topiramate from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of topiramate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Topiramate related compound A	0.66	1.1	0.5
Topiramate	1.0	—	—
Individual unspecified degradation product	—	—	0.2
Total impurities	—	—	0.7

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in tightly closed containers at controlled room temperature, protected from moisture.
- **LABELING:** Topiramate Capsules may be swallowed whole or may be administered by carefully opening the Capsule and sprinkling the entire contents on a teaspoon of soft food. This drug-food mixture should be swallowed immediately and not chewed. It should not be stored for future use. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Topiramate RS
  USP Topiramate Related Compound A RS
  2,3:4,5-Bis-*O*-(1-methylethylidene)-β-D-fructopyranose.
  $C_{12}H_{20}O_6$ 260.28

Topiramate Tablets

DEFINITION

Topiramate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of topiramate ($C_{12}H_{21}NO_8S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197F▲ (CN 1-May-2020)
  Wavenumber range: 4000–650 cm^{-1}
  Standard solution: 20 mg/mL of USP Topiramate RS in acetone
  Sample solution: Grind an appropriate number of Tablets to prepare a 20-mg/mL topiramate solution in acetone. Shake the solution for 30 min, and centrifuge for 10 min. Then pass an aliquot of the clear supernatant through a suitable nylon filter of 0.45-µm pore size, and use the filtrate for analysis.
  Analysis: Apply 50 µL of the *Standard solution* to a sodium chloride plate, allow the solution to dry, and then obtain the IR spectrum. Wash the window with acetone, and repeat the same procedure using the *Sample solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 1.54 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.0.
  Diluent: Methanol and water (1:4)
  Mobile phase: Methanol and *Buffer* (1:4)
  Standard solution: 6 mg/mL of USP Topiramate RS in *Diluent*
  Sample solution: 6 mg/mL of topiramate in *Diluent* from NLT 12 Tablets, based on the label claim. [NOTE—Shake vigorously for at least 30 min, and pass a portion through a chemical-resistant filter (PTFE) of 0.45-µm pore size.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Refractive index
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Temperature
  Column: 35°
  Detector: 35°
  Flow rate: 1.5 mL/min
  Injection size: 100 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of topiramate ($C_{12}H_{21}NO_8S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of topiramate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm
  Time: 20 min
  Mobile phase: 0.1% trifluoroacetic acid in water and methanol (1:1)
  Standard solution: 0.1 mg/mL of USP Topiramate RS in *Medium*
  Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Refractive index
  Guard column: 4.0-mm × 1-cm
  Column: 4.6-mm × 25-cm; 5-µm packing L11

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Temperature
Column: 40°
Detector: 40°
Flow rate: 1.2 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of topiramate ($C_{12}H_{21}NO_8S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of topiramate is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 40 min
Standard solution: (L/900) mg/mL of USP Topiramate RS in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first few mL.
Mobile phase: Water and acetonitrile (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperature
Column: 30°
Detector: 50°
Flow rate: 1.0 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 5000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of topiramate ($C_{12}H_{21}NO_8S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of topiramate is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Diluent: Acetonitrile and water (1:1)
Mobile phase: Water and acetonitrile (55:45)
Standard solution: 1.1 mg/mL of USP Topiramate RS in *Diluent*. Dilute with *Medium* to obtain a final concentration of about (L/900) mg/mL, where L is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first few mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperature
Column: 50°
Detector: 50°
Flow rate: 1.2 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of topiramate ($C_{12}H_{21}NO_8S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of topiramate is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **RELATED COMPOUNDS**
Diluent, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1.2 mg/mL of USP Topiramate RS and 0.6 mg/mL of USP Topiramate Related Compound A RS in *Diluent*
Peak identification solution: 0.6 mg/mL each of USP Topiramate RS and USP Topiramate Related Compound A RS in *Diluent*
System suitability
Samples: *Standard solution* and *Peak identification solution*
[NOTE—Identify the peaks due to topiramate related compound A and topiramate using the relative retention times given in *Table 1*.]
Suitability requirements
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for the individual impurity from the *Sample solution*
r_S = peak response of topiramate from the *Standard solution*
C_S = concentration of USP Topiramate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of topiramate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Topiramate related compound A	0.66	1.1	0.5
Topiramate	1.0	—	—
Individual unspecified degradation product	—	—	0.2
Total impurities	—	—	0.7

SPECIFIC TESTS

- **LIMIT OF SULFAMATE AND SULFATE**

[NOTE—Use water with resistivity NLT 18 megohm-cm for preparation of the *Mobile phase*, *Standard solution*, and *Sample solution*.]

Buffer: 0.8 g/L of *p*-hydroxybenzoic acid in water
Mobile phase: Methanol and *Buffer* (2.5: 97.5). Adjust with sodium hydroxide solution to a pH of 9.4 ± 0.5.
Standard solution: 0.015 mg/mL each of sodium sulfate and sulfamic acid in *Mobile phase* from anhydrous sodium sulfate and sulfamic acid, respectively
Sample solution: Transfer a suitable amount of ground powder from NLT 20 Tablets to a suitable volumetric flask to obtain a nominal concentration of 6 mg/mL of topiramate. Add 80% of the flask volume of *Mobile phase*, and shake for 30 min. Sonicate for 10 min, and dilute with *Mobile phase* to volume. Centrifuge, and pass through a polyethersulfone membrane filter of 0.45-µm pore size, discarding the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4.6-mm × 15-cm; 5-µm packing L47
Detector temperature: 30°
Flow rate: 1.5 mL/min
[NOTE—A suitable background suppression unit may be used.]
Injection size: 70 µL
System suitability
Sample: *Standard solution*
[NOTE—The approximate relative retention time of the sulfamate ion peak is 0.44 relative to the sulfate ion peak.]
Suitability requirements
Relative standard deviation: NMT 15.0% for the sulfamate and sulfate peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sulfate ion in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sulfate ion from the *Sample solution*
r_S = peak response of sulfate ion from the *Standard solution*
C_S = concentration of sodium sulfate in the *Standard solution* (mg/mL)
C_U = concentration of topiramate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of the sulfate anion, 96.04
M_{r2} = molecular weight of anhydrous sodium sulfate, 142.04

Calculate the percentage of sulfamate ion in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of sulfamate ion from the *Sample solution*
r_S = peak response of sulfamate ion from the *Standard solution*
C_S = concentration of sulfamic acid in the *Standard solution* (mg/mL)
C_U = concentration of topiramate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of the sulfamate anion, 96.09
M_{r2} = molecular weight of sulfamic acid, 97.09

Acceptance criteria: NMT 0.25% of sulfate ion; NMT 0.25% of sulfamate ion

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in tightly closed containers at controlled room temperature, protected from moisture.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Topiramate RS
2,3:4,5-Di-*O*-isopropylidene-β-D-fructopyranose sulfamate.
$C_{12}H_{21}NO_8S$ 339.36
USP Topiramate Related Compound A RS
2,3:4,5-Bis-*O*-(1-methylethylidene)-β-D-fructopyranose.
$C_{12}H_{20}O_6$ 260.28

Topiramate Compounded Oral Suspension

DEFINITION

Topiramate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of topiramate ($C_{12}H_{21}NO_8S$).

Prepare Topiramate Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Topiramate tablets[a] or powder, equivalent to	2 g
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Topiramate 200-mg tablets, Torrent Pharmaceuticals LTD, Kalamazoo, MI.
[b] Perrigo, Minneapolis, MN.

USP Monographs

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If using tablets, comminute the tablets to a fine powder in a suitable mortar or other mechanical means, or add *Topiramate powder* to the mortar. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make the mortar contents pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: Weigh 6.24 g of monobasic sodium phosphate dihydrate, and transfer to a 1-L volumetric flask. Dissolve in 800 mL of water, add 30 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.8. Dilute with water to volume, and mix thoroughly.

Solution B: Weigh 6.25 g of boric acid and 7.5 g of potassium chloride, and transfer to a 1-L volumetric flask. Dissolve in 900 mL of water, and adjust with potassium hydroxide to a pH of 7.8. Dilute with water to volume, and mix thoroughly.

Solution C: Transfer 50 mg of 9-fluorenylmethyl chloroformate to a 10-mL volumetric flask, and dilute with acetonitrile to volume. Store at 4°, and protect from light.

Solution D: Transfer 100 mg of glycine to a 10-mL volumetric flask, and dilute with water to volume.

Mobile phase: Acetonitrile and *Solution A* (48:52). Filter, and degas.

Standard stock solution: 1 mg/mL of USP Topiramate RS in acetonitrile

Standard solution: Transfer 1 mL of *Standard stock solution* into a 10-mL volumetric flask, dilute with *Solution B* to volume, and mix well.

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 0.5 mL of the Oral Suspension into a 10-mL volumetric flask, add 2 mL of water and 5 mL of acetonitrile, and sonicate for 10 min. Dilute with water to volume, and mix well. Pass through a PVDF filter of 0.45-µm pore size, discarding the first 3 mL of filtrate. Mix 1 mL of the filtrate with 9 mL of *Solution B*. Transfer 100 µL of the resulting solution into a HPLC vial, add 50 µL of *Solution C*, and mix on a vortex mixer for 20 s. Incubate at 50° for 15 min. Immediately add 100 µL of *Solution D* to the vial to terminate the reaction. Mix on a vortex mixer for 10 s, and allow to stand for at least 1 min prior to analysis.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 264 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 52°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for topiramate is about 8.8 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of topiramate ($C_{12}H_{21}NO_8S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of topiramate from the *Sample solution*

r_S = peak response of topiramate from the *Standard solution*

C_S = concentration of topiramate in the *Standard solution* (mg/mL)

C_U = nominal concentration of topiramate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.9–4.9

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

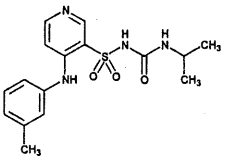
• **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Topiramate RS

Torsemide



$C_{16}H_{20}N_4O_3S$ 348.42

3-Pyridinesulfonamide, *N*-[[(1-methylethyl)amino]carbonyl]-4-[(3-methylphenyl)amino]-.

1-Isopropyl-3-[(4-*m*-toluidino-3-pyridyl)sulfonyl]urea [56211-40-6].

» Torsemide contains not less than 98.0 percent and not more than 102.0 percent of $C_{16}H_{20}N_4O_3S$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Torsemide RS

(Form 1)

USP Torsemide Related Compound A RS

4-[(3-Methylphenyl)amino]-3-pyridinesulfonamide.

$C_{12}H_{13}N_3O_2S$ 263.32

USP Torsemide Related Compound B RS

N-[(*n*-Butylamino)carbonyl]-4-[(3-methylphenyl)amino]-3-pyridinesulfonamide].

$C_{17}H_{22}N_4O_3S$ 362.45

USP Torsemide Related Compound C RS

N-[(Ethylamino)carbonyl]-4-[(3-methylphenyl)amino]-3-pyridinesulfonamide.

$C_{15}H_{18}N_4O_3S$ 334.39

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Water Determination, *Method I* ⟨921⟩: not more than 1.0%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—

0.02 M Potassium phosphate buffer and *Mobile phase*—Prepare as directed in the *Assay.*

Resolution solution—Transfer about 3 mg each of USP Torsemide RS and USP Torsemide Related Compound A RS to a 10-mL volumetric flask, add 3 mL of methanol, mix, and sonicate for not less than 8 minutes. Add 4.5 mL of *0.02 M Potassium phosphate buffer,* cool to room temperature, dilute with *Mobile phase* to volume, and mix.

Standard solution—Transfer about 8 mg each of USP Torsemide Related Compound A RS, USP Torsemide Related Compound B RS, and USP Torsemide Related Compound C RS, accurately weighed, to a 100-mL volumetric flask, add 30 mL of methanol, mix, and sonicate for not less than 8 minutes. Add 45 mL of *0.02 M Potassium phosphate buffer,* cool to room temperature, dilute with *Mobile phase* to volume, and mix. Quantitatively dilute a portion of this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.0019 mg per mL.

Test solution—Use the *Assay preparation.*

Chromatographic system—Prepare as directed in the *Assay.* Chromatograph the *Resolution solution* and the *Standard solution,* and record the peak responses over a period three times the retention time of torsemide as directed for *Procedure:* the resolution, *R,* between torsemide and torsemide related compound A is not less than 1.0; the tailing factors are not more than 2.0; and the relative standard deviation for replicate injections is not more than 10.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas for torsemide related compound A, torsemide related compound B, and torsemide related compound C. Calculate the percentage of each related compound, if present, in the portion of Torsemide taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard solution;* C_U is the concentration of Torsemide, in mg per mL, in the *Test solution;* and r_U and r_S are the peak areas for the relevant torsemide related compound obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.2% of torsemide related compound C, not more than 0.3% of torsemide related compound B, and not more than 0.5% of torsemide related compound A are found. Calculate the percentage of any other impurity in the portion of Torsemide taken by the formula:

$$100(r_i/r_S)$$

in which r_i is the peak response for each other impurity obtained from the *Test solution;* and r_S is the sum of the responses of all the peaks obtained from the *Test solution:* not more than 0.1% of any other impurity is found, not more than 0.2% of total other impurities is found, and not more than 1.0% of total impurities (including torsemide related compounds A, B, and C) is found.

Assay—

0.02 M Potassium phosphate buffer—Dissolve 2.7 g of monobasic potassium phosphate in about 900 mL of water. Adjust with phosphoric acid to a pH of 3.5, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *0.02 M Potassium phosphate buffer* and methanol (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 19 mg of USP Torsemide RS, accurately weighed, to a 50-mL volumetric flask, add 15 mL of methanol, mix, and sonicate for not less than 8 minutes. Add 22.5 mL of *0.02 M Potassium phosphate buffer,* cool to room temperature, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer about 38 mg of Torsemide, accurately weighed, to a 100-mL volumetric flask, add 30 mL of methanol, mix, and sonicate for not less than 8 minutes. Add 45 mL of *0.02 M Potassium phosphate buffer,* cool to room temperature, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 288-nm detector and a 4.6-mm × 15-cm column that contains 7-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount, in mg, of $C_{16}H_{20}N_4O_3S$ in the portion of Torsemide taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Torsemide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Add the following:

▲Torsemide Compounded Oral Suspension

DEFINITION

Torsemide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of torsemide ($C_{16}H_{20}N_4O_3S$).

Prepare Torsemide Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Torsemide Tablets, equivalent to[a]	500 mg of torsemide
Sodium Hydroxide (2 N)	Adjust to pH of 8.3

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Vehicle: 1:1 mixture of Ora-Sweet SF[b] and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Torsemide 10-mg tablets, Camber, Piscataway, NJ.
[b] Perrigo, Allegan, MI.

Place the *Torsemide Tablets* in a suitable container and triturate to a fine powder. Add a small amount of *Vehicle* and mix well to form a smooth paste. Add a sufficient amount of *Vehicle* to make the container contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container. Add sufficient *Vehicle* to bring to final volume. Shake to mix well. Add *Sodium Hydroxide (2 N)* dropwise to adjust the pH to 8.3.

ASSAY

• **PROCEDURE**

Solution A: 10 mM of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 4

Mobile phase: Methanol and *Solution A* (45:55)

Diluent: 50% methanol in water

Standard solution: Transfer 20 mg of USP Torsemide RS into a 200-mL volumetric flask. Add approximately 150 mL of *Diluent* and sonicate for 15 min, then dilute with *Diluent* to volume.

Sample solution: Transfer 1 mL of Oral Suspension into a 50-mL volumetric flask. Add approximately 40 mL of *Diluent* and sonicate for 15 min, then dilute with *Diluent* to volume. Filter 1 mL of this solution, discard the first 3 drops, then transfer into an HPLC vial.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Temperatures

Autosampler: 4°

Column: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for torsemide is about 9.6 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of torsemide ($C_{16}H_{20}N_4O_3S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of torsemide from the *Sample solution*

r_S = peak response of torsemide from the *Standard solution*

C_S = concentration of USP Torsemide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of torsemide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 8.0–9.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant plastic containers. Store at controlled room temperature or in a refrigerator.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator; NMT 30 days after the date on which it was compounded when stored at controlled room temperature.

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Torsemide RS▲ (USP 1-May-2020)

Torsemide Tablets

DEFINITION

Torsemide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of torsemide ($C_{16}H_{20}N_4O_3S$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**

Buffer: 2.72 g/L of monobasic potassium phosphate▲▲ 2S (USP41)

Solution A: Acetonitrile and methanol (10:90)

Mobile phase: *Buffer* and *Solution A* (50:50). Adjust with diluted phosphoric acid (1 in 10 v/v) to a pH of 4.0.

Standard solution: 0.4 mg/mL of USP Torsemide RS prepared as follows. To a quantity of USP Torsemide RS in a suitable flask, add methanol to 30% of the flask volume and sonicate for NLT 8 min. Add *Buffer* to fill 75% of the flask volume, cool, and dilute with *Mobile phase*. Pass through a membrane filter of 0.45-µm pore size.

Sample solution: Nominally 0.4 mg/mL of torsemide prepared as follows. Place ▲▲ 2S (USP41) 40 mg ▲of torsemide▲ 2S (USP41) from NLT 20 powdered Tablets in a 100-mL volumetric flask. Add methanol to 30% of the flask volume and sonicate for NLT 8 min. Add *Buffer* to fill 75% of the flask volume, cool, and dilute with *Mobile phase*. Pass through a membrane filter of 0.45-µm pore size. ▲The *Sample solution* is not stable at room temperature but is stable for 12 h at 6°.▲ 2S (USP41)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 288 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ 2S (USP41)

Column: 4.6-mm × 15-cm; 5-µm packing L1

Temperatures

▲**Autosampler:** 6°▲ 2S (USP41)

Column: 30°

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Flow rate: 1 mL/min
Injection volume: 20 µL
▲Run time: NLT 2 times the retention time of torsemide▲ 2S (USP41)
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of torsemide ($C_{16}H_{20}N_4O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of torsemide from the *Sample solution*
r_S = peak response of torsemide from the *Standard solution*
C_S = concentration of USP Torsemide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of torsemide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Buffer, Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard stock solution: 0.55 mg/mL ▲of USP Torsemide RS▲ 2S (USP41) prepared as follows. Transfer a quantity of USP Torsemide RS to a suitable volumetric flask. Add methanol to 30% of the flask volume and sonicate until dissolved. Add *Buffer* to fill 75% of the flask volume, cool to room temperature, and dilute with *Mobile phase* to volume.
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/900) mg/mL, where *L* is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of torsemide ($C_{16}H_{20}N_4O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \text{ ▲▲ 2S (USP41)} \times V \times 100$$

r_U = peak response of torsemide from the *Sample solution*
r_S = peak response of torsemide from the *Standard solution*
C_S = concentration of USP Torsemide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
▲▲ 2S (USP41)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of torsemide ($C_{16}H_{20}N_4O_3S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard stock solution: 0.11 mg/mL of USP Torsemide RS in *Medium*
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/900) mg/mL, where *L* is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 285 nm
Cell: 1.0 cm for 5-, 10-, and 20-mg Tablets and 0.1 cm for 100-mg Tablets
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of torsemide ($C_{16}H_{20}N_4O_3S$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Torsemide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of torsemide ($C_{16}H_{20}N_4O_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Buffer and **Solution A:** Prepare as directed in the *Assay*.
Mobile phase: *Buffer* and *Solution A* (55:45). Adjust with diluted phosphoric acid (1 in 10 v/v) to a pH of 4.0.
▲Standard stock solution A:▲ 2S (USP41) 0.1 mg/mL of USP Torsemide Related Compound A RS and 0.02 mg/mL of USP Torsemide Related Compound E RSUSP Torsemide Related Compound A RSUSP Torsemide Related Compound E RSmethanol to 32% of the flask volume and sonicate to dissolve. Dilute with *Mobile phase*
System suitability solution: ▲ 0.4 mg/mL of USP Torsemide RS, 4 µg/mL of USP Torsemide Related Compound A RS, and 0.8 µg/mL of USP Torsemide Related Compound E RS prepared as follows. To a quantity of USP Torsemide RS in a suitable flask add methanol to 30% of the flask volume and sonicate to dissolve. Add *Buffer* to fill 75% of the flask volume, and cool. Add a suitable volume of *Standard stock solution A* and dilute with *Mobile phase* to volume.
Standard stock solution B: 0.4 mg/mL each of USP Torsemide RS prepared as follows. To a suitable amount of USP Torsemide RS in a suitable flask, add methanol to 30% of the flask volume and sonicate for NLT 8 min. Add *Buffer* to fill 75% of the flask volume, cool, and dilute with *Mobile phase* to volume.
Sensitivity solution: 0.4 µg/mL of USP Torsemide RS in *Mobile phase* from *Standard stock solution B*▲ 2S (USP41)

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Standard solution: 4 µg/mL each of USP Torsemide RS and USP Torsemide Related Compound A RS and 0.8 µg/mL of USP Torsemide Related Compound E RS in *Mobile phase* from ▲*Standard stock solution A* and *Standard stock solution B*▲ 2S (USP41)

Sample solution: Nominally 0.4 mg/mL of torsemide prepared as follows. Weigh 40 mg of torsemide from NLT 20 powdered Tablets into a 100-mL volumetric flask. Add methanol to 30% of the flask volume, mix, and sonicate for NLT 8 min. Add *Buffer* to fill 75% of the flask volume, cool to room temperature, dilute with *Mobile phase* to volume, and mix. ▲The *Sample solution* is not stable at room temperature, but is stable for 15 h at 6°.▲ 2S (USP41)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 288 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
▲Autosampler temperature: 6°▲ 2S (USP41)
Flow rate: 0.8 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution*, ▲*Sensitivity solution*,▲ 2S (USP41) and *Standard solution*
▲[NOTE—See *Table 1* for relative retention times.]▲ 2S (USP41)
Suitability requirements
Resolution: NLT 2.5 between torsemide related compound A and torsemide related compound E, *System suitability solution*
Tailing factor: NMT 2.0 ▲for the torsemide peak, *System suitability solution*▲ 2S (USP41)
Relative standard deviation: NMT 5.0% ▲for the torsemide peak,▲ 2S (USP41) *Standard solution*
▲Signal-to-noise ratio: NLT 10.0, *Sensitivity solution*▲ 2S (USP41)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of torsemide related compound A or torsemide related compound E in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of torsemide related compound A or torsemide related compound E from the *Sample solution*
r_S = peak response of torsemide related compound A or torsemide related compound E from the *Standard solution*
C_S = concentration of USP Torsemide Related Compound A RS or USP Torsemide Related Compound E RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of torsemide in the *Sample solution* (mg/mL)

Calculate the percentage of any ▲unspecified degradation product▲ 2S (USP41) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any ▲unspecified degradation product▲ 2S (USP41) from the *Sample solution*
r_S = peak response of torsemide from the *Standard solution*
C_S = concentration of USP Torsemide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of torsemide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Torsemide related compound A▲▲ 2S (USP41)	0.39	0.6
Torsemide related compound E▲▲ 2S (USP41)	0.50	0.3
Torsemide related compound C▲a,b▲ 2S (USP41)	0.62	—
Torsemide ▲related compound▲ 2S (USP41) D▲b, c▲ 2S (USP41)	0.75	—
Torsemide	1.00	—
Torsemide related compound B▲b, d▲ 2S (USP41)	1.96	—
Any ▲unspecified degradation product▲ 2S (USP41)	—	0.2
Total impurities	—	1.1

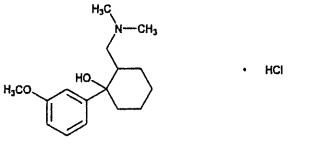
▲[a] *N*-(Ethylcarbamoyl)-4-(3-tolylamino)pyridine-3-sulfonamide. ▲ 2S (USP41)
▲[b] Process-related impurity controlled in the drug substance.▲ 2S (USP41)
▲[c] Ethyl {[4-(3-tolylamino)pyridin-3-yl]sulfonyl}carbamate.▲ 2S (USP41)
▲[d] *N*-(Butylcarbamoyl)-4-(3-tolylamino)pyridine-3-sulfonamide.▲ 2S (USP41)

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
- **LABELING:** The labeling indicates the *Dissolution* test with which the product complies, if *Test 1* is not used.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Torsemide RS
USP Torsemide Related Compound A RS
4-[(3-Methylphenyl)amino]-3-pyridinesulfonamide.
$C_{12}H_{13}N_3O_2S$ 263.32
USP Torsemide Related Compound E RS
▲4-(3-Tolyl)-2*H*-pyrido[4,3-*e*][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide.▲ 2S (USP41)
$C_{13}H_{11}N_3O_3S$ 289.31

Tramadol Hydrochloride



$C_{16}H_{25}NO_2 \cdot HCl$ 299.84
(±)-*cis*-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride;
(±)-*cis*-2-[(Dimethylamino)methyl]-1-(*m*-methoxyphenyl) cyclohexanol hydrochloride [36282-47-0].

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DEFINITION

Tramadol Hydrochloride contains NLT 98.0% and NMT 102.0% of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: An aqueous solution (1 in 100) meets the requirements.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 2 mL of trifluoroacetic acid in 1000 mL of water.

Mobile phase: Acetonitrile and *Solution A* (30:70)

System suitability solution: 0.05 mg/mL each of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Mobile phase*

Standard solution: 1.5 mg/mL of USP Tramadol Hydrochloride RS in *Mobile phase*

Sample solution: 1.5 mg/mL of Tramadol Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for tramadol related compound A and tramadol are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between tramadol related compound A and tramadol

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the portion of Tramadol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tramadol from the *Sample solution*

r_S = peak response of tramadol from the *Standard solution*

C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Tramadol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **CONTENT OF CHLORIDE**

Sample solution: 150 mg of Tramadol Hydrochloride in 40 mL of water

Analysis: To the *Sample solution* add, with stirring, 7.5 mL of 4 N nitric acid and 15.0 mL of 0.1 N silver nitrate, and titrate with 0.1 N ammonium thiocyanate VS, determining the endpoint potentiometrically, and using a silver–glass electrode system. Each mL of 0.1 N ammonium thiocyanate is equivalent to 3.545 mg of chloride.

Acceptance criteria: 11.6%–12.1% of chloride is found.

- **LIMIT OF TRAMADOL RELATED COMPOUND B**

(2-[(Dimethylamino)methyl]cyclohexanone hydrochloride)

Standard solution: 0.1 mg/mL of USP Tramadol Related Compound B RS in methanol

Sample solution: 50 mg/mL of Tramadol Hydrochloride in methanol

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Developing solvent system: Toluene, isopropyl alcohol, and 25% ammonia water (80:19:1)

Sodium nitrite solution: 50 mg/mL of sodium nitrite in water

Analysis: Place the plate in a chromatographic chamber saturated with ammonia vapor from stronger ammonia water, and allow to stand for NLT 20 min. Separately apply about 10 µL each of the *Sample solution* and the *Standard solution* to the plate, and develop the plate until the solvent front is NLT 10 cm above the line of application. Remove the plate, spray with Dragendorff's TS, and air-dry for 5 min. Spray the dried plate with *Sodium nitrite solution* until the spot from tramadol related compound B in the *Standard solution* is visible. Any secondary spot from the *Sample solution* corresponding to tramadol related compound B is not more intense than a corresponding spot from the *Standard solution*.

Acceptance criteria: NMT 0.2%

- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tramadol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Tramadol related compound A: NMT 0.2%

Individual impurities: NMT 0.1%

Total impurities: NMT 0.4%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ia* ⟨921⟩: NMT 0.5%
- **ACIDITY**

Sample solution: 500 mg of Tramadol Hydrochloride in 10 mL of water

Analysis: To the *Sample solution* add 0.2 mL of methyl red TS and 0.2 mL of 0.01 N hydrochloric acid VS, and titrate with 0.01 N sodium hydroxide VS.

Acceptance criteria: NMT 0.4 mL of 0.01 N sodium hydroxide VS is required to produce a yellow color.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tramadol Hydrochloride RS
USP Tramadol Related Compound A RS
(*RS,SR*)-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl) cyclohexanol hydrochloride.
$C_{16}H_{25}NO_2 \cdot HCl$ 299.84
USP Tramadol Related Compound B RS
(2-[(Dimethylamino)methyl]cyclohexanone hydrochloride).
$C_9H_{17}NO \cdot HCl$ 191.70

Tramadol Hydrochloride Compounded Oral Suspension

DEFINITION

Tramadol Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$).

Prepare Tramadol Hydrochloride Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Tramadol Hydrochloride tablets[a] equivalent to	500 mg of tramadol hydrochloride
Vehicle: a 1:1 mixture of Ora-Sweet[b] (sugar-free) and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Ultram 50-mg tablets, Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Tramadol Hydrochloride tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tramadol hydrochloride liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**
Solution A: 20 mM of phosphoric acid and 4 g/L of sodium 1-hexane sulfonate
Mobile phase: Acetonitrile and *Solution A* (50:50). Filter and degas.
Diluent: Acetonitrile and water (50:50)
Standard solution: 0.25 mg/mL of USP Tramadol Hydrochloride RS in *Diluent*
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.25 mg/mL of tramadol hydrochloride from Oral Suspension and *Diluent*, and centrifuge.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for tramadol hydrochloride is about 6 min.]
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.8–4.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or controlled room temperature
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tramadol Hydrochloride RS

Tramadol Hydrochloride Compounded Oral Suspension, Veterinary

DEFINITION

Tramadol Hydrochloride Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$).

Prepare Tramadol Hydrochloride Compounded Oral Suspension, Veterinary 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Tramadol Hydrochloride	2.0 g
Sodium Chloride	0.5 g
Ora-Blend[a], a sufficient quantity to make	100 mL

[a] Perrigo Pharmaceuticals, Allegan, MI.

Add *Tramadol Hydrochloride* and *Sodium Chloride* to a suitable mortar, and triturate to a fine powder. Add a small amount of *Ora-Blend*, and triturate to make a smooth paste. Add increasing volumes of *Ora-Blend* to make a tramadol hydrochloride liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Ora-Blend* to bring to final volume, and mix well. [NOTE—*Sodium Chloride* is used to mask bitterness.]

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ASSAY

• **PROCEDURE**

Solution A: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water. Add 2.0 g of sodium 1-octanesulfonate, and stir until dissolved. Adjust with phosphoric acid to a pH of 2.0.

Mobile phase: Methanol, acetonitrile, tetrahydrofuran, and *Solution A* (5:5:15:75)

Standard solution: 0.4 mg/mL of tramadol hydrochloride prepared from USP Tramadol Hydrochloride RS in *Solution A*

Sample solution: Transfer 1 mL of Oral Suspension, Veterinary into a 50-mL volumetric flask, dilute with *Solution A* to volume, and mix well.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for tramadol hydrochloride is about 13.4 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tramadol hydrochloride from the *Sample solution*

r_S = peak response of tramadol hydrochloride from the *Standard solution*

C_S = concentration of tramadol hydrochloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **PH** ⟨791⟩: 3.4–4.4

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.

• **BEYOND-USE DATE:** NMT 180 days after the day on which it was compounded when stored at controlled room temperature

• **LABELING:** Label it to indicate that it is for veterinary use only. Label it to state that it is to be well-shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Tramadol Hydrochloride RS

Tramadol Hydrochloride Tablets

DEFINITION

Tramadol Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample solution: Transfer powdered Tablets, equivalent to 200 mg of tramadol hydrochloride, into a 50-mL volumetric flask, add 20 mL of dichloromethane, and sonicate. Filter, and transfer the clear supernatant to a separating funnel. Extract the dichloromethane layer with two 10-mL portions of 2 N sodium hydroxide, and discard the aqueous layer. Dry the dichloromethane layer over anhydrous sodium sulfate, and filter. Evaporate this solution to dryness under a stream of nitrogen.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Dissolve 5 mL of perchloric acid in 950 mL of water in a 1-L volumetric flask. Add 4 mL of 25% ammonia water, dilute with water to volume, and mix. Adjust with 25% ammonia water to a pH of 2.2 ± 0.2.

Mobile phase: Acetonitrile and *Solution A* (23:77)

Standard solution: 0.1 mg/mL of USP Tramadol Hydrochloride RS in 0.1 N hydrochloric acid

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of tramadol hydrochloride, into a 100-mL volumetric flask. Add 70 mL of 0.1 N hydrochloric acid, sonicate for 5 min, and shake for 10 min. Dilute with 0.1 N hydrochloric acid to volume, and mix. Pass a portion of this solution through a suitable filter, discarding the first 20 mL of the filtrate. Transfer 10 mL of the clear filtrate into a 50-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 273 nm

Column: 3.9-mm × 15-cm; packing L7

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Solution A, Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.055 mg/mL of USP Tramadol Hydrochloride RS in *Medium*
Sample solution: Withdraw 9 mL from the dissolution vessel, and pass through a suitable filter. Discard the first 3 mL of the filtrate.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

Procedure for content uniformity
Solution A, Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Transfer 1 whole Tablet to a 100-mL volumetric flask, add 70 mL of 0.1 N hydrochloric acid, sonicate until the Tablet is completely disintegrated, and shake for 10 min. Dilute with 0.1 N hydrochloric acid to volume, and mix. Pass a portion of this solution through a suitable filter, discarding the first 20 mL of the filtrate. Transfer 10 mL of the clear filtrate into a 50-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Solution A and **Mobile phase:** Prepare as directed in the *Assay*.
System suitability solution: 0.2 mg/mL each of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Mobile phase*
Standard solution: 6 µg/mL of USP Tramadol Hydrochloride RS in *Mobile phase*
System sensitivity solution: Transfer 5 mL of the *Standard solution* into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 200 mg of tramadol hydrochloride, into a 50-mL volumetric flask. Add about 35 mL of *Mobile phase*, sonicate for 5 min, and shake for 10 min. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable filter, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 273 nm
Column: 3.9-mm × 15-cm; packing L7
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution, Standard solution,* and *System sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between tramadol related compound A and tramadol, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*; NMT 10%, *System sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of tramadol from the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

[NOTE—Disregard any peak that is shown to be due to solvents or excipients.]

Acceptance criteria
Individual unspecified impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
RS,SR-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl)cyclohexanol hydrochloride[a]	0.85	1.0	0.2
Tramadol hydrochloride	1.00	—	—
1-(3-Methoxyphenyl)-2-(dimethylaminomethyl) cyclohex-1-ene hydrochloride	5.27	1.0	0.2
1-(3-Methoxyphenyl)-2-(dimethylaminomethyl) cyclohex-6-ene hydrochloride	4.27	1.27	0.2

USP Monographs

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Impurity Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.7%

[a] Tramadol related compound A.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tramadol Hydrochloride RS
  USP Tramadol Related Compound A RS

Tramadol Hydrochloride Extended-Release Tablets

DEFINITION

Tramadol Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: Use the *Sample solution* from the *Assay*.
  Analysis: Using separate 1-cm cells, record the UV spectrum of the *Sample solution* and *Standard solution*.
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelength as that of a similar solution of the *Standard solution*.

ASSAY

- **PROCEDURE**
  Mobile phase: Tetrahydrofuran, trifluoroacetic acid, triethylamine, and water (10: 0.1: 0.1: 90). [NOTE—Maintain at a pH range of 2.2–2.4.]
  Standard stock solution: 1 mg/mL of USP Tramadol Hydrochloride RS prepared by dissolving in 20% of the flask volume of methanol. Sonicate if necessary, and dilute with water to volume.
  Standard solution: 0.13 mg/mL of USP Tramadol Hydrochloride RS in *Mobile phase*, from the *Standard stock solution*
  Sample solution: Nominally 0.13 mg/mL of tramadol hydrochloride in *Mobile phase*. Prepare by dissolving 10 Tablets in 20% of the flask volume of methanol, in a water bath for 60 min, at about 60° with intermittent shaking. Sonicate for 10 min. Add 40% of the flask volume of water, and sonicate for 30 min. If all Tablets are not fully disintegrated, then continue to sonicate until disintegration is completed. Shake the flask vigorously for 10 min using a mechanical shaker, and dilute with water to volume. Centrifuge a portion of the solution, pass through a suitable nylon filter, and collect the filtrate after discarding the first 2 mL. Pipet 5.0 mL of the filtrate into a 200-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 216 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L11
  Column temperature: 40°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 2000 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 1: 75 rpm
  Times: 2, 4, 8, 10, and 16 h
  Standard solution: (*L*/900) mg/mL of USP Tramadol Hydrochloride RS in *Medium*, where *L* is the label claim in mg/Tablet
  Sample solution: Withdraw 10 mL of the solution under test, and pass through a suitable filter of 0.45-µm pore size, discarding the first 4 mL of the filtrate. Replace the volume withdrawn with the same volume of *Medium* preheated at 37.0 ± 0.5°.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: 271 nm
  Cell: ▲5 mm▲ (ERR 1-May-2019)
  Blank: *Medium*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the concentration (C_i), in mg/mL, of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)

USP Monographs

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Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) dissolved at each time point (i):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$

$$Result_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$Result_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$Result_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$Result_5 = \{(C_5 \times V) + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of tramadol hydrochloride in the portion of the sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	NMT 15
2	4	10–40
3	8	50–85
4	10	65–95
5	16	NLT 80

The percentages of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 75 rpm
Times: 2, 4, 8, 10, and 16 h
Standard stock solution: 5 mg/mL of USP Tramadol Hydrochloride RS in *Medium*. Sonicate if necessary.
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a concentration of USP Tramadol Hydrochloride RS (see *Table 2*).

Table 2

Label Claim (mg/Tablet)	Concentration of USP Tramadol Hydrochloride RS (mg/mL)
100	0.075
200	0.100
300	0.200

Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Analytical wavelength: 271 nm
Cell
For Tablets labeled to contain 100 mg: 1 cm
For Tablets labeled to contain 200 and 300 mg: 0.5 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) dissolved at each time point (i):

$$Result_i = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	NMT 15
2	4	10–30
3	8	47–72
4	10	60–85
5	16	NLT 80

The percentages of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 75 rpm
Times: 2, 4, 8, and 16 h
Buffer: Trifluoroacetic acid and water (2:1000)
Mobile phase: Acetonitrile and *Buffer* (30:70)
Standard stock solution: 0.55 mg/mL of USP Tramadol Hydrochloride RS in water
Standard solution: (L/900) mg/mL of USP Tramadol Hydrochloride RS in *Medium* from the *Standard stock solution*, where L is the label claim of tramadol hydrochloride, in mg/Tablet. Pass the solution through a suitable filter of 0.45-µm pore size. Discard the first 5 mL of filtrate.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the portion of solution withdrawn with an equal volume of *Medium*. Discard the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 25°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0

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Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i), in mg/mL, of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of tramadol from the *Sample solution*

r_S = peak response of tramadol from the *Standard solution*

C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of tramadol hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of medium, 900 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (h)	Amount Dissolved (%)	
		100 mg/Tablet and 300 mg/Tablet	200 mg/Tablet
1	2	NMT 40	NMT 35
2	4	45–75	32–62
3	8	NLT 70	NLT 70
4	16	NLT 85	NLT 85

The percentages of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 75 rpm

Times: 2, 4, 8, 10, and 16 h

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (20:80)

Standard solution: 0.22 mg/mL of USP Tramadol Hydrochloride RS in *Medium*. Sonication may be necessary for complete dissolution.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 5-cm; 3-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: NLT 6 times the retention time of tramadol

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i), in mg/mL, of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of tramadol from the *Sample solution*

r_S = peak response of tramadol from the *Standard solution*

C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_5 = (\{C_5 \times [V - (4 \times V_S)]\} + [(C_4 + C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of tramadol hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	NMT 35
2	4	35–60
3	8	60–85
4	10	NLT 65
5	16	NLT 75

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The percentages of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 75 rpm
Times: 2, 4, 8, 10, and 16 h
Standard solution: (L/900) mg/mL of USP Tramadol Hydrochloride RS in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Withdraw NLT 8 mL of the solution under test, and pass through a suitable filter of 0.45-µm pore size, discarding the first 4 mL of the filtrate. Replace the volume withdrawn with the same volume of *Medium*.
Instrumental conditions
Mode: UV
Analytical wavelength: 271 nm
Cell
For Tablets labeled to contain 100 mg: 1 cm
For Tablets labeled to contain 200 and 300 mg: 0.5 cm
Blank: *Medium*
System suitability
Sample: *Standard solution*
Suitability requirement
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i), in mg/mL, of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{(C_5 \times V) + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of tramadol hydrochloride in the portion of the sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	NMT 15
2	4	7–37
3	8	49–74
4	10	64–89
5	16	NLT 85

The percentages of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase: Acetonitrile, trifluoroacetic acid, and water (20: 0.1: 80)
Diluent: Methanol and water (1:4)
System suitability stock solution: 0.05 mg/mL each of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Diluent* prepared by dissolving in 20% of the flask volume of methanol. Sonicate if necessary, and dilute with water to volume.
System suitability solution: 2.5 µg/mL each of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Diluent*, from the *System suitability stock solution*
Standard stock solution: 0.05 mg/mL of USP Tramadol Hydrochloride RS in *Diluent* prepared by dissolving in 20% of the flask volume of methanol. Sonicate if necessary, and dilute with water to volume.
Standard solution: 2.5 µg/mL of USP Tramadol Hydrochloride RS in *Diluent*, from the *Standard stock solution*
Sample solution: Nominally 1.2 mg/mL of tramadol hydrochloride in *Diluent*. Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 300 mg of tramadol hydrochloride, to a 250-mL volumetric flask. Add about 50 mL of methanol and heat in a water bath for 20 min at about 60°, with intermittent shaking to disperse the powder. Sonicate for 10 min. Add 100 mL of water, and sonicate with intermittent shaking for 10 min. Shake the flask vigorously for 10 min using a mechanical shaker. Dilute with water to volume, pass through a suitable nylon filter, and collect the filtrate after discarding the first 4 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 216 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 50°
Flow rate: 0.6 mL/min
Injection volume: 3 µL
Run time: 6 min
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between tramadol related compound A and tramadol, *System suitability solution*
Column efficiency: NLT 5000 theoretical plates, *Standard solution*
Capacity factor, k': NLT 1.5, *Standard solution*

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Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 6.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of tramadol from the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 7*)

Acceptance criteria: See *Table 7*.

Table 7

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desmethyl tramadol (impurity D)[a]	0.57	1.0	0.20
Tramadol related compound A[b]	0.84	1.0	0.2
Tramadol hydrochloride	1.00	—	—
1,6 Olefin[c]	2.78	3.0	—
1,2 Olefin[d]	3.28	2.2	—
Individual unspecified impurity	—	1.0	0.20
Total impurities	—	—	0.60

[a] 3-{(1*RS*,2*RS*)-2-[(Dimethylamino)methyl]-1-hydroxycyclohexyl}phenol.
[b] *RS*,*SR*-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl)cyclohexanol hydrochloride.
[c] 1-(3-Methoxyphenyl)-2-(dimethylaminomethyl) cyclohex-6-ene hydrochloride (identified and reported as an individual unspecified impurity if present).
[d] 1-(3-Methoxyphenyl)-2-(dimethylaminomethyl) cyclohex-1-ene hydrochloride (identified and reported as an individual unspecified impurity if present).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one test for *Dissolution* is given, the labeling states the test for *Dissolution* used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tramadol Hydrochloride RS
  (±)-*cis*-2-[(Dimethylamino)methyl]-1-(*m*-methoxyphenyl)cyclohexanol hydrochloride.
  $C_{16}H_{25}NO_2 \cdot HCl$ 299.84
  USP Tramadol Related Compound A RS
  RS,*SR*-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl) cyclohexanol hydrochloride.
  $C_{16}H_{25}NO_2 \cdot HCl$ 299.84

Tramadol Hydrochloride and Acetaminophen Tablets

DEFINITION

Tramadol Hydrochloride and Acetaminophen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) and acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

- **A.** The retention times of the *Tramadol sample solution* and the *Acetaminophen sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Tetrahydrofuran, triethylamine, water, and trifluoroacetic acid (8: 0.1: 92: 0.1). The apparent pH of the final solvent mixture should be between 2.2 and 2.4.
Diluent: Methanol and water (1:9)
Standard solution: 0.065 mg/mL of USP Acetaminophen RS and 0.075 mg/mL of USP Tramadol Hydrochloride RS in *Diluent*. Sonication may be used to aid dissolution.
Sample stock solution: Weigh NLT 20 Tablets, and determine the average Tablet weight. Grind the Tablets into a fine powder, and transfer an amount equivalent to one Tablet to a 50-mL volumetric flask. Add 30 mL of *Diluent* with continuous shaking to disperse the powder. Sonicate for 15 min with intermittent shaking, and shake the flask on a mechanical shaker for 30 min. Dilute with *Diluent* to volume, and mix well. Centrifuge the suspension, and use the supernatant for subsequent dilutions.
Tramadol sample solution: Nominally 75 µg/mL of tramadol hydrochloride in *Diluent* from the *Sample stock solution*
Acetaminophen sample solution: Nominally 65 µg/mL of acetaminophen in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 216 nm for tramadol hydrochloride and UV 249 nm for acetaminophen
Column: 4.6-mm × 15-cm; 5-µm packing L11
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 4 times the retention time of acetaminophen
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 10.0 between acetaminophen and tramadol
Tailing factor: NMT 2.0 for each analyte
Relative standard deviation: NMT 2.0% for each analyte
Analysis
Samples: *Standard solution*, *Tramadol sample solution*, and *Acetaminophen sample solution*
Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tramadol from the *Tramadol sample solution*
r_S = peak response of tramadol from the *Standard solution*

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C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tramadol hydrochloride in the *Tramadol sample solution* (mg/mL)

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Acetaminophen sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Acetaminophen sample solution* (mg/mL)

Acceptance criteria
Tramadol hydrochloride: 90.0%–110.0% of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$)
Acetaminophen: 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer solution: 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.50.
Mobile phase: Acetonitrile and *Buffer solution* (1:4)
Standard solution: 0.36 mg/mL of USP Acetaminophen RS and 0.04 mg/mL of USP Tramadol Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 272 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 25°
Flow rate: 1 mL/min
Injection volume: 25 µL
Run time: 2 times the retention time of tramadol
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for acetaminophen and tramadol are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between the acetaminophen and tramadol peaks
Relative standard deviation: NMT 2.0% for both the acetaminophen and tramadol peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) and tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of acetaminophen or tramadol from the *Sample solution*
r_S = peak response of acetaminophen or tramadol from the *Standard solution*
C_S = concentration of USP Acetaminophen RS or USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim for acetaminophen or tramadol hydrochloride (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) and tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 20 min
Buffer solution, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in *Dissolution Test 1*.
Tolerances: NLT 80% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) and tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **4-Aminophenol in Acetaminophen-Containing Drug Products** ⟨227⟩**:** Meet the requirements
• **Organic Impurities**
Mobile phase, Diluent, and **Sample stock solution:** Proceed as directed in the *Assay*.
Standard solution: 0.75 µg/mL each of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Diluent*
Sample solution: Pass a suitable volume of *Sample stock solution* through a nylon membrane filter of 0.45-µm pore size. Use the filtrate after discarding the first 4 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 216 nm
Column: 4.6-mm × 15-cm; 5-µm packing L11
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between tramadol related compound A and tramadol hydrochloride
Relative standard deviation: NMT 6.0% for tramadol hydrochloride
Analysis
Samples: *Diluent*, *Standard solution*, and *Sample solution*.
Disregard the peaks due to the *Diluent*.
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of tramadol from the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (µg/mL)

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C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
O-Desmethyl-tramadol[a]	0.60	0.2
Tramadol related compound A	0.80	0.2
Tramadol	1.0	—
Acetaminophen	0.38	—
Any individual, unspecified degradation product	—	0.2
Total degradation products	—	0.8

[a] 3-[(1*RS*,2*RS*)-2-[(Dimethylamino)methyl]-1-hydroxycyclohexyl]phenol.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acetaminophen RS
  USP 4-Aminophenol RS
  4-Amino-1-hydroxybenzene.
  C_6H_7NO 109.13
  USP Tramadol Hydrochloride RS
  (±)-*cis*-2-[(Dimethylamino)methyl]-1-(*m*-methoxyphenyl)cyclohexanol hydrochloride.
  $C_{16}H_{25}NO_2 \cdot HCl$ 299.84
  USP Tramadol Related Compound A RS
  RS,*SR*-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl) cyclohexanol hydrochloride.

Tramadol Hydrochloride and Acetaminophen Compounded Oral Suspension

DEFINITION

Tramadol Hydrochloride and Acetaminophen Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amounts of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) and acetaminophen ($C_8H_9NO_2$).

Prepare Tramadol Hydrochloride and Acetaminophen Compounded Oral Suspension containing 7.5 mg/mL of tramadol hydrochloride and 65 mg/mL of acetaminophen as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Tramadol Hydrochloride and Acetaminophen tablets[a]	750 mg of tramadol hydrochloride and 6500 mg of acetaminophen
Vehicle: a 1:1 mixture of Ora-Sweet[b] (sugar-free) and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Ultracet 37.5-mg/325-mg tablets, Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Tramadol Hydrochloride and Acetaminophen tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tramadol hydrochloride and acetaminophen liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

- **TRAMADOL HYDROCHLORIDE**

Solution A: 20 mM of phosphoric acid and 4 g/L of sodium 1-hexane sulfonate
Mobile phase: Acetonitrile and *Solution A* (50:50). Filter and degas.
Diluent: Acetonitrile and water (50:50)
Standard solution: 0.15 mg/mL of USP Tramadol Hydrochloride RS in *Diluent*
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.15 mg/mL of tramadol hydrochloride from Oral Suspension and *Diluent*, and centrifuge.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for tramadol hydrochloride is about 6 min.]
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tramadol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

- **ACETAMINOPHEN**

Mobile phase: Acetonitrile and water (70:30). Filter and degas.
Standard solution: 65 µg/mL of USP Acetaminophen RS in *Mobile phase*
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 65 µg/mL of acetaminophen from Oral Suspension and *Mobile phase*, and centrifuge.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min

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Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for acetaminophen is about 2 min.]
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

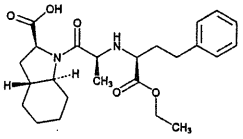
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **PH** ⟨791⟩**:** 3.8–4.8

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature
• **LABELING:** Label to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Tramadol Hydrochloride RS

Trandolapril



$C_{24}H_{34}N_2O_5$ 430.54
1*H*-Indole-2-carboxylic acid, octahydro *N*-[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]-1-alanyl (2*S*,3a*R*,7a*S*);
(2*S*,3a*R*,7a*S*)-1-[(*S*)-*N*-[(*S*)-1-Carboxy-3-phenylpropyl]alanyl] hexahydro-2-indolinecarboxylic acid, 1-ethyl ester [87679-37-6].

DEFINITION
Trandolapril contains NLT 98.0% and NMT 102.0% of trandolapril ($C_{24}H_{34}N_2O_5$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020) or ⟨197M⟩

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard stock solution: 0.1 mg/mL of USP Trandolapril RS in *Mobile phase*
Standard solution: 0.002 mg/mL ▲of USP Trandolapril RS▲ (USP 1-May-2019) in *Mobile phase* from *Standard stock solution*
Sample stock solution: ▲0.1▲ (USP 1-May-2019) mg/mL of Trandolapril in *Mobile phase*
Sample solution: 0.002 mg/mL of Trandolapril in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: NLT 5.5 times the retention time of trandolapril
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT ▲0.73%▲ (USP 1-May-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trandolapril ($C_{24}H_{34}N_2O_5$) in the portion of Trandolapril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response ▲of trandolapril▲ (USP 1-May-2019) from the *Sample solution*
r_S = peak response ▲of trandolapril▲ (USP 1-May-2019) from the *Standard solution*
C_S = concentration of USP Trandolapril RS in the *Standard solution* (mg/mL)
C_U = concentration of Trandolapril in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Change to read:

• ▲▲ (USP 1-May-2019) **RESIDUE ON IGNITION** ⟨281⟩
Sample: 2.0 g
Acceptance criteria: NMT 0.1%

Change to read:

• ▲**ORGANIC IMPURITIES**▲ (USP 1-May-2019)
Buffer: 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.5 ± 0.1.

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Solution A: Acetonitrile and *Buffer* (25:75), filtered and degassed
Solution B: Acetonitrile and *Buffer* (50:50), filtered and degassed
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	95	5
35	5	95
45	5	95
46	95	5
60	95	5

System suitability solution: 0.05 mg/mL each of USP Trandolapril Related Compound C RS and USP Trandolapril Related Compound D RS in *Solution A*
Standard solution: 0.0025 mg/mL of USP Trandolapril RS in *Solution A*
Sample solution: 2.5 mg/mL of Trandolapril in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; ▲3.5-µm▲ (USP 1-May-2019) packing L1
Column temperature: 40°
Flow rate: 1.3 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4 between trandolapril related compound C and trandolapril related compound D, *System suitability solution*
Tailing factor: ▲NMT▲ (USP 1-May-2019) 1.5, *Standard solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each ▲specified or any unspecified▲ (USP 1-May-2019) impurity in the portion of Trandolapril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each ▲specified or unspecified▲ (USP 1-May-2019) impurity from the *Sample solution*
r_S = peak response of trandolapril from the *Standard solution*
C_S = concentration of ▲USP Trandolapril RS in▲ (USP 1-May-2019) the *Standard solution* (mg/mL)
C_U = concentration of ▲Trandolapril in▲ (USP 1-May-2019) the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. [NOTE—Do not include trandolapril related compound D in the total impurities.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Trandolapril related compound C▲ (USP 1-May-2019)	2.1	0.45	0.2
Trandolapril related compound D▲ (USP 1-May-2019)	2.5	1.0	0.5
▲Any unspecified impurity▲ (USP 1-May-2019)	—	1.0	0.1
Total impurities	—	—	0.5

▲ (USP 1-May-2019)

SPECIFIC TESTS
• **OPTICAL ROTATION,** ⟨781S⟩ *Procedures, Specific Rotation*
Sample: 0.02 g/mL in dehydrated alcohol
Acceptance criteria: −16.5° to −18.5° at 20°
• **WATER DETERMINATION,** ⟨921⟩ *Method I, Method Ic*
Sample: 2 g
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Trandolapril RS
USP Trandolapril Related Compound C RS
(2*S*,3a*R*,7a*S*)-1-[*N*-[(*S*)-1-Carboxy-3-cyclohexylpropyl]-L-alanyl]hexahydro-2-indolinecarboxylic acid 1-ethyl ester.
$C_{24}H_{40}N_2O_5$ 436.58
USP Trandolapril Related Compound D RS
(*S*)-Ethyl 2-[(3*S*,5a*S*,9a*R*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl)]-4-phenylbutanoate.
$C_{24}H_{32}N_2O_4$ 412.52

Trandolapril Tablets

DEFINITION
Trandolapril Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: To 1 L of 8.9 g/L of dibasic sodium phosphate anhydrous, add 1 mL of triethylamine and adjust with phosphoric acid to a pH of 3.5. Pass through a suitable filter of 0.45-µm pore size.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Diluent: Acetonitrile and water (40:60)
Standard solution: 40 µg/mL of USP Trandolapril RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Trandolapril RS to a suitable volumetric flask. Add *Diluent* up to 70% of the flask volume. Sonicate if necessary. Dilute with *Diluent* to volume.

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Sample solution: Nominally 40 µg/mL of trandolapril in *Diluent* prepared as follows. Transfer a suitable quantity of trandolapril from NLT 10 Tablets to a suitable volumetric flask. Add *Diluent* up to 70% of the flask volume. Sonicate up to 30 min, if necessary, with occasional swirling. Dilute with *Diluent* to volume. Pass a portion of the solution through a membrane filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 216 nm. For *Identification B*, use a diode array detector in the range of 190–400 nm.
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of trandolapril

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trandolapril from the *Sample solution*
r_S = peak response of trandolapril from the *Standard solution*
C_S = concentration of USP Trandolapril RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of trandolapril in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water, deaerated; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase and **System suitability:** Proceed as directed in the *Assay*.
Standard stock solution: 0.2 mg/mL of USP Trandolapril RS prepared as follows. Transfer a suitable quantity of USP Trandolapril RS to a suitable volumetric flask. Add acetonitrile to about 5% of the flask volume, sonicate to dissolve, and dilute with *Medium* to volume.
Standard solution: (*L*/500) mg/mL of USP Trandolapril RS from the *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system: Proceed as directed in the *Assay*, except for the *Injection volume*.
Injection volume: 100 µL

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of trandolapril from the *Sample solution*
r_S = peak response of trandolapril from the *Standard solution*
C_S = concentration of USP Trandolapril RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer: To 1 L of 7.0 g/L of sodium perchlorate monohydrate in water (0.05 M), add 2 g of octanesulfonic acid sodium salt followed by 1 mL of triethylamine. Adjust with diluted perchloric acid (1 in 10) to a pH of 2.0. Pass through a membrane filter of 0.45-µm pore size.
Solution A: Acetonitrile, tetrahydrofuran, and *Buffer* (11:1:28)
Solution B: Acetonitrile and *Buffer* (75:25)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
25	85	15
50	50	50
55	85	15
65	85	15

Diluent: Acetonitrile and *Buffer* (40:60)
Standard stock solution 1: 0.06 mg/mL each of USP Trandolapril Related Compound C RS and USP Trandolapril Related Compound D RS in acetonitrile. Sonicate to dissolve.
System suitability solution: 0.6 mg/mL of USP Trandolapril RS and 6 µg/mL each of USP Trandolapril Related Compound C RS and USP Trandolapril Related Compound D RS from *Standard stock solution 1* in *Diluent*. Add *Diluent* up to 70% of the flask volume, sonicate to dissolve, and cool to room temperature. Dilute with *Diluent* to volume.
Standard stock solution 2: 0.3 mg/mL of USP Trandolapril RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Trandolapril RS to a suitable volumetric flask. Add *Diluent* up to 70% of the flask volume, sonicate, and cool to room temperature. Dilute with *Diluent* to volume.
Standard solution: 6 µg/mL of USP Trandolapril RS in *Diluent* from *Standard stock solution 2*.
Sample solution: Nominally 600 µg/mL of trandolapril in *Diluent* prepared as follows. Transfer a suitable quantity of trandolapril from NLT 15 Tablets to a suitable volumetric flask. Add *Diluent* up to 50% of the flask volume and sonicate for 30 min. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL

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System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between trandolapril related compound C and trandolapril related compound D, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified and unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified and unspecified degradation product from the *Sample solution*
r_S = peak response of trandolapril from the *Standard solution*
C_S = concentration of USP Trandolapril RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of trandolapril in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Reporting threshold: 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Trandolaprilat[a]	0.41	1.0	2.0
Trandolapril	1.00	1.0	—
Trandolapril related compound C	1.84	—	—[b]
Trandolapril related compound D	1.96	0.78	5.0
Any unspecified degradation product	—	1.00	1.0
Total degradation products	—	—	7.0

[a] (2*S*,3a*R*,7a*S*)-1-[*N*-[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]hexahydro-2-indolinecarboxylic acid.
[b] Process-related impurity used for resolution measurement only.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Trandolapril RS
  USP Trandolapril Related Compound C RS
  (2*S*,3a*R*,7a*S*)-1-[*N*-[(*S*)-1-Carboxy-3-cyclohexylpropyl]-L-alanyl]hexahydro-2-indolinecarboxylic acid 1-ethyl ester hydrochloride.
  $C_{24}H_{40}N_2O_5 \cdot HCl$ 473.05
  USP Trandolapril Related Compound D RS
  (*S*)-Ethyl 2-[(3*S*,5a*S*,9a*R*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl)]-4-phenylbutanoate.
  $C_{24}H_{32}N_2O_4$ 412.52

Trandolapril and Verapamil Hydrochloride Extended-Release Tablets

DEFINITION

Trandolapril and Verapamil Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) and verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the trandolapril and verapamil peaks of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peaks from the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer 1: 7 mL/L of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0.
  Buffer 2: 0.82 mg/mL of sodium acetate anhydrous in acetic acid
  Mobile phase: Acetonitrile, methanol, and *Buffer 1* (240:160:600)
  Diluent 1: Methanol and water (80:20)
  Diluent 2: Acetonitrile, 2-aminoheptane, and *Buffer 2* (30: 0.5: 70). Adjust with 10 M sodium hydroxide to a pH of 5.0.
  Standard solution: 20 µg/mL of USP Trandolapril RS and 24 µg/mL of USP Verapamil Hydrochloride RS in *Diluent 1* prepared as follows. Add *Diluent 1* to USP Trandolapril RS and USP Verapamil Hydrochloride RS to fill about 70% of the volume of the flask, sonicate to facilitate dissolution, and then dilute with *Diluent 1* to volume.
  Sample solution 1: Nominally 20 µg/mL of trandolapril from NLT 5 Tablets in *Diluent 1* prepared as follows. Add *Diluent 1* to the sample, to fill about 50% of the volume of the flask, and sonicate for 45 min with occasional swirling. Cool and dilute with *Diluent 1* to volume. Pass this solution through a suitable filter of 0.45-µm pore size.
  Alternatively, *Sample solution 1* can be prepared as follows. Suspend NLT 20 Tablets in 950 mL of *Diluent 2* taken in a 1-L volumetric flask. Stir for about 2 h with a stir bar at NLT 800 rpm until the Tablets are completely disintegrated. [NOTE—Ensure that the Tablets do not adhere to the bottom of the vessel.] Remove the stir bar and rinse with *Diluent 2*. Dilute with *Diluent 2* to volume and shake thoroughly. Centrifuge a portion of the suspension for 10 min, pipet a portion of the supernatant, and pass through a suitable filter. Discard the first 5 mL of the filtrate.
  Sample solution 2: Nominally 24 µg/mL of verapamil hydrochloride in *Diluent 1* or *Diluent 2* from *Sample solution 1*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 216 nm. For *Identification B*, use a diode array detector in the range of UV 200–400 nm.
  Column: 4.6-mm × 15-cm; 3-µm packing L1
  Column temperature: 40°
  Flow rate: 1.3 mL/min
  Injection volume: 20 µL
  Run time: NLT 1.5 times the retention time of the trandolapril peak

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System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both the trandolapril and verapamil peaks
Relative standard deviation: NMT 2.0% for both the trandolapril and verapamil peaks
Analysis
Samples: *Standard solution*, *Sample solution 1*, and *Sample solution 2*
Calculate the percentage of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) and verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trandolapril from *Sample solution 1* or verapamil from *Sample solution 2*
r_S = peak response of trandolapril or verapamil from the *Standard solution*
C_S = concentration of USP Trandolapril RS or USP Verapamil Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of trandolapril in *Sample solution 1* or verapamil hydrochloride in *Sample solution 2* (µg/mL)

Acceptance criteria
Trandolapril: 90.0%–110.0%
Verapamil hydrochloride: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩**: TEST 1**
For trandolapril
Medium: Water; 500 mL
Apparatus 2: 50 rpm
Time: 60 min
Buffer 1, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* except for the following.
Flow rate: 1 mL/min
Injection volume: 100 µL
Mobile phase: Acetonitrile and *Buffer 1* (400:600)
Standard stock solution: Prepare 0.2 mg/mL of USP Trandolapril RS as follows. Transfer USP Trandolapril RS to a suitable volumetric flask, add about 5% of the volume of the flask of acetonitrile, sonicate to dissolve, and dilute with *Medium*.
Standard solution: (*L*/500) mg/mL in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V) \times (1/L) \times 100$$

r_U = peak response of trandolapril from the *Sample solution*
r_S = peak response of trandolapril from the *Standard solution*
C_S = concentration of USP Trandolapril RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) is dissolved.
For verapamil hydrochloride
Acid stage medium: Simulated gastric fluid (without enzyme); 900 mL
Buffer stage medium: Simulated intestinal fluid (without enzyme); 900 mL
Apparatus 2: 50 rpm, with a sinker (see *Dissolution* ⟨711⟩, *Apparatus, Figure 2a*)
Times: 1 h in *Acid stage medium*; 2, 3.5, 5, and 8 h in *Buffer stage medium*
Mobile phase: Acetonitrile and *Buffer 1* (400:600)
Buffer 1, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* except for the following.
Detector: UV 278 nm
Flow rate: 1 mL/min
Injection volume: 10 µL
Standard stock solution: Prepare 1.34 mg/mL of USP Verapamil Hydrochloride RS as follows. Transfer USP Verapamil Hydrochloride RS to a suitable volumetric flask, add about 20% of the volume of the flask of methanol, sonicate to dissolve, cool to room temperature, and dilute with water to volume.
Standard solution: (*L*/900) mg/mL in the respective *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Withdraw 10 mL of solution at each time interval, and centrifuge at 2500 rpm for 5 min.
Analysis
Samples: *Standard solution* and *Sample solutions*
Place 1 Tablet in the sinker and place the sinker in the vessel. Prepare *Samples* as directed under *Sample solutions*.
Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved in the acid stage:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of verapamil from the *Sample solution*
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution*
V = volume of *Acid stage medium*, 900 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the sample withdrawn from the vessel at each buffer stage time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of verapamil from the *Sample solution* at each time point (*i*)
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

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$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_5 = (\{C_5 \times [V - (4 \times V_S)]\} + [(C_4 + C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of verapamil hydrochloride in the *Sample solution* at the specified time point (i) (mg/mL)
V = volume of *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point in the buffer stage (mL)

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	7–20
2	15–40
3.5	40–70
5	NLT 60
8	NLT 85

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **DISSOLUTION** ⟨711⟩: **TEST 2**

[NOTE—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.]

For trandolapril and verapamil hydrochloride (acid stage)

[NOTE—During analysis, place the bi-layer Tablet in the dissolution vessel such that the trandolapril layer faces the bottom of the vessel.]

Medium: Simulated gastric fluid (without enzyme); 900 mL
Apparatus 2: 50 rpm, suitable wire helix sinkers
Times
Trandolapril: 45 min
Verapamil hydrochloride: 1 h
Buffer: Dissolve 6.8 g of monobasic potassium phosphate and the contents from one vial of paired ion chromatography reagent into a 1-L volumetric flask. Dissolve in approximately 950 mL of water. Adjust with dilute potassium hydroxide to a pH of 6.0 and then dilute with water to volume.
Solution A: Acetonitrile and *Buffer* (15:85)
Solution B: Acetonitrile and *Buffer* (60:40)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
6	0	100
13	0	100
14	100	0
20	100	0

Standard stock solution: 0.56 mg/mL of USP Trandolapril RS in methanol
Standard solution 1: (L/900) mg/mL of USP Trandolapril RS in 0.1 N hydrochloric acid from the *Standard stock solution*, where L is the label claim in mg/Tablet. [NOTE—For better recoveries during analysis, suitable quantities of an excipient mixture used in the formulation are mixed with the *Standard solution 1* before dilution as follows: 0.85 mg of excipient mixture per milliliter of *Standard solution 1* for trandolapril/verapamil hydrochloride Tablet strength 2/180; 1.05 mg of excipient mixture per milliliter of *Standard solution 1* for trandolapril/verapamil hydrochloride Tablet strengths 1/240, 2/240, and 4/240.]
Standard solution 2: (L/900) mg/mL of USP Verapamil Hydrochloride RS in 0.1 N hydrochloric acid, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system: Procedure for trandolapril
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 2-mm × 30-mm; 7-µm packing L1
Flow rate: 1 mL/min
Injection volume: 250 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution 1* and *Sample solution*
Use a portion of the sample withdrawn at 45 min to analyze the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) dissolved and save the rest for analyzing verapamil hydrochloride.
Calculate the percentage of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) dissolved:

$$Result = (r_U/r_S) \times (C_S \times V) \times (1/L) \times 100$$

r_U = peak response of trandolapril from the *Sample solution*
r_S = peak response of trandolapril from *Standard solution 1*
C_S = concentration of USP Trandolapril RS in *Standard solution 1* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Instrumental conditions: Procedure for verapamil hydrochloride
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Wavelength range: 250–300 nm
Cell: 1 cm
Analysis
Samples: *Standard solution 2* and *Sample solution*

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For the total labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved in the acid stage, combine the results from the analysis of the sample withdrawn at 45 min and also at 1 h.

Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at each time point using a suitable multicomponent analysis software.

Tolerances

Trandolapril: NLT 80% (*Q*) of the labeled amount of trandolapril ($C_{24}H_{34}N_2O_5$) is dissolved.

Verapamil hydrochloride: See *Table 3* at 1 h.

For verapamil hydrochloride (buffer stage)

Buffer stage medium: Simulated intestinal fluid TS (without enzyme); 900 mL. Adjust with 0.2 N sodium hydroxide to a pH of 7.5.

Apparatus 2: 50 rpm

Times: 2, 3.5, 5, and 8 h

Sample solutions: Pass portions of the solution under test at each time interval through a suitable filter of 0.45-µm pore size.

Instrumental conditions and **Analysis:** Proceed as directed under verapamil hydrochloride (acid stage).

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved for Tablet Strength Trandolapril 2/ Verapamil Hydrochloride 180 (mg/mg) (%)	Amount Dissolved for All Other Tablet Strengths (%)
1	6–17	4–16
2	14–30	9–25
3.5	36–66	30–60
5	57–90	51–90

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements

IMPURITIES

• **TRANDOLAPRIL RELATED IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the type of formulation, perform either *Trandolapril Related Impurities, Procedure 1* and *Content of Trandolapril Related Compound E*, or *Trandolapril Related Impurities, Procedure 2*. *Trandolapril Related Impurities, Procedure 2* is recommended when the formulation is a bi-layered Tablet.]

Buffer 1: Prepare a mixture of 2.72 g/L of monobasic potassium phosphate and 3.5 g/L of sodium 1-decane sulfonate. The pH of the resulting solution is about 4.68.

Buffer 2: Prepare a mixture of 2.72 g/L of monobasic potassium phosphate and 3 g/L of sodium 1-octane sulfonate. Adjust with triethylamine to a pH of 6.6.

Diluent: Acetonitrile, methanol, and water (25:25:50)

Solution A: Acetonitrile and *Buffer 1* (15:85)

Solution B: Acetonitrile, methanol, and *Buffer 2* (70:7:30)

Solution C: Acetonitrile, methanol, and *Buffer 2* (70:4:30)

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	74	26	0
22	74	26	0
29	53	47	0
35	46	54	0
37	45	0	55
70	30	0	70
75	0	100	0
95	0	100	0
100	74	26	0
110	74	26	0

Trandolapril related compound A stock solution: 5 µg/mL of USP Trandolapril Related Compound A RS prepared as follows. Transfer USP Trandolapril Related Compound A RS to a suitable volumetric flask, add about 10% of the volume of the flask of methanol, and sonicate to dissolve. Cool and dilute with *Diluent* to volume.

System suitability solution: 0.125 mg/mL of USP Trandolapril RS and 0.15 µg/mL of USP Trandolapril Related Compound A RS in *Diluent* prepared as follows. Add about 30% of the volume of the flask of methanol to USP Trandolapril RS, and sonicate to dissolve. Cool, add appropriate quantities of *Trandolapril related compound A stock solution*, and dilute with *Diluent* to volume.

Standard solution: 1.2 µg/mL of USP Trandolapril RS in *Diluent*

Sample solution: Nominally 0.125 mg/mL of trandolapril in *Diluent* prepared as follows. Transfer an equivalent to 6.25 mg of trandolapril, from finely powdered Tablets (NLT 10), to a 50-mL volumetric flask, add 30 mL of *Diluent*, and sonicate for 15 min with intermittent and vigorous shaking. Cool and dilute with *Diluent* to volume. Centrifuge the resulting solution at 2500 rpm for 20 min, and pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run times: NLT 1.5 times the retention time of trandolapril for the *Standard solution*, and NLT 7.0 times for the *System suitability solution* and the *Sample solution*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between trandolapril and trandolapril related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for trandolapril, *System suitability solution*

Relative standard deviation: NMT 5.0% for trandolapril, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of trandolapril from the *Standard solution*
C_S = concentration of USP Trandolapril RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of trandolapril in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Trandolapril related compound E[a]	0.16	—
Trandolapril related compound A[b]	0.59	—
Trandolapril	1.00	—
Trandolapril isopropyl ester[b, c]	1.98	—
Trandolapril related compound C[b, d]	2.15	—
Trandolapril related compound D	3.79	3.5
Any unspecified impurity	—	0.5
Total impurities[e]	—	2.0

[a] This impurity is controlled using the procedure described under *Content of Trandolapril Related Compound E*.
[b] Process related impurity and is controlled in the drug substance.
[c] (2*S*,3a*R*,7a*S*)-1-[*N*-[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]hexahydro-2-indolinecarboxylic acid 1-isopropyl ester.
[d] (2*S*,3a*R*,7a*S*)-1-[*N*-[(*S*)-1-Carboxy-3-cyclohexylpropyl]-L-alanyl]hexahydro-2-indolinecarboxylic acid 1-ethyl ester.
[e] Total impurities include all process related and degradation impurities from both *Trandolapril Related Impurities* and *Verapamil Hydrochloride Related Impurities* procedures except for trandolapril related compound D and do not include the content of trandolapril related compound E.

• **TRANDOLAPRIL RELATED IMPURITIES, PROCEDURE 2**

[NOTE—*Trandolapril Related Impurities, Procedure 2* is recommended when the formulation is a bi-layered Tablet.]

Diluent: Ethanol and water (50:50)
Solution A: Acetonitrile and isopropanol (25:75)
Solution B: *Solution A*, triethylamine, phosphoric acid, and water (20: 0.1: 0.1: 80)
Solution C: *Solution A*, triethylamine, phosphoric acid, and water (40: 0.1: 0.1: 60)
Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution B (%)	Solution C (%)
0	100	0
30	0	100
32	100	0
40	100	0

Standard stock solution: 0.05 mg/mL each of USP Trandolapril Related Compound D RS and USP Trandolapril Related Compound E RS prepared as follows. Add about 50% of the volume of the flask of alcohol and then dilute with water to volume.
System suitability solution: 0.8 mg/mL of USP Verapamil Hydrochloride RS and 4 µg/mL each of USP Trandolapril Related Compound D RS and USP Trandolapril Related Compound E RS prepared as follows. Transfer a suitable quantity of USP Verapamil Hydrochloride RS and a suitable quantity of *Standard stock solution* to a suitable volumetric flask, and dilute with *Diluent* to volume.
Standard solution: 2 µg/mL each of USP Trandolapril Related Compound D RS and USP Trandolapril Related Compound E RS from the *Standard stock solution* in *Diluent*
Sample solution: Cut off the trandolapril layer of a sufficient number of Tablets with a tablet cutter or scalpel. Finely powder the trandolapril layer mixture using a mortar and pestle and transfer a suitable quantity of the powder to a suitable volumetric flask as described in *Table 7*.

Table 7

Strength of Tablet [Trandolapril (mg)/ Verapamil Hydrochloride (mg)]	Amount of Trandolapril Layer Mixture (mg)	Initial Volume of the Diluent (mL)	Volume of the Flask (mL)
1/240	1050	25	25
2/180	1050	50	50
2/240	1050	50	50
4/240	1050	100	100

Dissolve the mixture in a volume of *Diluent* per *Table 7* and stir for about 10 min. Further sonication may be necessary for complete dissolution. Remove and rinse the stir bar with *Diluent* and dilute with *Diluent* to volume. Centrifuge the contents for about 10 min and use the supernatant. Pass through a suitable filter of 0.45-µm size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 and 278 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Temperatures
Autosampler: 2°–8°
Column: 45°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: NLT 3.0 times the retention time of trandolapril
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between verapamil and trandolapril related compound E, *System suitability solution*
Relative standard deviation: NMT 2.0% for both peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Overlay the chromatograms at 215 nm and at 278 nm. Disregard any peaks detected in the chromatogram at 278 nm as verapamil related impurity peaks.
Calculate the percentage of trandolapril related compound D and trandolapril related compound E impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

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r_S = peak response of trandolapril related compound D or trandolapril related compound E from the *Standard solution*
C_S = concentration of USP Trandolapril Related Compound D RS or USP Trandolapril Related Compound E RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of trandolapril in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of trandolapril related compound D from the *Standard solution*
C_S = concentration of USP Trandolapril Related Compound D RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of trandolapril in the *Sample solution* (µg/mL)
F = relative response factor, 1.2

Acceptance criteria: See *Table 8*.

Table 8

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Trandolapril related compound E	0.66	2.0
Trandolapril	1.00	—
Trandolapril related compound D	1.97	3.5
Any unspecified impurity	—	0.5
Total impurities[a]	—	1.0

[a] Total impurities include all process related and degradation impurities from *Trandolapril Related Impurities, Procedure 2* except for trandolapril related compound D and trandolapril related compound E.

• **CONTENT OF TRANDOLAPRIL RELATED COMPOUND E**

[NOTE—The *Content of Trandolapril Related Compound E* procedure is recommended when *Trandolapril Related Impurities, Procedure 1* is used.]

Buffer: 2.72 g/L of monobasic potassium phosphate and 1.0 g/L of octane sulphonic acid. Adjust with 2% (v/v) phosphoric acid to a pH of 4.0.
Diluent 1: Acetonitrile and water (90:10)
Diluent 2: Acetonitrile and *Buffer* (20:80)
Mobile phase: See *Table 9*.

Table 9

Time (min)	Buffer (%)	Acetonitrile (%)
0	84	16
20	84	16
22	20	80
32	20	80
35	84	16
45	84	16

Standard stock solution: 0.05 mg/mL of USP Trandolapril Related Compound E RS prepared as follows. Transfer a suitable amount of USP Trandolapril Related Compound E RS to a suitable volumetric flask and add methanol to about 5% of the total volume. Sonicate to dissolve, cool, and dilute with *Diluent 2* to volume.
Standard solution: 0.5 µg/mL of USP Trandolapril Related Compound E RS in *Diluent 2* from *Standard stock solution*
Sample stock solution: Nominally 0.3 mg/mL of trandolapril prepared as follows. Transfer a quantity nominally equivalent to 7.5 mg of trandolapril, from finely powdered Tablets (NLT 10), to a 25-mL volumetric flask, add 15 mL of *Diluent 1*, and sonicate for 15 min with intermittent swirling. Cool and dilute with *Diluent 1* to volume.
Sample solution: Nominally 0.075 mg/mL of trandolapril in *Buffer* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run times: NLT 1.5 times and NLT 3.5 times the retention time of trandolapril related compound E for the *Standard solution* and the *Sample solution*, respectively
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trandolapril related compound E in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trandolapril related compound E from the *Sample solution*
r_S = peak response of trandolapril related compound E from the *Standard solution*
C_S = concentration of USP Trandolapril Related Compound E RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of trandolapril in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 2.0%. Disregard peaks below 0.045%.

• **VERAPAMIL HYDROCHLORIDE RELATED IMPURITIES**

Buffer: 6.8 g/L of monobasic potassium phosphate and 1.0 g/L of sodium 1-octane sulfonate. Adjust with phosphoric acid to a pH of 2.5.
Diluent: Acetonitrile, methanol, and water (25:25:50)
Mobile phase: Acetonitrile and *Buffer* (33:67)
Verapamil related compound B stock solution: 0.05 mg/mL of USP Verapamil Related Compound B RS in *Diluent*
System suitability solution: 1.25 mg/mL of USP Verapamil Hydrochloride RS and 5 µg/mL of USP Verapamil Related Compound B RS in *Diluent*. Initially add *Diluent* to USP Verapamil Hydrochloride RS to fill about 50% of the volume of the flask, and sonicate to dissolve. Cool and add appropriate quantities of *Verapamil related compound B stock solution*, then dilute with *Diluent* to volume.
Standard stock solution: 0.3 mg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*. Initially add *Mobile phase*

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to about 70% of the volume of the flask, and sonicate to dissolve. Cool, and dilute with *Mobile phase* to volume.

Standard solution: 0.12 mg/mL of USP Verapamil Hydrochloride RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Nominally 1.2 mg/mL of verapamil hydrochloride in *Mobile phase* prepared as follows. Transfer a quantity equivalent to 240 mg of verapamil hydrochloride, from finely powdered Tablets (NLT 10), to a 200-mL volumetric flask, add 150 mL of *Mobile phase*, and sonicate for 30 min with intermittent vigorous shaking. Cool, and dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run times: NLT 1.6 times the retention time of verapamil for the *Standard solution*, and NLT 3.0 times for the *System suitability solution* and the *Sample solution*

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between verapamil related compound B and verapamil, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

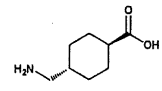
r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Disregard peaks below 0.02%.
Any other individual unknown impurity: NMT 0.5%
Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in a well-closed container at 15°–25°.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Trandolapril RS
  USP Trandolapril Related Compound A RS
  (2*S*,3a*R*,7a*S*)-1-[*N*-[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]hexahydro-2-indolinecarboxylic acid 1-methyl ester.
  $C_{23}H_{32}N_2O_5$ 416.51
  USP Trandolapril Related Compound D RS
  (*S*)-Ethyl 2-[(3*S*,5a*S*,9a*R*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl)]-4-phenylbutanoate.
  $C_{24}H_{32}N_2O_4$ 412.52
  USP Trandolapril Related Compound E RS
  (2*S*,3a*R*,7a*S*)-1-[*N*-[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]hexahydro-2-indolinecarboxylic acid.
  $C_{22}H_{30}N_2O_5$ 402.48
  USP Verapamil Hydrochloride RS
  USP Verapamil Related Compound B RS
  α-[2-[[2-(3,4-Dimethoxyphenyl)-ethyl]methylamino] ethyl]-3,4-dimethoxy-α-(1-methylethyl)-benzeneacetonitrile monohydrochloride.
  $C_{26}H_{36}N_2O_4 \cdot HCl$ 477.05

Tranexamic Acid



$C_8H_{15}NO_2$ 157.21
trans-4-(Aminomethyl)cyclohexanecarboxylic acid; Cyclohexanecarboxylic acid, 4-(aminomethyl)-, *trans* [1197-18-8].

DEFINITION

Tranexamic Acid contains NLT 99.0% and NMT 101.0% of $C_8H_{15}NO_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample solution: 140 mg of Tranexamic Acid in 20 mL of glacial acetic acid

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric

Analysis
Sample: *Sample solution*
Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Carry out a blank titration.
Each mL of 0.1 N perchloric acid is equivalent to 15.72 mg of $C_8H_{15}NO_2$.

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%; 1-g sample is used
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩: A 0.51-g portion shows no more chloride than corresponds to 0.1 mL of 0.020 N hydrochloric acid (0.014%).

ORGANIC IMPURITIES

- **Procedure**

Mobile phase: Dissolve 11.0 g of anhydrous monobasic sodium phosphate in 500 mL of water, and add 5 mL of triethylamine, followed by 1.4 g of sodium lauryl sulfate. Adjust with diluted phosphoric acid (10% w/w) to a pH of 2.5, and dilute with water to 600 mL. Mix this solution with 400 mL of methanol.

System suitability solution: 0.2 mg/mL of USP Tranexamic Acid RS and 0.002 mg/mL of USP Tranexamic Acid Related Compound C RS in water

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Standard solution: 50 µg/mL of USP Tranexamic Acid RS in water
Sample solution: 10 mg/mL of Tranexamic Acid in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: 3 times the retention time of tranexamic acid
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between tranexamic acid and 0.002 mg/mL of tranexamic acid related compound C
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Tranexamic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (0.1F)$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for tranexamic acid from the *Standard solution*
C_S = concentration of USP Tranexamic Acid RS in the *Standard solution* (µg/mL)
C_U = concentration of Tranexamic Acid in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.2%
[NOTE—Disregard any peak less than 0.025%.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tranexamic acid related compound A[a]	2.1	1	0.1
Tranexamic acid related compound B[b]	1.5	1.2	0.2
Tranexamic acid related compound C[c]	1.1	0.005	0.1
Tranexamic Acid	1.0	1.0	—
Tranexamic acid related compound D[d]	1.3	0.006	0.1

[a] *trans,trans*-4,4′-(Iminodimethylene)di(cyclohexanecarboxylic)acid.
[b] *cis*-4-(Aminomethyl)cyclohexanecarboxylic acid.
[c] (*RS*)-4-(Aminomethyl) cyclohex-1-enecarbocylic acid.
[d] 4-Aminomethyl benzoic acid.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩**:** Dry 1.00 g at 105° under vacuum for 2 h. It loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature not exceeding 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tranexamic Acid RS
USP Tranexamic Acid Related Compound C RS
(*RS*)-4-(Aminomethyl)cyclohex-1-enecarbocylic acid.
$C_8H_{13}NO_2$ 155

Add the following:

▲Tranexamic Acid Injection

DEFINITION
Tranexamic Acid Injection is a sterile solution of Tranexamic Acid in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer the content of Injection into a beaker and heat on a hot plate at 100° for 30 min, until a paste appears. Then heat it in an oven to complete dryness at 110° for about 30 min.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: Dissolve 11 g of monobasic sodium phosphate in 500 mL of water, and add 5 mL of triethylamine. Add 1.4 g of sodium lauryl sulfate, adjust with diluted phosphoric acid (10% w/w) to a pH of 2.5, and dilute with water to 600 mL.
Mobile phase: Methanol and *Buffer* (40:60)
Standard solution: 1 mg/mL of USP Tranexamic Acid RS in water
Sample solution: Nominally 1 mg/mL of tranexamic acid prepared as follows. Transfer an accurately measured volume of Injection from a composite of contents from NLT 3 vials to a suitable volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing *L1*
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of tranexamic acid
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tranexamic acid from the *Sample solution*
r_S = peak response of tranexamic acid from the *Standard solution*
C_S = concentration of USP Tranexamic Acid RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of tranexamic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

System suitability solution: 0.2 mg/mL of USP Tranexamic Acid RS and 2 µg/mL of USP Tranexamic Acid Related Compound C RS in water

Sensitivity solution: 0.01 mg/mL of USP Tranexamic Acid RS in water

Standard solution: 0.05 mg/mL of USP Tranexamic Acid RS in water

Sample solution: Nominally 10 mg/mL of tranexamic acid prepared as follows. Transfer an accurately measured volume of Injection to an appropriate volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing *L1*

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 3.3 times the retention time of tranexamic acid

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between tranexamic acid and tranexamic acid related compound C, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of tranexamic acid from the *Standard solution*

C_S = concentration of USP Tranexamic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tranexamic acid in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.03%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tranexamic acid	1.0	—
Tranexamic acid related compound C[a]	1.1	—
Aminomethylbenzoic acid[a, b]	1.3	—
cis-Tranexamic acid[a, c]	1.5	—
Ditranexamic acid amine[a, d]	2.1	—
Any unspecified impurity	—	0.1
Total impurities	—	0.5

[a] For identification only. These are process impurities monitored in the drug substance and are not included in the total impurities.

[b] 4-(Aminomethyl)benzoic acid.

[c] *cis*-4-(Aminomethyl)cyclohexanecarboxylic acid.

[d] *trans,trans*-4,4′-[Iminobis(methylene)]dicyclohexanecarboxylic acid.

SPECIFIC TESTS

• **pH** ⟨791⟩: 6.5–8.0

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.

Change to read:

• **▲STERILITY TESTS** ⟨71⟩:▲ (ERR 1-Oct-2018) Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mg of tranexamic acid

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Tranexamic Acid RS

USP Tranexamic Acid Related Compound C RS

(*RS*)-4-(Aminomethyl) cyclohex-1-enecarboxylic acid.

$C_8H_{13}NO_2$ 155.19▲ 1S (USP41)

Add the following:

▲Tranexamic Acid Tablets

DEFINITION

Tranexamic Acid Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Finely powder 1 Tablet. Transfer a portion of the powdered Tablet, equivalent to 75 mg of tranexamic acid, to a suitable vial. Add 1 mL of water, mix on a vortex mixer for a few seconds, and sonicate for 1 min. Pass the suspension through a suitable filter onto a suitable watchglass. Evaporate the filtrate in an oven at 60° for 2 h, and then stir gently with a glass rod. Dry in an oven at 60° for another 1 h.

Acceptance criteria: The IR spectrum of the *Sample* corresponds to that of USP Tranexamic Acid RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

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ASSAY

• **PROCEDURE**

Solution A: Dissolve 10.5 g of monobasic sodium phosphate monohydrate in 1000 mL of water, and add 8 mL of triethylamine followed by 2.3 g of sodium dodecyl sulfate. Adjust with 85% phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Solution A* (15:85)

Standard solution: 2.6 mg/mL of USP Tranexamic Acid RS in water. Sonicate, if needed.

Sample solution: Nominally 2.6 mg/mL of tranexamic acid prepared as follows. Transfer a portion of finely powdered Tablets (NLT 20), equivalent to 650 mg of tranexamic acid, to a 250-mL volumetric flask. Add about 200 mL of water, sonicate for about 20 min with occasional shaking, and dilute with water to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; 3.5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of tranexamic acid

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tranexamic acid from the *Sample solution*

r_S = peak response of tranexamic acid from the *Standard solution*

C_S = concentration of USP Tranexamic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tranexamic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Solution A and **Chromatographic system:** Proceed as directed in the *Assay*.

Mobile phase: Acetonitrile and *Solution A* (20:80)

Standard solution: 0.72 mg/mL of USP Tranexamic Acid RS in water. Sonicate, if needed. Pass the solution through a suitable filter of 0.45-µm pore size.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of tranexamic acid from the *Sample solution*

r_S = peak response of tranexamic acid from the *Standard solution*

C_S = concentration of USP Tranexamic Acid RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim of tranexamic acid (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A and **Mobile phase:** Prepare as directed in the *Assay*.

System suitability solution: 20 µg/mL of USP Tranexamic Acid RS and 2 µg/mL of USP Tranexamic Acid Related Compound C RS in *Mobile phase*

Standard solution: 0.01 mg/mL of USP Tranexamic Acid RS in *Mobile phase*

Sample solution: Nominally 10 mg/mL of tranexamic acid in *Mobile phase* prepared as follows. Transfer a portion of finely powdered Tablets (NLT 20), equivalent to 500 mg of tranexamic acid, to a 50-mL volumetric flask. Add about 40 mL of *Mobile phase*, sonicate for about 20 min with occasional shaking, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 3.5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 5.3 times of the retention time of tranexamic acid

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between tranexamic acid and tranexamic acid related compound C, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified and any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified or any unspecified degradation product from the *Sample solution*

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r_S = peak response of tranexamic acid from the *Standard solution*
C_S = concentration of USP Tranexamic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tranexamic acid in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tranexamic acid	1.0	—
Tranexamic acid related compound C[a]	1.1	—
Tranexamic acid related compound D[a,b]	1.2	—
Tranexamic acid related compound B[c]	1.6	0.3
Tranexamic acid related compound A[d]	2.3	0.2
Any unspecified degradation product	—	0.10
Total degradation products	—	0.5

[a] Process impurity controlled in the drug substance. It is included for identification purposes only. It should not be reported for the drug product, and should not be included in the total degradation products.
[b] 4-(Aminomethyl)benzoic acid.
[c] *cis*-4-(Aminomethyl)cyclohexanecarboxylic acid.
[d] *trans,trans*-4,4′-[Iminobis(methylene)]dicyclohexanecarboxylic acid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in well-closed containers, at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tranexamic Acid RS
  USP Tranexamic Acid Related Compound C RS
  (*R,S*)-4-(Aminomethyl)cyclohex-1-enecarboxylic acid.
  $C_8H_{13}NO_2$ 155.19 ▲(USP 1-Aug-2019)

Tranylcypromine Tablets

DEFINITION

Tranylcypromine Tablets contain an amount of tranylcypromine sulfate $[(C_9H_{11}N)_2 \cdot H_2SO_4]$ equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of tranylcypromine ($C_9H_{11}N$).

IDENTIFICATION

Change to read:

- **▲A.▲ 1S (USP41)** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained by the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 1S (USP41)

ASSAY

Change to read:

- **PROCEDURE**

Buffer: Transfer 3.4 g of monobasic ammonium phosphate into a 1-L volumetric flask containing about 900 mL of water. Adjust the solution with phosphoric acid to a pH of 2.2 ± 0.1. Dilute with water to volume, and mix well.
Mobile phase: Methanol and *Buffer* (30:70)
▲▲ (ERR 1-Oct-2018)
Diluent: Methanol, water, and 0.05 N sulfuric acid ▲VS▲ 1S (USP41) (20:60:20)
Standard stock solution: 0.4 mg/mL of USP Tranylcypromine Sulfate RS prepared as follows. Transfer a suitable quantity of USP Tranylcypromine Sulfate RS to a suitable volumetric flask. Add 60% of the flask volume of methanol and 0.05 N sulfuric acid ▲VS▲ 1S (USP41) (50:50), sonicate to dissolve, and dilute with *Diluent* to volume.
Standard solution: 0.04 mg/mL of USP Tranylcypromine Sulfate RS in *Diluent*, from the *Standard stock solution*
Sample stock solution: Nominally 0.5 mg/mL of tranylcypromine prepared as follows. Transfer a suitable number of Tablets to an appropriate volumetric flask, add 30% of the flask volume of 0.05 N sulfuric acid ▲VS▲ 1S (USP41) and sonicate for 10 min with intermittent shaking. Add 30% of the flask volume of methanol and sonicate for 10 min with intermittent shaking. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix. Centrifuge a portion of the solution and pass the supernatant through a filter of 0.45-µm pore size, discarding the first 2 mL.
Sample solution: Nominally 0.03 mg/mL of tranylcypromine in *Diluent*, from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ 1S (USP41)
Column: 4.6-mm × 25-cm; 4-µm packing L11
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
▲Run time: NLT 2 times the retention time of tranylcypromine▲ 1S (USP41)
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tranylcypromine ($C_9H_{11}N$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tranylcypromine in the *Sample solution* (mg/mL)
M = number of moles of tranylcypromine per mole of tranylcypromine sulfate, 2
M_{r1} = molecular weight of tranylcypromine, 133.19
M_{r2} = molecular weight of tranylcypromine sulfate, 364.46

Acceptance criteria: 90.0%–110.0%

USP Monographs

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PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid VS; 500 mL, deaerated
Apparatus 1: 100 rpm
Time: 45 min
Buffer: Transfer 6.94 g of sodium perchlorate monohydrate to a 1000-mL volumetric flask containing 900 mL of water and mix until dissolved. Adjust with perchloric acid to a pH of 2.50 and dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (15:85)
Standard stock solution: 0.54 mg/mL of USP Tranylcypromine Sulfate RS in water. [NOTE—Sonicate as needed.]
Standard solution: 0.027 mg/mL of USP Tranylcypromine Sulfate RS in *Medium*, from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 15-cm; 5-µm packing *L1*
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
▲▲▲ 1S (USP41)▲ (ERR 1-Oct-2018)
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tranylcypromine ($C_9H_{11}N$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times [M \times (M_{r1}/M_{r2})] \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
M = number of moles of tranylcypromine per mole of tranylcypromine sulfate, 2
M_{r1} = molecular weight of tranylcypromine, 133.19
M_{r2} = molecular weight of tranylcypromine sulfate, 364.46
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of tranylcypromine ($C_9H_{11}N$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Buffer▲ ▲ 1S (USP41) and **Diluent:** *Assay*
Solution A: Methanol and *Buffer* (15:85)
Solution B: Methanol and *Buffer* (30:70)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	100	0
25	0	100
37	0	100
39	100	0
45	100	0

Standard stock solution: 70 µg/mL of USP Tranylcypromine Sulfate RS and 280 µg/mL of USP Tranylcypromine Related Compound A RS in *Diluent*. [NOTE—Sonicate as needed.]
Standard solution: 0.7 µg/mL of USP Tranylcypromine Sulfate RS and 2.8 µg/mL of USP Tranylcypromine Related Compound A RS prepared as follows. Transfer a suitable volume of the *Standard stock solution* to a suitable volumetric flask, add 30% each of the flask volume of 0.05 N sulfuric acid ▲VS▲ 1S (USP41) and methanol, and dilute with *Diluent* to volume.
Sample solution: Use the *Sample stock solution* prepared as directed in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing *L1*
Column temperature: 35°
Flow rate: 1.2 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between tranylcypromine and tranylcypromine related compound A
Tailing factor: NMT 2.0
Relative standard deviation: NMT 6.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each ▲degradation product▲ 1S (USP41) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak response of the ▲degradation product▲ 1S (USP41) from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tranylcypromine in the *Sample solution* (mg/mL)
M = number of moles of tranylcypromine per mole of tranylcypromine sulfate, 2
M_{r1} = molecular weight of tranylcypromine, 133.19
M_{r2} = molecular weight of tranylcypromine sulfate, 364.46

Acceptance criteria
Individual ▲degradation product:▲ 1S (USP41) NMT 0.2%
Total ▲degradation products:▲ 1S (USP41) NMT 1.2%

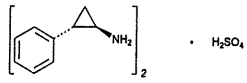
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tranylcypromine Sulfate RS
USP Tranylcypromine Related Compound A RS
(±)-*cis*-2-Phenylcyclopropanamine hydrochloride;
cis-Tranylcypromine hydrochloride.
$C_9H_{11}N \cdot HCl$ 169.65

Tranylcypromine Sulfate



$(C_9H_{11}N)_2 \cdot H_2SO_4$ 364.46
Cyclopropanamine, 2-phenyl-, *trans*-(±)-, sulfate (2:1);
(±)-*trans*-2-Phenylcyclopropylamine sulfate (2:1) [13492-01-8].

DEFINITION
Tranylcypromine Sulfate contains NLT 98.0% and NMT 102.0% of $(C_9H_{11}N)_2 \cdot H_2SO_4$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY
• **PROCEDURE**
Buffer: Dissolve 3.4 g of monobasic ammonium phosphate in about 900 mL of water in a 1-L volumetric flask. Adjust with phosphoric acid to a pH of 2.2 ± 0.1, and dilute with water to volume.
Mobile phase: Methanol and *Buffer* (3:7)
0.05 N sulfuric acid: Cautiously add 1.3 mL of sulfuric acid to 100 mL of water, cool to room temperature, and dilute to 1000 mL.
Diluent: Methanol, water, and *0.05 N sulfuric acid* (1:3:1)
Standard stock solution: Using a sonicator, dissolve USP Tranylcypromine Sulfate RS in *0.05 N sulfuric acid* and methanol (about 30% of the final volume of each solvent). Dilute with *Diluent* to obtain a 400 µg/mL solution.
Standard solution: 40 µg/mL of USP Tranylcypromine Sulfate RS in *Diluent*, prepared from the *Standard stock solution*
Sample stock solution: Using a sonicator, dissolve Tranylcypromine Sulfate in methanol and *0.05 N sulfuric acid* (about 30% of the final volume of each solvent). Dilute with *Diluent* to obtain a 400 µg/mL solution.
Sample solution: 40 µg/mL tranylcypromine sulfate in *Diluent*, prepared from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 4-µm packing L11
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $(C_9H_{11}N)_2 \cdot H_2SO_4$ in the portion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (µg/mL)
C_U = concentration of tranylcypromine sulfate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.1%
ORGANIC IMPURITIES
• **Procedure**
Buffer, 0.05 N sulfuric acid, and **Diluent:** Proceed as directed in the *Assay*.
Solution A: Methanol and *Buffer* (3:17)
Solution B: Methanol and *Buffer* (3:7)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	100	0
25	0	100
37	0	100
39	100	0
45	100	0

Standard stock solution: 14 µg/mL of USP Tranylcypromine Sulfate RS, and 60 µg/mL each of USP Tranylcypromine Related Compound A RS and USP Tranylcypromine Related Compound B RS in *Diluent*. [NOTE—Sonicate as needed.]
Standard solution: Transfer a portion of the *Standard stock solution* to a suitable volumetric flask containing methanol and *0.05 N sulfuric acid* (30% of the final volume of each solvent). Dilute with *Diluent* to volume to obtain a solution containing 0.7 µg/mL of USP Tranylcypromine Sulfate RS and 3.0 µg/mL each of USP Tranylcypromine Related Compound A RS and USP Tranylcypromine Related Compound B RS.
Sample solution: Using a sonicator, dissolve Tranylcypromine Sulfate in methanol and *0.05 N sulfuric acid* (about 30% of the final volume of each solvent). Dilute with *Diluent* to obtain a solution containing 680 µg/mL of tranylcypromine sulfate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 249 nm for tranylcypromine related compound B; 220 nm for all other specified and unspecified impurities
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1.2 mL/min

USP Monographs

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Injection size: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between tranylcypromine and tranylcypromine related compound A, determined at 220 nm

Tailing factor: NMT 2.0 for tranylcypromine, determined at 220 nm

Relative standard deviation: NMT 6.0% for tranylcypromine and tranylcypromine related compound A, determined at 220 nm; NMT 6.0% for tranylcypromine related compound B, determined at 249 nm.

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Disregard the signal for tranylcypromine related compound B observed at 220 nm.]

Identify tranylcypromine related compound A, *cis*-hydrazide, *trans*-hydrazide, and any unspecified impurity using the data in *Impurity Table 1*. Calculate the percentage of each impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

r_U = peak response of the impurity from the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of USP Tranylcypromine Sulfate RS in the *Standard solution* (µg/mL)

C_U = concentration of Tranylcypromine Sulfate in the *Sample solution* (µg/mL)

F = response factor relative to tranylcypromine sulfate

Calculate the percentage of tranylcypromine related compound B in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response at 249 nm of tranylcypromine related compound B from the *Sample solution*

r_S = peak response at 249 nm of tranylcypromine related compound B from the *Standard solution*

C_S = concentration of USP Tranylcypromine Related Compound B RS in the *Standard solution* (µg/mL)

C_U = concentration of Tranylcypromine Sulfate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of 3-phenylallylamine free base, 133.19

M_{r2} = molecular weight of 3-phenylallylamine hydrochloride (USP Tranylcypromine Related Compound B RS), 169.66

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

[NOTE—Tranylcypromine related compound B is determined at 249 nm. All other specified and unspecified impurities are determined at 220 nm.]

Total impurities: NMT 1.0% for sum of tranylcypromine related compound A, tranylcypromine related compound B, *cis*-hydrazide, *trans*-hydrazide, and any unspecified impurity.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tranylcypromine related compound A[a]	0.82	0.74	0.50
Tranylcypromine sulfate	1.0	1.0	—
Tranylcypromine related compound B[b]	1.2	—	0.50
cis-Hydrazide[c]	1.4	0.86	0.50
trans-Hydrazide[d]	2.5	1.1	0.50
Any unspecified impurity	—	1.0	0.10

[a] (±)-*cis*-2-Phenylcyclopropanamine (*cis*-Cypromine).

[b] 3-Phenylallylamine (Cinnamylamine), monitored at 249 nm.

[c] (±)-*cis*-2-Phenylcyclopropanecarbohydrazide.

[d] (±)-*trans*-2-Phenylcyclopropanecarbohydrazide.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩: Dry a sample in vacuum at 60° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

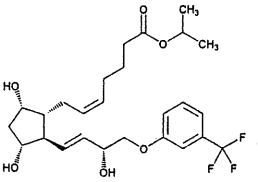
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Tranylcypromine Sulfate RS

USP Tranylcypromine Related Compound A RS
(±)-*cis*-2-Phenylcyclopropanamine hydrochloride; *cis*-tranylcypromine hydrochloride.
$C_9H_{11}N \cdot HCl$ 169.65

USP Tranylcypromine Related Compound B RS
3-Phenylallylamine hydrochloride; cinnamylamine hydrochloride.
$C_9H_{11}N \cdot HCl$ 169.65

Travoprost



$C_{26}H_{35}F_3O_6$ 500.55

[1*R*-[1α(*Z*),2β(1*E*,3*R**),3α,5α]]-7-[3,5-Dihydroxy-2-[3-hydroxy-4-[3-(trifluoromethyl)phenoxy]-1-butenyl] cyclopentyl]-5-heptenoic acid, 1-methylethyl ester;
Isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(1*E*,3*R*)-3-hydroxy-4-[(α,α,α-trifluoro-*m*-tolyl)oxy]-1-butenyl] cyclopentyl]-5-heptenoate [157283-68-6].

DEFINITION

Travoprost contains NLT 96.0% and NMT 102.0% of travoprost ($C_{26}H_{35}F_3O_6$), calculated on the anhydrous and solvent-free basis.

[**CAUTION**—Great care should be taken to avoid contact with the body.]

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: Use USP Travoprost RS.

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Sample solution: Prepare as directed in the *Assay*.
Chromatographic system
Adsorbent: Chromatographic plate coated with silica gel that contains 20% silver nitrate
Application volume: 5 µL. [NOTE—To keep the spot size small, it is usually necessary to apply approximately 1–2 µL at a time, allowing the spot to dry between each application.]
Developing solvent system: Ethyl acetate and ethanol (4:1)
Spray reagent: 20% phosphomolybdic acid in ethanol
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter. Spray the plate with *Spray reagent*, and heat it in an oven at 80°–100°. The travoprost will appear as black spots.
Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Add 2.0 mL of phosphoric acid to 1 L of water. Adjust with sodium hydroxide to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (3:7)
Standard solution: Use USP Travoprost RS without dilution (0.5 mg/mL).
Sample solution: Transfer 25 mg of Travoprost to a 50-mL volumetric flask, and dissolve in 15 mL of acetonitrile. Add 25 mL of water, mix, and wait until the solution reaches room temperature. Dilute with water to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 5-cm; packing L1
Flow rate: 3.0 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—USP Travoprost RS contains a small percentage of the 5,6-*trans* isomer. The relative retention times for travoprost and the 5,6-*trans* isomer are about 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between travoprost and the 5,6-*trans* isomer
Column efficiency: NLT 1500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of travoprost ($C_{26}H_{35}F_3O_6$) in the portion of Travoprost taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Travoprost RS in the *Standard solution* (mg/mL)
C_U = concentration of Travoprost in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Travoprost taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response for each individual impurity
r_T = sum of the responses of all the peaks
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Travoprost related compound A	0.11	1.0	0.2
Epoxide derivative[a]	0.55	1.0	0.4
15-*epi* Diastereomer[b]	0.90	1.1	0.1
5,6-*trans* Isomer[c]	1.16	1.0	3.5
15-Keto derivative[d]	1.45	1.6	0.3
Any other individual impurity	—	—	0.1
Total impurities	—	—	4.0

[a] (5*Z*)-(9*S*,11*R*,15*S*)-9,11,15-Trihydroxy-13,14-epoxy-16-(*m*-trifluoromethylphenoxy)-17,18,19,20-tetranor-5-prostadienoic acid, isopropyl ester.
[b] (5*Z*,13*E*)-(9*S*,11*R*,15*S*)-9,11,15-Trihydroxy-16-(*m*-trifluoromethylphenoxy)-17,18,19,20-tetranor-5,13-prostadienoic acid, isopropyl ester.
[c] (5*E*,13*E*)-(9*S*,11*R*,15*R*)-9,11,15-Trihydroxy-16-(*m*-trifluoromethylphenoxy)-17,18,19,20-tetranor-5,13-prostadienoic acid, isopropyl ester.
[d] (5*Z*,13*E*)-(9*S*,11*R*)-9,11-Dihydroxy-15-oxo-16-(*m*-trifluoromethylphenoxy)-17,18,19,20-tetranor-5,13-prostadienoic acid, isopropyl ester.

• **LIMIT OF ETHYL ACETATE**

Standard solution: 50 µg/mL of ethyl acetate in *N,N*-dimethylacetamide
Sample solution: 0.02 g/mL of Travoprost in *N,N*-dimethylacetamide
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m; 1-µm coating G16
Carrier gas: Helium
Temperatures
Injector port: 140°
Detector: 240°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
55	0	55	6
55	25	240	20

Flow rate: 4 mL/min
Injection volume: 1 µL

USP Monographs

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System suitability
Sample: *Standard solution*
[NOTE—The retention time for ethyl acetate is about 2–5 min.]
Suitability requirements
Resolution: NLT 1.5 between ethyl acetate and any adjacent peak
Relative standard deviation: NMT 15.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, in ppm, of ethyl acetate in the portion of Travoprost taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of ethyl acetate in the *Standard solution* (µg/mL)
C_U = concentration of Travoprost in the *Sample solution* (g/mL)

Acceptance criteria: NMT 5000 ppm

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL in dehydrated alcohol
Acceptance criteria: +52.0° to +58.0° at 365 nm
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩
Sample: 0.2 g
Solvent: Acetonitrile and methanol (1:1)
Titrant: Use a titrant for which 1 mL is equivalent to 2 mg of water.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve at –25° to –15° in tight, light-resistant containers under a nitrogen atmosphere.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Travoprost RS

Travoprost Ophthalmic Solution

DEFINITION
Travoprost Ophthalmic Solution is a sterile buffered aqueous solution of Travoprost. It contains NLT 90.0% and NMT 110.0% of the labeled amount of travoprost ($C_{26}H_{35}F_3O_6$). It may contain suitable stabilizers, buffers, and antimicrobial agents.
[**CAUTION**—Great care should be taken when handling the active ingredient to avoid contact with the body.]

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**
Buffer: 2.18 mg/mL of sodium 1-octanesulfonate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer* (17:33)
Standard solution: 0.04 mg/mL of travoprost from USP Travoprost RS in a mixture of acetonitrile and water (3:7)
Sample solution: Use Ophthalmic Solution without dilution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. ▲For *Identification B*, use a diode array detector in the range of 190–400 nm.▲ 2S (USP41)
Column: 4.6-mm × 15-cm; ▲5-µm▲ 2S (USP41) packing L1
Flow rate: 2.0 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—USP Travoprost RS contains a small percentage of the 5,6-*trans* isomer. The relative retention times for travoprost and the 5,6-*trans* isomer are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between travoprost and the 5,6-*trans* isomer
▲▲ 2S (USP41)
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of travoprost ($C_{26}H_{35}F_3O_6$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of travoprost in the *Standard solution* (mg/mL)
C_U = nominal concentration of travoprost in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

• **LIMIT OF TRAVOPROST RELATED COMPOUND A**
Buffer: Add 1.0 mL of phosphoric acid to 1.0 L of water, and adjust with sodium hydroxide to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (6:19)
Standard solution: 0.3 µg/mL of USP Travoprost Related Compound A RS in a mixture of acetonitrile and water (1:4)
Sample solution: Use Ophthalmic Solution without dilution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 5-cm; ▲3-µm▲ 2S (USP41) packing L1
Flow rate: 3.0 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*

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Suitability requirements
▲▲ 2S (USP41)
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of travoprost related compound A in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Travoprost Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of travoprost in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%
• **LIMIT OF DEGRADATION PRODUCTS**
Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of travoprost from the *Standard solution*
C_S = concentration of USP Travoprost RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of travoprost in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5,6-*trans* Isomer[a]	1.1	1.0	5.0
15-Keto derivative[b]	1.4	1.7	1.0
Total impurities[c]	—	—	5.5

[a] (5*E*,13*E*)-(9*S*,11*R*,15*R*)-9,11,15-Trihydroxy-16-(*m*-trifluoromethylphenoxy)-17,18,19,20-tetranor-5,13-prostadienoic acid, isopropyl ester.
[b] (5*Z*,13*E*)-(9*S*,11*R*)-9,11-Dihydroxy-15-oxo-16-(*m*-trifluoromethylphenoxy)-17,18,19,20-tetranor-5,13-prostadienoic acid, isopropyl ester.
[c] It is the sum of all degradation products, including travoprost related compound A, obtained in the test for *Limit of Travoprost Related Compound A*.

SPECIFIC TESTS

Change to read:

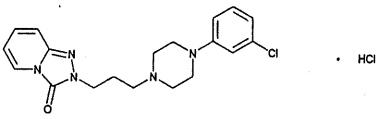
• **STERILITY TESTS** ⟨71⟩: Meets the requirements▲▲ 2S (USP41)
• **pH** ⟨791⟩: 5.5–6.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 2° and 25°.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Travoprost RS
USP Travoprost Related Compound A RS
(5*Z*,13*E*)-(9*S*,11*R*,15*R*)-9,11,15-Trihydroxy-16-(*m*-trifluoromethylphenoxy)-17,18,19,20-tetranor-5,13-prostadienoic acid
or
(*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-{(*R*,*E*)-3-hydroxy-4-[3-(trifluoromethyl)phenoxy]but-1-enyl}cyclopentyl)hept-5-enoic acid.
$C_{23}H_{29}F_3O_6$ ▲458.47▲ 2S (USP41)

Trazodone Hydrochloride



$C_{19}H_{22}ClN_5O \cdot HCl$ 408.32
1,2,4-Triazolo[4,3-*a*]pyridin-3(2*H*)-one, 2-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-, monohydrochloride; 2-[3-[4-(*m*-Chlorophenyl)-1-piperazinyl]propyl]-*s*-triazolo[4,3-*a*]-pyridin-3(2*H*)-one monohydrochloride [25332-39-2].

DEFINITION
Trazodone Hydrochloride contains NLT 98.0% and NMT 102.0% of trazodone hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 0.01% (v/v) of triethylamine in water
Solution B: 0.01% (v/v) of triethylamine in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
12	32	68
12.01	80	20
15	80	20

Diluent: *Solution A* and *Solution B* (80:20)
System suitability solution: 1 µg/mL each of USP Trazodone Related Compound C RS and USP Trazodone Related Compound D RS, and 0.1 mg/mL of USP Trazodone Hydrochloride RS in *Diluent*
Standard solution: 1 mg/mL of USP Trazodone Hydrochloride RS in *Diluent*

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Sample solution: 1 mg/mL of Trazodone Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
[NOTE—A mixture of acetonitrile, 2-propanol, acetone, and formic acid (400:300:300:2) is recommended for injector wash to minimize the sample carry-over.]
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between trazodone related compound C and trazodone; NLT 2.8 between trazodone and trazodone related compound D, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trazodone hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$) in the portion of Trazodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trazodone from the *Sample solution*
r_S = peak response of trazodone from the *Standard solution*
C_S = concentration of USP Trazodone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Trazodone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1 µg/mL each of USP Trazodone Related Compound C RS and USP Trazodone Related Compound D RS in the *Standard solution*
Standard solution: 1 µg/mL of USP Trazodone Hydrochloride RS in *Diluent*
Sample solution: 1 mg/mL of Trazodone Hydrochloride in *Diluent*
System suitability
Sample: *System suitability solution*
[NOTE—Refer to *Table 2* for the relative retention times.]
Suitability requirements
Relative standard deviation: NMT 5.0% for the trazodone peak
Resolution: NLT 1.5 between trazodone related compound C and trazodone; NLT 2.8 between trazodone and trazodone related compound D
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Trazodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of trazodone from the *Standard solution*
C_S = concentration of USP Trazodone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Trazodone Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Triazolopyridinone[a]	0.1	0.48	0.10
Trazodone *N*-oxide[b]	0.40	1.0	0.1
Deschloro trazodone[c]	0.65	0.71	0.1
Trazodone related compound C	0.96	1.0	0.1
Trazodone	1.0	—	—
Trazodone related compound D	1.1	1.0	0.1
4-Ethyl trazodone[d]	1.4	1.0	0.1
Trazodone isobutyl ether analog[e]	2.0	1.0	0.1
Bispiperazine analog[f]	2.1	1.3	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] [1,2,4]Triazolo[4,3-*a*]pyridin-3(2*H*)-one.
[b] 4-(3-Chlorophenyl)-1-[3-(3-oxo-[1,2,4]triazolo[4,3-*a*]pyridin-2(3*H*)-yl) propyl]piperazine 1-oxide.
[c] 2-[3-(4-Phenylpiperazin-1-yl)propyl]-[1,2,4]triazolo[4,3-*a*]pyridin-3(2*H*)-one.
[d] 2-{3-[4-(3-Chloro-4-ethylphenyl)piperazin-1-yl]propyl}-[1,2,4]triazolo[4,3-*a*] pyridin-3(2*H*)-one.
[e] 1-(3-Chlorophenyl)-4-(3-isobutoxypropyl)piperazine.
[f] 1,3-Bis(4-(3-chlorophenyl)piperazin-1-yl)propane.

• **LIMIT OF TRAZODONE RELATED COMPOUND F AND CYCLOPHOSPHAMIDE RELATED COMPOUND A**
[NOTE—Perform this test only if trazodone related compound F and cyclophosphamide related compound A are known process impurities.]
Solution A: 5 mM ammonium bicarbonate solution
Solution B: Acetonitrile
Diluent: Acetonitrile, water, and formic acid (100:900:1)
Standard solution: 0.025 µg/mL each of USP Trazodone Related Compound F RS and USP Cyclophosphamide Related Compound A RS, in *Diluent*
Sample solution: 0.01 g/mL of Trazodone Hydrochloride in *Diluent*
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
6.5	20	80

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Table 3 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
6.51	90	10

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: MS/MS (tandem mass spectrometer)
MS conditions
Ionization: Triple quadrupole ionization in positive ion mode
Acquisition mode: Multiple reaction monitoring (MRM) of the following mass transitions:
Cyclophosphamide related compound A 142 → 63
Trazodone related compound F 273 → 120
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Flow rate to ion source: 0.5 mL/min
Injection volume: Adjust to between 5 and 50 µL, depending on the mass spectrometer. [NOTE—A mixture of 2-propanol, water, and formic acid (800:200:1) is recommended for the injector wash to minimize the sample carry-over.]
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times of cyclophosphamide related compound A and trazodone related compound F are 0.4 and 1.0, respectively.]
Suitability requirements
Signal-to-noise ratio: NLT 100 for the trazodone related compound F peak and NLT 50 for the cyclophosphamide related compound A peak
Relative standard deviation: NMT 15.0% each for trazodone related compound F and cyclophosphamide related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Under the chromatographic conditions, the elution order is cyclophosphamide related compound A, trazodone, and trazodone related compound F. Use of an appropriate switching valve program in order to completely divert the trazodone peak to waste between the elution times of the two impurities is recommended.]
Calculate, in µg/g, the amount of trazodone related compound F and cyclophosphamide related compound A in the portion of Trazodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U)$$

r_U = peak response of trazodone related compound F or cyclophosphamide related compound A from the *Sample solution*
r_S = peak response of trazodone related compound F or cyclophosphamide related compound A from the *Standard solution*
C_S = concentration of USP Trazodone Related Compound F RS or USP Cyclophosphamide Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Trazodone Hydrochloride in the *Sample solution* (g/mL)

Acceptance criteria: NMT 2.5 µg/g each of trazodone related compound F and cyclophosphamide related compound A

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at a pressure of 50 mm of mercury at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyclophosphamide Related Compound A RS
Bis(2-chloroethyl)amine hydrochloride.
$C_4H_{10}Cl_3N$ 178.48
USP Trazodone Hydrochloride RS
USP Trazodone Related Compound C RS
2-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}-[1,2,4]triazolo[4,3-*a*]pyridin-3(2*H*)-one hydrochloride.
$C_{19}H_{22}ClN_5O \cdot HCl$ 408.32
USP Trazodone Related Compound D RS
2-{3-[4-(3-Bromophenyl)piperazin-1-yl]propyl}-[1,2,4]triazolo[4,3-*a*]pyridin-3(2*H*)-one hydrochloride.
$C_{19}H_{22}BrN_5O \cdot HCl$ 452.78
USP Trazodone Related Compound F RS
1-(3-Chlorophenyl)-4-(3-chloropropyl)piperazine hydrochloride.
$C_{13}H_{18}Cl_2N_2 \cdot HCl$ 309.66

Trazodone Hydrochloride Tablets

DEFINITION
Trazodone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of trazodone hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$).

IDENTIFICATION
• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 1.15 g/L of monobasic ammonium phosphate, adjusted with sodium hydroxide to a pH of 6.0
Mobile phase: Methanol and *Buffer* (75:25)
Standard solution: 0.1 mg/mL of USP Trazodone Hydrochloride RS in 0.01 N hydrochloric acid TS
Sample solution: Nominally 0.1 mg/mL of trazodone hydrochloride from NLT 20 finely powdered Tablets. Transfer a suitable quantity of the powder to a suitable volumetric flask. Dissolve in 0.01 N hydrochloric acid TS and dilute with 0.01 N hydrochloric acid TS to volume. Sonicate for about 30 min, and pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 5-mm × 10-cm; 4-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 4.5 times the retention time of trazodone
System suitability
Sample: *Standard solution*

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Suitability requirements
Column efficiency: NLT 900 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trazodone hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trazodone from the *Sample solution*
r_S = peak response of trazodone from the *Standard solution*
C_S = concentration of USP Trazodone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of trazodone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
▲Test 1▲ (RB 1-Apr-2018)
Medium: 0.01 N hydrochloric acid TS; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Buffer, Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trazodone hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of trazodone from the *Sample solution*
r_S = peak response of trazodone from the *Standard solution*
C_S = concentration of USP Trazodone Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of trazodone hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$) is dissolved.
▲Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.01 N hydrochloric acid TS; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: To each liter of water add 5 mL of triethylamine, and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (25:75)
Standard solution: (L/900) mg/mL of USP Trazodone Hydrochloride RS in *Medium*. Sonicate if necessary.
Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size. Discard the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 1.6 times the retention time of trazodone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trazodone hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of trazodone from the *Sample solution*
r_S = peak response of trazodone from the *Standard solution*
C_S = concentration of USP Trazodone Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of trazodone hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$) is dissolved.▲ (RB 1-Apr-2018)
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A: 6.75 g/L of monobasic potassium phosphate. Add 1.0 mL of triethylamine for each liter of the solution, and mix.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
30	60	40
35	60	40
60	42	58
63	30	70
78	30	70
78.1	90	10
90	90	10

Diluent: Methanol, water, and hydrochloric acid (650:350:3)
System suitability solution: 0.7 µg/mL of USP Trazodone Hydrochloride RS and 1.5 µg/mL of USP Trazodone Related Compound C RS in *Diluent*

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Standard solution: 0.7 µg/mL of USP Trazodone Hydrochloride RS in *Diluent*

Sample solution: Nominally 500 µg/mL of trazodone from finely powdered Tablets (NLT 20) prepared as follows. Transfer a portion of powdered Tablets (NLT 50 mg) to a suitable volumetric flask. Add about 80% of the flask volume of *Diluent*, and sonicate for 10 min. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.0-mm × 15-cm; 3-µm packing L1
Flow rate: 0.7 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.5 between the trazodone related compound C and trazodone peaks, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of trazodone from the *Standard solution*
C_S = concentration of USP Trazodone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of trazodone in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Triazolopyridinone[a, b]	0.1	—
Chlorophenylpiperazine[a, c]	0.6	—
Hydroxypropyl chlorophenylpiperazine[a, d]	0.7	—
Isotrazodone[a, e]	0.8	—
Trazodone related compound C[a]	0.97	—
Trazodone hydrochloride	1.0	—
Trazodone dimer[a, f]	1.5	—
Trazodone related compound F[a, g]	1.6	—
Bispiperazine analog[a, h]	1.8	—
Bis(3-chlorophenyl)piperazine[a, i]	2.2	—
Any individual unspecified degradation product	—	1.0
Total degradation products	—	2.0

[a] Process impurity included for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.
[b] [1,2,4]Triazolo[4,3-*a*]pyridin-3(2*H*)-one.
[c] 1-(3-Chlorophenyl)piperazine.
[d] 3-[4-(3-Chlorophenyl)piperazin-1-yl]propan-1-ol.
[e] 1-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-[1,2,4]triazolo[4,3-*a*]pyridin-1-ium-3-olate.
[f] 1,1-Bis{2-chloro-[4-(3-{1,2,4-triazolo[4,3-*a*]pyridin-3-(2*H*)-on-2-yl}propyl)piperazine-1-yl]phenyl}ethane trihydrochloride.
[g] 1-(3-Chlorophenyl)-4-(3-chloropropyl)piperazine.
[h] 1,3-Bis(4-(3-chlorophenyl)piperazin-1-yl)propane.
[i] 1,4-Bis(3-chlorophenyl)piperazine.

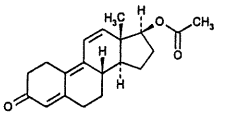
ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

Add the following:

▲• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. ▲ (RB 1-Apr-2018)

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Trazodone Hydrochloride RS
USP Trazodone Related Compound C RS
2-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}-[1,2,4]triazolo[4,3-*a*]pyridin-3(2*H*)-one hydrochloride.
$C_{19}H_{22}ClN_5O \cdot HCl$ 408.32

Trenbolone Acetate



$C_{20}H_{24}O_3$ 312.40
Estra-4,9,11-trien-3-one, 17-(acetyloxy)-, (17β)-; 17β-Hydroxyestra-4,9,11-trien-3-one, acetate [10161-34-9].

DEFINITION
Trenbolone Acetate contains NLT 97.0% and NMT 103.0% of $C_{20}H_{24}O_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Diluent: Acetonitrile, methanol, water, and acetic acid (36.5: 30: 33.5: 0.1)
Solution A: Acetonitrile, methanol, and water (36.5: 30: 33.5)

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Solution B: Acetonitrile and methanol (9:1)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6	100	0
16	0	100
26	0	100
26.1	100	0
30	100	0

Standard solution: 1 mg/mL of USP Trenbolone Acetate RS in *Diluent*. Sonicate, if necessary, to dissolve.
Sample solution: 1 mg/mL of Trenbolone Acetate in *Diluent*. Sonicate, if necessary, to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 229 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection size: 5 µL
System suitability
Samples: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{20}H_{24}O_3$ in the portion of Trenbolone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of Trenbolone Acetate from the *Sample solution*
r_S = peak response of trenbolone acetate from the *Standard solution*
C_S = concentration of USP Trenbolone Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Trenbolone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0%, on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

• **Procedure**
Diluent, Solution A, Solution B, Mobile phase, Standard solution, Sample solution and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1 mg/mL of USP Trenbolone Acetate System Suitability Mixture RS in *Diluent*. Sonicate, if necessary, to dissolve.
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for trenbolone acetate related compound A and trenbolone acetate are 1.2 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between trenbolone acetate and trenbolone acetate related compound A, *System suitability solution*
Column efficiency: NLT 8000 theoretical plates for the trenbolone acetate peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Trenbolone Acetate taken:

$$\text{Result} = (100 \times r_U)/[(r_S + S) \times F]$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of Trenbolone Acetate from *Sample solution*
S = sum of the peak responses of each impurity, each divided by their respective response factor
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total specified and unspecified impurities: NMT 2.0%
Reporting level for impurities: NMT 0.10%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Trenbolone related compound B[a]	0.4	1.04	1.0
Trenbolone related compound C[b]	0.8	1.10	0.5
Trenbolone acetate	1.0	—	—
Related compound A[c]	1.2	1.0	0.5
Any unspecified impurity	—	1.00[d]	0.5

[a] Trenbolone.
[b] Trenbolone acetate 17α-isomer.
[c] Conjugated dihydrotrenbolone acetate, or 11,12-dihydrotrenbolone acetate.
[d] Unless determined otherwise.

SPECIFIC TESTS

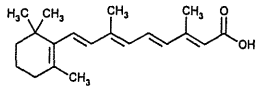
• **ABSORBANCE**
Sample solution: 100 mg/mL in dehydrated alcohol
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Analytical wavelength: 440 nm
Cell: 2 cm
Blank: Dehydrated alcohol
Analysis
Samples: *Sample solution* and *Blank*
Acceptance criteria: NMT 0.3
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩**:** +39° to +43°
Sample solution: 5 mg/mL in methanol
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample in a vacuum at 60° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a refrigerator.
• **LABELING:** Label it to indicate that it is for veterinary use only.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Trenbolone Acetate RS
USP Trenbolone Acetate System Suitability Mixture RS
Mixture containing trenbolone and conjugated dihydrotrenbolone acetate in a matrix of trenbolone acetate.

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Tretinoin



$C_{20}H_{28}O_2$ 300.44
Retinoic acid;
all-*trans*-Retinoic acid [302-79-4].

DEFINITION
Tretinoin contains NLT 97.0% and NMT 103.0% of tretinoin ($C_{20}H_{28}O_2$), calculated on the dried basis.
Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedures.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Change to read:

• **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelength: 352 nm
Medium: Dilute 1 mL of 0.01 N hydrochloric acid with isopropyl alcohol to 1000 mL.
Sample solution: 4 µg/mL in *Medium*
Acceptance criteria: Absorptivities do not differ by more than 3.0%, calculated on the dried basis.

ASSAY
• **Procedure**
Sample: 240 mg of Tretinoin
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium methoxide VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 50 mL of dimethylformamide, and add 3 drops of a 1-in-100 solution of thymol blue in dimethylformamide. Titrate with *Titrant* to a greenish endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 30.04 mg of tretinoin ($C_{20}H_{28}O_2$).
Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Limit of Isotretinoin**
Mobile phase: Isooctane, isopropyl alcohol, and glacial acetic acid (99.65: 0.25: 0.1)
System suitability stock solution: 250 µg/mL of USP Tretinoin RS in isooctane prepared as follows. Dissolve a quantity of USP Tretinoin RS in a minimum amount of methylene chloride, and add a suitable amount of isooctane to the known concentration.
Standard stock solution: 250 µg/mL of USP Isotretinoin RS in isooctane prepared as follows. Dissolve a quantity of USP Isotretinoin RS in a minimum amount of methylene chloride, and add a suitable amount of isooctane to the known concentration.
System suitability solution: Transfer 5 mL of *Standard stock solution* into a 100-mL volumetric flask, and add *System suitability stock solution* to volume.
Standard solution: 12.5 µg/mL of USP Isotretinoin RS in isooctane from *Standard stock solution*
Sample solution: Transfer 25 mg of Tretinoin into a 100-mL volumetric flask. Dissolve in a minimum amount of methylene chloride, and add isooctane to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 352 nm
Column: 4.0-mm × 25-cm; packing L3
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[Note—The relative retention times for isotretinoin and tretinoin are about 0.84 and 1.00, respectively.]
Suitability requirements
Resolution: NLT 2.0 between isotretinoin and tretinoin
Relative standard deviation: NMT 2.0% for the isotretinoin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of isotretinoin in the portion of Tretinoin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isotretinoin from the *Sample solution*
r_S = peak response of isotretinoin from the *Standard solution*
C_S = concentration of USP Isotretinoin RS in the *Standard solution* (µg/mL)
C_U = concentration of Tretinoin in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 5.0%

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample under vacuum at room temperature for 16 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, preferably under an atmosphere of an inert gas, protected from light.
• **USP Reference Standards** ⟨11⟩
USP Isotretinoin RS
USP Tretinoin RS

Tretinoin Cream

DEFINITION
Tretinoin Cream contains NLT 90.0% and NMT 120.0% of the labeled amount of tretinoin ($C_{20}H_{28}O_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedure. Use

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stabilized tetrahydrofuran in the preparation of the *Standard solution* and the *Sample solution*.
Diluted phosphoric acid: Dilute 10 mL of phosphoric acid with water to 100 mL.
Buffer: 1.38 g/L of monobasic sodium phosphate in water. Adjust with *Diluted phosphoric acid* to a pH of 3.0.
Diluent A: Water and *Diluted phosphoric acid* (9:1)
Diluent B: Tetrahydrofuran and *Diluent A* (3:2)
Mobile phase: Tetrahydrofuran and *Buffer* (42:58)
Standard stock solution: 0.4 mg/mL of USP Tretinoin RS in tetrahydrofuran
Standard solution: 4 µg/mL of USP Tretinoin RS in *Diluent B* from *Standard stock solution*
Sample stock solution: Nominally 20 µg/mL of tretinoin in tetrahydrofuran prepared as follows. Transfer a quantity of Cream, equivalent to 1.0 mg of tretinoin, into a 50-mL volumetric flask, and add 20.0 mL of tetrahydrofuran. Shake the flask to disperse the cream, dilute with tetrahydrofuran to volume, mix, and filter if necessary.
Sample solution: 4 µg/mL of tretinoin in *Diluent B* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tretinoin ($C_{20}H_{28}O_2$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tretinoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tretinoin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in collapsible tubes or in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Tretinoin RS

Tretinoin Gel

DEFINITION
Tretinoin Gel contains NLT 90.0% and NMT 130.0% of the labeled amount of $C_{20}H_{28}O_2$.

IDENTIFICATION
• The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
[Note—Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedure.]
Mobile phase: Prepare a mixture of acetonitrile and water (17:3). Add 5 mL of glacial acetic acid to each L of the mixture, mix well, filter, and degas.
Standard solution: 0.02 mg/mL of USP Tretinoin RS in methanol. [Note—Sonicate if needed.]
Sample solution: Transfer Tretinoin Gel, equivalent to 2 mg of tretinoin, to a 100-mL volumetric flask. Add about 70 mL of methanol, sonicate with intermittent shaking for 30 min, and dilute with methanol to volume. Centrifuge a portion of this solution at 3000 rpm for 10 min. Use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 353 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
Autosampler temperature: 15°
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Tailing factor: NMT 2.0
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{20}H_{28}O_2$ in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tretinoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tretinoin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–130.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, protected from light.
• **USP Reference Standards** ⟨11⟩
USP Tretinoin RS

Tretinoin Topical Solution

DEFINITION
Tretinoin Topical Solution is a solution of Tretinoin in a suitable nonaqueous, hydrophilic solvent. It contains NLT 90.0% and NMT 135.0% of the labeled amount of tretinoin ($C_{20}H_{28}O_2$) (w/w).

IDENTIFICATION
• **A.**
Sample solution: Mix 5 mL of Topical Solution and 5 mL of water.
Acceptance criteria: A voluminous yellow precipitate is formed.

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• **B.**
Sample solution: Proceed as directed for the *Sample solution* in the *Assay*.
Analysis: Measure the absorbance between 300 and 450 nm.
Acceptance criteria: The *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Tretinoin RS, concomitantly measured.

ASSAY
• **PROCEDURE**
Avoid exposure to strong light, and use low-actinic glassware.
Acidified isopropyl alcohol: Dilute 1 mL of 0.01 N hydrochloric acid with isopropyl alcohol to make 1000 mL.
Standard solution: 3.75 µg/mL of USP Tretinoin RS in *Acidified isopropyl alcohol*
Sample solution: Nominally 3.75 µg/mL of tretinoin in *Acidified isopropyl alcohol* from Topical Solution
Instrumental conditions
Mode: UV
Analytical wavelength: 352 nm
Cell: 1 cm
Blank: *Acidified isopropyl alcohol*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tretinoin ($C_{20}H_{28}O_2$) in the portion of Topical Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

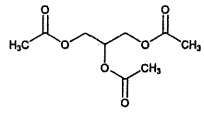
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tretinoin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tretinoin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–135.0%

OTHER COMPONENTS
• **ALCOHOL DETERMINATION** ⟨611⟩**:** 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tretinoin RS

Triacetin



$C_9H_{14}O_6$ 218.21
1,2,3-Propanetriol triacetate;
Triacetin;
Glyceryl triacetate [102-76-1].

DEFINITION
Triacetin contains NLT 97.0% and NMT 102.0% of triacetin ($C_9H_{14}O_6$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)
• **B.** The retention time of the triacetin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Internal standard solution: 0.1 mg/mL of benzyl alcohol in 2-propanol
Standard solution: 0.25 mg/mL of USP Triacetin RS in *Internal standard solution*
Sample solution: 0.25 mg/mL of Triacetin in *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 30-m; coated with a 0.25-µm film of phase G46
Temperatures
Injection port: 200°
Detector: 250°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	1
100	30	230	1

Carrier gas: Hydrogen
Flow rate: 1.5 mL/min
Injection volume: 1 µL
Injection type: Split ratio, 40:1
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzyl alcohol and triacetin are 0.7 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for the triacetin peak
Relative standard deviation: NMT 1.0%, peak response ratio of triacetin to benzyl alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of triacetin ($C_9H_{14}O_6$) in the portion of Triacetin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of triacetin to benzyl alcohol from the *Sample solution*
R_S = peak response ratio of triacetin to benzyl alcohol from the *Standard solution*
C_S = concentration of USP Triacetin RS in the *Standard solution* (mg/mL)
C_U = concentration of Triacetin in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

USP Monographs

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IMPURITIES

• **ORGANIC IMPURITIES**

Internal standard solution: 0.02 mg/mL of benzyl alcohol in 2-propanol

Peak identification solution: 0.25 mg/mL each of glycerol, monoacetin, diacetin, and USP Triacetin RS in *Internal standard solution*

Standard solution: 0.02 mg/mL of USP Triacetin RS in *Internal standard solution*

Sample solution: 20 mg/mL of Triacetin in *Internal standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m; coated with a 0.25-µm film of phase G46

Temperatures

Injection port: 200°

Detector: 250°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	—	50	4
50	30	140	13
140	30	230	2

Carrier gas: Hydrogen

Flow rate: 1.5 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 40:1

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for benzyl alcohol and triacetin are 0.62 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for the triacetin peak

Relative standard deviation: NMT 5.0%, peak response ratio of triacetin to benzyl alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Triacetin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (1/F) \times 100$$

R_U = peak response ratio of each impurity to benzyl alcohol from the *Sample solution*

R_S = peak response ratio of triacetin to benzyl alcohol from the *Standard solution*

C_S = concentration of USP Triacetin RS in the *Standard solution* (mg/mL)

C_U = concentration of Triacetin in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Reporting threshold: 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Acetic acid	0.24	0.62	0.1
Glycerol	0.65	0.54	0.1
1-Monoacetin	0.75	1.0	0.1
2-Monoacetin	0.82	1.0	0.1
1,3-Diacetin	0.90	1.0	0.1
1,2-Diacetin	0.92	0.73	0.1
Triacetin	1.0	—	—
Individual, unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.2

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.2%

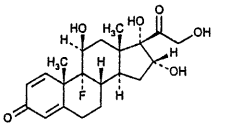
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Triacetin RS

Triamcinolone



$C_{21}H_{27}FO_6$ 394.43

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,16,17,21-tetrahydroxy-, (11β,16α).

9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione [124-94-7].

» Triamcinolone contains not less than 97.0 percent and not more than 102.0 percent of $C_{21}H_{27}FO_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Triamcinolone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution: 20 µg per mL.

Medium: methanol.

Absorptivities at 238 nm, calculated on the dried basis, do not differ by more than 3.0%.

Specific rotation ⟨781S⟩: between +65° and +72°.

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Test solution: 2 mg per mL, in dimethylformamide.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 2.0% of its weight.
Residue on ignition ⟨281⟩: 0.5%.

Assay—
Mobile phase—Prepare a degassed solution containing about 60 volumes of methanol and 40 volumes of water such that the retention times for triamcinolone and hydrocortisone are about 5 and 10 minutes, respectively.
Internal standard solution—Dissolve hydrocortisone in *Mobile phase* to obtain a solution having a concentration of about 0.3 mg per mL.
Standard preparation—Transfer about 10 mg of USP Triamcinolone RS, accurately weighed, to a 50-mL volumetric flask, dissolve in *Internal standard solution*, dilute with the same solvent to volume, and mix.
Assay preparation—Using about 10 mg of Triamcinolone, accurately weighed, prepare as directed under *Standard preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%, and the resolution factor between triamcinolone and hydrocortisone is not less than 3.0.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{27}FO_6$ in the portion of Triamcinolone taken by the formula:

$$50C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Triamcinolone RS in the *Standard preparation*, and R_U and R_S are the peak response ratios of triamcinolone to hydrocortisone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Triamcinolone Tablets

» Triamcinolone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{27}FO_6$.

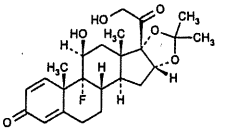
Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Triamcinolone RS
Identification—Powder a number of Tablets, equivalent to about 25 mg of triamcinolone, and digest with 25 mL of acetone for 15 minutes. Filter through a fine-porosity, sintered-glass filtering funnel into about 100 mL of solvent hexane, swirl the liquid, and allow to stand for 30 minutes. Collect the crystals that form, wash the crystals with three 10-mL portions of water followed by 2 mL of acetone, and dry at 60° for 1 hour: the dried crystals so obtained respond to *Identification* test *A* under *Triamcinolone.*
Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{21}H_{27}FO_6$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 238 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Triamcinolone RS in the same *Medium.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{21}H_{27}FO_6$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase , *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Triamcinolone.*
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of triamcinolone, to a suitable container. Add 50.0 mL of *Internal standard solution*, and shake vigorously by mechanical means for 10 minutes. Centrifuge for 10 minutes or until a clear supernatant is obtained.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Triamcinolone.* The relative retention times are about 1.0 for triamcinolone and 1.9 for hydrocortisone. Calculate the quantity, in mg, of $C_{21}H_{27}FO_6$ in the portion of Tablets taken by the formula:

$$50C(R_U/R_S)$$

in which the terms are as defined therein.

Triamcinolone Acetonide



$C_{24}H_{31}FO_6$ 434.50
Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11β,16α)-.
9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone [76-25-5].

» Triamcinolone Acetonide contains not less than 97.0 percent and not more than 102.0 percent of $C_{24}H_{31}FO_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Fluoxymesterone RS
USP Triamcinolone Acetonide RS

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy, 197K*▲ (CN 1-May-2020): recrystallized from methanol.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: methanol.
Specific rotation ⟨781S⟩**:** between +118° and +130°.
Test solution: 5 mg per mL, in dimethylformamide.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 1.5% of its weight.

Chromatographic purity—
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (17:8). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Test solution—Transfer about 25 mg of Triamcinolone Acetonide, accurately weighed, to a 50-mL volumetric flask; dissolve in 25 mL of methanol, shake vigorously to aid dissolution; dilute with *Mobile phase* to volume; and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Test solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between triamcinolone acetonide and any impurity peak is not less than 1.0.
Procedure—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatogram for not less than four times the retention time of triamcinolone acetonide, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Triamcinolone Acetonide taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 0.3% of any individual impurity is found, and not more than 0.8% of total impurities is found.

Assay—
Mobile phase—Prepare a solution of acetonitrile in water containing approximately 30% (v/v) of acetonitrile.
Internal standard solution—Dissolve USP Fluoxymesterone RS in methanol to obtain a solution having a concentration of about 50 µg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Triamcinolone Acetonide RS in *Internal standard solution* to obtain a solution having a known concentration of about 75 µg per mL. Mix an accurately measured volume of the resulting solution with an equal volume of *Mobile phase* to obtain a *Standard preparation* containing about 37.5 µg of USP Triamcinolone Acetonide RS per mL.
Assay preparation—Using about 37 mg of Triamcinolone Acetonide, accurately weighed, proceed as directed for *Standard preparation.*
Procedure—Introduce equal volumes (between 15 µL and 25 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) operated at room temperature, by means of a suitable microsyringe or sampling valve. Adjust the operating parameters with *Mobile phase* on the column so that the separation of triamcinolone acetonide and internal standard is optimized, with a retention time of about 14.5 minutes for triamcinolone acetonide. Typically, the apparatus is fitted with a 4-mm × 30-cm column containing packing L1 and is equipped with a UV detector capable of monitoring absorbance at 254 nm, and a suitable recorder. In a suitable chromatogram, the coefficient of variation for five replicate injections of a single specimen is not more than 3.0%; and the resolution factor, *R* (see *Chromatography* ⟨621⟩), between the peaks for triamcinolone acetonide and fluoxymesterone is not less than 2.0. Measure the heights of the internal standard and triamcinolone acetonide peaks at the same retention times obtained from the *Assay preparation* and the *Standard preparation.* Calculate the quantity, in mg, of $C_{24}H_{31}FO_6$ in the portion of Triamcinolone Acetonide taken by the formula:

$$1000C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Triamcinolone Acetonide RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak heights of triamcinolone acetonide to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Triamcinolone Acetonide Topical Aerosol

» Triamcinolone Acetonide Topical Aerosol is a solution of Triamcinolone Acetonide in a suitable propellant in a pressurized container. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{24}H_{31}FO_6$.

Packaging and storage—Preserve in pressurized containers, and avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Triamcinolone Acetonide RS
Identification—Apply 20 µL of a solution prepared as directed for *Assay preparation* in the *Assay* but without the addition of the *Internal standard solution,* and 20 µL of a solution of USP Triamcinolone Acetonide RS in methanol containing 30 µg per mL, to a line parallel to and about 1.5 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream,* beginning with "Place the plate in a developing chamber." The specified result is obtained.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Other requirements—It meets the requirements for *Pressure Test, Minimum Fill,* and *Leakage Test* under *Topical Aerosols* ⟨603⟩.

Assay—
Mobile phase—Prepare a degassed solution of water and acetonitrile (70:30).
Internal standard solution—Dissolve fluoxymesterone in methanol to obtain a solution having a concentration of about 25 µg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Triamcinolone Acetonide RS in methanol to

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obtain a solution having a concentration of about 100 µg per mL. Transfer 15.0 mL of this solution to a 50-mL volumetric flask, add 25.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix. This solution has a known concentration of about 30 µg per mL.

Assay preparation—Fit the valve of a previously weighed Triamcinolone Acetonide Aerosol container with a suitable tube assembly so that the contents can be sprayed directly into the bulb portion of a 100-mL volumetric flask containing 50.0 mL of *Internal standard solution* and 20 mL of methanol. Spray a portion of the contents, equivalent to about 3 mg of triamcinolone acetonide, into the flask, determining the exact amount sprayed by difference. Place in a sonic bath for about 5 minutes to expel the propellant. Dilute with methanol to volume, and mix. [NOTE—The propellant is extremely flammable. When evaporating, observe proper precautions and work under an explosion-proof hood.]

Procedure—Introduce equal volumes (between 15 µL and 25 µL) of the *Assay preparation* and the *Standard preparation* into a chromatograph (see *Chromatography* ⟨621⟩) operated at room temperature and fitted with a 3.9-mm × 30-cm column, packed with packing L1, and equipped with a 254-nm detector. Adjust the operating parameters and the *Mobile phase* composition such that the separation of triamcinolone acetonide and internal standard is optimized, with a retention time of about 14 minutes for triamcinolone acetonide. In a suitable system, the relative standard deviation for five replicate injections of the *Standard preparation* is not more than 3.0%. Measure the responses of the internal standard and triamcinolone acetonide peaks at the same retention times obtained from the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in µg, of $C_{24}H_{31}FO_6$ in the portion of Topical Aerosol taken by the formula:

$$100C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Triamcinolone Acetonide RS in the *Standard preparation*, and R_U and R_S are the ratios of the peak responses of triamcinolone acetonide to internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Triamcinolone Acetonide Cream

» Triamcinolone Acetonide Cream is Triamcinolone Acetonide in a suitable cream base. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{24}H_{31}FO_6$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Triamcinolone Acetonide RS

Identification—Place a 2-g quantity of Cream in a conical flask, add 50 mL of chloroform and 15 g of anhydrous sodium sulfate, and swirl to dissolve the specimen. Filter the solution and clarify the filtrate, if necessary, by the further addition of anhydrous sodium sulfate and a second filtration. Evaporate the filtrate to near dryness, and dissolve the residue in chloroform to obtain a solution containing about 100 µg per mL. Apply 10 µL of this solution and 10 µL of a solution of USP Triamcinolone Acetonide RS in chloroform containing 100 µg per mL, on a line parallel to and about 1.5 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in a developing chamber containing and equilibrated with a mixture of chloroform, benzene, and methanol (100:40:20). Develop the chromatogram until the solvent front has moved about 12 cm above the line of application. Remove the plate, allow the solvent to evaporate, and spray with a mixture of equal volumes of sodium hydroxide solution (1 in 5) and a 1 in 500 solution of blue tetrazolium in methanol: the intensity of the blue color and the R_F of the spot obtained with the solution under test are similar to those of the spot obtained with the Standard solution.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Minimum fill ⟨755⟩: meets the requirements.

Assay—

Mobile phase—Prepare a solution of acetonitrile in water containing approximately 30% (v/v) of acetonitrile.

Internal standard solution—Dissolve fluoxymesterone in isopropyl alcohol to obtain a solution having a concentration of about 50 µg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Triamcinolone Acetonide RS in *Internal standard solution* to obtain a solution having a known concentration of about 75 µg per mL. Mix an accurately measured volume of the resulting solution with an equal volume of *Mobile phase* to obtain a *Standard preparation* containing about 37.5 µg of USP Triamcinolone Acetonide RS per mL.

Assay preparation—Transfer an accurately weighed quantity of Cream, equivalent to about 1.5 mg of triamcinolone acetonide, to a screw-cap tube. Add 20.0 mL of *Internal standard solution*, and cap securely. Heat for 5 minutes at 60°, then swirl vigorously for not less than 30 seconds. Repeat the heating and swirling sequence three times. Cool in a methanol-ice bath for 15 to 20 minutes, then centrifuge for 15 minutes at −5°. Dilute an accurately measured volume of the supernatant with an equal volume of *Mobile phase*. Cool in a methanol-ice bath for 10 to 15 minutes, with occasional agitation. Filter first through a pledget of glass wool or a prefilter disk and then through a 0.45-µm porosity membrane to obtain a clear solution.

Procedure—Introduce equal volumes (between 15 and 25 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩), operated at room temperature, by means of a suitable microsyringe or sampling valve. Adjust the operating parameters with *Mobile phase* on the column, such that the separation of triamcinolone acetonide and internal standard is optimized, with a retention time of about 14.5 minutes for triamcinolone acetonide. Typically, the apparatus is fitted with a 30-cm × 4-mm column containing packing L1, and is equipped with a UV detector capable of monitoring absorbance at 254 nm, and a suitable recorder. In a suitable chromatogram, the coefficient of variation for five replicate injections of a single specimen is not more than 3.0%, and the resolution factor, R (see *Chromatography* ⟨621⟩), between the peaks for triamcinolone acetonide and fluoxymesterone is not less than 2.0. Measure the heights of the internal standard and triamcinolone acetonide peaks, at the same retention times obtained from the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of $C_{24}H_{31}FO_6$ in the portion of Cream taken by the formula:

$$40C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Triamcinolone Acetonide RS in the *Standard preparation*, and

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R_U and R_S are the ratios of the peak heights of triamcinolone acetonide to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Triamcinolone Acetonide Lotion

» Triamcinolone Acetonide Lotion is Triamcinolone Acetonide in a suitable lotion base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{24}H_{31}FO_6$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Triamcinolone Acetonide RS
Identification—It responds to the *Identification* test under *Triamcinolone Acetonide Cream.*
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Minimum fill ⟨755⟩: meets the requirements.
Assay—Proceed with Lotion as directed in the *Assay* under *Triamcinolone Acetonide Cream*, except to read "Lotion" in place of "Cream" throughout.

Triamcinolone Acetonide Ointment

» Triamcinolone Acetonide Ointment is Triamcinolone Acetonide in a suitable ointment base. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{24}H_{31}FO_6$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Triamcinolone Acetonide RS
Identification—Place 2 g of Ointment in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg per mL. Apply 10 µL of this solution, and 10 µL of a solution of USP Triamcinolone Acetonide RS in alcohol containing 250 µg per mL, on a line parallel to and about 1.5 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in a developing chamber containing and equilibrated with a mixture of chloroform, benzene, and methanol (100:40:20). Develop the chromatogram until the solvent front has moved about 12 cm above the line of application. Remove the plate, allow the solvent to evaporate, and spray with a mixture of equal volumes of sodium hydroxide solution (1 in 5) and a 1 in 500 solution of blue tetrazolium in methanol: the intensity of the blue color and the R_F of the spot obtained from the solution under test are similar to those of the spot obtained from the Standard solution.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Minimum fill ⟨755⟩: meets the requirements.
Assay—Proceed with Ointment as directed in the *Assay* under *Triamcinolone Acetonide Cream*, except to read "Ointment" in place of "Cream" throughout.

Triamcinolone Acetonide Dental Paste

» Triamcinolone Acetonide Dental Paste is Triamcinolone Acetonide in a suitable emollient paste. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{24}H_{31}FO_6$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Triamcinolone Acetonide RS
Identification—It responds to the *Identification* test under *Triamcinolone Acetonide Cream.*
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Mobile phase—Prepare as directed in the *Assay* under *Triamcinolone Acetonide Cream.*
Internal standard solution and *Standard preparation*—Prepare as directed in the *Assay* under *Triamcinolone Acetonide Cream.*
Assay preparation—Transfer an accurately weighed quantity of Dental Paste, equivalent to about 1.5 mg of triamcinolone acetonide, to a screw-cap tube. Add 20.0 mL of *Internal standard solution*, cap, and place in a sonic bath for 15 to 20 minutes. Place in a water bath at 70° for 5 minutes, then swirl for 1 minute. Repeat the heating and swirling sequence once. Cool in an ice–methanol bath for 15 minutes, then centrifuge for 10 minutes at –5°. Mix an accurately measured volume of the supernatant with an equal volume of *Mobile phase.* Place in an ice–methanol bath for 15 minutes, then centrifuge for 15 minutes. Draw off and discard the upper phase. Filter the lower phase to obtain a clear solution.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Triamcinolone Acetonide Cream.*

Triamcinolone Acetonide Nasal Spray

DEFINITION
Triamcinolone Acetonide Nasal Spray is an aqueous suspension of Triamcinolone Acetonide. It is supplied in a form suitable for nasal administration. It contains NLT 90.0% and NMT 110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

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ASSAY

• **PROCEDURE**

Buffer A: 3.4 g/L of monobasic potassium phosphate prepared as follows. Dissolve 3.4 g of monobasic potassium phosphate in 900 mL of water, adjust with 5 M sodium hydroxide to a pH of 7.0, and dilute with water to 1000 mL.

Buffer B: 3.4 g/L of monobasic potassium phosphate prepared as follows. Dissolve 3.4 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.0, and dilute with water to 1000 mL.

Solution A: Acetonitrile and *Buffer A* (27.5: 72.5)

Solution B: Acetonitrile and *Buffer A* (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	60	40
30.1	0	100
44	0	100
44.1	100	0
52	100	0

Diluent: Acetonitrile and *Buffer B* (27.5: 72.5)

Standard stock solution: 0.4 mg/mL of USP Triamcinolone Acetonide RS in acetonitrile. Sonication for 15 min may be used to aid in dissolution.

Standard solution: 40 µg/mL of USP Triamcinolone Acetonide RS from *Standard stock solution* in *Diluent*

System suitability stock solution: 0.04 mg/mL of USP Triamcinolone Acetonide Related Compound B RS and USP Triamcinolone Acetonide Related Compound C RS in *Diluent*

System suitability solution: 40 µg/mL of USP Triamcinolone Acetonide RS and 0.8 µg/mL each of USP Triamcinolone Acetonide Related Compound B RS and USP Triamcinolone Acetonide Related Compound C RS in suitable volumes of *Standard stock solution* and *System suitability stock solution* in *Diluent*

Sample solution: Nominally 40 µg/mL of triamcinolone acetonide prepared as follows. Transfer a portion of the Nasal Spray, equivalent to 4 mg of triamcinolone acetonide, to a 100-mL volumetric flask. Dissolve in 28 mL of acetonitrile with the aid of sonication. Allow to cool to room temperature and dilute with *Buffer B* to volume. Centrifuge and use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.75 mL/min
Injection volume: 100 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 4* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between triamcinolone acetonide related compound C and triamcinolone acetonide related compound B; NLT 3.0 between triamcinolone acetonide related compound B and triamcinolone acetonide, *System suitability solution*
Tailing factor: NMT 1.3 for triamcinolone acetonide, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Triamcinolone Acetonide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of triamcinolone acetonide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF EDETATE DISODIUM**

Perform this test if edetate disodium is a known component in the Nasal Spray.

Buffer: Add 990 mL of water into a 1000-mL beaker, followed by 10.0 mL of 1.0 M tetrabutylammonium hydroxide in methanol. Adjust with phosphoric acid to a pH of 7.0.

Mobile phase: Acetonitrile and *Buffer* (15:85)

Solution A: 40 g/L of sodium chloride and 2 g/L of sodium acetate. Adjust with glacial acetic acid to a pH of 5.5.

Solution B: 1.0 g/L of cupric sulfate in water

Diluent: Acetonitrile and water (50:50)

Standard stock solution: 0.5 mg/mL of edetate disodium in water. Sonication may be used to aid in dissolution.

Standard solution: 0.05 mg/mL of edetate disodium. Transfer 5.0 mL of *Standard stock solution* to a 50-mL volumetric flask. Next add 10 mL of *Solution A* and then add 5.0 mL of acetonitrile. Mix the resulting solution and then add 20.0 mL of *Solution B*, dilute with *Diluent* to volume, and mix.

Sample solution: Combine the contents of NLT 5 bottles of Nasal Spray and mix the contents to obtain a composite suspension. Transfer a 5.0-g portion of the Nasal Spray to a 50-mL volumetric flask. Add 10 mL of *Solution A* and 5.0 mL of acetonitrile. Mix and sonicate for 10 min and allow the solution to equilibrate to room temperature. Add 20.0 mL of *Solution B*, and sonicate for 10 min. Allow the sample to equilibrate to room temperature and dilute with *Diluent* to volume. Centrifuge a portion for 15 min, and use the supernatant. [NOTE—Centrifuging at 4000 rpm for 15 min may be suitable.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.1-mm × 15-cm; 5-µm packing L21
Flow rate: 1 mL/min
Injection volume: 25 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of edetate disodium ($C_{10}H_{14}N_2Na_2O_8$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

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r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of edetate disodium in the *Standard solution* (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Nasal Spray in the *Sample solution* (mg)

Acceptance criteria: 0.045%–0.055%

Change to read:

- **CONTENT OF BENZALKONIUM CHLORIDE**

Perform this test if benzalkonium chloride is a known component in the Nasal Spray.
Buffer: Dissolve 10.8 g of monobasic sodium phosphate dihydrate in 90 mL of water, and adjust with phosphoric acid a pH of 2.5. Dilute with water to 100 mL.
Solution A: Mix 50 mL of *Buffer*, 750 mL of water, and 200 mL of methanol. Add 5 mL of triethylamine. Mix and adjust with phosphoric acid to a pH of 2.5.
Solution B: Methanol and phosphoric acid prepared as follows. Mix 1 L of methanol with 50 mL of phosphoric acid.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	55	45
3.0	5	95
3.2	55	45
5.0	55	45

Diluent: 1% (v/v) hydrochloric acid in methanol
System suitability solution: ▲0.04 mg/mL of USP Benzalkonium Chloride RS in *Diluent* prepared as follows. Transfer a suitable volume of USP Benzalkonium Chloride RS to a suitable volumetric flask and dilute with *Diluent* to volume. ▲ (USP 1-May-2019)
Standard stock solution: 0.2 mg/mL of USP Benzalkonium Bromide RS in water. [NOTE—A few drops of methanol may be used to resolve the formation of foam prior to dilution.]
Standard solution: 0.04 mg/mL of USP Benzalkonium Bromide RS. Transfer an aliquot of *Standard stock solution* to a suitable volumetric flask, and add water equal to 30% of the flask volume. Dilute with *Diluent* to volume.
Sample solution: Combine the contents of NLT 5 bottles of Nasal Spray and mix the contents to obtain a composite suspension. Transfer a 5.0-g portion of the Nasal Spray to a 10-mL volumetric flask. Dilute with *Diluent* to volume. Centrifuge and use the supernatant. [NOTE—Centrifuging at 4000 rpm for 15 min may be suitable. Supernatant may be passed through a suitable filter of NMT 0.2-µm pore size.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 3.0-cm; 2.6-µm packing L1
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—*Table 3* for the relative retention times. The peak due to the C10 analog may not be visible due to its low concentration in the *System suitability solution*▲The *Standard solution* may contain only one peak as it is predominantly the C12 analog.▲ (USP 1-May-2019)]
Suitability requirements
Resolution: NLT 2.5 between the pairs of C12 and C14 homologs and C14 and C16 homologs of benzalkonium, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the sum of each corrected benzalkonium peak response (r_U) as follows:

$$\text{Result} = \Sigma(r_U) \times (1/F)$$

r_U = peak response of each benzalkonium homolog from the *Sample solution*
F = relative response factor of the corresponding benzalkonium homolog relative to benzalkonium bromide (see *Table 3*)

Table 3

Benzalkonium Chloride Analog	Relative Retention Time	Relative Response Factor
C10	0.65	1.3
C12	1.0	1.2
C14	1.35	1.0
C16	1.59	0.98

Calculate the percentage of benzalkonium chloride in the portion of Nasal Spray taken:

$$\text{Result} = (\Sigma r_U / r_S) \times C_S \times (V/W) \times 100$$

Σr_U = sum of the corrected peak responses of the benzalkonium homologs from the *Sample solution*
r_S = peak response of benzalkonium from the *Standard solution*
C_S = concentration of USP Benzalkonium Bromide RS in the *Standard solution* (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Nasal Spray in the *Sample solution* (mg)

Acceptance criteria: 0.0135%–0.0165%

PERFORMANCE TESTS

Change to read:

- **DELIVERED DOSE UNIFORMITY (BETWEEN CONTAINERS)**

Buffer: 7.0 g/L of sodium perchlorate prepared as follows. Dissolve 7.0 g of sodium perchlorate in 900 mL of water, adjust with perchloric acid to a pH of 3.0, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (50:50)
Standard solution: 40 µg/mL of USP Triamcinolone Acetonide RS in *Mobile phase*
Sample solution: Nominally 40 µg/mL of triamcinolone acetonide prepared as follows. Transfer a portion of Nasal Spray, equivalent to 4.0 mg of triamcinolone acetonide, to a suitable volumetric flask. Add *Mobile phase* to 80% of the flask volume. Sonicate for 15 min. Allow to equilibrate to room temperature. Dilute with *Mobile phase* to volume. Centrifuge and pass the supernatant through a filter of

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0.45-µm pore size. [NOTE—Centrifuging at 4000 rpm for 45 min may be suitable.]
Repeat this procedure with 9 additional ▲containers.▲ (USP 1-May-2019)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 40 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 2%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲the labeled amount of▲ (USP 1-May-2019) triamcinolone acetonide ($C_{24}H_{31}FO_6$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Triamcinolone Acetonide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of triamcinolone acetonide in the *Sample solution* (µg/mL)

Acceptance criteria
Tier 1: The content of each of the 10 ▲containers▲ (USP 1-May-2019) is within 90.0%–110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
If the criterion in *Tier 1* cannot be met, proceed to *Tier 2*.
Tier 2: If the content of 1 ▲container▲ (USP 1-May-2019) is outside 90.0%–110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$) and the content of none of the ▲containers▲ (USP 1-May-2019) is outside 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$), test an additional 20 ▲containers.▲ (USP 1-May-2019) All the 30 results (including the results from *Tier 1*) meet the following acceptance criteria.

1. The content of each of 29 out of 30 ▲containers▲ (USP 1-May-2019) is within 90.0%–110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
2. The content of each of the 30 ▲containers▲ (USP 1-May-2019) is within 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Change to read:

• **DELIVERED DOSE UNIFORMITY (WITHIN CONTAINER)**
Buffer: 7.0 g/L of sodium perchlorate prepared as follows. Dissolve 7.0 g of sodium perchlorate in 900 mL of water, adjust with perchloric acid to a pH of 3.0, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* ▲(50:50)▲ (USP 1-May-2019)
Standard solution: 4.8 µg/mL of USP Triamcinolone Acetonide RS in *Mobile phase*
Beginning sample solution (BOU): Hold the pump upright, actuate 5 times, and wipe the nosepiece dry. Hold a 25-mL volumetric flask in an inverted position and actuate the pump. Quickly turn the flask upright, wait 10 s, and repeat the process. Add 15 mL of *Mobile phase* while rinsing the neck of the flask and sonicate for 15 min. Allow the flask to equilibrate to room temperature, and dilute with *Mobile phase* to volume. Centrifuge and use the clear supernatant. [NOTE—Centrifuging at 4000 rpm for 15 min may be suitable.]
Middle sample solution (MOU): Using the same pump as above, discharge an appropriate number of actuations to arrive at 50% of the labeled number of actuations to waste and wipe the nosepiece dry. Hold a 25-mL volumetric flask in an inverted position and actuate the pump to collect the next actuation. Quickly turn the flask upright, wait 10 s, and repeat the process to collect the next actuation. Add 15 mL of *Mobile phase* while rinsing the neck of the flask and sonicate for 15 min. Allow the flask to equilibrate to room temperature, and dilute with *Mobile phase* to volume. Centrifuge and use the clear supernatant. This is the MOU sample. [NOTE—Centrifuging at 4000 rpm for 15 min may be suitable.]
End sample solution (EOU): Using the same pump as above, discharge the next appropriate number of actuations to arrive at 100% of the labeled number of actuations to waste, and wipe the nosepiece dry. Hold a 25-mL volumetric flask in an inverted position and actuate the pump to collect the next actuation. Quickly turn the flask upright, wait 10 s, and repeat the process to collect the next actuation. Add 15 mL of *Mobile phase* while rinsing the neck of the flask and sonicate for 15 min. Allow the flask to equilibrate to room temperature, and dilute with *Mobile phase* to volume. Centrifuge and use the clear supernatant. This is the EOU sample. [NOTE—Centrifuging at 4000 rpm for 15 min may be suitable.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 200 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution*, *Beginning sample solution*, *Middle sample solution*, and *End sample solution*
Calculate the percentage of the labeled amount of the triamcinolone acetonide ($C_{24}H_{31}FO_6$) delivered dose:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the appropriate *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Triamcinolone Acetonide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of triamcinolone acetonide in the appropriate *Sample solution* (µg/mL)

Acceptance criteria
Calculate the mean delivered dose from the BOU results from all 10 containers.
Calculate the mean delivered dose from the MOU results from all 10 containers.
Calculate the mean delivered dose from the EOU results from all 10 containers.

USP Monographs

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Calculate the ▲container delivery mean▲ (USP 1-May-2019) from the BOU, MOU, and EOU results from each of the 10 containers.

Tier 1

1. Mean delivered dose of BOU samples from all 10 ▲containers▲ (USP 1-May-2019) is within 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
2. Mean delivered dose of MOU samples from all 10 ▲containers▲ (USP 1-May-2019) is within 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
3. Mean delivered dose of EOU samples from all 10 ▲containers▲ (USP 1-May-2019) is within 85.0%–115.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
4. NMT 1 ▲container delivery mean from the 10 containers▲ (USP 1-May-2019) is outside 80.0%–120.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
5. None of the ▲container delivery means from the 10 containers▲ (USP 1-May-2019) is outside 75.0%–125.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

▲*Tier 1* criteria 1–3 must be met. If criterion 4 or 5 cannot be met, proceed to *Tier 2*.▲ (USP 1-May-2019)

Tier 2: ▲If NMT 3 container delivery means are outside 80.0%–120.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$) and none of the container delivery means is outside 75.0%–125.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$), test an additional 20 containers. All 30 container delivery means (including the results from *Tier 1*) meet the following acceptance criteria.▲ (USP 1-May-2019)

1. NMT 3 of 30 ▲container delivery means▲ (USP 1-May-2019) are outside 80.0%–120.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).
2. None of the 30 ▲container delivery means▲ (USP 1-May-2019) is outside 75.0%–125.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

IMPURITIES

Change to read:

• **Organic Impurities**

Buffer A, Buffer B, Solution A, Solution B, Mobile phase, Diluent, Standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each degradation product in the portion of Nasal Spray taken:

$$\text{Result} = (r_i/r_U) \times 100$$

r_i = peak response of each degradation product from the *Sample solution*

r_U = peak response of triamcinolone acetonide from the *Sample solution*

Acceptance criteria: See *Table 4*. Disregard any peak below 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Triamcinolone acetonide ketoacid derivative[a]	0.4	▲0.3▲ (RB 1-May-2019)
Triamcinolone acetonide related compound C	0.83	▲2.8▲ (RB 1-May-2019)
Triamcinolone acetonide related compound B	0.91	0.4
Triamcinolone acetonide	1.0	—
Any other individual degradation product	—	0.1
Total degradation products	—	▲3.4▲ (RB 1-May-2019)

[a] 9-Fluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-(11β,16α)-3,20-dioxopregna-1,4-diene-21-oic acid.

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.5–6.0

Delete the following:

▲• **Osmolality and Osmolarity** ⟨785⟩: 280–380 mOsmol/kg▲ (USP 1-May-2019)

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, and *Pseudomonas aeruginosa*. The total aerobic microbial count is NMT 10^1 cfu/mL and the total combined molds and yeasts count is NMT 10^1 cfu/mL.

• **Particle Size**

Analysis: Shake the Nasal Spray and prime the pump by spraying 3–4 times. Actuate the spray and collect the sample on a glass microscope slide held above the nozzle, and repeat to prepare a second slide. Using light microscopy, determine the dimension of NLT 200 particles of triamcinolone acetonide by measuring NLT 100 particles from 20 random fields of view for each slide prepared. Repeat the procedure using a second container of Nasal Spray.

Acceptance criteria: See *Table 5*.

Table 5

Particle Size (µm)	Acceptance Criteria (%)
<1	NMT 3
1–6	70–95
>9	NMT 4

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in a tight, light-resistant container, and store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Benzalkonium Bromide RS

USP Benzalkonium Chloride RS

USP Triamcinolone Acetonide RS

USP Triamcinolone Acetonide Related Compound B RS

9-Fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene) bis(oxy)]-(11β,16α)-pregna-1,4,14-triene-3,20-dione.

$C_{24}H_{29}FO_6$ 432.48

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USP Triamcinolone Acetonide Related Compound C RS
9-Fluoro-11,21,21-trihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-(11β,16α)-pregna-1,4-diene-3,20-dione.
$C_{24}H_{31}FO_7$ 450.50

Triamcinolone Acetonide Injectable Suspension

DEFINITION
Triamcinolone Acetonide Injectable Suspension is a sterile suspension of Triamcinolone Acetonide in a suitable aqueous medium. It contains NLT 90.0% and NMT 115.0% of the labeled amount of $C_{24}H_{31}FO_6$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Sample: Extract a volume of Injectable Suspension, equivalent to 50 mg of triamcinolone acetonide, with two 10-mL portions of peroxide-free ether, and discard the ether extracts. Filter with the aid of suction, wash with small portions of water, and dry the precipitate at 105° for 1 h.
Acceptance criteria: Meets the requirements

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲** (CN 1-May-2020)

Sample solution: 20 µg/mL in methanol, using the triamcinolone acetonide obtained from *Identification* test *A*
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (30:70)
Internal standard solution: 84 µg/mL of fluoxymesterone in methanol
Standard stock solution: 200 µg/mL of USP Triamcinolone Acetonide RS in methanol
Standard solution: 80 µg/mL of USP Triamcinolone Acetonide RS from the *Standard stock solution* diluted with *Internal standard solution*
Sample stock solution: Dissolve a volume of freshly mixed Injectable Suspension in methanol to obtain a solution containing 200 µg/mL of triamcinolone acetonide.
Sample solution: 80 µg/mL of triamcinolone acetonide from the *Sample stock solution* diluted with *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 1.0 mL/min
Injection size: 15–25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between triamcinolone acetonide and fluoxymesterone
Relative standard deviation: NMT 3.0% for five replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{31}FO_6$ in the portion of Injectable Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the triamcinolone acetonide peak to the internal standard peak from the *Sample solution*
R_S = peak response ratio of the triamcinolone acetonide peak to the internal standard peak from the *Standard solution*
C_S = concentration of USP Triamcinolone Acetonide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of triamcinolone acetonide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 4.4 USP Endotoxin Units/mg of triamcinolone acetonide.
- **pH ⟨791⟩:** 5.0–7.5
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS ⟨1⟩:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature. Do not freeze.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Triamcinolone Acetonide RS

Triamcinolone Diacetate

$C_{25}H_{31}FO_8$ 478.51
Pregna-1,4-diene-3,20-dione, 16,21-bis(acetyloxy)-9-fluoro-11,17-dihydroxy-, (11β,16α)-.
9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione 16,21-diacetate [67-78-7].

» Triamcinolone Diacetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{25}H_{31}FO_8$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Triamcinolone Diacetate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K▲* (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U▲* (CN 1-May-2020)—

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Solution: 20 µg per mL.
Medium: dehydrated alcohol.
Absorptivities at 238 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.
Specific rotation ⟨781S⟩: between +39° and +45°.
Test solution: 5 mg per mL, in dimethylformamide.
Water Determination, *Method I* ⟨921⟩: not more than 6.0%.
Residue on ignition ⟨281⟩: not more than 0.5%.

Assay—
0.005 M Monobasic sodium phosphate solution—Dissolve monobasic sodium phosphate in water to obtain a solution containing 690 µg per mL.
Mobile phase—Prepare a mixture of *0.005 M Monobasic sodium phosphate solution*, acetonitrile, and tetrahydrofuran (62:37:1), filter through a 0.45-µm solvent-resistant filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Triamcinolone Diacetate RS in *Mobile phase*, and dilute quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 40 µg per mL.
Assay preparation—Transfer about 50 mg of Triamcinolone Diacetate, accurately weighed, to a 50-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Pipet 2 mL of this solution into a second 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
System suitability preparation—Dissolve suitable quantities of USP Triamcinolone Diacetate RS and propylparaben in *Mobile phase* to obtain a solution containing about 40 µg per mL and 15 µg per mL, respectively.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are 1.0 for triamcinolone diacetate and about 1.1 for propylparaben, the resolution, *R*, between the triamcinolone diacetate and propylparaben peaks is not less than 1.7, and the tailing factor, *T*, for the analyte peak is not more than 1.5. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{25}H_{31}FO_8$ in the portion of Triamcinolone Diacetate taken by the formula:

$$1.25C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Triamcinolone Diacetate RS in the *Standard preparation*, and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Triamcinolone Diacetate Oral Solution

» Triamcinolone Diacetate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone diacetate ($C_{25}H_{31}FO_8$). It contains a suitable preservative.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Triamcinolone Diacetate RS
Identification—Transfer a quantity of Oral Solution, equivalent to about 10 mg of triamcinolone diacetate, to a separator, and extract with three 10-mL portions of chloroform. Evaporate the combined chloroform extracts on a steam bath to dryness, and dissolve the residue in 5.0 mL of chloroform. Apply 10 µL each of this solution and a solution of USP Triamcinolone Diacetate RS in chloroform containing 2 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate and chloroform (9:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2) and heating on a hot plate or under a lamp until spots appear: the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Assay—
0.005 M Monobasic sodium phosphate solution, Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Triamcinolone Diacetate.*
Assay preparation—Quantitatively transfer an accurately measured portion of Oral Solution, equivalent to about 50 mg of triamcinolone diacetate to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pipet 4 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer about 25 mL of this solution to a 50-mL, glass-stoppered centrifuge tube, and centrifuge at high speed for 10 minutes.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Triamcinolone Diacetate.* Calculate the quantity, in mg, of triamcinolone diacetate ($C_{25}H_{31}FO_8$) in the portion of Oral Solution taken by the formula:

$$1.25C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Triamcinolone Diacetate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Triamcinolone Diacetate Injectable Suspension

» Triamcinolone Diacetate Injectable Suspension is a sterile suspension of Triamcinolone Diacetate in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{25}H_{31}FO_8$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Triamcinolone Diacetate RS

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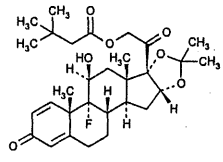
Identification—Filter a volume of Injectable Suspension, equivalent to about 50 mg of triamcinolone diacetate, through a medium-porosity, sintered-glass funnel, wash with water, and dry the crystals in vacuum at 60° for 1 hour. Dissolve 2 mg of the dried crystals in 1 mL of methanol in a small mortar. Evaporate with the aid of gentle heat and a stream of nitrogen to dryness: the crystals so obtained respond to *Identification* test *A* under *Triamcinolone Diacetate.*
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 7.1 USP Endotoxin Units per mg of triamcinolone diacetate.
Uniformity of dosage units ⟨905⟩: meets the requirements.
pH ⟨791⟩: between 4.5 and 7.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
0.005 M Monobasic sodium phosphate solution, Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Triamcinolone Diacetate.*
Assay preparation—Quantitatively transfer an accurately measured portion of Injectable Suspension, equivalent to about 50 mg of triamcinolone diacetate, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pipet 2 mL of this solution into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Triamcinolone Diacetate.* Calculate the quantity, in mg, of $C_{25}H_{31}FO_8$ in the portion of Injectable Suspension taken by the formula:

$$1.25C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Triamcinolone Diacetate RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Triamcinolone Hexacetonide



$C_{30}H_{41}FO_7$ 532.64
Pregna-1,4-diene-3,20-dione, 21-(3,3-dimethyl-1-oxobutoxy)-9-fluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11β,16α)-;
9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone 21-(3,3-dimethylbutyrate) [5611-51-8].

DEFINITION
Triamcinolone Hexacetonide contains NLT 97.0% and NMT 102.0% of $C_{30}H_{41}FO_7$, calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Mobile phase: Methanol and water (3:1)
System suitability solution: 0.4 mg/mL each of USP Triamcinolone Acetonide RS and USP Triamcinolone Hexacetonide RS in methanol
Standard solution: 0.4 mg/mL of USP Triamcinolone Hexacetonide RS in methanol
Sample solution: 0.4 mg/mL of Triamcinolone Hexacetonide in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for triamcinolone acetonide and triamcinolone hexacetonide are 0.27 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 7.5 between triamcinolone acetonide and triamcinolone hexacetonide
Tailing factor: NMT 1.3 for triamcinolone hexacetonide
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{30}H_{41}FO_7$ in the portion of Triamcinolone Hexacetonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Triamcinolone Hexacetonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Triamcinolone Hexacetonide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES
ORGANIC IMPURITIES
• **Procedure: Limit of Triamcinolone Acetonide**
Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*
Standard solution: 0.004 mg/mL of USP Triamcinolone Acetonide RS in methanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of triamcinolone acetonide in the portion of Triamcinolone Hexacetonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triamcinolone acetonide from the *Sample solution*
r_S = peak response of triamcinolone acetonide from the *Standard solution*
C_S = concentration of USP Triamcinolone Acetonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Triamcinolone Hexacetonide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

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SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in chloroform
  Acceptance criteria: +91° to +98°
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample in a vacuum at 60° for 4 h: it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Triamcinolone Acetonide RS
  USP Triamcinolone Hexacetonide RS

Triamcinolone Hexacetonide Injectable Suspension

DEFINITION
Triamcinolone Hexacetonide Injectable Suspension is a sterile suspension of Triamcinolone Hexacetonide in a suitable aqueous medium. It contains NLT 90.0% and NMT 115.0% of the labeled amount of triamcinolone hexacetonide ($C_{30}H_{41}FO_7$).

IDENTIFICATION

Change to read:

- **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Place a volume of Injectable Suspension, equivalent to 25 mg of triamcinolone hexacetonide, and 2 mL of water in a membrane filter of 0.20-µm pore size. Apply vacuum to the filter, wash the residue with two 5-mL portions of water, and air-dry the filter and the precipitate. Place the dried filter and precipitate in a small beaker with 5 mL of alcohol, and dissolve the precipitate. Decant the alcohol solution into a small beaker and evaporate with the aid of low heat and a current of air to dryness.
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Mobile phase: Methanol and water (3:1)
  System suitability solution: 0.3 mg/mL of amcinonide and 0.4 mg/mL of USP Triamcinolone Hexacetonide RS in methanol
  Standard solution: 0.4 mg/mL of USP Triamcinolone Hexacetonide RS in methanol
  Sample solution: Using a "to contain" pipet, transfer a volume of Injectable Suspension, equivalent to 40 mg of triamcinolone hexacetonide, to a 100-mL volumetric flask. Rinse the pipet with methanol, collecting the rinse in the volumetric flask. Dilute with methanol to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 1.4 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for amcinonide and triamcinolone hexacetonide are about 0.50 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 10 between amcinonide and triamcinolone hexacetonide
  Tailing factor: NMT 1.2 for triamcinolone hexacetonide
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{30}H_{41}FO_7$ in the portion of Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triamcinolone hexacetonide from the *Sample solution*
r_S = peak response of triamcinolone hexacetonide from the *Standard solution*
C_S = concentration of USP Triamcinolone Hexacetonide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of triamcinolone hexacetonide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES
ORGANIC IMPURITIES
- **Procedure: Limit of Triamcinolone Acetonide**

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.4 mg/mL each of USP Triamcinolone Acetonide RS and USP Triamcinolone Hexacetonide RS in methanol
Standard solution: 0.004 mg/mL of USP Triamcinolone Acetonide RS in methanol
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 7.5 between triamcinolone acetonide and triamcinolone hexacetonide
Tailing factor: NMT 1.2 for triamcinolone hexacetonide
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of triamcinolone acetonide in the portion of Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triamcinolone acetonide from the *Sample solution*
r_S = peak response of triamcinolone acetonide from the *Standard solution*
C_S = concentration of USP Triamcinolone Acetonide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of triamcinolone hexacetonide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

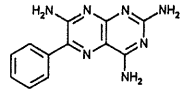
SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 17.2 USP Endotoxin Units/mg of triamcinolone hexacetonide.
- **pH** ⟨791⟩**:** 4.0–8.0
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at controlled room temperature. Do not freeze.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Triamcinolone Acetonide RS
  USP Triamcinolone Hexacetonide RS

Triamterene



$C_{12}H_{11}N_7$ 253.26

2,4,7-Pteridinetriamine, 6-phenyl-;
2,4,7-Triamino-6-phenylpteridine;
6-Phenylpteridine-2,4,7-triamine [396-01-0].

DEFINITION

Triamterene contains NLT 98.0% and NMT 102.0% of triamterene ($C_{12}H_{11}N_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197M▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: *Acetonitrile*, methanol, butylamine, and water (140:140:2:720). Sonicate to mix and adjust with acetic acid to a pH of 5.3.

Standard solution: 0.05 mg/mL of USP Triamterene RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Triamterene in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 355 nm
Column: 4.0-mm × 25-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of triamterene

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of triamterene ($C_{12}H_{11}N_7$) in the portion of Triamterene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triamterene from the *Sample solution*
r_S = peak response of triamterene from the *Standard solution*
C_S = concentration of USP Triamterene RS in the *Standard solution* (mg/mL)
C_U = concentration of Triamterene in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Diluent: To 50 mL of 0.1 N sodium hydroxide in a 500-mL volumetric flask, add 250 mL of acetonitrile and dilute with water to volume.

Standard stock solution 1: 0.25 mg/mL of USP Triamterene RS in *Mobile phase*

Standard stock solution 2: 0.1 mg/mL of USP Triamterene Related Compound A RS in *Diluent*. Sonication may be required for complete dissolution.

Standard stock solution 3: 0.1 mg/mL of USP Triamterene Related Compound B RS in *Diluent*. Sonication may be required for complete dissolution.

Standard stock solution 4: 0.1 mg/mL of USP Triamterene Related Compound C RS in *Diluent*. Sonication may be required for complete dissolution.

System suitability solution: 0.1 mg/mL of USP Triamterene RS from *Standard stock solution 1* and 0.01 mg/mL of USP Triamterene Related Compound B RS from *Standard stock solution 3* in *Mobile phase*

Sensitivity solution: 0.5 µg/mL of USP Triamterene RS in *Mobile phase* from *Standard stock solution 1*

Standard solution: 0.001 mg/mL each of USP Triamterene RS, USP Triamterene Related Compound A RS, USP Triamterene Related Compound B RS, and USP Triamterene Related Compound C RS from the respective *Standard stock solution* in *Mobile phase*

Sample solution: 1 mg/mL of Triamterene in *Mobile phase*. [NOTE—Sonication may be required to aid the dissolution.]

System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—See *Table 1* for the relative retention times for triamterene related compound B and triamterene.]
Suitability requirements
Resolution: NLT 2.0 between triamterene related compound B and triamterene, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of triamterene related compound A, triamterene related compound B, and triamterene related compound C in the portion of Triamterene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each corresponding specified impurity from the *Sample solution*
r_S = peak response of each corresponding specified impurity from the *Standard solution*
C_S = concentration of each corresponding specified impurity in the *Standard solution* (mg/mL)
C_U = concentration of Triamterene in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Triamterene taken:

USP Monographs

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of triamterene from the *Standard solution*
C_S = concentration of USP Triamterene RS in the *Standard solution* (mg/mL)
C_U = concentration of Triamterene in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Reporting threshold: 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Triamterene related compound A	0.4	0.1
Triamterene related compound B	0.8	0.15
Triamterene	1.0	—
Triamterene related compound C	2.8	0.15
Any unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 105° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Triamterene RS
USP Triamterene Related Compound A RS
5-Nitrosopyrimidine-2,4,6-triamine.
$C_4H_6N_6O$ 154.13
USP Triamterene Related Compound B RS
2,7-Diamino-6-phenylpteridin-4-ol.
$C_{12}H_{10}N_6O$ 254.25
USP Triamterene Related Compound C RS
2,4-Diamino-6-phenylpteridin-7-ol.
$C_{12}H_{10}N_6O$ 254.25

Triamterene Capsules

» Triamterene Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of triamterene ($C_{12}H_{11}N_7$).

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
USP Reference standards ⟨11⟩—
USP Triamterene RS
USP Triamterene Related Compound A RS
2,4,6-Triamino-5-nitrosopyrimidine.
$C_4H_6N_6O$ 154.13
USP Triamterene Related Compound B RS
2,7-Diamino-4-hydroxy-6-phenylpteridine.
$C_{12}H_{10}N_6O$ 254.25
USP Triamterene Related Compound C RS
2,4-Diamino-7-hydroxy-6-phenylpteridine.
$C_{12}H_{10}N_6O$ 254.25

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—Transfer a portion of the contents of the Capsules, equivalent to about 0.1 g of triamterene, to a 250-mL volumetric flask. Add 100 mL of methoxyethanol, shake until dissolved, dilute with water to volume, and mix. Transfer 5 mL of this solution to a 200-mL volumetric flask, add 5 mL of formic acid, and dilute with water to volume. Prepare a solution of USP Triamterene RS in the manner described above to obtain a Standard solution with a final concentration of about 10 µg per mL. Determine the UV spectrum from 280 nm to 420 nm.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
TEST 1—
Medium: 1% w/v of polysorbate 20 in 0.1 N acetic acid; 900 mL.
Apparatus 2: 100 rpm.
Time: 120 minutes.
Procedure—Proceed as directed for *Test 2*.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{12}H_{11}N_7$ is dissolved in 120 minutes.
TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{12}H_{11}N_7$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 357 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Triamterene RS in the same *Medium*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{12}H_{11}N_7$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Related compounds—
Buffer solution, *Mobile phase*, *System suitability solution* and *Chromatographic system*—Proceed as directed in the *Assay*.
Standard solution—Transfer a known quantity of about 50 mg, accurately weighed, of each of USP Triamterene RS, USP Triamterene Related Compound A RS, USP Triamterene Related Compound B RS, and USP Triamterene Related Compound C RS into a 100-mL volumetric flask. Add approximately 4 mL of acetonitrile, and swirl the flask to wet the contents. Add 4 mL of water and swirl the flask to create a suspension. Add 2 drops (approximately 0.1 mL) of phosphoric acid directly into the suspension, followed by the addition of 50 mL of water. Shake for 15 minutes on a mechanical shaker. Warm the contents of the flask in a hot water bath (not higher than 60°) until the bulk of the material has dissolved (about 3 minutes or longer). Dissolve and dilute

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with water to volume, and mix well. Pass through a 0.45-µm suitable membrane filter. Dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.5 µg per mL of each of the reference standards.

Test solution—Use the *Assay preparation.*

Procedure—Inject equal quantities of about 10 µL of the *Standard solution* and the *Test solution* into the chromatograph, and measure the peak responses. Calculate the percentage of known triamterene related compounds A, B, and C in each Capsule taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of the respective USP Triamterene Related Compound RS in the *Standard solution;* C_U is the concentration, in mg per mL, of triamterene in the *Test solution;* and r_U and r_S are the peak responses of each impurity obtained from the *Test solution* and the *Standard solution*, respectively. Calculate any other unknown impurity in the portion of the Capsule taken using the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of the USP Triamterene RS in the *Standard solution;* C_U is the concentration, in mg per mL, of triamterene in the *Test solution;* r_U is the peak response of any other unknown impurity obtained from the *Test solution;* and r_S is the peak response of the USP Triamterene RS in the *Standard solution:* not more than 0.2% of triamterene related compound A is found; not more than 0.2% of triamterene related compound B is found; not more than 0.2% of triamterene related compound C is found; not more than 0.2% of any other unknown individual impurity is found; and the sum of all impurities is not more than 1.0%.

Assay—

Buffer solution—Dissolve 6.8 g of sodium phosphate monobasic monohydrate (taken in a 1-L flask) in about 900 mL of water. To this solution, add 1.43 g of propylamine hydrochloride, and adjust the pH to 5.5 with 1 M sodium hydroxide. Dilute with water to volume, and mix well.

Mobile phase—Prepare a filtered and degassed mixture of the *Buffer solution* and acetonitrile (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Blank solution—Place 4 mL of acetonitrile and 4 mL of water in a 100-mL flask. Add 2 drops (approximately 0.1 mL) of phosphoric acid. Dilute with water to volume, and mix well. Pass through a suitable 0.45-µg membrane filter.

System suitability solution—Accurately weigh about 5 mg of USP Triamterene Related Compound C RS and 50 mg of USP Triamterene RS into two separate 100-mL volumetric flasks. Add approximately 4 mL of acetonitrile into each flask to wet the material. Add approximately 4 mL of water into each flask to create a suspension. Add 2 drops (approximately 0.1 mL) of phosphoric acid directly into the suspension in each flask. Add approximately 50 mL of water into each flask. Shake for 15 minutes on a mechanical shaker. Warm the contents of the flasks in a hot water bath (not higher than 60°), until the bulk of the material has dissolved (about 3 minutes or longer). Dissolve and dilute with water to volume, and mix well. Dilute with water appropriate portions of these solutions taken in a suitable volumetric flask, quantitatively and stepwise if necessary, to obtain a solution having a known concentration of 500 µg of USP Triamterene RS and 0.5 µg of USP Triamterene Related Compound C RS. Pass through a suitable 0.45-µm membrane filter.

Standard preparation—Accurately weigh about 50 mg of USP Triamterene RS into a 100-mL volumetric flask. Add approximately 4 mL of acetonitrile, and swirl to wet the material. Add 4 mL of water, and swirl to create a suspension. Add 2 drops of phosphoric acid into the suspension.

[**CAUTION**—Make sure that the phosphoric acid is not just adhering to the sides of the flask.]

Add approximately 50 mL of water into the flask, and shake for 15 minutes on a mechanical shaker. Warm the contents of the flasks in a hot water bath (not higher than 60°) until the bulk of the material has dissolved (about 3 minutes or longer). Dissolve and dilute with water to volume, and mix well. Pass through a suitable 0.45-µm membrane filter.

Assay preparation—Remove, as completely as possible, the contents of not fewer than 10 Capsules, and weigh. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of triamterene, to a 1000-mL volumetric flask. Wash the empty shells with 40 mL of acetonitrile, and add to the volumetric flask. Swirl the flask to wet the powder. Add 20 mL of water, and swirl the flask to create a suspension. Add 20 drops (approximately 1 mL) of phosphoric acid directly into the suspension. Add approximately 500 mL of water, and mix well. Shake well for 15 minutes on a mechanical shaker. Place in a hot water bath (not higher than 60°), until the bulk of the material has dissolved (about 3 minutes or longer). Cool to room temperature slowly (do not place in cold water bath). Dissolve and dilute with water to volume, and mix well. (The final solution could be slightly cloudy). Pass the solution through a suitable 0.45-µm membrane filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 300-mm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution* and the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution between triamterene and triamterene related compound C is not less than 3; the tailing factor for triamterene is not more than 2.5; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

Procedure—Inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of $C_{12}H_{11}N_7$ in the portion of Tablets taken by the formula:

$$100(r_U/r_S)(C_S/C_U)$$

in which r_U is the peak response of triamterene obtained from the *Assay preparation;* r_S is the response of the corresponding peak in the *Standard preparation;* and C_S and C_U are the concentrations, in mg per mL, of triamterene in the *Standard preparation* and the *Assay preparation,* respectively.

Triamterene and Hydrochlorothiazide Capsules

DEFINITION

Triamterene and Hydrochlorothiazide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

[NOTE—The Capsule and Tablet dosage forms should not be considered bioequivalent. If patients are to be

USP Monographs

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transferred from one dosage form to the other, retitration and appropriate changes in dosage may be necessary.]

IDENTIFICATION

Change to read:

- **A.** ▲The UV spectra of the major peaks of the *Diluted sample solution* correspond to those of the *Diluted standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Buffer: Transfer 6.9 g of monobasic sodium phosphate and 1.43 g of propylamine hydrochloride to a 1-L volumetric flask. Dissolve in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 5.5, and dilute with water to volume.

Mobile phase: Acetonitrile and *Buffer* (20:80)

▲Diluent: Acetonitrile, glacial acetic acid, and water (10:5:85)▲ (USP 1-Aug-2019)

Standard solution: ▲0.25 mg/mL of USP Hydrochlorothiazide RS and 0.25(*J*) mg/mL of USP Triamterene RS prepared as follows.▲ (USP 1-Aug-2019) Transfer 25 mg of USP Hydrochlorothiazide RS into a 100-mL volumetric flask. Add 25*J* mg of USP Triamterene RS, *J* being the ratio of the labeled amount, in milligrams, of triamterene to the labeled amount, in milligrams, of hydrochlorothiazide/Capsule. Add 10 mL of acetonitrile, 10 mL of water, and 5 mL of glacial acetic acid, sonicating for 2–3 min after each addition. Cool to room temperature, and dilute with water to volume.

▲Diluted standard solution: 0.125 mg/mL of USP Hydrochlorothiazide RS and 0.125(*J*) mg/mL of USP Triamterene RS from *Standard solution* in *Diluent*▲ (USP 1-Aug-2019)

Sample solution: ▲Nominally 0.25 mg/mL of hydrochlorothiazide prepared as follows.▲ (USP 1-Aug-2019) Transfer a portion nominally equivalent to 50 mg of hydrochlorothiazide from NLT 20 Capsules (remove, as completely as possible, the contents of Capsules) to a 200-mL volumetric flask. Add 20 mL of acetonitrile, and sonicate for 10 min. Add 20 mL of boiling water, sonicate for 5 min, and mix. Add 10 mL of acetic acid, sonicate for 10 min, and mix. Add 140 mL of water, mix, and allow to cool to room temperature. Dilute with water to volume, mix, and filter, discarding the first 3 mL of the filtrate.

▲Diluted sample solution: Nominally 0.125 mg/mL of hydrochlorothiazide from *Sample solution* in *Diluent*▲ (USP 1-Aug-2019)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. ▲For *Identification A*, use a diode array detector in the range of 190–400 nm.▲ (USP 1-Aug-2019)

Column: 3.9-mm × 30-cm; ▲10-µm▲ (USP 1-Aug-2019) packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

▲Run time: NLT 2 times the retention time of triamterene▲ (USP 1-Aug-2019)

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for hydrochlorothiazide and triamterene are 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between hydrochlorothiazide and triamterene

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution*, ▲*Diluted standard solution*, *Sample solution*, and *Diluted sample solution*. [NOTE—The *Diluted standard solution* and *Diluted sample solution* are used for *Identification A*.]▲ (USP 1-Aug-2019)

Calculate the percentage of the labeled amount of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triamterene or hydrochlorothiazide from the *Sample solution*

r_S = peak response of triamterene or hydrochlorothiazide from the *Standard solution*

C_S = concentration of ▲USP Triamterene RS or USP Hydrochlorothiazide RS▲ (USP 1-Aug-2019) in the *Standard solution* (mg/mL)

C_U = nominal concentration of triamterene or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

Medium: 0.1 M acetic acid containing 1% polysorbate 20; 900 mL

Apparatus 2: 100 rpm

Time: 120 min

Standard solution: A known concentration of USP Triamterene RS and USP Hydrochlorothiazide RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with water as needed in comparison with the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelengths: 357 nm for triamterene; 271 nm for hydrochlorothiazide (corrected for interference from triamterene on the basis of the absorbances of triamterene at 271 and 357 nm)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved. ▲▲ (USP 1-Aug-2019)

Tolerances: NLT 80% (*Q*) ▲▲ (USP 1-Aug-2019) of the labeled amounts of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 4.0% tetrasodium ethylenediaminetetraacetate, 2.0% polysorbate 40, and 0.05% pancreatin prepared as follows. Add the polysorbate 40 and tetrasodium ethylenediaminetetraacetate to water, and mix thoroughly. Adjust with phosphoric acid to a pH of 8.0

USP Monographs

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± 0.05. Heat to 37°, and add the pancreatin powder. Mix thoroughly, and transfer immediately to the dissolution vessel; 900 mL
Apparatus 1 (use 10-mesh baskets): 100 rpm
Time: 8 h
Buffer: 0.08 M monobasic sodium phosphate
Mobile phase: Methanol and *Buffer* (25:75)
Standard stock solution: 0.22 mg/mL of USP Triamterene RS and 0.11 mg/mL of USP Hydrochlorothiazide RS prepared as follows. To the triamterene and hydrochlorothiazide in a suitable volumetric flask add methanol to fill about 1/5 of the flask volume, sonicate for 10 min, and heat in a steam bath until completely dissolved. Dilute with *Medium* to volume.
Standard solution: ▲0.055 mg/mL of USP Triamterene RS in *Medium* prepared as follows.▲ (USP 1-Aug-2019) Transfer 25 mL of *Standard stock solution* into a 100-mL volumetric flask, dilute with *Medium* to volume, and mix gently to minimize foaming.
Sample solution: Pass a portion of the solution under test through a suitable filter. [NOTE—Do not use nylon filters.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Columns
Guard: Packing L7
Analytical: 3.9-mm × 30-cm; packing L11
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
▲[NOTE—The relative retention times for hydrochlorothiazide and triamterene are about 1.0 and 3.6, respectively.]▲ (USP 1-Aug-2019)
Suitability requirements
Resolution: NLT 2.0 for the triamterene and hydrochlorothiazide peaks
Relative standard deviation: NMT 2.0% for the triamterene and hydrochlorothiazide peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of the labeled amount of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of triamterene or hydrochlorothiazide from the *Sample solution*
r_S = peak response of triamterene or hydrochlorothiazide from the *Standard solution*
C_S = concentration of ▲USP Triamterene RS or USP Hydrochlorothiazide RS▲ (USP 1-Aug-2019) in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (*Q*) of the labeled amount of triamterene ($C_{12}H_{11}N_7$) and NLT 80% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Analysis: Proceed as directed in *Test 1*.
Tolerances: NLT 75% (*Q*) of the labeled amounts of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements with respect to triamterene and hydrochlorothiazide

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Solution A: 6.8 g/L of sodium acetate trihydrate in water. Adjust with glacial acetic acid to a pH of 5.0.
Solution B: Acetonitrile and methanol (75:25)
Mobile phase: *Solution B* and *Solution A* (10:90)
Standard stock solution: 0.15 mg/mL of USP Benzothiadiazine Related Compound A RS in acetonitrile
Standard solution: ▲0.015 mg/mL of USP Benzothiadiazine Related Compound A RS prepared as follows.▲ (USP 1-Aug-2019) Transfer 10.0 mL of ▲*Standard stock solution*▲ (USP 1-Aug-2019) to a 100-mL volumetric flask, add 50 mL of acetonitrile, and 6 mL of glacial acetic acid, and dilute with water to volume.
Sample solution: ▲Nominally 1.5 mg/mL of hydrochlorothiazide prepared as follows.▲ (USP 1-Aug-2019) Transfer a portion nominally equivalent to 150 mg of hydrochlorothiazide from Capsules (remove, as completely as possible, the contents of NLT 20 Capsules) to a 100-mL volumetric flask. Add 60 mL of acetonitrile and 6 mL of glacial acetic acid, and sonicate for 10 min. Cool, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 273 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
▲[NOTE—The relative retention times for benzothiadiazine related compound A, hydrochlorothiazide, and triamterene are about 1.0, 1.5, and 10, respectively.]▲ (USP 1-Aug-2019)
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of benzothiadiazine related compound A in the hydrochlorothiazide contained in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A from the *Standard solution*
C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0% of benzothiadiazine related compound A

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ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store at controlled room temperature.▲ (USP 1-Aug-2019)
- **LABELING:** Label the Capsules to indicate the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzothiadiazine Related Compound A RS
  4-Amino-6-chloro-1,3-benzenedisulfonamide.
  $C_6H_8ClN_3O_4S_2$ 285.73
  USP Hydrochlorothiazide RS
  USP Triamterene RS

Triamterene and Hydrochlorothiazide Tablets

DEFINITION

Triamterene and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

[NOTE—The Capsules and Tablets dosage forms should not be considered bioequivalent. If patients are to be transferred from one dosage form to the other, retitration and appropriate changes in dosage may be necessary.]

IDENTIFICATION

Change to read:

- **A.** The retention ▲times of the major peaks▲ (USP 1-Dec-2019) of the *Sample solution* ▲correspond to those of▲ (USP 1-Dec-2019) the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B.** ▲The UV spectra of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

- **PROCEDURE**

Buffer: ▲Dissolve 0.82 g of sodium acetate anhydrous in 1000 mL of water, and adjust with glacial acetic acid to a pH of 4.5.▲ (USP 1-Dec-2019)

Mobile phase: Acetonitrile and *Buffer* ▲(35:65)▲ (USP 1-Dec-2019)

Diluent: Acetonitrile, glacial acetic acid, and water ▲(350:35:650)

System suitability stock solution: 1.5 µg/mL of USP Benzothiadiazine Related Compound A RS in *Diluent*. Sonicate the solution to aid the dissolution.

Standard stock solution: 0.75 mg/mL of USP Triamterene RS and 0.50 mg/mL of USP Hydrochlorothiazide RS in *Diluent*. Sonicate the solution to aid the dissolution.

System suitability solution: 22.5 µg/mL of USP Triamterene RS, 15 µg/mL of USP Hydrochlorothiazide RS, and 0.075 µg/mL of USP Benzothiadiazine Related Compound A RS from the *Standard stock solution* and *System suitability stock solution*, diluted with *Mobile phase*

Standard solution: 0.023 mg/mL of USP Triamterene RS and 0.015 mg/mL of USP Hydrochlorothiazide RS in *Mobile phase* from *Standard stock solution*

Sample stock solution: Nominally 0.5 mg/mL of hydrochlorothiazide in *Diluent* prepared as follows. Transfer a suitable number of Tablets (NLT 10) to a suitable volumetric flask. Add *Diluent* to 80% of the flask volume and sonicate for 30 min. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.▲ (USP 1-Dec-2019)

Sample solution: ▲Nominally 0.015 mg/mL of hydrochlorothiazide from the *Sample stock solution*, diluted with *Mobile phase*▲ (USP 1-Dec-2019)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: ▲UV 272 nm. For *Identification B*, use a diode array detector in the range of 190–400 nm.▲ (USP 1-Dec-2019)
Column: ▲4.6-mm × 25-cm; 5-µm packing L1▲ (USP 1-Dec-2019)
Flow rate: ▲1 mL/min▲ (USP 1-Dec-2019)
Injection volume: ▲20 µL
Run time: NLT 2 times the retention time of hydrochlorothiazide▲ (USP 1-Dec-2019)

System suitability
▲Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for benzothiadiazine related compound A and hydrochlorothiazide are 0.93 and 1.0, respectively.▲ (USP 1-Dec-2019)]
Suitability requirements
Resolution: ▲NLT 1.5 between hydrochlorothiazide and benzothiadiazine related compound A, *System suitability solution*▲ (USP 1-Dec-2019)
Relative standard deviation: NMT 2.0%, ▲*Standard solution*▲ (USP 1-Dec-2019)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triamterene or hydrochlorothiazide from the *Sample solution*
r_S = peak response of triamterene or hydrochlorothiazide from the *Standard solution*
C_S = concentration of ▲USP Triamterene RS or USP Hydrochlorothiazide RS▲ (USP 1-Dec-2019) in the *Standard solution* (mg/mL)
C_U = nominal concentration of triamterene or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
▲Buffer: Transfer 6.9 g of monobasic sodium phosphate and 1.43 g of propylamine hydrochloride to a 1-L

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volumetric flask. Dissolve in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 5.5, and dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (20:80)▲ (USP 1-Dec-2019)
Standard solution: USP Triamterene RS and USP Hydrochlorothiazide RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
▲Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for hydrochlorothiazide and triamterene are 0.65 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between hydrochlorothiazide and triamterene
Relative standard deviation: NMT 2.0%▲ (USP 1-Dec-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{▲Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of triamterene or hydrochlorothiazide from the *Sample solution*
r_S = peak response of triamterene or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Triamterene RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL▲ (USP 1-Dec-2019)

Tolerances: NLT 80% (*Q*) of the labeled amounts of triamterene ($C_{12}H_{11}N_7$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Change to read:

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements▲ (USP 1-Dec-2019)

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Solution A: 6.8 mg/mL of sodium acetate trihydrate in water. Adjust with glacial acetic acid to a pH of 5.0.
Solution B: Acetonitrile and methanol (75:25)
Mobile phase: *Solution B* and *Solution A* (10:90)
Standard stock solution: ▲ (USP 1-Dec-2019) 0.15 mg/mL of USP Benzothiadiazine Related Compound A RS in acetonitrile
Standard solution: ▲0.015 mg/mL of USP Benzothiadiazine Related Compound A RS prepared as follows.▲ (USP 1-Dec-2019) Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 50 mL of acetonitrile and 6 mL of glacial acetic acid, and dilute with water to volume.
Sample solution: ▲Nominally 1.5 mg/mL of hydrochlorothiazide prepared as follows.▲ (USP 1-Dec-2019) Transfer an equivalent to 150 mg of hydrochlorothiazide from powdered Tablets (NLT 20) to a 100-mL volumetric flask. Add 60 mL of acetonitrile and 6 mL of glacial acetic acid, and sonicate for 10 min. Cool, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 273 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ▲benzothiadiazine related compound A, hydrochlorothiazide, and triamterene are about 1.0, 1.5, and 10, respectively.▲ (USP 1-Dec-2019)]
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of benzothiadiazine related compound A in the hydrochlorothiazide contained in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A from the *Standard solution*
C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the ▲*Sample solution*▲ (USP 1-Dec-2019) (mg/mL)

Acceptance criteria: NMT 1.0% of benzothiadiazine related compound A

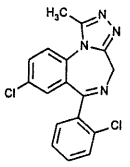
ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store at controlled room temperature.▲ (USP 1-Dec-2019)
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73
USP Hydrochlorothiazide RS
USP Triamterene RS

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Triazolam



$C_{17}H_{12}Cl_2N_4$ 343.21

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine, 8-chloro-6-(2-chlorophenyl)-1-methyl-;

8-Chloro-6-(*o*-chlorophenyl)-1-methyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepine [28911-01-5].

DEFINITION

Triazolam contains NLT 97.0% and NMT 103.0% of $C_{17}H_{12}Cl_2N_4$, calculated on the dried basis.

[**CAUTION**—Exercise care to prevent inhaling particles of triazolam and to prevent its contacting any part of the body.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Acetonitrile and water (45:55)
Buffer: 1.4 g/L monobasic potassium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (45:55)
System suitability solution: 0.02 mg/mL each of USP Triazolam RS and USP Alprazolam Related Compound A RS in *Diluent*
Standard solution: 0.025 mg/mL of USP Triazolam RS in *Diluent*
Sample solution: 0.025 mg/mL of Triazolam in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: 3 times the retention time of triazolam
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for alprazolam related compound A and triazolam are 0.77 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between alprazolam related compound A and triazolam, *System suitability solution*
Tailing:
NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of triazolam ($C_{17}H_{12}Cl_2N_4$) in the portion of Triazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triazolam from the *Sample solution*
r_S = peak response of triazolam from the *Standard solution*
C_S = concentration of USP Triazolam RS in the *Standard solution* (mg/mL)
C_U = concentration of Triazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
- **ORGANIC IMPURITIES**

Diluent, Buffer, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.25 µg/mL of USP Triazolam RS in *Diluent*
Sample solution: 0.25 mg/mL of Triazolam in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for alprazolam related compound A and triazolam are 0.77 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between alprazolam related compound A and triazolam, *System suitability solution*
Tailing: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Sample: *Sample solution*
Calculate the total percentage of impurities in the portion of Triazolam taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the areas of each of the minor component peaks detected
r_T = sum of all minor component peak areas and the area of the major component peak

Acceptance criteria
Total impurities: NMT 1.5%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 60° and at a pressure not exceeding 5 mm of mercury for 16 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Triazolam RS
USP Alprazolam Related Compound A RS
2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3*H*-1,4-benzodiazepine.
$C_{17}H_{15}ClN_4O$ 326.78

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Triazolam Tablets

» Triazolam Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{12}Cl_2N_4$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Triazolam RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* obtained as directed in the *Assay* corresponds to that of the *Standard preparation*, relative to the internal standard.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Stock standard solution—Weigh accurately about 5 mg of USP Triazolam RS, dissolve in and dilute with methanol to 200.0 mL, and mix.
Working standard solution—For each 0.125 mg of the labeled amount of triazolam per Tablet, add 2.0 mL of *Stock standard solution* to a 200-mL volumetric flask. Dilute with water to volume, and mix.
Procedure—After 30 minutes, withdraw a portion of the solution under test, and filter immediately. Inject equal volumes (about 200 µL) of this solution and the *Working standard solution* into a liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a detector capable of monitoring UV absorbance at 222 nm and a 4.6-mm × 10-cm stainless steel column containing packing L7. The mobile phase is a mixture of water and acetonitrile (60:40). The flow rate is about 1 mL per minute. The relative standard deviation of the peak response for the *Working standard solution* is not more than 3.0%, and the number of theoretical plates is not less than 500. Calculate the percentage of $C_{17}H_{12}Cl_2N_4$ dissolved by the formula:

$$50{,}000(r_U/r_S)(C/L)$$

in which r_U and r_S are the peak responses of the solution under test and the *Working standard solution*, respectively, C is the concentration, in mg per mL, of USP Triazolam RS in the *Working standard solution*, and L is the labeled amount, in mg, of triazolam in the Tablet.
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{17}H_{12}Cl_2N_4$ is dissolved in 30 minutes.

Change to read:

Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—
Mobile phase and *Chromatographic system*—Proceed as directed ▲ (ERR 1-Jan-2019) in the *Assay* ▲ (ERR 1-Jan-2019).
Internal standard solution—Prepare a solution of alprazolam in acetonitrile having a concentration of about 0.025 mg per mL.
Standard preparation—Dissolve about 3.2 mg of USP Triazolam RS, accurately weighed, in 100.0 mL of *Internal standard solution*, and mix.
Test preparation—Transfer 1 Tablet to a container, add about 0.4 mL of water directly onto the Tablet, allow to stand for about 2 minutes, and swirl the container to disperse the Tablet. For each 0.25 mg of the labeled amount of triazolam in the Tablet, add 10.0 mL of *Internal standard solution* to the container. Shake, and centrifuge if necessary.
Procedure—Proceed as directed ▲ (ERR 1-Jan-2019) in the *Assay* ▲ (ERR 1-Jan-2019). Calculate the quantity, in mg, of $C_{17}H_{12}Cl_2N_4$ in the Tablet taken by the formula:

$$CV(R_U/R_S)$$

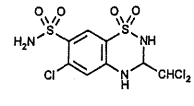
in which V is the volume, in mL, of *Internal standard solution* in the *Test preparation*.

Assay—
Mobile phase —Prepare a filtered and degassed mixture of acetonitrile, chloroform, butyl alcohol, water, and glacial acetic acid (850: 80: 50: 20: 0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Prepare a solution of alprazolam in acetonitrile, having a concentration of 0.1 mg per mL.
Standard stock solution—Dissolve an accurately weighed quantity of USP Triazolam RS in acetonitrile, and dilute with acetonitrile to obtain a solution having a known concentration of about 0.1 mg per mL.
Standard preparation—Transfer 8.0 mL of *Standard stock solution* to a 200-mL volumetric flask, add 8.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 0.8 mg of triazolam, to a 200-mL volumetric flask. Add 2 mL of water, mix, and allow to stand for 10 minutes. Mix vigorously for 10 seconds, and add 8.0 mL of *Internal standard solution*, shake vigorously for 10 minutes, dilute with acetonitrile to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L3. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are 1 for triazolam and about 1.4 for the internal standard, the resolution, R, between the internal standard and triazolam is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{12}Cl_2N_4$ in the portion of Tablets taken by the formula:

$$200C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Triazolam RS in the *Standard preparation*, and R_U and R_S are the ratios of the triazolam peak area to the internal standard peak area obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Trichlormethiazide



$C_8H_8Cl_3N_3O_4S_2$ 380.66
2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3-(dichloromethyl)-3,4-dihydro-, 1,1-dioxide, (±)-.

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(±)-6-Chloro-3-(dichloromethyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [133-67-5].

» Trichlormethiazide, dried at 105° for 3 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_8H_8Cl_3N_3O_4S_2$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73
USP Trichlormethiazide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: Prepare a solution in a mixture of equal volumes of toluene and alcohol containing 1 mg per mL. Apply 10 µL of this solution and 10 µL of a Standard solution of USP Trichlormethiazide RS in the same medium having a known concentration of 1 mg per mL to a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram, using ethyl acetate as the solvent, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots using short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Selenium ⟨291⟩: 0.003%.

Diazotizable substances—

Standard solution—Prepare a solution in a mixture of equal volumes of toluene and alcohol containing 250 µg of USP Benzothiadiazine Related Compound A RS in each mL.

Test solution—Transfer 100.0 mg of Trichlormethiazide, accurately weighed, to a 10-mL volumetric flask, dissolve in 1 mL of acetone, dilute with a mixture of equal volumes of toluene and alcohol to volume, and mix.

Procedure—Apply 5 µL each of the *Standard solution* and of the *Test solution* to a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography*, under *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in ethyl acetate until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and locate the spots on the plate by spraying first with a 1 in 20 solution of sodium nitrite in dilute hydrochloric acid (1 in 12), and then with a 1 in 1000 solution of *N*-(1-naphthyl) ethylenediamine dihydrochloride in alcohol: after 3 minutes, any spot from the *Test solution*, occurring at the R_F value corresponding to that produced by the *Standard solution*, is not greater in size or intensity than the spot produced by the *Standard solution*, corresponding to not more than 2.5% of diazotizable substances.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.05 M monobasic potassium phosphate and methanol (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer about 120 mg of methylparaben to a 50-mL volumetric flask, add methanol to volume, and mix.

Standard preparation—Transfer about 25 mg of USP Trichlormethiazide RS, accurately weighed, to a 50-mL volumetric flask, add 4.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.5 mg of USP Trichlormethiazide RS per mL.

Assay preparation—Transfer about 50 mg of Trichlormethiazide, accurately weighed, to a 100-mL volumetric flask, add 8.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2.3 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the analyte and internal standard peaks is not less than 2.0, and the relative standard deviation for the response ratios calculated for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (5 to 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.56 for trichlormethiazide and 1.0 for methylparaben. Calculate the quantity, in mg, of $C_8H_8Cl_3N_3O_4S_2$ in the portion of Trichlormethiazide taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Trichlormethiazide RS in the *Standard preparation*, and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Delete the following:

▲Trichlormethiazide Tablets

» Trichlormethiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_8H_8Cl_3N_3O_4S_2$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Trichlormethiazide RS

Identification—Evaporate 25 mL of the combined ethyl acetate extracts obtained in the *Assay* on a steam bath to dryness, and dissolve the residue in 1 mL of a mixture of equal volumes of benzene and alcohol: a 10-µL portion of this solution responds to *Identification* test *B* under *Trichlormethiazide*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.

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Procedure— [NOTE—Conduct the analysis of the specimen promptly after the specimen aliquot is withdrawn from the vessel, to minimize hydrolysis.] Determine the amount of $C_8H_8Cl_3N_3O_4S_2$ dissolved, employing the procedure set forth in the *Assay*, making any necessary volumetric adjustments.

Tolerances—Not less than 65% (*Q*) of the labeled amount of $C_8H_8Cl_3N_3O_4S_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨ 905 ⟩ : meet the requirements.

Assay—

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay* under *Trichlormethiazide*.

Acidic methanol—Add 2.8 mL of sulfuric acid to 100 mL of water, mix, and cool. Dilute with methanol to 1000 mL, and mix.

Internal standard solution—Transfer about 20 mg of methylparaben to a 200-mL volumetric flask, add *Acidic methanol* to volume, and mix.

Standard preparation—Transfer about 20 mg of USP Trichlormethiazide RS, accurately weighed, to a 50-mL volumetric flask, add *Acidic methanol* to volume, and mix. Transfer 5.0 mL of this solution to a centrifuge tube, add 5.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Trichlormethiazide RS per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 2 mg of trichlormethiazide, to a centrifuge tube. Add 5.0 mL of *Acidic methanol* and 5.0 mL of *Internal standard solution*, and mix to disperse the powder. Insert the stopper in the tube, rotate by mechanical means for 20 minutes, and centrifuge to obtain a clear supernatant.

Procedure—Separately inject equal volumes (5 µL to 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.58 for trichlormethiazide and 1.0 for methylparaben. Calculate the quantity, in mg, of $C_8H_8Cl_3N_3O_4S_2$ in the portion of Tablets taken by the formula:

$$10C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Trichlormethiazide RS in the *Standard preparation*, and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively. ▲ 2S (USP41)

Tricitrates Oral Solution

DEFINITION

Tricitrates Oral Solution is a solution of Sodium Citrate, Potassium Citrate, and Citric Acid in a suitable aqueous medium. It contains, in each 100 mL, NLT 2.23 g and NMT 2.46 g of sodium (Na), equivalent to NLT 9.5 g and NMT 10.5 g of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$); NLT 3.78 g and NMT 4.18 g of potassium (K), equivalent to NLT 10.45 g and NMT 11.55 g of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$); NLT 12.20 g and NMT 13.48 g of citrate ($C_6H_5O_7$) as sodium citrate and potassium citrate; and NLT 6.34 g and NMT 7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$). [NOTE—The sodium and potassium ion contents of Tricitrates Oral Solution are each approximately 1 mEq/mL.]

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** ⟨191⟩
Sample: 2 mL Oral Solution
Acceptance criteria: Meets the requirements for test *A* under *Sodium*

• **B.**
Sample solution: Oral Solution (1 in 20)
Analysis: Add 5 mL of sodium cobaltinitrite TS to 2 mL of *Sample solution*.
Acceptance criteria: A yellow precipitate is formed immediately (presence of potassium).

• **C.** The retention time of the citrate peak of the *Sample solution* corresponds to that of *Standard solution 1*, as obtained in the *Assay* for *Citric Acid*.

ASSAY

• **SODIUM AND POTASSIUM**
Sodium stock solution: Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.
Potassium stock solution: Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.
Diluent: Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, then add water to volume. This solution contains 15 mEq of Li/1000 mL.
Standard solution: Pipet 50 mL of *Sodium stock solution* and 50 mL of *Potassium stock solution* into a 500-mL volumetric flask, and dilute with water to volume. Each mL of this solution contains 0.1 mEq of sodium (Na) and 0.1 mEq of potassium (K). Transfer 50 µL of this solution to a 10-mL volumetric flask, and dilute with *Diluent* to volume.
Sample solution: Transfer a volume of Oral Solution, equivalent to 2 g of combined citrates, to a 100-mL volumetric flask, and dilute with water to volume. Transfer 50 µL of this solution to a 10-mL volumetric flask, and dilute with *Diluent* to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelengths
Sodium emission line: 589 nm
Potassium emission line: 766 nm
Blank: *Diluent*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the quantity, in g, of sodium (Na) in the portion of Oral Solution taken:

$$\text{Result} = (A_r/M_r) \times (R_{U,Na}/R_{S,Na}) \times (W/F)$$

A_r = atomic weight of sodium, 22.99
M_r = molecular weight of sodium chloride, 58.44
$R_{U,Na}$ = sodium emission reading from the *Sample solution*
$R_{S,Na}$ = sodium emission reading from the *Standard solution*
W = weight of sodium chloride in the *Sodium stock solution*, 14.61 g
F = conversion factor, 25

Calculate the quantity, in g, of potassium (K) in the portion of Oral Solution taken:

$$\text{Result} = (A_r/M_r) \times (R_{U,K}/R_{S,K}) \times (W/F)$$

A_r = atomic weight of potassium, 39.10
M_r = molecular weight of potassium chloride, 74.55
$R_{U,K}$ = potassium emission reading from the *Sample solution*

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$R_{S,K}$ = potassium emission reading from the *Standard solution*
W = weight of potassium chloride in the *Potassium stock solution*, 18.64 g
F = conversion factor, 25

Acceptance criteria: 2.23–2.46 g/100 mL of sodium and 9.5–10.5 g/100 mL of potassium

• **CITRATE**

Mobile phase, Standard solution 1, Chromatographic system, and **System suitability:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution: Transfer an appropriate volume of Oral Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate)*.

Analysis: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure*.

Calculate the concentration, in g/100 mL, of citrate ($C_6H_5O_7$) in the portion of Oral Solution taken:

$$\text{Result} = [(r_U/r_S) \times (C_S/C_U) - A(M_{r1}/M_{r2})] \times L$$

r_U = citrate peak area from the *Sample solution*
r_S = citrate peak area from *Standard solution 1*
C_S = concentration of citrate in *Standard solution 1* (µg/mL)
C_U = nominal concentration of *Sample solution*
A = concentration of citric acid monohydrate determined in the *Assay* for *Citric Acid* (mg/mL)
M_{r1} = molecular weight of citrate, 189.10
M_{r2} = molecular weight of citric acid monohydrate, 210.14
L = Oral Solution label claim (g/100 mL)

Acceptance criteria: 12.20–13.48 g/100 mL as sodium citrate and potassium citrate

• **CITRIC ACID**

Sample solution: Transfer 15 mL of Oral Solution into a 250-mL volumetric flask, and dilute with water to volume.

Analysis: Transfer 5 mL of the *Sample solution* into a suitable flask, and add 25 mL of water and 5 drops of phenolphthalein TS. Titrate with 0.02 N sodium hydroxide VS to a pink endpoint. Each mL of 0.02 N sodium hydroxide is equivalent to 1.401 mg of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$).

Acceptance criteria: 6.34–7.02 g/100 mL

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 4.9–5.4

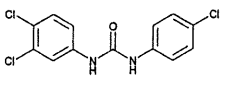
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Citric Acid RS

Triclocarban



$C_{13}H_9Cl_3N_2O$ 315.58

Urea, *N*-(4-chlorophenyl)-*N*′-(3,4-dichlorophenyl)-; 3,4,4′-Trichlorocarbanilide; 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)urea [101-20-2].

DEFINITION

Triclocarban contains NLT 97.0% and NMT 103.0% of triclocarban ($C_{13}H_9Cl_3N_2O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, methanol, water, and glacial acetic acid (400: 500: 300: 1.5)

System suitability solution: 0.1 mg/mL each of USP Triclocarban RS, USP Triclocarban Related Compound A RS, and USP Triclocarban Related Compound C RS in acetonitrile

Standard solution: 0.1 mg/mL of USP Triclocarban RS in acetonitrile. The solution is stable for 12 h.

Sample solution: 0.1 mg/mL of Triclocarban in acetonitrile. The solution is stable for 12 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for triclocarban related compound A, triclocarban, and triclocarban related compound C are 0.7, 1.0, and 1.6, respectively.]

Suitability requirements

Resolution: NLT 2.0 between triclocarban related compound A and triclocarban, NLT 2.0 between triclocarban and triclocarban related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of triclocarban ($C_{13}H_9Cl_3N_2O$) in the portion of Triclocarban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Triclocarban RS in the *Standard solution* (mg/mL)
C_U = concentration of Triclocarban in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase: Acetonitrile, methanol, water, and glacial acetic acid (370:380:250:1)

Internal standard solution: 0.12 mg/mL of *p*-nitrochlorobenzene in methanol

Standard solution: 2 µg/mL each of USP Triclocarban RS, USP Triclocarban Related Compound A RS, USP

USP Monographs

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Triclocarban Related Compound C RS, USP Triclocarban Related Compound D Mixture RS, and 2.4 µg/mL of *p*-nitrochlorobenzene from the *Internal standard solution*. Dissolve and dilute with methanol to volume.

Sample solution: 0.2 mg/mL of Triclocarban and 2.4 µg/mL of *p*-nitrochlorobenzene from the *Internal standard solution*. Dissolve and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Signal-to-noise ratio: NLT 10 for triclocarban related compound A, triclocarban related compound C, and triclocarban related compound D mixture

Relative standard deviation: NMT 2.0% for triclocarban

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Triclocarban taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of each impurity to the internal standard from the *Sample solution*

R_S = peak response ratio of corresponding specified impurity to the internal standard from the *Standard solution* (use the triclocarban peak response ratio to calculate for any individual impurity)

C_S = concentration of each impurity in the *Standard solution* (µg/mL)

C_U = concentration of Triclocarban in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
p-Nitrochlorobenzene[a]	0.45	—
Triclocarban related compound A[b]	0.64	1.0
Triclocarban	1.00	—
Triclocarban related compound C[c]	1.66	1.0
Triclocarban related compound D mixture[d]	2.72 and 3.25	1.0
Any other individual impurity	—	1.0
Total impurities	—	2.0

[a] Internal standard—for information only.

[b] 1,3-Bis(4-chlorophenyl)urea.

[c] 1,3-Bis(3,4-dichlorophenyl)urea.

[d] Consists of two components, triarylbiuret 1 and triarylbiuret 2. Triarylbiuret 1 is [3,5-bis(4-chlorophenyl)-1-(3,4-dichlorophenyl)biuret] with RRT of 2.72. Triarylbiuret 2 is [1,5-bis(4-chlorophenyl)-3-(3,4-dichlorophenyl)biuret] with RRT of 3.25. The acceptance criteria is the sum of triarylbiuret 1 and triarylbiuret 2.

• **CHLOROANILINES**

Dimethylformamide solution: 5% dimethylformamide in 1 N hydrochloric acid

Sodium nitrite solution: 2.5 mg/mL of sodium nitrite in water

Ammonium sulfamate solution: 25 mg/mL of ammonium sulfamate in water

***N*-(1-naphthyl)ethylenediamine dihydrochloride solution:** 10 mg/mL in water

Standard stock solution 1: 560 µg/mL of 3,4-dichloroaniline and 440 µg/mL of *p*-chloroaniline dissolved in 2% of the final volume with the *Dimethylformamide solution*. Dilute with 0.2 N hydrochloric acid to volume.

Standard stock solution 2: 56 µg/mL of 3,4-dichloroaniline and 44 µg/mL of *p*-chloroaniline in 0.2 N hydrochloric acid from *Standard stock solution 1*

Standard stock solution 3: 5.6 µg/mL of 3,4-dichloroaniline and 4.4 µg/mL of *p*-chloroaniline in 0.2 N hydrochloric acid from *Standard stock solution 2*

Standard stock solution 4: 0.56 µg/mL of 3,4-dichloroaniline and 0.44 µg/mL of *p*-chloroaniline in 0.2 N hydrochloric acid from *Standard stock solution 3*

Standard solutions: Prepare *Standard solutions 1–9* as listed in *Table 2*. Transfer the aliquot of the *Standard stock solution* to each individual 25-mL volumetric flask. Place all flasks in an ice bath at 0° to 5° for 10 min. Add to each volumetric flask 1 mL of *Sodium nitrite solution*, shake well, and let the solution stand for 10 min in the ice bath. Add to each flask 1 mL of the *Ammonium sulfamate solution*, shake well, and allow the reaction to take place for 10 min inside the ice bath. Add 2 mL of the *N-(1-naphthyl) ethylenediamine dihydrochloride solution* to each *Standard solution 1–8*, and remove all volumetric flasks from the ice bath. Do not add the *N-(1-naphthyl)ethylenediamine dihydrochloride solution* to *Standard solution 9*. Dilute all solutions with 0.2 N hydrochloric acid to volume. Allow to stand for at least 30 s, and measure the absorbance within 15 min.

Table 2

Standard Solution	Standard Stock Solution Used	Volume of Standard Stock Solution (mL)	Concentration of Total Chloroaniline (ppm)
1	Standard stock solution 4	2	20
2	Standard stock solution 4	5	50
3	Standard stock solution 4	10	100
4	Standard stock solution 4	15	150
5	Standard stock solution 3	2	200
6	Standard stock solution 3	3	300
7	Standard stock solution 3	4	400
8	Standard stock solution 3	5	500
9	Standard stock solution 3	5	0

Sample stock solution: Dissolve 500 mg of Triclocarban with 5 mL of the *Dimethylformamide solution* in a 50-mL beaker, and shake the solution. Continue shaking the solution while adding 15 mL of 0.2 N hydrochloric acid to precipitate the product, and shake for 15 min. Filter the

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suspension into a 50-mL volumetric flask, and dilute with 0.2 N hydrochloric acid to volume.

Sample solution: Transfer 10 mL of the *Sample stock solution* to a 25-mL volumetric flask, and place in an ice bath at 0° to 5° for 10 min. Add 1 mL of the *Sodium nitrite solution*, shake well, and let the solution stand for 10 min in the ice bath. Add 1 mL of the *Ammonium sulfamate solution*, shake well, and allow to stand for 10 min. Add 1 mL of the *N-(1-naphthyl)ethylenediamine dihydrochloride solution*, and remove the solution from the ice bath. Dilute with 0.2 N hydrochloric acid to volume. Allow to stand for at least 30 s, and measure the absorbance within 15 min.

Sample solution blank: Follow the procedure described in the preparation of the *Sample solution* but do not add the *N-(1-naphthyl)ethylenediamine dihydrochloride solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 552 nm

Analysis

Samples: *Standard solutions* and *Sample solution*

Calibrate the absorbance to zero with *Standard solution 9*. Generate a calibration curve of absorbance against concentration of total chloroaniline using the data from *Standard solutions 1–8*. Determine the concentration, C_S, in µg/mL, of chloroanilines in the *Sample solution* using the calibration curve. Calculate the concentration, in ppm, of chloroanilines in the portion of Triclocarban taken:

$$\text{Result} = (C_S/C_U) \times F$$

C_S = concentration of chloroanilines in the *Sample solution* (µg/mL)

C_U = concentration of Triclocarban in the *Sample solution* (µg/mL)

F = conversion factor to ppm (10^6)

Acceptance criteria: NMT 400 ppm

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Sample: Dry a sample at 110° for 4 h.

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed container. Store at ambient temperature and humidity.
- **USP REFERENCE STANDARDS** ⟨11⟩

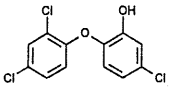
USP Triclocarban RS

USP Triclocarban Related Compound A RS
1,3-Bis(4-chlorophenyl)urea.
$C_{13}H_{10}Cl_2N_2O$ 281.14

USP Triclocarban Related Compound C RS
1,3-Bis(3,4-dichlorophenyl)urea.
$C_{13}H_8Cl_4N_2O$ 350.03

USP Triclocarban Related Compound D Mixture RS
Mixture of triarylbiuret 1 (3,5-bis(4-chlorophenyl)-1-(3,4-dichlorophenyl)biuret and triarylbiuret 2 (1,5-bis(4-chlorophenyl)-3-(3,4-dichlorophenyl)biuret).
$C_{20}H_{13}Cl_4N_3O_2$ 469.15

Triclosan



$C_{12}H_7Cl_3O_2$ 289.54

Phenol, 5-chloro-2-(2,4-dichlorophenoxy)-.
2,4,4′-Trichloro-2′-hydroxydiphenyl ether [3380-34-5].

» Triclosan contains not less than 97.0 percent and not more than 103.0 percent of $C_{12}H_7Cl_3O_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP 2,4-Dichlorophenol RS
USP Parachlorophenol RS
USP Triclosan RS
USP Triclosan Related Compounds Mixture A RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Water Determination, *Method I* ⟨921⟩**:** not more than 0.1%.

Completeness of solution ⟨641⟩—A solution of 1.40 g of Triclosan in 10 mL of acetone is clear.

Related compounds—

Chromatographic system—Proceed as directed in the *Assay*.

Test solution—Use the *Assay preparation*.

Procedure—Inject a volume (about 2.0 µL) of the *Test solution* into the chromatograph, increase the column temperature by 20° per minute to 140°, then increase column temperature by 4° per minute to 240°, maintain this temperature for not less than 5 minutes, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Triclosan taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all of the peaks: not more than 0.1% of any individual impurity is found; and not more than 0.5% of total impurities is found.

Limit of monochlorophenols and 2,4-dichlorophenol —

Phosphate buffer—Transfer about 1.38 g of anhydrous monobasic sodium phosphate and about 1.42 g of dibasic sodium phosphate to a 1-L volumetric flask, dissolve in and dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Phosphate buffer* (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Quantitatively dissolve accurately weighed quantities of USP Parachlorophenol RS and USP 2,4-Dichlorophenol RS in acetonitrile, dilute with an equal

USP Monographs

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volume of water, and mix. Transfer a portion of this solution to a suitable container, and dilute quantitatively, and stepwise if necessary, with a mixture of acetonitrile and water (1:1) to obtain a solution having known concentrations of about 0.5 µg of parachlorophenol and 0.1 µg of 2,4-dichlorophenol per mL.

Test solution—Transfer about 250 mg of Triclosan, accurately weighed, to a 25-mL low-actinic volumetric flask, dissolve in 20 mL of acetonitrile, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a coulometric electrochemical detector with electrode 1 set at 0.45 V and electrode 2 set at 0.75 V, both having a positive (oxidative) polarity and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 9.0% for 2,4-dichlorophenol.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The peak responses for parachlorophenol and 2,4-dichlorophenol in the chromatogram of the *Test solution* are not greater than the corresponding peaks in the chromatogram of the *Standard solution.*

Limit of 1,3,7-trichlorodibenzo- *p*-dioxin, 2,8-dichlorodibenzo- *p*-dioxin, 2,8-dichlorodibenzofuran, and 2,4,8-trichlorodibenzofuran—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (70: 30: 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Test solution—Transfer about 2.0 g of Triclosan, accurately weighed, to a screw-capped centrifuge tube, add 5 mL of 2 N potassium hydroxide, and shake for 10 minutes to dissolve. Add 3 mL of *n*-hexane, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to a suitable container, add another 3 mL of *n*-hexane to the aqueous layer, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to the previous extract, discard the aqueous layer, add 3 mL of 2 N potassium hydroxide to the combined organic layers, shake for 10 minutes, and allow the phases to separate. Discard the aqueous layer, add another 3 mL of 2 N potassium hydroxide to the combined organic layers, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to a suitable container, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of methanol, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the USP Triclosan Related Compounds Mixture A RS, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.59 for 2,8-dichlorodibenzofuran, 0.71 for 2,8-dichlorodibenzo-*p*-dioxin, 0.88 for 2,4,8-trichlorodibenzofuran, and 1.0 for 1,3,7-trichlorodibenzo-*p*-dioxin; and the relative standard deviation for replicate injections is not more than 15.0%, determined from the 2,8-dichlorodibenzo-*p*-dioxin peak.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the concentration of each analyte in the portion of Triclosan taken by the formula:

$$(C/W)(r_i/r_s)$$

in which C is the concentration, in µg per mL, of the respective analyte in the USP Triclosan Related Compounds Mixture A RS; W is the weight, in g, of Triclosan taken; and r_i and r_s are the peak responses for the respective analyte obtained from the *Test solution* and the USP Triclosan Related Compounds Mixture A RS, respectively: not more than 0.25 ppm of 2,8-dichlorodibenzofuran is found; not more than 0.5 ppm of 2,4,8-trichlorodibenzofuran is found; not more than 0.25 ppm of 1,3,7-trichlorodibenzo-*p*-dioxin is found; and not more than 0.5 ppm of 2,8-dichlorodibenzo-*p*-dioxin is found.

Limit of 2,3,7,8-tetrachlorodibenzo- *p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran—

[CAUTION—2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran are extremely toxic substances. Exercise all necessary precautions in the conduct of this procedure.]

Stationary phase A—Transfer about 10 g of silica gel to a suitable container, add about 3 mL of 1 N sodium hydroxide, and mix.

Stationary phase B—Transfer about 60 g of silica gel to a suitable container, add about 74 mL of concentrated sulfuric acid, and mix.

Chromatographic column A—Transfer 5.1 g of *Stationary phase A,* 0.5 g of silica gel, 6.2 g of *Stationary phase B,* and 3.2 g of sodium sulfate to a glass chromatographic column having an internal diameter of 10 mm. Wash the column with 50 mL of *n*-hexane, and discard the eluate.

Chromatographic column B—Transfer 2.5 g of alumina and 2.5 g of sodium sulfate to a glass chromatographic column having an internal diameter of 6 mm. Wash the column with 30 mL of *n*-hexane, and discard the eluate.

Internal standard solution—Transfer accurately measured quantities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled, and 2,3,7,8-tetrachlorodibenzofuran, ^{13}C-labeled, in nonane, and dilute quantitatively, and stepwise if necessary, with 2,2,4-trimethylpentane to obtain a solution having known concentrations of about 1.0 pg of each per µL.

Test solution—Transfer about 30 g of Triclosan, accurately weighed, to a separatory funnel, add 30 µL of *Internal standard solution,* dissolve in 200 mL of 1 N sodium hydroxide, extract with four 30-mL portions of *n*-hexane, and combine the extracts. Wash the combined extracts with 20 mL of water, extract the washing with 15 mL of *n*-hexane, and add the extract to the other combined extracts. Add about 3 g of anhydrous sodium sulfate to the combined extracts, allow to stand for 30 minutes, quantitatively transfer to an appropriate round-bottom flask, and distill, using a distillation apparatus with a vigreux column, until about 1 mL remains. Transfer this solution to the top of *Chromatographic column A,* and elute with 50 mL of *n*-hexane. Collect the eluate on top of *Chromatographic column B,* and elute with 30 mL of a mixture of *n*-hexane and methylene chloride (98:2), discarding the eluate. Elute with 40 mL of a mixture of *n*-hexane and methylene chloride (1:1), collecting the eluates in a round-bottom flask. Distill the combined eluates, using a distillation apparatus with a vigreux column, until about 1 mL remains. Further concentrate this solution with the aid of a stream of nitrogen to about 50 µL, evaporate at room temperature to dryness, and dissolve in 10 µL of 2,2,4-trimethylpentane.

Chromatographic system (see *Chromatography* ⟨621⟩ and *Mass Spectrometry* ⟨736⟩)—The gas chromatograph is equipped with a high-resolution mass spectrograph with an electron-impact ionization source and a 0.25-mm × 60-m capillary column coated with phase G48. The carrier gas is

USP Monographs

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helium. The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 80°, then, 1 minute after the injection, the temperature is increased at a rate of 20° per minute to 220°, then increased at a rate of 2° per minute to 270°, and maintained at 270° for not less than 20 minutes. The injection port temperature is maintained at 280°. Chromatograph the *Internal standard solution*, and record the peak responses as directed for *Procedure:* the signal-to-noise ratio at a mass-to-charge ratio of 321.89 is not less than 50.

Procedure—Inject a volume (about 1 µL) of the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses at mass-to-charge ratios of 319.90, 321.89, 331.88, 333.93, 303.90, 305.90, 315.94, and 317.94. The peak response for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin at a mass-to-charge ratio of 319.90 is not more than the peak response of the associated internal standard at a mass-to-charge ratio of 331.88; the peak response for 2,3,7,8-tetrachlorodibenzofuran at a mass-to-charge ratio of 303.90 is not more than the peak response of the associated internal standard at a mass-to-charge ratio of 315.94.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Triclosan RS in ethyl acetate, and dilute quantitatively, and stepwise if necessary, with ethyl acetate to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer about 40 mg of Triclosan, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with ethyl acetate to volume, and mix.

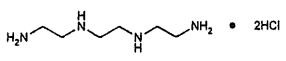
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 15-m capillary column with phase G3. The carrier gas is helium maintained at about 6 psi. The injection port temperature is maintained at 34° and is increased rapidly to 200° immediately after the injection, the column temperature is maintained at 34°, and the detector temperature is maintained at 260°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 2.0 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, increase the column temperature by 20° per minute to 140°, then increase the column temperature by 4° per minute to 240°, maintain this temperature for not less than 5 minutes, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{12}H_7Cl_3O_2$ in the portion of Triclosan taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Triclosan RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Trientine Hydrochloride



$C_6H_{18}N_4 \cdot 2HCl$ 219.16

1,2-Ethanediamine, *N,N′*-bis(2-aminoethyl)-, dihydrochloride.
Triethylenetetramine dihydrochloride [38260-01-4].

» Trientine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_6H_{18}N_4 \cdot 2HCl$, calculated on the dried basis.

Packaging and storage—Preserve under an inert gas in tight, light-resistant containers, and store in a refrigerator.
USP Reference standards ⟨11⟩—
USP Trientine Hydrochloride RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197M▲ (CN 1-May-2020)
pH ⟨791⟩: between 7.0 and 8.5, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 40° for 4 hours: it loses not more than 2.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.15%.
Chromatographic purity—The sum of the intensities of all secondary spots obtained from the *Test preparation* in *Part I* and *Part II* corresponds to not more than 2.0%.

Part I—

Spray reagent—Dissolve 300 mg of ninhydrin in a mixture of 100 mL of butyl alcohol and 3 mL of glacial acetic acid.

Standard preparation A— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Trientine Hydrochloride RS in methanol to obtain a solution containing 10 mg per mL.

Standard preparation B— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of diethylenetriamine in methanol to obtain a solution containing 1.0 mg per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Standard preparation C— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of 1-(2-aminoethyl)piperazine in methanol to obtain a solution containing 1.0 mg per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Standard preparation D— [NOTE—Use low-actinic glassware.] Transfer 5.0 mL of *Standard preparation C* to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

Test preparation— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of Trientine Hydrochloride in methanol to obtain a solution containing 10 mg per mL.

Procedure—Apply separately 3 µL each of the *Test preparation*, of *Standard preparation B*, and of *Standard preparation C* to a suitable unwashed, high performance thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) having a 1.5-cm preadsorbent zone and coated with a 0.15-mm layer of chromatographic silica gel mixture. To a fourth spot, apply 3 µL each of *Standard preparations A, B,* and *C.* To a fifth spot, apply 3 µL each of *Standard preparations A, B,* and *D.* Allow the spots to dry, place the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of isopropyl alcohol and ammonium hydroxide (3:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate with the aid of a current of air. Spray the plate with *Spray reagent,* dry at 105° for 5 minutes, and observe the plate under long-wavelength UV light. Determine

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the locus of the diethylenetriamine and the 1-(2-aminoethyl) piperazine spots from the chromatograms of *Standard preparations B* and *C*, respectively. Determine the concentration of diethylenetriamine in the *Test preparation* by comparing the size and intensity of any secondary spot from the chromatogram of the *Test preparation* having an R_F value corresponding to the R_F value of diethylenetriamine with the diethylenetriamine spots obtained from the chromatograms of the *Standard preparation* mixtures. Determine the concentration of any other observed impurities in the *Test preparation* by comparing the size and intensity of any other secondary spots from the chromatogram of the *Test preparation* with the 1-(2-aminoethyl)piperazine spots obtained from the chromatograms of the *Standard preparation* mixtures.

Part II—

Spray reagent—Dissolve 200 mg of ninhydrin in 100 mL of alcohol.

Tris (2-aminoethyl)amine stock solution— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of tris(2-aminoethyl)amine in methanol to obtain a solution containing 1.0 mg per mL.

Standard preparation A— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Trientine Hydrochloride RS in methanol to obtain a solution containing 10 mg per mL.

Standard preparation B— [NOTE—Use low-actinic glassware.] Transfer 1.0 mL of *Tris(2-aminoethyl)amine stock solution* to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

Standard preparation C— [NOTE—Use low-actinic glassware.] Transfer 0.5 mL of *Tris(2-aminoethyl)amine stock solution* to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

Test preparation— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of Trientine Hydrochloride in methanol to obtain a solution containing 10 mg per mL.

Procedure—Apply separately 3 µL each of the *Test preparation* and of *Standard preparation A* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously washed with methanol. To a third spot apply 3 µL each of *Standard preparations A* and *B*. To a fourth spot, apply 3 µL each of *Standard preparations A* and *C*. Allow the spots to dry, place the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of ammonium hydroxide and alcohol (2:1) at a temperature of 2° to 6° until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate with the aid of a current of air. Spray the plate with *Spray reagent*, dry at 105° for 5 minutes, and observe the plate under long-wavelength UV light. Determine the concentration of tris(2-aminoeth yl)amine in the *Test preparation* by comparing the size and intensity of any secondary spot from the chromatogram of the *Test preparation* having an R_F value corresponding to the R_F value of tris(2-aminoethyl)amine with the tris(2-aminoethyl)amine spots obtained from the chromatograms of the *Standard preparation* mixtures.

Assay—Dissolve about 220 mg of Trientine Hydrochloride, accurately weighed, in 150 mL of water in a 250-mL beaker. Adjust with hydrochloric acid to a pH of 2.0; then adjust with ammonium hydroxide to a pH of 9.5 ± 0.5; and then adjust with glacial acetic acid to a pH of 5.0. Heat the solution to 90°, and while hot, titrate with 0.1 N cupric nitrate VS, determining the endpoint potentiometrically, using an electrode system consisting of a cupric ion-selective electrode and a calomel reference electrode with an outer filling solution of 1 M potassium nitrate. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary correction. Each mL of 0.1 N cupric nitrate is equivalent to 21.92 mg of $C_6H_{18}N_4 \cdot 2HCl$.

Trientine Hydrochloride Capsules

» Trientine Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_{18}N_4 \cdot 2HCl$.

Packaging and storage—Preserve in tight containers, and store in a refrigerator.

USP Reference standards ⟨11⟩—
USP Trientine Hydrochloride RS

Identification—Triturate an amount of the contents of Capsules, equivalent to about 1.5 mg of trientine hydrochloride, with 0.5 mL of acetone in an agate or mullite mortar. Evaporate in a gentle current of air to dryness. Repeat the acetone addition, trituration, and drying steps: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Trientine Hydrochloride RS.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
pH 8.2 buffer—Prepare as directed under *Assay*.
Copper sulfate reagent—Mix 10 mL of copper sulfate solution (5 g copper sulfate pentahydrate in 100 mL of water) with 40 mL of *pH 8.2 buffer*. [NOTE—The solution must be clear.]

Standard preparation—Dissolve an accurately weighed quantity of USP Trientine Hydrochloride RS in water to obtain a solution having a known concentration of about 0.5 mg per mL.

Procedure—Pipet an aliquot of a filtered portion of the solution under test, estimated to contain about 5 mg of trientine hydrochloride, into a 50-mL centrifuge tube. Into a similar centrifuge tube, pipet an equivalent volume of water to provide a reagent blank, and into a third centrifuge tube pipet 10 mL of *Standard preparation*. Into each tube, pipet 5 mL of *Copper sulfate reagent*, stopper, and mix immediately using a vortex mixer. Determine the absorbances of the solutions from the *Standard preparation* and the test solution at 580 and 410 nm, with a suitable spectrophotometer, against the reagent blank. Calculate the quantity in mg of trientine hydrochloride dissolved by the formula:

$$5000(C/V)[(A_U - A_{UX})/(A_S - A_{SX})]$$

in which C is the concentration, in mg per mL, of USP Trientine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of the aliquot of test solution used, A_U and A_S are the absorbances at 580 nm of test and standard solutions, respectively, and A_{UX} and A_{SX} are the absorbances at 410 nm of test and standard solutions, respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_6H_{18}N_4 \cdot 2HCl$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

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Assay—

Copper reagent—Dissolve 5 g of copper sulfate pentahydrate in water to make 100 mL, and mix.

pH 8.2 buffer—Dissolve 20.74 g of anhydrous dibasic sodium phosphate, 6.72 g of anhydrous citric acid, and 0.535 g of monobasic sodium phosphate in 400 mL of water, adjust with sodium hydroxide solution (1 in 2) to a pH of 8.2 ± 0.05, dilute with water to make 500 mL, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Trientine Hydrochloride RS in methanol to obtain a solution having a known concentration of about 2.5 mg per mL. Transfer 5.0 mL of this solution to a glass-stoppered, 50-mL conical flask.

Assay preparation—Remove, as completely as possible, the contents of not less than 20 Capsules. Weigh the contents, and determine the average weight per capsule. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 250 mg of trientine hydrochloride, to a 100-mL volumetric flask. Add about 70 mL of methanol, and shake or sonicate to dissolve. Dilute with methanol to volume, mix, and filter, discarding the first few mL of the filtrate. Transfer 5.0 mL of this solution to a glass-stoppered, 50-mL conical flask.

Procedure—To each of the flasks containing the *Standard preparation* and the *Assay preparation* and to a similar flask containing 5.0 mL of methanol to provide the blank, add 10.0 mL of *pH 8.2 buffer* and 1.0 mL of *Copper reagent*, and mix. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 580 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_6H_{18}N_4 \cdot 2HCl$ in the portion of Capsules taken by the formula:

$$100C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Trientine Hydrochloride RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Trifluoperazine Oral Solution

» Trifluoperazine Oral Solution contains an amount of trifluoperazine hydrochloride ($C_{21}H_{24}F_3N_3S \cdot 2HCl$) equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of trifluoperazine ($C_{21}H_{24}F_3N_3S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Trifluoperazine Hydrochloride RS
[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—*Solution*: Prepared as directed in the *Assay*.

B: Mix 1 mL of Oral Solution with 5 mL of methanol: a 5-µL portion of this solution meets the requirements of *Identification* test *D* under *Trifluoperazine Hydrochloride*.

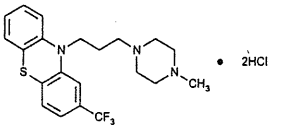
pH ⟨791⟩: between 2.0 and 3.2.

Assay— [NOTE—Use low-actinic glassware.] Transfer an accurately measured volume of Oral Solution, equivalent to about 50 mg of trifluoperazine, to a 250-mL separator with the aid of about 100 mL of water. Add 10 mL of sodium hydroxide solution (1 in 10), and extract with three 50-mL portions of cyclohexane. Wash the combined cyclohexane extracts with about 20 mL of water, and discard the water washing. Extract the combined cyclohexane extracts with four 50-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 500-mL volumetric flask. Dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Trifluoperazine Hydrochloride RS in the same medium having a known concentration of about 12 µg per mL in 1-cm cells at 278 nm and at the wavelength of maximum absorbance at about 255 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of trifluoperazine ($C_{21}H_{24}F_3N_3S$) in each mL of the Oral Solution taken by the formula:

$$(407.51/480.43)(5C/V)(A_{255} - A_{278})_U/(A_{255} - A_{278})_S$$

in which 407.51 and 480.43 are the molecular weights of trifluoperazine and trifluoperazine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Trifluoperazine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Oral Solution taken; and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the assay solution (U) and the Standard solution (S), respectively.

Trifluoperazine Hydrochloride



$C_{21}H_{24}F_3N_3S \cdot 2HCl$ 480.42
10*H*-Phenothiazine, 10-[3-(4-methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-, dihydrochloride.
10-[3-(4-Methyl-1-piperazinyl)propyl]-2-(trifluoromethyl) phenothiazine dihydrochloride [440-17-5].

» Trifluoperazine Hydrochloride, dried in vacuum at 60° for 4 hours, contains not less than 98.0 percent and not more than 101.0 percent of $C_{21}H_{24}F_3N_3S \cdot 2HCl$.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Trifluoperazine Hydrochloride RS
[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and

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solutions containing them, by conducting the procedures without delay, under subdued light, or by using low-actinic glassware.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 255 nm, calculated on the dried basis, do not differ by more than 2.0%.

C: A solution (1 in 100) responds to the tests for *Chloride* ⟨191⟩.

D: Prepare a solution in methanol containing 1.2 mg per mL. Apply 5 µL each of this solution and a solution of USP Trifluoperazine Hydrochloride RS in methanol containing 1.2 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of acetone and ammonium hydroxide (200:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with a solution of iodoplatinic acid prepared by dissolving 100 mg of chloroplatinic acid in 1 mL of 1 N hydrochloric acid, adding 25 mL of potassium iodide solution (1 in 25), diluting with water to 100 mL, and then adding 0.5 mL of formic acid: the R_F value of the principal spot from the test solution corresponds to that from the Standard solution.

pH ⟨791⟩**:** between 1.7 and 2.6, in a solution (1 in 20).

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 1.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Assay—Dissolve about 500 mg of Trifluoperazine Hydrochloride, previously dried and accurately weighed, in 50 mL of glacial acetic acid, and add crystal violet TS and 15 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 24.02 mg of $C_{21}H_{24}F_3N_3S \cdot 2HCl$.

Trifluoperazine Hydrochloride Injection

» Trifluoperazine Hydrochloride Injection is a sterile solution of Trifluoperazine Hydrochloride in Water for Injection. It contains an amount of trifluoperazine hydrochloride ($C_{21}H_{24}F_3N_3S \cdot 2HCl$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of trifluoperazine ($C_{21}H_{24}F_3N_3S$).

Packaging and storage—Preserve in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Trifluoperazine Hydrochloride RS
[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—
A: The solution employed for measurement of absorbance in the *Assay* exhibits UV maxima and minima at the same wavelengths as that of a similar solution of USP Trifluoperazine Hydrochloride RS, concomitantly measured.

B: Mix 5 mL of it with 5 mL of methanol: a 5-µL portion of this solution responds to *Identification* test *D* under *Trifluoperazine Hydrochloride.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 172.0 USP Endotoxin Units per mg of trifluoperazine.

pH ⟨791⟩: between 4.0 and 5.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay— [NOTE—Use low-actinic glassware.] Transfer an accurately measured volume of Injection, equivalent to about 20 mg of trifluoperazine, to a 250-mL separator. Add 10 mL of 4 N sulfuric acid, and extract with three 25-mL portions of carbon tetrachloride. Discard the carbon tetrachloride after each extraction. Add 10 mL of ammonium hydroxide, and extract with five 40-mL portions of cyclohexane. Extract the combined cyclohexane extracts with five 50-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 500-mL volumetric flask. Dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 25.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Trifluoperazine Hydrochloride RS in the same medium having a known concentration of about 12 µg per mL in 1-cm cells at 278 nm and at the maximum at about 255 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of trifluoperazine ($C_{21}H_{24}F_3N_3S$) in each mL of the Injection taken by the formula:

$$(407.51/480.43)(2C/V)(A_{255} - A_{278})_U/(A_{255} - A_{278})_S$$

in which 407.51 and 480.43 are the molecular weights of trifluoperazine and trifluoperazine hydrochloride, respectively, C is the concentration, in µg per mL, of USP Trifluoperazine Hydrochloride RS in the Standard solution, V is the volume, in mL, of Injection taken, and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the assay solution (U) and the Standard solution (S), respectively.

Trifluoperazine Hydrochloride Tablets

» Trifluoperazine Hydrochloride Tablets contain an amount of trifluoperazine hydrochloride ($C_{21}H_{24}F_3N_3S \cdot 2HCl$) equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of trifluoperazine ($C_{21}H_{24}F_3N_3S$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

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USP Reference standards ⟨11⟩—
USP Trifluoperazine Hydrochloride RS
[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—
A: The UV absorption spectrum of the solution employed for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Trifluoperazine Hydrochloride RS, concomitantly measured.
B: Triturate a portion of powdered Tablets, equivalent to about 10 mg of trifluoperazine, with 10 mL of methanol, and centrifuge. A 5-µL portion of this solution responds to *Identification* test *D* under *Trifluoperazine Hydrochloride.*

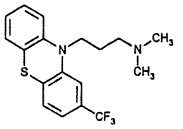
Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 1: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of trifluoperazine ($C_{21}H_{24}F_3N_3S$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 255 nm (determine the analytical value to be used for the absorbance at 255 nm by subtracting the absorbance at 278 nm from the observed maximum absorbance at 255 nm), using filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Trifluoperazine Hydrochloride RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{21}H_{24}F_3N_3S$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Content Uniformity.*

Assay—
Mobile phase—To 2.9 g of *dl*-10-camphorsulfonic acid, add 200 mL of water and stir until solution is complete. Adjust with 1 N sodium hydroxide to a pH of 3.0, dilute with methanol to 1000 mL, mix, and filter through a 0.45-µm membrane filter.
Standard preparation—Dissolve an accurately weighed quantity of USP Trifluoperazine Hydrochloride RS in methanol to obtain a solution having a known concentration of about 12 µg of trifluoperazine hydrochloride per mL (10 µg of trifluoperazine per mL).
Assay preparation—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of trifluoperazine, add to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with methanol to volume, and mix. Filter the solution through a 0.45-µm filter.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 262-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%, and the tailing factor for the trifluoperazine peak is not more than 2.0.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{24}F_3N_3S$, in the portion of Tablets taken by the formula:

$$2(407.51/480.43)C(r_U/r_S)$$

in which 407.51 and 480.43 are the molecular weights of trifluoperazine and trifluoperazine hydrochloride, respectively, C is the concentration, in mg per mL, of USP Trifluoperazine Hydrochloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Triflupromazine



$C_{18}H_{19}F_3N_2S$ 352.42
10*H*-Phenothiazine-10-propanamine, *N,N*-dimethyl-2-(trifluoromethyl)-.
10-3-(Dimethylamino)propyl-2-(trifluoro methyl) phenothiazine [146-54-3].

» Triflupromazine contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{19}F_3N_2S$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Triflupromazine Hydrochloride RS
[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—
A: It meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩, USP Triflupromazine Hydrochloride RS being used, and 0.01 N hydrochloric acid being used in place of water to dissolve the specimen.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 7 µg per mL.
Medium: 0.5 N sulfuric acid.
Absorptivities at 255 nm do not differ by more than 3.0%.
Residue on ignition ⟨281⟩: not more than 0.2%.

Ordinary impurities ⟨466⟩—
Test solution: acetone.
Standard solution: acetone.
Eluant: a mixture of chloroform and methanol (4:1).
Visualization: 1.
Assay—Dissolve about 800 mg of Triflupromazine, accurately weighed, in 100 mL of glacial acetic acid. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 35.24 mg of $C_{18}H_{19}F_3N_2S$.

USP Monographs

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Triflupromazine Oral Suspension

» Triflupromazine Oral Suspension contains an amount of triflupromazine ($C_{18}H_{19}F_3N_2S$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triflupromazine hydrochloride ($C_{18}H_{19}F_3N_2S \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant glass containers.

USP Reference standards ⟨11⟩—
USP Triflupromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: Transfer about 1.0 mL of Oral Suspension, accurately weighed, to a glass-stoppered, low-actinic 35-mL centrifuge tube; add 10.0 mL of methanol; and shake vigorously by mechanical means for 3 minutes. Centrifuge for 5 minutes, and use the methanol layer for the test. Apply 50 µL of the test solution in streaks 4 to 5 cm in length and 0.2 cm in width and 50 µL of a Standard solution containing 1 mg of USP Triflupromazine Hydrochloride RS per mL of methanol to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the streaks to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and methanol (4:1) until the solvent front has moved about four-fifths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the streaks by viewing the plate under short-wavelength and long-wavelength UV light: the R_F value and fluorescence of the streak obtained from the test solution correspond to those obtained from the Standard solution.

B: The UV absorption spectrum of the solution employed for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Triflupromazine Hydrochloride RS, concomitantly measured.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay—

Mixed solvent—Mix 25 mL of isoamyl alcohol with 10 mL of sodium hydroxide solution (1 in 25) in a separator, shake, and discard the aqueous washing. Add 10 mL of 0.1 N hydrochloric acid, shake, and discard the aqueous washing. Prepare 500 mL of a 3 in 100 solution of the washed isoamyl alcohol in *n*-heptane.

pH 5.6 Acetate buffer—Dissolve 1.4 g of sodium acetate in 100 mL of water, and adjust by the addition of glacial acetic acid to a pH of 5.6 ± 0.1.

Procedure—Transfer an accurately measured volume of well-mixed Oral Suspension, equivalent to about 20 mg of triflupromazine hydrochloride, to a glass-stoppered, 150-mL centrifuge bottle. In a second, similar bottle dissolve about 20 mg of USP Triflupromazine Hydrochloride RS, accurately weighed, in 1.0 mL of water; then add 1.0 mL of sodium hydroxide solution (1 in 25); and mix. To a third, similar bottle add 2 mL of water to provide the blank. Treat the two preparations and the blank as follows. Add 100.0 mL of ether, shake by mechanical means for 15 minutes, and allow the layers to separate. Transfer 50.0 mL of the ether layer to another glass-stoppered, 150-mL centrifuge bottle, and evaporate on a water bath maintained at about 35°, with the aid of a current of air, to dryness. Add 5.0 mL of sodium hydroxide solution (1 in 50), mix, add 100.0 mL of *Mixed solvent,* shake by mechanical means for 10 minutes, and centrifuge. Transfer 20.0 mL of the nonaqueous phase to a glass-stoppered centrifuge tube, add 10.0 mL of *pH 5.6 Acetate buffer,* shake by mechanical means for 5 minutes, and centrifuge. Transfer 2.0 mL of the nonaqueous phase to a glass-stoppered centrifuge tube containing 25.0 mL of 0.5 N sulfuric acid, shake by mechanical means for 5 minutes, and centrifuge. Concomitantly determine the absorbances of the aqueous solutions in 1-cm cells at the wavelength of maximum absorbance at about 255 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of triflupromazine hydrochloride ($C_{18}H_{19}F_3N_2S \cdot HCl$) equivalent to the triflupromazine ($C_{18}H_{19}F_3N_2S$) in each mL of the Oral Suspension taken by the formula:

$$(W/V)(A_U/A_S)$$

in which W is the weight, in mg, of USP Triflupromazine Hydrochloride RS taken; V is the volume, in mL, of Oral Suspension taken; and A_U and A_S are the absorbances of the solution from the Oral Suspension and the Standard solution, respectively.

Triflupromazine Hydrochloride

$C_{18}H_{19}F_3N_2S \cdot HCl$ 388.88

10*H*-Phenothiazine-10-propanamine, *N*,*N*-dimethyl-2-(trifluoromethyl)-, monohydrochloride.
10-[3-(Dimethylamino)propyl]-2-(trifluoromethyl) phenothiazine monohydrochloride [1098-60-8].

» Triflupromazine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{19}F_3N_2S \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant glass containers.

USP Reference standards ⟨11⟩—
USP Triflupromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or by using low-actinic glassware.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

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Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: 0.5 N sulfuric acid.
Absorptivities at 255 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Prepare a solution of Triflupromazine Hydrochloride in methanol containing 10 mg per mL. Apply 10 µL each of this solution and a Standard solution of USP Triflupromazine Hydrochloride RS in methanol containing 10 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol, water, and ammonium hydroxide (88:11:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying lightly with dilute methanolic sulfuric acid (4 in 10) and then heating for 15 minutes: the R_F value and color (pink-orange) of the principal spot obtained from the test solution correspond to that obtained from the Standard solution.

Loss on drying ⟨731⟩—Dry it at 100° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Ordinary impurities ⟨466⟩—
Test solution: acetone.
Standard solution: acetone.
Eluant: a mixture of chloroform and methanol (4:1).
Visualization: 1.

Assay—Dissolve about 800 mg of Triflupromazine Hydrochloride, accurately weighed, in 100 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 38.89 mg of $C_{18}H_{19}F_3N_2S \cdot HCl$.

Triflupromazine Hydrochloride Injection

» Triflupromazine Hydrochloride Injection is a sterile solution of Triflupromazine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 112.0 percent of the labeled amount of $C_{18}H_{19}F_3N_2S \cdot HCl$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Triflupromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: Place a volume of Injection, equivalent to about 100 mg of triflupromazine hydrochloride, in a test tube, add 5 mL of 8 N nitric acid, and mix: a peach to amber color develops, quickly turns dark brown, and then changes to a clear solution having a yellow tint.

B: A volume of Injection, equivalent to about 50 mg of triflupromazine hydrochloride, meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

C: The UV absorption spectrum of the *Assay preparation*, prepared as directed in the *Assay*, exhibits maxima and minima at the same wavelengths as that of the *Standard preparation*, prepared as directed in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.8 USP Endotoxin Units per mg of triflupromazine hydrochloride.

pH ⟨791⟩: between 3.5 and 5.2.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Standard preparation—Transfer about 50 mg of USP Triflupromazine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 0.5 N sulfuric acid, dilute with 0.5 N sulfuric acid to volume, and mix. Proceed as directed under *Assay preparation*, beginning with "Transfer 10.0 mL of this solution to a 100-mL volumetric flask." The concentration of USP Triflupromazine Hydrochloride RS in the *Standard preparation* is about 5 µg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of triflupromazine hydrochloride, to a 50-mL volumetric flask, dilute with 0.5 N sulfuric acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with the same acid to volume, and mix. Transfer 10.0 mL of this solution to a glass-stoppered, 50-mL centrifuge tube containing 10 mL of ether previously chilled in an ice bath, insert the stopper, shake for 3 minutes, and centrifuge at 1500 rpm for 5 minutes. Transfer 5.0 mL of the aqueous layer to a 100-mL volumetric flask, dilute with 0.5 N sulfuric acid to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 255 nm, with a suitable spectrophotometer, using 0.5 N sulfuric acid as the blank. Calculate the quantity, in mg, of $C_{18}H_{19}F_3N_2S \cdot HCl$ in each mL of the Injection taken by the formula:

$$(10C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Triflupromazine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Triflupromazine Hydrochloride Tablets

» Triflupromazine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{18}H_{19}F_3N_2S \cdot HCl$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Triflupromazine Hydrochloride RS

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[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: Triturate a portion of powdered Tablets, equivalent to about 50 mg of triflupromazine hydrochloride, with 5 mL of methanol, and centrifuge. A 10-µL portion of the supernatant meets the requirements of *Identification* test *C* under *Triflupromazine Hydrochloride*.

B: The solution prepared from the Tablets for measurement of absorbance in the *Assay* exhibits an absorbance maximum at 255 ± 2 nm.

Dissolution ⟨711⟩—

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{18}H_{19}F_3N_2S \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 305 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Triflupromazine Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{18}H_{19}F_3N_2S \cdot HCl$ is dissolved in 45 minutes.

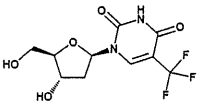
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of triflupromazine hydrochloride, to a separator, add 10 mL of 0.1 N hydrochloric acid and 20 mL of water, and mix. Add 6 N ammonium hydroxide to render the mixture alkaline to litmus, add 1 mL in excess, and extract with five 50-mL portions of chloroform, passing each extract through anhydrous sodium sulfate into a 250-mL volumetric flask. Dilute with chloroform to volume, and mix. Evaporate 10.0 mL of this solution under reduced pressure to dryness, and dissolve the residue in 0.1 N hydrochloric acid to make 100.0 mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Triflupromazine Hydrochloride RS in the same medium having a known concentration of about 8 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 255 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{18}H_{19}F_3N_2S \cdot HCl$ in the portion of Tablets taken by the formula:

$$2.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Triflupromazine Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

Trifluridine



$C_{10}H_{11}F_3N_2O_5$ 296.20

Thymidine, α,α,α-trifluoro-;
2′-Deoxy-5-(trifluoromethyl)uridine [70-00-8].

DEFINITION

Trifluridine contains NLT 98.0% and NMT 102.0% of trifluridine ($C_{10}H_{11}F_3N_2O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 25 µg/mL in 0.1 N hydrochloric acid

Acceptance criteria: Meets the requirements

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: 0.15% sodium citrate solution. Adjust with 1 N hydrochloric acid to a pH of 6.8.

Standard stock solution: 1 mg/mL of USP Trifluridine RS and 0.01 mg/mL each of USP Trifluridine Related Compound A RS and 5-(trifluoromethyl)uracil in water. [NOTE—This stock preparation may be stored at 0°–5° for 3 months.]

Standard solution: 0.2 mg/mL of USP Trifluridine RS and 0.002 mg/mL each of USP Trifluridine Related Compound A RS and 5-(trifluoromethyl)uracil in water from the *Standard stock solution*

Sample solution: 0.2 mg/mL of Trifluridine in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: ▲4.6-mm▲ (USP 1-Dec-2019) × 25-cm; ▲10-µm▲ (USP 1-Dec-2019) packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

▲Run time: NLT 3.75 times the retention time of trifluridine▲ (USP 1-Dec-2019)

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between 5-(trifluoromethyl)uracil and trifluridine related compound A; NLT 4.0 between trifluridine related compound A and trifluridine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of trifluridine ($C_{10}H_{11}F_3N_2O_5$) in the portion of Trifluridine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response ▲of trifluridine▲ (USP 1-Dec-2019) from the *Sample solution*

r_S = peak response ▲of trifluridine▲ (USP 1-Dec-2019) from the *Standard solution*

C_S = concentration of USP Trifluridine RS in the *Standard solution* (mg/mL)

C_U = concentration of Trifluridine in the *Sample solution* (mg/mL)

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Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of trifluridine related compound A and 5-(trifluoromethyl)uracil in the portion of Trifluridine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trifluridine related compound A or 5-(trifluoromethyl)uracil from the *Sample solution*

r_S = peak response of trifluridine related compound A or 5-(trifluoromethyl)uracil from the *Standard solution*

C_S = concentration of USP Trifluridine Related Compound A RS▲or▲ (USP 1-Dec-2019) 5-(trifluoromethyl)uracil in the *Standard solution* (mg/mL)

C_U = concentration of Trifluridine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0% of each related compound

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 30 mg/mL in water

Acceptance criteria: +47° to +51°

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

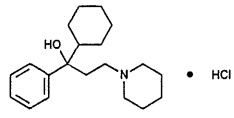
USP Trifluridine RS

USP Trifluridine Related Compound A RS

5-Carboxy-2′-deoxyuridine.

$C_{10}H_{12}N_2O_7$ 272.22

Trihexyphenidyl Hydrochloride



$C_{20}H_{31}NO \cdot HCl$ 337.93

1-Piperidinepropanol, α-cyclohexyl-α-phenyl-, hydrochloride, (±)-;

(±)-α-Cyclohexyl-α-phenyl-1-piperidinepropanol hydrochloride [52-49-3].

DEFINITION

Trihexyphenidyl Hydrochloride contains NLT 98.0% and NMT 102.0% of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Add 0.2 mL of triethylamine in 200 mL of water and mix. Adjust with phosphoric acid to a pH of 4.0.

Mobile phase: Acetonitrile and *Solution A* (75:25)

Standard solution: 0.2 mg/mL of USP Trihexyphenidyl Hydrochloride RS in acetonitrile

Sample solution: 0.2 mg/mL of Trihexyphenidyl Hydrochloride in acetonitrile

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 3 times the retention time of trihexyphenidyl

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1300 theoretical plates

Tailing factor: NMT 3.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$) in the portion of Trihexyphenidyl Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Trihexyphenidyl Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Trihexyphenidyl Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.02 mg/mL of USP Trihexyphenidyl Hydrochloride RS in *Mobile phase*

System suitability solution: 0.01 mg/mL of USP Trihexyphenidyl Related Compound A RS in *Standard stock solution*

Standard solution: 0.002 mg/mL of USP Trihexyphenidyl Hydrochloride RS from *Standard stock solution* in *Mobile phase*

Sample solution: 2 mg/mL of Trihexyphenidyl Hydrochloride in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

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[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between trihexyphenidyl and trihexyphenidyl related compound A, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Signal-to-noise ratio: NLT 50, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Trihexyphenidyl Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of trihexyphenidyl hydrochloride from the *Standard solution*
C_S = concentration of USP Trihexyphenidyl Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Trihexyphenidyl Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peaks below 0.02%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Trihexyphenidyl related compound A	0.47	0.5
Trihexyphenidyl	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%
• **pH** ⟨791⟩
Sample solution: 10 mg/mL in water
Acceptance criteria: 5.2–6.2

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Trihexyphenidyl Hydrochloride RS
USP Trihexyphenidyl Related Compound A RS
1-Phenyl-3-(piperidin-1-yl)propan-1-one hydrochloride.
$C_{14}H_{19}NO \cdot HCl$ 253.77

Trihexyphenidyl Hydrochloride Extended-Release Capsules

» Trihexyphenidyl Hydrochloride Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Trihexyphenidyl Hydrochloride RS

Identification—
A: Reduce the contents of a number of Capsules, equivalent to 20 mg of trihexyphenidyl hydrochloride, to a fine powder, and triturate with 25 mL of chloroform. Filter the mixture, and evaporate the filtrate, by gently heating, to about 10 mL. Add the solution to 100 mL of *n*-hexane: a white precipitate is formed. Allow the mixture to stand for 30 minutes, and collect the precipitate on a solvent-resistant membrane filter to 1-µm pore size. Wash the crystals with a small portion of *n*-hexane, and air-dry: the IR absorption spectrum of a potassium bromide dispersion of the crystals so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Trihexyphenidyl Hydrochloride RS.
B: The precipitate obtained in *Identification* test *A* responds to the tests for *Chloride* ⟨191⟩.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 1: 100 rpm.
Times: 3, 6, and 12 hours.
Determine the amount of $C_{20}H_{31}NO \cdot HCl$ dissolved, using the following method.
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and triethylamine (920: 80: 0.2), and adjust with phosphoric acid to a pH of 4.0.
Standard solution—Dissolve an accurately weighed quantity of USP Trihexyphenidyl Hydrochloride RS in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 5 µg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 8.3-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor for the trihexyphenidyl peak is not more than 2.8, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Dilute the *Standard solution* and the solution under test with acetonitrile (1:1). Separately inject equal volumes (about 20 µL) of these solutions into the chromatograph, record the chromatograms, and measure the peak responses for trihexyphenidyl. Calculate the percentage of the labeled amount of $C_{20}H_{31}NO \cdot HCl$ dissolved.
Tolerances—The percentages of the labeled amount of $C_{20}H_{31}NO \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
3	between 20% and 50%
6	between 40% and 70%
12	not less than 70%

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase—Prepare a mixture of acetonitrile, water, and triethylamine (920: 80: 0.2), adjust with phosphoric acid to a pH of 4.0, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Trihexyphenidyl Hydrochloride RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

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Assay preparation—Fill a 500-mL volumetric flask with *Mobile phase* to volume. Transfer to this flask the accurately weighed contents of a counted number of Capsules, equivalent to about 100 mg of trihexyphenidyl hydrochloride. Mix, sonicate, with occasional shaking for 45 minutes, allow to stand for 15 minutes, and filter, discarding the first 5 mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 8-cm column that contains 3-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1300 theoretical plates, the tailing factor for the analyte peak is not more than 3.0, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$) in each of the Capsules taken by the formula:

$$(500C/N)(r_U/r_S)$$

in which N is the number of Capsules taken, C is the concentration, in mg per mL, of USP Trihexyphenidyl Hydrochloride RS in the *Standard preparation*, r_U and r_S are the trihexyphenidyl peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Trihexyphenidyl Hydrochloride Oral Solution

» Trihexyphenidyl Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Trihexyphenidyl Hydrochloride RS

Identification—
A: To 50 mL of Oral Solution add 50 mL of water and 50 mL of 1 N sodium hydroxide, and stir. Cool the mixture at 4° to 5° for 30 minutes: a white precipitate or cloudiness is observed. Add 100 mL of water to the cooled mixture, stir, and filter by means of vacuum through a 47-mm membrane filter of 1-µm pore size. Wash the crystals with about 100 mL of water, and allow to air-dry: the IR absorption spectrum of a potassium bromide dispersion of the crystals so obtained exhibits maxima only at the same wavelengths as that of the crystalline base obtained from about 20 mg of USP Trihexyphenidyl Hydrochloride RS, similarly prepared and measured.

B: The retention time exhibited by trihexyphenidyl hydrochloride in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

pH ⟨791⟩: between 2.0 and 3.0.

Alcohol Determination ⟨611⟩: between 90.0% and 110.0% of the labeled amount of C_2H_5OH.

Assay—
Mobile phase—Prepare a mixture of acetonitrile, water, and triethylamine (920: 80: 0.2), adjust with phosphoric acid to a pH of 4.0, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Trihexyphenidyl Hydrochloride RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.08 mg per mL.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 2 mg of trihexyphenidyl hydrochloride, to a 25-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 8-cm column that contains 3-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1300 theoretical plates, the tailing factor for the analyte peak is not more than 3.0, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$) in each mL of the Oral Solution taken by the formula:

$$(25C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*, C is the concentration, in mg per mL, of USP Trihexyphenidyl Hydrochloride RS in the *Standard preparation*, r_U and r_S are the trihexyphenidyl peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Trihexyphenidyl Hydrochloride Tablets

DEFINITION
Trihexyphenidyl Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$).

IDENTIFICATION
- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 1.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0. Pass the solution through a suitable filter of 0.22-µm pore size.
Solution B: 0.5 mL of phosphoric acid in 1 L of acetonitrile
Mobile phase: See *Table 1*.

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	60	40
22	60	40
22.1	95	5
24	95	5

Diluent: Methanol and water (80:20)
Standard solution: 0.1 mg/mL of USP Trihexyphenidyl Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 0.5 mg/mL of trihexyphenidyl hydrochloride prepared as follows. Transfer a suitable amount of Tablets (NLT 10), equivalent to 50 mg of trihexyphenidyl hydrochloride, to a 100-mL volumetric flask. Add 10 mL of 0.1 N hydrochloric acid and sonicate. Add another 80 mL of *Solution B* and sonicate again. Dilute with *Solution B* to volume. Centrifuge and use the supernatant.
Sample solution: Nominally 0.1 mg/mL of trihexyphenidyl hydrochloride in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm. For *Identification* test *A*, use a diode array detector in the range of 190–300 nm.
Column: 2.1-mm × 10-cm; 2.6-µm packing L1
Column temperature: 30°
Flow rate: 0.3 mL/min
Injection volume: 3.0 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 3.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trihexyphenidyl from the *Sample solution*
r_S = peak response of trihexyphenidyl from the *Standard solution*
C_S = concentration of USP Trihexyphenidyl Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of trihexyphenidyl hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: pH 4.5 acetate buffer, prepared by mixing 3.0 g of sodium acetate and 1.7 mL of glacial acetic acid with water to obtain 1000 mL of solution with a pH of 4.50 ± 0.05; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: A known concentration of USP Trihexyphenidyl Hydrochloride RS in *Medium*
Sample solution: Filtered portion of the solution under test
Bromocresol green solution: Dissolve 250 mg of bromocresol green in a mixture of 15 mL of water and 5 mL of 0.1 N sodium hydroxide, dilute with *Medium* to 500 mL, and mix. Extract 250 mL portions of this solution with two 100 mL portions of chloroform and discard the chloroform extracts.
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 415 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Transfer an accurately measured volume of the *Sample solution*, estimated to contain about 50 µg of trihexyphenidyl hydrochloride, to a 50-mL centrifuge tube. Transfer an equal, accurately measured volume of the *Standard solution* to a second 50-mL centrifuge tube. Transfer an equal, accurately measured volume of *Medium* to a third 50-mL centrifuge tube to provide a *Blank*. Add 5 mL of the *Bromocresol green solution* and 10.0 mL of chloroform to each tube, insert the stoppers into the tubes, and shake vigorously for NLT 20 s. Centrifuge the mixtures to separate the layers. Aspirate and discard the upper aqueous layers. Filter each chloroform layer through a separate phase-separating filter paper. Determine the amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$) dissolved from the absorbance of the filtrate from the *Sample solution* in comparison with that from the *Standard solution*. The filtrate from the *Blank* is used to set the instrument.
Tolerances: NLT 75% (*Q*) of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.1 mg/mL of USP Trihexyphenidyl Hydrochloride RS and 0.01 mg/mL of USP Trihexyphenidyl Related Compound A RS in *Diluent*
Standard solution: 0.001 mg/mL of USP Trihexyphenidyl Hydrochloride RS in *Diluent*
Sample solution: Nominally 1 mg/mL of trihexyphenidyl hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Tablets (NLT 10), equivalent to 100 mg of trihexyphenidyl hydrochloride, to a 100-mL volumetric flask. Add 10 mL of 0.1 N hydrochloric acid and sonicate. Add another 80 mL of *Solution B* and sonicate again. Dilute with *Solution B* to volume. Centrifuge and use the supernatant.
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 50, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of trihexyphenidyl from the *Standard solution*
C_S = concentration of USP Trihexyphenidyl Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of trihexyphenidyl hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.10%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Trihexyphenidyl related compound A[a]	0.4	—
Trihexyphenidyl	1.0	—
Any individual unspecified degradation product	—	1
Total degradation products	—	2

[a] This impurity included for identification only and is not to be included in the calculation of total degradation products.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Trihexyphenidyl Hydrochloride RS
  USP Trihexyphenidyl Related Compound A RS
  1-Phenyl-3-(piperidin-1-yl)propan-1-one hydrochloride.
  $C_{14}H_{19}NO \cdot HCl$ 253.77

Trikates Oral Solution

» Trikates Oral Solution is a solution of Potassium Acetate, Potassium Bicarbonate, and Potassium Citrate in Purified Water. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of potassium.

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—

A: Two mL, diluted with water to 12 mL, responds to test *B* under *Identification Tests—General* ⟨191⟩, *Acetate*.

B: It responds to tests for bicorbonate under *Identification Tests—General* ⟨191⟩, *Bicarbonate*.

C: To 5 mL add 1 mL of hydrochloric acid, and heat to near boiling for 5 minutes to evolve carbon dioxide. Cool, add 1 mL of calcium chloride TS, and render just alkaline to bromothymol blue TS with 1 N sodium hydroxide. Boil for 3 minutes with gentle agitation: a white, crystalline precipitate appears that is insoluble in 1 N sodium hydroxide but dissolves in 3 N hydrochloric acid.

Assay—

Potassium stock solution—Dissolve, in water, 191 mg of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 100.0 mL of this solution to a second 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 µg of potassium per mL.

Standard preparations—To separate 100-mL volumetric flasks transfer 10.0 mL and 13.0 mL, respectively, of the *Potassium stock solution*. To each flask add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix. The *Standard preparations* contain 1.0 µg and 1.3 µg of potassium per mL, respectively.

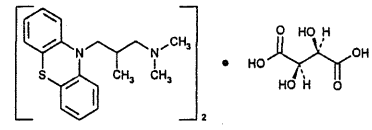
Assay preparation—Transfer 1.0 mL of Oral Solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a third 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a potassium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of potassium. From the graph so obtained, determine the concentration, *C*, in µg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mEq, of potassium in the Oral Solution taken by the formula:

$$100C/39.10$$

in which 39.10 is the atomic weight of potassium.

Trimeprazine Tartrate



$(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$ 746.98

10*H*-Phenothiazine-10-propanamine *N*,*N*,β-trimethyl-, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (2:1).
10-[3-(Dimethylamino)-2-methylpropyl]phenothiazine tartrate (2:1) [4330-99-8; 41375-66-0].

» Trimeprazine Tartrate contains not less than 98.0 percent and not more than 101.0 percent of $(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Trimeprazine Tartrate RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

USP Monographs

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

C: Prepare a solution of it in methanol containing 6 mg in each 5 mL. Proceed as directed under *Thin-layer Chromatographic Identification Test* ⟨201⟩, applying 5 µL of this solution and 5 µL of a similar solution of USP Trimeprazine Tartrate RS, using as the solvent system a mixture of 0.15 mL of ammonium hydroxide and 100 mL of acetone. Locate the spots on the plate by lightly spraying with iodoplatinic acid solution [prepared by dissolving 100 mg of chloroplatinic acid in 1 mL of 1 N hydrochloric acid, adding 25 mL of potassium iodide solution (1 in 25), diluting with water to 100 mL, and adding 0.5 mL of formic acid]: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Ordinary impurities ⟨466⟩—

Test solution: methanol.

Standard solution: methanol.

Eluant: a mixture of ethyl acetate saturated with ammonium hydroxide and ether (1:1).

Visualization: 1.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.005 M sodium 1-heptanesulfonate in methanol, water, and acetic acid (65:34:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Trimeprazine Tartrate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.031 mg per mL.

Assay preparation—Transfer about 62 mg of Trimeprazine Tartrate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume. Transfer 5 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, is not less than 2.0 and not more than 5.0, the column efficiency is not less than 1200 theoretical plates, the tailing factor is not more than 3.5, and the relative standard deviation for replicate injections is not more than 0.6%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$ in the portion of Trimeprazine Tartrate taken by the formula:

$$2000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Trimeprazine Tartrate RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Trimeprazine Oral Solution

» Trimeprazine Oral Solution contains an amount of trimeprazine tartrate $[(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6]$ equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of trimeprazine ($C_{18}H_{22}N_2S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Trimeprazine Tartrate RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Mix 10 mL of Oral Solution with about 30 mL of water in a separator, render the solution alkaline with 1 N sodium hydroxide, and extract with two 30-mL portions of ether. Transfer the ether extracts to a beaker, evaporate the ether by warming, and dissolve the residue in 5 mL of methanol: 5 µL of this solution meets the requirements of *Identification* test *C* under *Trimeprazine Tartrate*.

Alcohol Determination ⟨611⟩: between 4.5% and 6.5% of C_2H_5OH.

Limit of trimeprazine sulfoxide—

Mobile phase and *Chromatographic system* —Proceed as directed in the *Assay*.

Standard solution—Transfer about 60.6 mg of USP Trimeprazine Tartrate RS, accurately weighed, to a 50-mL volumetric flask. Add 5 mL of dilute hydrochloric acid (1 in 100) followed by 2 mL of 30 percent hydrogen peroxide, and heat at 60° for 10 minutes. Cool, dilute with 1 M sodium bisulfite to volume, and mix. Transfer 10.0 mL to a 60-mL separator, add 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the extracts through ether-wetted anhydrous sodium sulfate into a 250-mL conical flask. Cautiously evaporate the flask to dryness. Dissolve the residue in 10.0 mL of methanol, and filter if necessary. Each mL of this solution contains about 1 mg of trimeprazine sulfoxide. Transfer 1.0 mL of this solution to a 500-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution containing about 0.0024 mg per mL of trimeprazine sulfoxide, expressed as trimeprazine tartrate.

Test solution—Use the *Assay preparation* as directed in the *Assay*.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks. The *Test solution* may exhibit a minor peak whose retention time corresponds to the peak exhibited by the *Standard solution* and whose retention time is about 0.6 relative to the main peak. Calculate the concentration, in mg

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per mL, of trimeprazine sulfoxide, in the portion of Oral Solution taken by the formula:

$$100(C/V)(596.89/746.98)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of trimeprazine tartrate in the *Standard solution*; V is the volume, in mL, of Oral Solution taken; 596.89 and 746.98 are the molecular weights of trimeprazine and trimeprazine tartrate, respectively, and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 0.036 mg per mL is found.

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Trimeprazine Tartrate.*

Assay preparation—Using a "to contain" pipet, transfer an accurately measured volume of Oral Solution, equivalent to about 2.5 mg of trimeprazine, to a 100-mL volumetric flask containing 50 mL of *Mobile phase.* Rinse the pipet with *Mobile phase,* collecting the rinses in the volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of trimeprazine ($C_{18}H_{22}N_2S$) in each mL of Oral Solution taken by the formula:

$$100(C/V)(596.89/746.98)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Trimeprazine Tartrate RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; 596.89 and 746.98 are the molecular weights of trimeprazine and trimeprazine tartrate, respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Trimeprazine Tartrate Tablets

» Trimeprazine Tartrate Tablets contain an amount of trimeprazine tartrate [$(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$] equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of trimeprazine ($C_{18}H_{22}N_2S$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Trimeprazine Tartrate RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* obtained as directed in the *Assay.*

B: Triturate a portion of powdered Tablets, equivalent to about 10 mg of trimeprazine, with 10 mL of methanol, and centrifuge: 5 µL of this solution meets the requirements of *Identification* test *C* under *Trimeprazine Tartrate.*

Dissolution ⟨711⟩—

Medium: 0.01 N hydrochloric acid; 500 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{18}H_{22}N_2S$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 251 nm on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Trimeprazine Tartrate RS in the same *Medium.*

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{18}H_{22}N_2S$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Transfer 1 Tablet to a 100-mL volumetric flask, add about 50 mL of 0.1 N hydrochloric acid, and shake by mechanical means until the tablet is completely disintegrated. Add 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate, quantitatively and stepwise if necessary, with 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 5 µg of trimeprazine per mL. Concomitantly determine the absorbances of this solution and a solution of USP Trimeprazine Tartrate RS in the same medium, having a known concentration of about 6 µg per mL, in 1-cm cells, at 276 nm and at the wavelength of maximum absorbance at about 251 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of trimeprazine in the Tablet by the formula:

$$(T/D)(0.7991C)(A_{251} - A_{276})_U/(A_{251} - A_{276})_S$$

in which T is the labeled quantity, in mg, of trimeprazine in the Tablet, D is the concentration, in µg per mL, of trimeprazine in the test solution, based on the labeled quantity per Tablet and the extent of dilution, C is the concentration, in µg per mL, of USP Trimeprazine Tartrate RS in the Standard solution, 0.7991 is the factor converting trimeprazine tartrate to trimeprazine, and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Tablets (U) and the Standard solution (S), respectively.

Assay—

Mobile phase , Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Trimeprazine Tartrate.*

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of trimeprazine, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, mix, and filter.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{22}N_2S$ in the portion of Tablets taken by the formula:

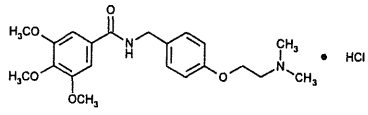
$$100C(596.89/746.98)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Trimeprazine Tartrate RS in the *Standard preparation,* 596.89 and 746.98 are the molecular weights of trimeprazine and trimeprazine tartrate, respectively, and r_U and r_S are the

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peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Trimethobenzamide Hydrochloride



$C_{21}H_{28}N_2O_5 \cdot HCl$ 424.92
Benzamide, *N*-[[4-[2-(dimethylamino)ethoxy]phenyl] methyl]-3,4,5-trimethoxy-, monohydrochloride.
N-[*p*-[2-(Dimethylamino)ethoxy]benzyl]-3,4,5-trimethoxy benzamide monohydrochloride [554-92-7].

» Trimethobenzamide Hydrochloride, dried at 105° for 4 hours, contains not less than 98.5 percent and not more than 100.5 percent of $C_{21}H_{28}N_2O_5 \cdot HCl$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Trimethobenzamide Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 258 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: It meets the requirements of the *Thin-layer Chromatographic Identification Test* ⟨201⟩. Prepare the test solution by dissolving 10 mg of Trimethobenzamide Hydrochloride in 10.0 mL of methanol. Apply 10-µL portions of the test solution and the Standard solution to the plate, and develop in a solvent system consisting of a mixture of ethyl acetate, alcohol, and ammonium hydroxide (90:10:5).

D: It meets the requirements of the tests for *Chloride* ⟨191⟩.
Melting range, *Class I* ⟨741⟩: between 186° and 190°.
Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Assay—Dissolve about 1.3 g of Trimethobenzamide Hydrochloride, previously dried and accurately weighed, in 80 mL of glacial acetic acid and 15 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically using suitable electrodes. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 42.49 mg of $C_{21}H_{28}N_2O_5 \cdot HCl$.

Trimethobenzamide Hydrochloride Capsules

» Trimethobenzamide Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{28}N_2O_5 \cdot HCl$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Trimethobenzamide Hydrochloride RS

Identification—

A: The UV absorption spectrum of the solution employed for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of the Standard solution.

B: Transfer a portion of the contents of Capsules, equivalent to about 20 mg of trimethobenzamide hydrochloride, to a suitable vessel, dissolve in 15 mL of 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a separator, and add 5 mL of 1 N sodium hydroxide. Extract with 15 mL of chloroform, filtering the chloroform extract through anhydrous sodium sulfate into a suitable vessel, and evaporate to dryness. Allow to cool to room temperature, add a small portion of ether, and evaporate at room temperature to dryness. Dry the residue at 60° for 1 hour: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Trimethobenzamide Hydrochloride RS.

C: Place a portion of the contents of Capsules, equivalent to about 25 mg of trimethobenzamide hydrochloride, in a 10-mL volumetric flask, add methanol to volume, mix, and filter: the filtrate so obtained responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, a solvent system consisting of a mixture of ethyl acetate, alcohol, and ammonium hydroxide (90:10:5) being used.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{21}H_{28}N_2O_5 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 258 nm of filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Trimethobenzamide Hydrochloride RS in the same *Medium.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{21}H_{28}N_2O_5 \cdot HCl$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Transfer, as completely as possible, the contents of not less than 20 Capsules to a suitable tared container, and determine the average weight per Capsule. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of trimethobenzamide hydrochloride, to a 100-mL volumetric flask. Add 50 mL of dilute hydrochloric acid (1 in 120), shake the mixture for several minutes, then add dilute hydrochloric acid (1 in 120) to volume, and mix. Filter through small retentive filter paper, discarding the first 20 mL of the filtrate. Transfer 4.0 mL of the subsequent filtrate to a 100-mL volumetric flask, add dilute hydrochloric acid (1 in 120) to volume, and mix. Concomitantly determine the absorbances of this solution

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and a Standard solution of USP Trimethobenzamide Hydrochloride RS in the same medium having a known concentration of about 20 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of $C_{21}H_{28}N_2O_5 \cdot HCl$ in the portion of Capsules taken by the formula:

$$2.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Trimethobenzamide Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Capsules and the Standard solution, respectively.

Trimethobenzamide Hydrochloride Injection

» Trimethobenzamide Hydrochloride Injection is a sterile solution of Trimethobenzamide Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{21}H_{28}N_2O_5 \cdot HCl$.

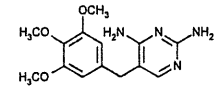
Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Trimethobenzamide Hydrochloride RS

Identification—
A: It meets the requirements of *Identification* test *A* under *Trimethobenzamide Hydrochloride Capsules.*
B: Transfer a volume of Injection, equivalent to about 100 mg of trimethobenzamide hydrochloride, to a separator containing 20 mL of water. Add 2 mL of 1 N sodium hydroxide, and proceed as directed in *Identification* test *B* under *Trimethobenzamide Hydrochloride Capsules*, beginning with "Extract with 15 mL of chloroform."
C: Dilute a portion of Injection quantitatively and stepwise with methanol to obtain a solution containing 2.5 mg of trimethobenzamide hydrochloride per mL: this solution meets the requirements of the *Thin-layer Chromatographic Identification Test* ⟨201⟩, a solvent system consisting of a mixture of ethyl acetate, alcohol, and ammonium hydroxide (90:10:5) being used.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.80 USP Endotoxin Units per mg of trimethobenzamide hydrochloride.
pH ⟨791⟩: between 4.5 and 5.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer to a suitable separator an accurately measured volume of Injection, equivalent to about 200 mg of trimethobenzamide hydrochloride. Add 5 mL of water and 3 mL of dilute hydrochloric acid (1 in 12), and extract with four 20-mL portions of ether, collecting the ether extracts in a second separator, and transferring the aqueous layer to a 500-mL volumetric flask. Wash the combined ether extracts with one 20-mL portion of water, transfer the aqueous layer to the 500-mL volumetric flask, dilute with water to volume, and mix. Dilute 5.0 mL of the solution with dilute hydrochloric acid (1 in 120) to 100.0 mL, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Trimethobenzamide Hydrochloride RS in the same medium having a known concentration of about 20 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of $C_{21}H_{28}N_2O_5 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(10C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Trimethobenzamide Hydrochloride RS in the Standard solution, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Trimethoprim



$C_{14}H_{18}N_4O_3$ 290.32
2,4-Pyrimidinediamine, 5-[(3,4,5-trimethoxyphenyl)methyl]-.
2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine [738-70-5].

» Trimethoprim contains not less than 98.5 percent and not more than 101.0 percent of $C_{14}H_{18}N_4O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at room temperature.
USP Reference standards ⟨11⟩—
USP Trimethoprim RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197S*▲ (CN 1-May-2020)—
Solution: 1 in 100.
Medium: chloroform.
B: Transfer about 100 mg of it, accurately weighed, to a 100-mL volumetric flask, and dissolve in 25 mL of alcohol. Dilute quantitatively and stepwise with sodium hydroxide solution (1 in 250) to obtain a 1 in 50,000 solution: the UV absorption spectrum of this solution exhibits maxima and minima only at the same wavelengths as that of a similar solution of USP Trimethoprim RS, concomitantly measured; and the respective absorptivities, calculated on the dried basis for the test sample only, at the wavelength of maximum absorbance at about 287 nm do not differ by more than 3.0%.
Melting range ⟨741⟩: between 199° and 203°.
Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Buffer solution—Prepare a 10 mM sodium perchlorate solution in water, adjust with phosphoric acid to a pH of 3.6, and mix.

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Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Dissolve accurately weighed quantities of USP Trimethoprim RS and diaveridine; and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 10 µg per mL and 5 µg per mL, respectively.

Test solution—Transfer about 25.0 mg of Trimethoprim, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains base-deactivated packing L1. The flow rate is 1.3 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the peaks for trimethoprim and diaveridine is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram for not less than 11 times the retention time of the trimethoprim peak, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Trimethoprim taken by the formula:

$$100\{Fr_i/[\Sigma(Fr_i) + Fr_T]\}$$

in which F is a relative response factor, and is equal to 0.5 for any peak having a relative retention time of 0.9, 2.3, 2.7, or 10.3, and is equal to 1.0 for all other peaks; r_i is the peak response for each impurity; and r_T is the peak response for trimethoprim obtained from the *Test solution:* not more than 0.1% of any individual impurity is found; and not more than 0.2% of total impurities is found.

Assay—Transfer about 300 mg of Trimethoprim, accurately weighed, to a conical flask, add 60 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 29.03 mg of $C_{14}H_{18}N_4O_3$.

Trimethoprim Tablets

» Trimethoprim Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{14}H_{18}N_4O_3$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Trimethoprim RS

Identification—Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of trimethoprim, with 2.5 mL of methanol. Add 2.5 mL of chloroform, triturate again, and centrifuge. Apply 25 µL of this test solution and 25 µL of a Standard solution of USP Trimethoprim RS in a mixture of methanol and chloroform (1:1) containing 20 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in an unsaturated chamber with a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (95: 7.5: 1), until the solvent front has moved approximately 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{14}H_{18}N_4O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 271 nm of filtered portions of the solution under test, suitably diluted with 0.01 N hydrochloric acid to a concentration of about 20 µg per mL, in comparison with a Standard solution having a known concentration of USP Trimethoprim RS in the same *Medium.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{14}H_{18}N_4O_3$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of 1% glacial acetic acid in water (v/v) and acetonitrile (21:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Using an accurately weighed quantity of USP Trimethoprim RS, prepare a solution in methanol having a known concentration of about 0.2 mg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of trimethoprim, to a 100-mL volumetric flask, add 50 mL of methanol, and sonicate for 5 minutes, with intermittent swirling. Dilute with methanol to volume, and mix. Centrifuge, pipet 10 mL of the supernatant into a 50-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.2-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph five replicate injections of the *Standard preparation,* and measure the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%.

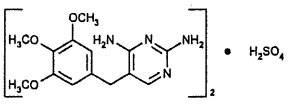
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the analyte peak. Calculate the quantity, in mg, of $C_{14}H_{18}N_4O_3$, in the portion of Tablets taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Trimethoprim RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Trimethoprim Sulfate



$(C_{14}H_{18}N_4O_3)_2 \cdot H_2SO_4$ 678.71

2,4-Pyrimidinediamine, 5-[(3,4,5-trimethoxyphenyl)methyl]-, sulfate (2:1) (salt).

2,4-Diamino-5-[(3,4,5-trimethoxybenzyl)pyrimidine]-, sulfate (2:1) (salt) [56585-33-2].

» Trimethoprim Sulfate contains not less than 98.5 percent and not more than 101.0 percent of $(C_{14}H_{18}N_4O_3)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Trimethoprim RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—*Solution*—Transfer about 100 mg of it, accurately weighed, to a 100-mL volumetric flask, dissolve in 25 mL of alcohol, dilute with 0.1 N sodium hydroxide to volume, and mix. *Medium*—Dilute the *Solution* quantitatively and stepwise with 0.1 N sodium hydroxide to obtain a solution containing a known concentration of about 20 µg per mL.

Absorptivity, at about 287 nm, calculated on the anhydrous basis, is between 83.0% and 86.4% of USP Trimethoprim RS.

B: It responds to the tests for *Sulfate* ⟨191⟩.

Melting range ⟨741⟩**:** between 210° and 215°.

pH ⟨791⟩**:** between 7.5 and 8.5, in a solution (0.5 mg per mL).

Water Determination, *Method I* ⟨921⟩ **:** not more than 3.0%.

Chromatographic purity—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Diluent—Prepare a mixture of chloroform and methanol (9:1).

Test solution—Transfer about 20 mg of Trimethoprim Sulfate, accurately weighed, to a 10-mL volumetric flask, add 4 mL of glacial acetic acid, and swirl to dissolve. Dilute with *Diluent* to volume, and mix.

Standard solution—Dissolve an accurately weighed quantity of USP Trimethoprim RS in *Diluent.* Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of 0.02 mg per mL.

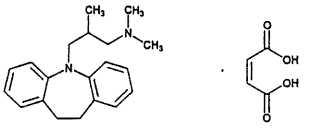
Application volume: 10 µL.

Developing solvent system: a mixture of chloroform, methanol, and 6 N ammonium hydroxide (95: 7.5: 1).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Spray the plate with a freshly prepared mixture of 1.9 g of ferric chloride in 20 mL of water and 0.5 g of potassium ferricyanide in 10 mL of water. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with that of the principal spot in the chromatogram of the *Standard solution:* no secondary spot in the chromatogram obtained from the *Test solution* is larger or more intense than the principal spot obtained from the *Standard solution* (0.1%); and the sum of the intensities of the secondary spots obtained from the *Test solution* corresponds to not more than 0.5%.

Assay—Transfer about 800 mg of Trimethoprim Sulfate, accurately weighed, to a 50-mL conical flask, add about 60 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 67.87 mg of $(C_{14}H_{18}N_4O_3)_2 \cdot H_2SO_4$.

Trimipramine Maleate



$C_{20}H_{26}N_2 \cdot C_4H_4O_4$ 410.51

5*H*-Dibenz[*b,f*]azepine-5-propanamine, 10,11-dihydro-*N,N,β*-trimethyl-, *(Z)*-2-butenedioate (1:1).

5-[3-(Dimethylamino)-2-methylpropyl]-10,11-dihydro-5*H*-dibenz[*b,f*]azepine maleate (1:1) [521-78-8].

» Trimipramine Maleate contains not less than 98.0 percent and not more than 102.0 percent of $C_{20}H_{26}N_2 \cdot C_4H_4O_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, and store at room temperature.

USP Reference standards ⟨11⟩—
USP Iminodibenzyl RS
$C_{14}H_{13}N$ 195.28
USP Imipramine Hydrochloride RS
USP Trimipramine Maleate RS
USP Trimipramine Related Compound A RS
5-[3-(Dimethylamino)-2-methylpropyl]-5*H*-dibenz[*b,f*]azepine.
$C_{20}H_{24}N_2$ 292.42

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Related compounds—

Mobile phase and *Standard stock preparation*—Prepare as directed in the *Assay.*

Impurity stock solution—Dissolve accurately weighed quantities of USP Imipramine Hydrochloride RS and USP Iminodibenzyl RS in a suitable volume of *Mobile phase* to obtain a solution having a known concentration of about 50 µg per mL of iminodibenzyl and 56.5 µg per mL of imipramine hydrochloride.

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Trimipramine related compound A solution—Dissolve a suitable quantity of USP Trimipramine Related Compound A RS in *Mobile phase* to obtain a solution having a concentration of about 50 µg per mL.

Trimipramine stock solution—Quantitatively dilute the *Standard stock preparation* with *Mobile phase* to obtain a solution having a known concentration of about 70 µg per mL of trimipramine maleate.

System suitability solution—Transfer about 7 mg of USP Trimipramine Maleate RS to a 10-mL volumetric flask, dissolve in a small amount of *Mobile phase,* add 0.1 mL each of the *Impurity stock solution* and the *Trimipramine related compound A solution,* and dilute with *Mobile phase* to volume.

Standard solution—Transfer 5.0 mL each of the *Impurity stock solution* and the *Trimipramine stock solution* with *Mobile phase* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Dilute the resulting solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a final solution having a known concentration of about 0.5 µg per mL each of iminodibenzyl, imipramine (free base), and trimipramine (free base). [NOTE—This solution is stable for one day at room temperature. The concentration of imipramine (free base), in µg per mL, can be calculated using the molecular weights of imipramine (282.41) and imipramine hydrochloride (318.88). The concentration of trimipramine (free base), in µg per mL, can be calculated using the molecular weights of trimipramine (294.43) and trimipramine maleate (410.51).]

Test solution—Use the *Assay stock preparation.*

Chromatographic system—Prepare as directed in the *Assay.* Chromatograph about 10 µL of the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between imipramine and trimipramine related compound A is not less than 1.5. [NOTE—For identification purposes, the approximate relative retention times of the specified impurities are given in *Table 1.*]

Procedure—Separately inject about 10 µL of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatogram for three times the retention time for trimipramine. Identify the components based on their relative retention times in *Table 1.* Measure the peak areas of all the peaks in the *Test solution.* Calculate the percentage of imipramine and iminodibenzyl in the portion of Trimipramine Maleate taken by the formula:

$$100(C_S/C_T)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of any given impurity (free base) in the *Standard solution;* C_T is the concentration of Trimipramine Maleate, in mg per mL, in the *Test solution;* r_U is the individual peak response of the given impurity obtained from the *Test solution;* and r_S is the corresponding response for the same impurity obtained from the *Standard solution.* Calculate the percentage of trimipramine related compound A in the portion of Trimipramine Maleate taken by the formula:

$$100(1/3.6)(C_S/C_T)(r_U/r_S)$$

in which 3.6 is the relative response factor for trimipramine related compound A; C_S is the concentration, in mg per mL, of trimipramine (free base) in the *Standard solution;* C_T is the concentration of Trimipramine Maleate, in mg per mL, in the *Test solution;* r_U is the peak response for trimipramine related compound A obtained from the *Test solution;* and r_S is the peak response of trimipramine obtained from the *Standard solution.* Calculate the percentage of each unknown impurity in the portion of Trimipramine Maleate taken by the formula:

$$100(C_S/C_T)(r_i/r_S)$$

in which C_S is the concentration, in mg per mL, of trimipramine (free base) in the *Standard solution;* C_T is the concentration of Trimipramine Maleate, in mg per mL, in the *Test solution;* r_i is the individual peak response of the given impurity obtained from the *Test solution;* and r_S is the response for trimipramine obtained from the *Standard solution:* the limits of the related compounds are given in *Table 1.* [NOTE—Disregard any peak due to the maleate counterion eluting at a relative retention time of about 0.13.]

Table 1

Peak Identification	Approximate Relative Retention Time (RRT)	Limit % (w/w)
Trimipramine *N*-oxide[1]	0.32	NMT 0.15
Iminodibenzyl[2]	0.49	NMT 0.20
Desmethyltrimipramine[3]	0.68	NMT 0.15
Imipramine[4]	0.72	NMT 0.20
Trimipramine related compound A[5]	0.80	NMT 0.10
Trimipramine diamine[6]	2.39	NMT 0.30
Any other individual impurity	—	NMT 0.10
Total impurites	—	NMT 1.0

[1] (2*RS*)-3-(10,11-Dihydro-5*H*-dibenzo[b,f]azepin-5-yl)-*N*,*N*,2-trimethylpropan-1-amine *N*-oxide.
[2] 10,11-Dihydro-5*H*-dibenzo[b,f]azepine.
[3] (2*RS*)-3-(10,11-Dihydro-5*H*-dibenzo[b,f]azepin-5-yl)-*N*,2-dimethylpropan-1-amine 6.
[4] (2*RS*)-3-(10,11-Dihydro-5*H*-dibenzo[b,f]azepin-5-yl)-*N*, *N*-dimethylpropan-1-amine.
[5] 5-[3-(Dimethylamino)-2-methylpropyl]-5*H*-dibenz[*b*,*f*]azepine.
[6] (2*RS*)-*N*1-((2*RS*)-3-(10,11-Dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-2-methylpropyl)-*N*1,*N*3,*N*3,2-tetramethylpropane-1,3-diamine.

Assay—

Buffer solution—Dissolve about 1.4 g of anhydrous dibasic sodium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 7.7.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and *Buffer solution* (18:12:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve an accurately weighed quantity of USP Trimipramine Maleate RS in a suitable volume of *Mobile phase* to obtain a solution having a known concentration of about 0.7 mg per mL.

Standard preparation—Transfer 3 mL of the *Standard stock preparation* to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a final solution having a known concentration of about 0.21 mg per mL of trimipramine maleate.

Assay stock preparation—Dissolve an accurately weighed quantity of Trimipramine Maleate in a suitable volume of *Mobile phase* to obtain a solution having a known concentration of about 0.7 mg per mL.

Assay preparation—Transfer 3 mL of the *Assay stock preparation* to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a final solution having a known concentration of about 0.21 mg per mL of trimipramine maleate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector

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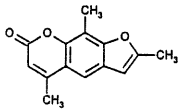
and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 30°. Chromatograph about 20 µL of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the trimipramine maleate peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for up to 1.5 times the retention time of trimipramine maleate, and measure the responses for the trimipramine maleate peak. Calculate the percentage of $C_{20}H_{26}N_2 \cdot C_4H_4O_4$, in the portion of Trimipramine Maleate taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Trimipramine Maleate RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of Trimipramine Maleate in the *Assay preparation;* and r_U and r_S are the trimipramine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Trioxsalen



$C_{14}H_{12}O_3$ 228.24

7H-Furo[3,2-*g*]1]benzopyran-7-one, 2,5,9-trimethyl-. 2,5,9-Trimethyl-7*H*-furo[3,2-*g*]1]benzopyran-7-one [3902-71-4].

» Trioxsalen contains not less than 97.0 percent and not more than 103.0 percent of $C_{14}H_{12}O_3$, calculated on the dried basis.
[**CAUTION**—Avoid exposing the skin to Trioxsalen.]

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Trioxsalen RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 5 µg per mL.
Medium: chloroform.

C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay.*

Loss on drying ⟨731⟩—Dry it at 105° for 6 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%.

Related compounds—In the chromatogram obtained from the *Assay preparation* in the *Assay,* the sum of the responses of any peaks detected, other than the major peak due to trioxsalen, is not more than 2.0% of the total of all the peak responses and the response of the peak occurring at retention time relative to trioxsalen of about 0.75 is not more than 1.5% of the total of all responses.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol and water (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Trioxsalen RS in tetrahydrofuran to obtain a solution having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer about 100 mg of Trioxsalen, accurately weighed, to a 100-mL volumetric flask, dissolve in tetrahydrofuran, dilute with tetrahydrofuran to volume, mix, and filter. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the trioxsalen peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{14}H_{12}O_3$ in the portion of Trioxsalen taken by the formula:

$$2000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Trioxsalen RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Trioxsalen Tablets

» Trioxsalen Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{14}H_{12}O_3$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Trioxsalen RS
Identification—Triturate an amount of finely powdered Tablets, equivalent to about 10 mg of trioxsalen, with 100 mL of chloroform, and filter. Apply 5 µL each of this solution and a Standard solution of USP Trioxsalen RS in chloroform having a known concentration of 100 µg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram, using methanol as the solvent, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the

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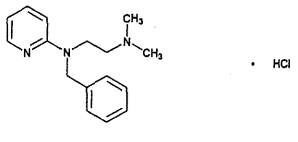
solvent to evaporate. Locate the spots on the plate by viewing under an UV lamp: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—
Apparatus 2: 100 rpm.
Time: 60 minutes.
Dilute simulated intestinal fluid—Prepare a 1 in 12 solution of simulated intestinal fluid TS and water.
Procedure—Assemble the apparatus, adding 225 mL of *Dilute simulated intestinal fluid* to each vessel, and operate the apparatus for 40 minutes. At the end of the 40 minutes, immediately add 675 mL of dehydrated alcohol to each of the vessels. Continue to operate the apparatus for an additional 20 minutes. Determine the amount of $C_{14}H_{12}O_3$ dissolved from UV absorbance determined at the wavelength of maximum absorbance at about 252 nm, filtered portions of the solution under test, in comparison with a Standard solution having a known concentration of USP Trioxsalen RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{14}H_{12}O_3$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of trioxsalen, to a separator containing 25 mL of water. Extract with three 25-mL portions of chloroform, filtering each extract into a 100-mL volumetric flask. Wash the filter with chloroform, dilute with chloroform to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with chloroform to volume, and mix. Concomitantly determine the absorbances of this solution and a solution of USP Trioxsalen RS in the same medium having a known concentration of about 5 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 252 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of $C_{14}H_{12}O_3$ in the portion of Tablets taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Trioxsalen RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

Tripelennamine Hydrochloride



$C_{16}H_{21}N_3 \cdot HCl$ 291.82
1,2-Ethanediamine, *N,N*-dimethyl-*N'*-(phenylmethyl)-*N'*-2-pyridinyl-, monohydrochloride;
2-[Benzyl[2-(dimethylamino)ethyl]amino]pyridine monohydrochloride [154-69-8].

DEFINITION
Tripelennamine Hydrochloride contains NLT 98.0% and NMT 102.0% of tripelennamine hydrochloride ($C_{16}H_{21}N_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

ASSAY
- **PROCEDURE**

Solution A: 29 mM sodium 1-octanesulfonate solution
Mobile phase: Methanol, *N,N*-dimethyloctylamine, and *Solution A* (530:1:430), prepared as follows. Transfer 530 mL of methanol to a suitable container, add 1.0 mL of *N,N*-dimethyloctylamine, and mix thoroughly. Add 430 mL of *Solution A*, mix, and adjust with phosphoric acid to a pH of 3.0.
Benzaldehyde stock solution: Transfer 1.0 mL of benzaldehyde to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Benzaldehyde solution: Transfer 5.0 mL of the *Benzaldehyde stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
System suitability stock solution: 0.5 mg/mL of 2-benzylaminopyridine in *Mobile phase* prepared as follows. Transfer 50 mg of 2-benzylaminopyridine to a 100-mL volumetric flask. Add 10 mL of methanol, sonicate to dissolve, and dilute with *Mobile phase* to volume.
System suitability solution: Transfer 5.0 mL of the *System suitability stock solution* to a 100-mL volumetric flask. Add 5.0 mL of *Benzaldehyde solution*, and dilute with *Mobile phase* to volume.
Standard solution: 0.5 mg/mL of USP Tripelennamine Hydrochloride RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Tripelennamine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 35°
[NOTE—New columns are conditioned with *Mobile phase* overnight before the initial use, and may be reconditioned, as necessary, thereafter.]
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for benzaldehyde and 2-benzylaminopyridine are 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.5 between benzaldehyde and 2-benzylaminopyridine, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tripelennamine hydrochloride ($C_{16}H_{21}N_3 \cdot HCl$) in the portion of Tripelennamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Tripelennamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Tripelennamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Solution A, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tripelennamine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Each individual impurity: NMT 0.1%
Total impurities: NMT 1.0%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tripelennamine Hydrochloride RS

Tripelennamine Hydrochloride Injection

» Tripelennamine Hydrochloride Injection is a sterile solution of Tripelennamine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of tripelennamine hydrochloride ($C_{16}H_{21}N_3 \cdot HCl$).

Packaging and storage—Preserve in tight, single-dose or multiple-dose containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Store at a controlled room temperature, and protect from light.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Tripelennamine Hydrochloride RS

Identification—

A: *Identification—Organic Nitrogenous Bases* ⟨181⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 4.6 USP Endotoxin Units per mg of tripelennamine hydrochloride.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 6.0 and 7.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Dissolve 4.8 g of monobasic potassium phosphate in 880 mL of water in a 2-liter cylinder. Add 720 mL of methanol and 400 mL of acetonitrile, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Tripelennamine Hydrochloride RS quantitatively in water to obtain a solution having a known concentration of about 0.02 mg per mL. Protect this solution from light.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 4 mg of tripelennamine hydrochloride, to a 200-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, a 3.9-mm × 30-mm guard column that contains packing L1, and a 3.9-mm × 30-cm analytical column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the tripelennamine peaks. Calculate the quantity, in mg, of tripelennamine hydrochloride ($C_{16}H_{21}N_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$200(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Tripelennamine Hydrochloride RS in the *Standard preparation; V* is the volume, in mL, of Injection taken to prepare the *Assay preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Tripelennamine Hydrochloride Tablets

» Tripelennamine Hydrochloride Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{16}H_{21}N_3 \cdot HCl$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Tripelennamine Hydrochloride RS

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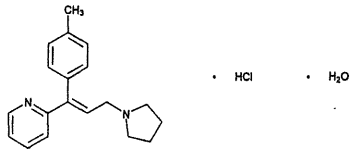
Identification—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{16}H_{21}N_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 306 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Tripelennamine Hydrochloride RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{16}H_{21}N_3 \cdot HCl$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Proceed with Tablets as directed under *Salts of Organic Nitrogenous Bases* ⟨501⟩, determining the absorbance at 313 nm. Calculate the quantity, in mg, of $C_{16}H_{21}N_3 \cdot HCl$ in the portion of Tablets taken by the formula:

$$50C(A_U/A_S)$$

in which C is the concentration, in mg per mL, calculated on the dried basis, of USP Tripelennamine Hydrochloride RS in the *Standard Preparation*.

Triprolidine Hydrochloride



$C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$ 332.87
$C_{19}H_{22}N_2 \cdot HCl$ 314.86
Pyridine, 2-[1-(4-methylphenyl)-3-(1-pyrrolidinyl)-1-propenyl]-, monohydrochloride, monohydrate, (*E*)-; (*E*)-2-[3-(1-Pyrrolidinyl)-1-*p*-tolylpropenyl]pyridine monohydrochloride monohydrate [6138-79-0].
Anhydrous [550-70-9].

DEFINITION
Triprolidine Hydrochloride contains NLT 98.0% and NMT 102.0% of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile, formic acid, and water (200:1:800) prepared as follows. Mix 200 mL of acetonitrile with 800 mL of water, add 1 mL of formic acid, and sonicate.
Diluent: Acetonitrile and water (20:80)
System suitability solution: 0.01 mg/mL each of USP Triprolidine Hydrochloride RS and USP Triprolidine Hydrochloride *Z*-Isomer RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Triprolidine Hydrochloride RS in *Diluent* prepared as follows. Transfer a suitable amount of USP Triprolidine Hydrochloride RS to a suitable volumetric flask and dissolve, by sonication, in about 60% of the flask volume of *Diluent*. Dilute with *Diluent* to volume.
Sample solution: 0.1 mg/mL of Triprolidine Hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Triprolidine Hydrochloride to a suitable volumetric flask and dissolve, by sonication, in about 60% of the flask volume of *Diluent*. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25.0-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 20 min
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between triprolidine hydrochloride and triprolidine hydrochloride *Z*-isomer, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl$) in the portion of Triprolidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Triprolidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Triprolidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 10 µg/mL each of USP Triprolidine Hydrochloride RS and USP Triprolidine Hydrochloride *Z*-Isomer RS in *Diluent*
Sample solution: 0.5 mg/mL of Triprolidine Hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Triprolidine Hydrochloride to a suitable volumetric flask and dissolve, by sonication, in about 60% of the flask volume of *Diluent*. Dilute with *Diluent* to volume.
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]

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Suitability requirements
Resolution: NLT 5.0 between triprolidine hydrochloride and triprolidine hydrochloride *Z*-isomer
Relative standard deviation: NMT 1.8% for each peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of triprolidine hydrochloride *Z*-isomer in the portion of Triprolidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of triprolidine hydrochloride *Z*-isomer from the *Sample solution*
r_S = peak response of triprolidine hydrochloride *Z*-isomer from the *Standard solution*
C_S = concentration of USP Triprolidine Hydrochloride *Z*-Isomer RS in the *Standard solution* (mg/mL)
C_U = concentration of Triprolidine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Triprolidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of triprolidine hydrochloride from the *Standard solution*
C_S = concentration of USP Triprolidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Triprolidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Triprolidine	1.0	—
Triprolidine hydrochloride *Z*-isomer	1.5	2.0
Any individual unspecified impurity	—	0.10
Total impurities	—	3.0

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: 4.0%–6.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Triprolidine Hydrochloride RS
USP Triprolidine Hydrochloride *Z*-Isomer RS
(*Z*)-2-[3-(1-Pyrrolidinyl)-1-*p*-tolylpropenyl]pyridine monohydrochloride.
$C_{19}H_{22}N_2 \cdot HCl$ 314.85

Triprolidine Hydrochloride Oral Solution

» Triprolidine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Triprolidine Hydrochloride RS

Identification—
A: Transfer a volume of Oral Solution, equivalent to about 12 mg of triprolidine hydrochloride, to a 125-mL separator, add 25 mL of water, then add 4 mL of sodium hydroxide solution (1 in 2), and mix. Add 10 mL of cyclohexane, shake, allow the phases to separate completely, and discard the aqueous layer. Transfer 8 mL of the cyclohexane solution to a glass-stoppered, 25-mL conical flask, evaporate on a steam bath with the aid of a current of air to dryness, and continue to heat the flask for about 1 minute after the solvent has completely evaporated. Cool, add 2 mL of cyclohexane, and mix: the IR absorption spectrum of the cyclohexane solution so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Triprolidine Hydrochloride RS.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
pH ⟨791⟩: between 5.6 and 6.6.
Alcohol Determination, *Method II* ⟨611⟩: between 3.0% and 5.0% of C_2H_5OH.

Assay—
Mobile phase—Prepare a suitable degassed and filtered mixture of alcohol and ammonium acetate solution (1 in 250) (17:3).
Standard preparation—Dissolve an accurately weighed quantity of USP Triprolidine Hydrochloride RS in 0.01 N hydrochloric acid, and dilute quantitatively and stepwise with 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 0.05 mg of anhydrous USP Triprolidine Hydrochloride RS per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 2.5 mg of triprolidine hydrochloride, to a 50-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.2-mm × 25-cm column that contains packing L3. The flow rate is about 1.5 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%; and the tailing factor is not more than 1.5.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$) in the portion of Oral Solution taken by the formula:

$$(332.88/314.86)(50C)(r_U/r_S)$$

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in which 332.88 and 314.86 are the molecular weights of triprolidine hydrochloride monohydrate and anhydrous triprolidine hydrochloride, respectively; *C* is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Triprolidine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Triprolidine Hydrochloride Tablets

» Triprolidine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Triprolidine Hydrochloride RS

Identification—

A: Weigh and finely powder not less than 20 Tablets. Transfer a portion of the powder, equivalent to about 20 mg of triprolidine hydrochloride, to a glass-stoppered test tube, add 20 mL of water, and shake for 3 minutes. Add 2 mL of 1 N sodium hydroxide, mix, then add 3 mL of cyclohexane, shake for 3 minutes, and centrifuge for 5 minutes: the IR absorption spectrum of the clear supernatant so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Triprolidine Hydrochloride RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay.*

Dissolution ⟨711⟩—

Medium: pH 4.0 ± 0.05 acetate buffer, prepared by mixing 4.9 g of glacial acetic acid and 2.45 g of sodium acetate trihydrate with water to obtain 1000 mL of solution; 500 mL.

Apparatus 1: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 277 nm of filtered portions of the solution under test, in comparison with a Standard solution having a known concentration of USP Triprolidine Hydrochloride RS in the same medium.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Transfer 1 Tablet to a 100-mL volumetric flask, add 70 mL of water, and sonicate, swirling the flask intermittently, until the tablet is dissolved. Dilute with water to volume, mix, and filter, discarding the first 50 mL of the filtrate. Dilute a portion of the filtrate quantitatively and stepwise with 0.1 N sulfuric acid to obtain a solution having a concentration of about 1.25 µg of triprolidine hydrochloride per mL. Concomitantly determine the fluorescence intensities of this solution and a similarly prepared Standard solution having a known concentration of about 1.25 µg of USP Triprolidine Hydrochloride RS per mL, at the excitation wavelength of 300 nm with a slit width of 2 mm, and an emission wavelength of 460 nm with a slit width of 2 mm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of $C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$ in the Tablet taken by the formula:

$$(332.88/314.86)(TC/D)(I_U/I_S)$$

in which 332.88 and 314.86 are the molecular weights of the monohydrate and anhydrous forms of triprolidine hydrochloride, respectively; *T* is the labeled quantity, in mg, of triprolidine hydrochloride in the Tablet; *C* is the concentration, in µg per mL, of USP Triprolidine Hydrochloride RS in the Standard solution; *D* is the concentration, in µg per mL, of triprolidine hydrochloride in the solution from the Tablet, on the basis of the labeled quantity per Tablet and the extent of dilution, and I_U and I_S are the fluorescence intensities of the solution from the Tablet and the Standard solution, respectively.

Assay—

Mobile phase and *Standard preparation*—Prepare as directed in the *Assay* under *Triprolidine Hydrochlorides Oral Solution.*

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5.0 mg of triprolidine hydrochloride, to a 100-mL volumetric flask. Add about 10 mL of 0.01 N hydrochloric acid, and sonicate for 10 minutes. Cool to room temperature. Dilute with 0.01 N hydrochloric acid to volume, mix, and filter.

Chromatographic system and *Procedure*—Proceed as directed in the *Assay* under *Triprolidine Hydrochlorides Oral Solution,* except to calculate the quantity, in mg, of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$) in the portion of Tablets taken by the formula:

$$(332.88/314.86)(100C)(r_U/r_S)$$

in which 332.88 and 314.86 are the molecular weights of triprolidine hydrochloride monohydrate and anhydrous triprolidine hydrochloride, respectively; *C* is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Triprolidine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Triprolidine and Pseudoephedrine Hydrochlorides Oral Solution

» Triprolidine and Pseudoephedrine Hydrochlorides Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Triprolidine Hydrochloride RS
USP Pseudoephedrine Hydrochloride RS

Identification—

A: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

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B: Transfer 10 mL of Oral Solution to a suitable glass-stoppered tube, add 10 mL of ether and 2 mL of 1 N sodium hydroxide, shake for 5 minutes, and allow the layers to separate. The ether layer is the test solution. Prepare a Standard solution in water of USP Pseudoephedrine Hydrochloride RS and USP Triprolidine Hydrochloride RS having known concentrations of 6 mg per mL and 250 µg per mL, respectively. Separately apply 10-µL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butyl alcohol, glacial acetic acid, and water (8:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength and long-wavelength UV light: the R_F values of the principal spots obtained from the test solution correspond to those obtained from the Standard solution.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of alcohol and 0.40% ammonium acetate solution (17:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Pseudoephedrine Hydrochloride RS and USP Triprolidine Hydrochloride RS in 0.01 N hydrochloric acid, and dilute quantitatively and stepwise with 0.01 N hydrochloric acid to obtain a solution having known concentrations of about 1.2 mg of USP Pseudoephedrine Hydrochloride RS per mL and about 0.05 mg of anhydrous USP Triprolidine Hydrochloride RS per mL, and filter.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 60 mg of pseudoephedrine hydrochloride, to a 50-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%; and the resolution factor between triprolidine and pseudoephedrine is not less than 2.0. The tailing factor for the triprolidine peak is not more than 2.0, and the pseudoephedrine peak is not more than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.68 for pseudoephedrine hydrochloride and 1.0 for triprolidine hydrochloride. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Oral Solution taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses for pseudoephedrine hydrochloride obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the quantity, in mg, of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$) in the portion of Oral Solution taken by the formula:

$$(332.88/314.86)(50C)(r_U/r_S)$$

in which 332.88 and 314.86 are the molecular weights of triprolidine hydrochloride monohydrate and anhydrous triprolidine hydrochloride, respectively; C is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Triprolidine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses for triprolidine hydrochloride obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Triprolidine and Pseudoephedrine Hydrochlorides Tablets

» Triprolidine and Pseudoephedrine Hydrochlorides Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Triprolidine Hydrochloride RS
USP Pseudoephedrine Hydrochloride RS

Identification—

A: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those of the *Standard preparation* as obtained in the *Assay.*

B: Transfer 1 Tablet to a suitable glass-stoppered tube, add 10 mL of water, shake for 5 minutes, and allow the solids to settle. Prepare a Standard solution in water of USP Pseudoephedrine Hydrochloride RS and USP Triprolidine Hydrochloride RS having known concentrations of 6 mg per mL and 250 µg per mL, respectively. Separately apply 10-µL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butyl alcohol, glacial acetic acid, and water (8:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, mark the solvent front, allow the solvent to evaporate, and examine the plate under short- and long-wavelength UV light: the R_F values of the principal spots obtained from the test solution correspond to those obtained from the Standard solution.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Determine the amounts of pseudoephedrine hydrochloride and triprolidine hydrochloride dissolved using the following method.

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Triprolidine and Pseudoephedrine Hydrochlorides Oral Solution.*

Procedure—Inject an accurately measured volume (about 200 µL) of a filtered portion of the solution under test into the chromatograph by means of a microsyringe or a sampling valve, record the chromatogram, and measure the responses for the major peaks. Calculate the quantities of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) and

USP Monographs

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triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$) dissolved in comparison with a Standard solution having known concentrations of USP Pseudoephedrine Hydrochloride RS and USP Triprolidine Hydrochloride RS in the same medium and similarly chromatographed.

Tolerances—Not less than 75% *(Q)* of the labeled amounts of $C_{10}H_{15}NO \cdot HCl$ and $C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Content Uniformity* with respect to triprolidine hydrochloride and to pseudoephedrine hydrochloride.

Assay—

Mobile phase and *Standard preparation*—Prepare as directed in the *Assay* under *Triprolidine and Pseudoephedrine Hydrochlorides Oral Solution*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 120 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask. Add about 10 mL of 0.01 N hydrochloric acid, and sonicate for 10 minutes. Cool to room temperature. Dilute with 0.01 N hydrochloric acid to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩) and *Procedure*—Proceed as directed in the *Assay* under *Triprolidine and Pseudoephedrine Hydrochlorides Oral Solution*, except to calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses for pseudoephedrine hydrochloride obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of triprolidine hydrochloride ($C_{19}H_{22}N_2 \cdot HCl \cdot H_2O$) in the portion of Tablets taken by the formula:

$$(332.88/314.86)(100C)(r_U/r_S)$$

in which 332.88 and 314.86 are the molecular weights of triprolidine hydrochloride monohydrate and anhydrous triprolidine hydrochloride, respectively; C is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Triprolidine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses for triprolidine hydrochloride obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Trisulfapyrimidines Oral Suspension

» Trisulfapyrimidines Oral Suspension contains, in each 100 mL, not less than 3.0 g and not more than 3.7 g of sulfadiazine ($C_{10}H_{10}N_4O_2S$), sulfamerazine ($C_{11}H_{12}N_4O_2S$), and sulfamethazine ($C_{12}H_{14}N_4O_2S$). It may contain either Sodium Citrate or Sodium Lactate, and it may contain a suitable antimicrobial agent.

Packaging and storage—Preserve in tight containers, at a temperature above freezing.

Labeling—Its label indicates the presence and proportion of any sodium citrate or sodium lactate and any antimicrobial agent.

USP Reference standards ⟨11⟩—
USP Sulfadiazine RS
USP Sulfamerazine RS
USP Sulfamethazine RS

Identification—The retention times of the three individual sulfapyrimidines obtained in the *Assay* correspond to the retention times of the respective USP Reference Standards.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay—

Mobile phase—Prepare a suitable degassed solution of water, acetonitrile, and glacial acetic acid (86:13:1) such that the relative retention times of sulfadiazine, sulfamerazine, and sulfamethazine are approximately 0.6, 0.8, and 1.0, respectively. (If the retention times are excessive, the concentration of acetonitrile may be increased.)

Standard preparation—Transfer 33 mg each of USP Sulfadiazine RS, USP Sulfamerazine RS, and USP Sulfamethazine RS, accurately weighed, to a 100-mL volumetric flask, dissolve in 25 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Pipet 3 mL into a 25-mL volumetric flask, dilute with water to volume, and mix to obtain a *Standard preparation* having a known concentration of about 40 µg of each USP Reference Standard per mL.

Assay preparation—Determine the specific gravity of the Oral Suspension, using a tared, 50-mL volumetric flask, by weighing 50 mL of Oral Suspension that previously has been shaken in the original container to ensure homogeneity, allowed to stand long enough for entrapped air to rise, and finally inverted carefully just prior to transfer to the volumetric flask. Transfer an accurately weighed quantity of Oral Suspension, well-shaken and free from entrapped air, equivalent to about 100 mg of total sulfapyrimidines, to a 100-mL volumetric flask, add 25 mL of 0.1 N sodium hydroxide, and swirl for several minutes to dissolve the sulfapyrimidines. Dilute with water to volume, and mix. Filter the mixture, discarding the first several mL of the filtrate. Pipet 3 mL of the clear filtrate into a 25-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph five replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%, and the resolution factors between sulfadiazine and sulfamerazine and between sulfamerazine and sulfamethazine are each not less than 3.0.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times for sulfadiazine, sulfamerazine, and sulfamethazine are approximately 0.6, 0.8, and 1.0, respectively. Calculate the quantity, in mg, of sulfadiazine in the portion of Oral Suspension taken by the formula:

$$0.833C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Sulfadiazine RS in the *Standard preparation;* and R_U and R_S are

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the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively. Similarly measure the responses of the sulfamerazine and sulfamethazine peaks, and calculate the quantity, in mg, of each in the portion of Oral Suspension taken.

Trisulfapyrimidines Tablets

» Trisulfapyrimidines Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of each of the sulfapyrimidines, consisting of equal amounts of sulfadiazine ($C_{10}H_{10}N_4O_2S$), sulfamerazine ($C_{11}H_{12}N_4O_2S$), and sulfamethazine ($C_{12}H_{14}N_4O_2S$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Sulfadiazine RS
USP Sulfamerazine RS
USP Sulfamethazine RS

Identification—The Tablets respond to the *Identification* test under *Trisulfapyrimidines Oral Suspension.*

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.
Procedure—Determine the amount of total sulfapyrimidines dissolved by employing UV absorption at the wavelength of maximum absorbance at about 254 nm on filtered portions of the solution under test, suitably diluted with 0.01 N sodium hydroxide, in comparison with a Standard solution having approximately equal, known, concentrations of USP Sulfadiazine RS, USP Sulfamerazine RS, and USP Sulfamethazine RS in the same media.
Tolerances—Not less than 70% *(Q)* of the labeled amount of total sulfapyrimidines is dissolved in 60 minutes.

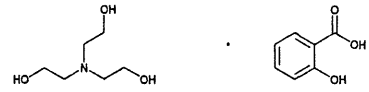
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase , *Standard preparation,* and *Chromatographic system*—Proceed as directed in the *Assay* under *Trisulfapyrimidines Oral Suspension.*
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of total sulfapyrimidines, to a 250-mL volumetric flask, add 50 mL of 0.1 N sodium hydroxide, swirl for several minutes to dissolve the sulfapyrimidines, dilute with water to volume, and mix. Filter the mixture, discarding the first several mL of the filtrate. Pipet 3 mL of the clear filtrate into a 25-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Trisulfapyrimidines Oral Suspension.* Calculate the quantity, in mg, of sulfadiazine in the portion of Tablets taken by the formula:

$$2.08C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Sulfadiazine RS in the *Standard preparation,* and R_U and R_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively. Similarly measure the responses of the sulfamerazine and sulfamethazine peaks, and calculate the quantity, in mg, of each in the portion of Tablets taken.

Trolamine Salicylate



$C_{13}H_{21}NO_6$ 287.31
Triethanolamine salicylate [2174-16-5].

DEFINITION
Trolamine Salicylate is a compounded mixture of Trolamine and Salicylic Acid in propylene glycol. It contains NLT 95.0% and NMT 105.0% of the labeled amount of trolamine salicylate ($C_{13}H_{21}NO_6$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 1 mg/mL. The *Sample solution* and the *Standard solution* contain the equivalent of 1 mg/mL of salicylic acid in *Medium.*
Medium: Methanol
Cell: 0.1 cm
Acceptance criteria: Absorptivities do not differ by more than 1.0%.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (3:7)
Standard solution: 48 µg/mL of USP Salicylic Acid RS in methanol
Sample solution: Transfer an amount equivalent to 300 mg of salicylic acid from a portion of Trolamine Salicylate, into a 250-mL volumetric flask, and dilute with methanol to volume. Transfer 2 mL of this solution to a 50-mL volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 308 nm
Column: 4.0-mm × 12.5-cm; packing L7
Flow rate: 1 mL/min
Column temperature: 30°
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 8000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trolamine salicylate ($C_{13}H_{21}NO_6$) in the portion of Trolamine Salicylate taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

Acceptance criteria: 95.0%–105.0%

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IMPURITIES

• **LIMIT OF FREE SALICYLIC ACID**

Standard solution 1: 0.2 mg/mL of USP Salicylic Acid RS in xylene

Standard solution 2: 0.1 mg/mL of USP Salicylic Acid RS from *Standard solution 1* in xylene

Sample solution: 0.96 mg/mL of Trolamine Salicylate in xylene

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Toluene, acetone, and glacial acetic acid (17: 8: 0.2)

Analysis: Proceed as directed in the chapter. Develop in a chamber previously equilibrated with *Developing solvent system*. Examine the plate under long-wavelength UV light.

Acceptance criteria: NMT 0.02%; any secondary spot of the *Sample solution* is not greater in size or intensity than the spot of *Standard solution 1*.

• **OTHER ORGANIC IMPURITIES**

Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: Use the *Sample solution* from the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Trolamine Salicylate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of all the peak responses

Acceptance criteria: NMT 1.0% of any individual impurity and NMT 2.0% of total impurities are found.

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩: 1.190–1.220

• **REFRACTIVE INDEX** ⟨831⟩: 1.505–1.535 at 20°

• **pH** ⟨791⟩

Solution: 50 mg/mL

Acceptance criteria: 6.5–7.5

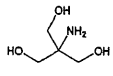
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers in a cool place.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Salicylic Acid RS

Tromethamine



$C_4H_{11}NO_3$ 121.14

1,3-Propanediol, 2-amino-2-(hydroxymethyl)-;

2-Amino-2-(hydroxymethyl)-1,3-propanediol [77-86-1].

DEFINITION

Tromethamine contains NLT 99.0% and NMT 101.0% of tromethamine ($C_4H_{11}NO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B.**

Sample solution: 1 in 5

Analysis: To 4.5 mL of a saturated solution of salicylaldehyde add 0.5 mL of glacial acetic acid, and mix. Add 4.0 mL of the *Sample solution*, and mix.

Acceptance criteria: A yellow color is produced.

• **C.**

Sample solution: 1 in 5

Analysis: To 0.5 mL of a solution (4 in 10) of ceric ammonium nitrate in 2 N nitric acid add 3 mL of water and 0.5 mL of the *Sample solution*, and mix.

Acceptance criteria: The color changes from light yellow to orange.

ASSAY

• **PROCEDURE**

Sample: 250 mg

Analysis: Dissolve the *Sample* in 100 mL of water, add bromocresol purple TS, and titrate with 0.1 N hydrochloric acid VS to a yellow endpoint. Each mL of 0.1 N hydrochloric acid is equivalent to 12.11 mg of tromethamine ($C_4H_{11}NO_3$).

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 168°–172°

• **pH** ⟨791⟩

Sample solution: 1 in 20

Acceptance criteria: 10.0–11.5

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Tromethamine RS

Tromethamine for Injection

» Tromethamine for Injection is a sterile, lyophilized mixture of tromethamine with Potassium Chloride and Sodium Chloride. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of tromethamine ($C_4H_{11}NO_3$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of potassium chloride (KCl) and of sodium chloride (NaCl).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

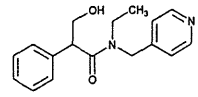
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

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Identification—
A: The IR absorption spectrum of a mineral oil dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Tromethamine RS.
B: A solution, prepared as directed in the labeling, responds to the tests for *Chloride* ⟨191⟩, for *Sodium* ⟨191⟩, and for *Potassium* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.03 USP Endotoxin Unit per mg of tromethamine.
pH ⟨791⟩: between 10.0 and 11.5, in a solution prepared as directed in the labeling.
Water Determination, *Method I* ⟨921⟩—Add 5 mL of glacial acetic acid prior to the titration: the content is not more than 1.0%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Potassium chloride content—
Standard solutions—Prepare five standard solutions (*1*, *2*, *3*, *4*, and *5*) each containing 0.60 mEq of sodium (35 mg of sodium chloride) per liter, and to the solutions add, respectively, 0-, 2-, 4-, 6-, and 8-mg supplements of potassium, in the form of the chloride, per L. If necessary, because of changes in the sensitivity of the photometer, vary the levels of concentration of the potassium, keeping the ratios between solutions approximately as given.
Standard graph—Set a suitable flame photometer for maximum emittance at a wavelength of 766 nm to 767 nm. (The exact wavelength setting will vary slightly with the instrument.) Adjust the instrument to zero emittance with solution *1*. Then adjust the instrument to 100% emittance with solution *5*. Read the percentage emittance of solutions *2*, *3*, and *4*. Plot the observed emittance of solutions *2*, *3*, *4*, and *5* as the ordinate and the concentration, in µg per mL, of potassium as the abscissa on arithmetic coordinate paper.
Procedure—Dissolve the entire contents of 1 container of Tromethamine for Injection in sufficient water, and dilute quantitatively and stepwise with water to obtain a solution containing about 4 µg of potassium per mL, or a quantity corresponding to the concentration of the *Standard solutions*. Adjust the instrument to zero emittance with solution *1* and to 100% emittance with solution *5*. Read the percentage emittance of the test solution. By reference to the *Standard graph*, determine the concentration, in µg per mL, of potassium in the test solution, apply the dilution factor, and calculate the quantity, in mg, of potassium in the container of Tromethamine for Injection. Each mg of potassium is equivalent to 1.907 mg of potassium chloride (KCl).
Sodium chloride content—Proceed as directed under *Potassium chloride content*, with the following modifications: (1) Prepare the *Standard solutions* to contain 0, 2, 4, 6, and 8 mg of sodium, in the form of the chloride, per 1000 mL, without added potassium; (2) prepare the *Standard graph* with the flame photometer set at 588 nm to 589 nm; and (3) under *Procedure* read "sodium" for "potassium" throughout. Each mg of sodium is equivalent to 2.542 mg of sodium chloride (NaCl).
Other requirements—It meets the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩, and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.
Assay for tromethamine—Dissolve the entire contents of 1 container of Tromethamine for Injection in sufficient water, diluting with water to an accurately measured volume to obtain a solution containing about 36 mg of tromethamine per mL. Transfer to a beaker an accurately measured volume of the solution, equivalent to about 180 mg of tromethamine, dilute with water to about 100 mL, add bromocresol purple TS, and titrate with 0.1 N hydrochloric acid VS to a yellow endpoint. Each mL of 0.1 N hydrochloric acid is equivalent to 12.11 mg of $C_4H_{11}NO_3$.

Tropicamide



$C_{17}H_{20}N_2O_2$ 284.35
Benzeneacetamide, *N*-ethyl-α-(hydroxymethyl)-*N*-(4-pyridinylmethyl)-, (±)-;
N-Ethyl-3-hydroxy-2-phenyl-*N*-(pyridin-4- ylmethyl) propanamide [1508-75-4].

DEFINITION
Tropicamide contains NLT 98.0% and NMT 102.0% of tropicamide ($C_{17}H_{20}N_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Dissolve 0.135 g of sodium dodecyl sulfate and 3.4 mL of phosphoric acid in 950 mL of water. Adjust with 10 M sodium hydroxide to a pH of 3.0, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (27:73)
Standard solution: 0.15 mg/mL of USP Tropicamide RS prepared as follows. Transfer USP Tropicamide RS into a suitable volumetric flask, and add acetonitrile equivalent to 6% of the final volume to dissolve. Dilute with water to volume.
Sample solution: 0.15 mg/mL of Tropicamide prepared as follows. Transfer Tropicamide into a suitable volumetric flask, and add acetonitrile equivalent to 6% of the final volume to dissolve. Dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 210 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tropicamide ($C_{17}H_{20}N_2O_2$) in the portion of Tropicamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tropicamide from the *Sample solution*
r_S = peak response of tropicamide from the *Standard solution*
C_S = concentration of USP Tropicamide RS in the *Standard solution* (mg/mL)

USP Monographs

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C_U = concentration of Tropicamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.

System suitability stock solution 1: 100 µg/mL each of USP Tropicamide Related Compound A RS, USP Tropicamide Related Compound C RS, and USP Tropicamide Related Compound D RS prepared as follows. Transfer USP Tropicamide Related Compound A RS, USP Tropicamide Related Compound C RS, and USP Tropicamide Related Compound D RS into a suitable volumetric flask, and add acetonitrile equivalent to 4% of the final volume to dissolve. Dilute with water to volume.

System suitability stock solution 2: 10 µg/mL each of USP Tropicamide Related Compound A RS, USP Tropicamide Related Compound C RS, and USP Tropicamide Related Compound D RS prepared as follows. Dilute 1 mL of *System suitability stock solution 1* with water to 10 mL.

System suitability stock solution 3: 0.5 mg/mL of USP Tropicamide RS and 20 µg/mL of USP Tropicamide Related Compound B RS prepared as follows. Transfer USP Tropicamide RS and USP Tropicamide Related Compound B RS into a suitable volumetric flask, and add acetonitrile equivalent to 10% of the final volume to dissolve. Dilute with water to volume.

System suitability solution: Mix 1 mL each of *System suitability stock solution 2* and *System suitability stock solution 3*.

Standard stock solution: 1 mg/mL of USP Tropicamide RS prepared as follows. Transfer USP Tropicamide RS into a suitable volumetric flask, and add acetonitrile equivalent to 6% of the final volume to dissolve. Dilute with water to volume.

Standard solution 1: 1 µg/mL of USP Tropicamide RS in water from *Standard stock solution*

Standard solution 2: 1.5 µg/mL each of USP Tropicamide Related Compound C RS and USP Tropicamide Related Compound D RS in water from *System suitability stock solution 1*

Sample solution: 1 mg/mL of Tropicamide prepared as follows. Transfer 50 mg of Tropicamide into a 50-mL volumetric flask, and add 3 mL of acetonitrile to dissolve. Dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 210 and 254 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 15 µL
Run time: NLT 3 times the retention time of the tropicamide peak

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution at 210 nm: NLT 2 between the tropicamide related compounds C and A peaks; NLT 2 between the tropicamide related compounds A and D peaks

Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of tropicamide related compound C and tropicamide related compound D in the portion of Tropicamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of relevant tropicamide related compound from the *Sample solution* at 210 nm
r_S = peak response of relevant tropicamide related compound from *Standard solution 2* at 210 nm
C_S = concentration of relevant tropicamide related compound in *Standard solution 2* (mg/mL)
C_U = concentration of Tropicamide in the *Sample solution* (mg/mL)

Calculate the percentage of other individual impurities in the portion of Tropicamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution* at 254 nm
r_S = peak response of USP Tropicamide RS from *Standard solution 1* at 254 nm
C_S = concentration of USP Tropicamide RS in *Standard solution 1* (mg/mL)
C_U = concentration of Tropicamide in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05% at 254 nm.

Table 1

Name	Relative Retention Time	Relative Response Factor	Detection Wavelength (nm)	Acceptance Criteria, NMT (%)
Tropicamide related compound C	0.4	—	210	0.15
Tropicamide related compound A	0.5	1.3	254	0.15
Tropicamide related compound D	0.8	—	210	0.15
Tropicamide	1.0	—	—	—
Tropicamide related compound B	2.3	1.7	254	0.3
Any individual unspecified impurity	—	1.0	254	0.10
Total impurities[a]	—	—	254	0.5

[a] Excluding tropicamide related compound C and tropicamide related compound D.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Sample: 500 mg
Analysis: Dry the *Sample* under vacuum over phosphorus pentoxide at 80° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

USP Monographs

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- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tropicamide RS
  USP Tropicamide Related Compound A RS
  N-(Pyridin-4-ylmethyl)ethanamine.
  $C_8H_{12}N_2$ 136.19
  USP Tropicamide Related Compound B RS
  N-Ethyl-2-phenyl-*N*-(pyridin-4-ylmethyl)acrylamide.
  $C_{17}H_{18}N_2O$ 266.34
  USP Tropicamide Related Compound C RS
  3-Hydroxy-2-phenylpropionic acid.
  $C_9H_{10}O_3$ 166.17
  USP Tropicamide Related Compound D RS
  2-Phenylacetic acid.
  $C_8H_8O_2$ 136.15

Tropicamide Ophthalmic Solution

DEFINITION

Tropicamide Ophthalmic Solution is a sterile, aqueous solution of Tropicamide. It contains NLT 95.0% and NMT 105.0% of the labeled amount of tropicamide ($C_{17}H_{20}N_2O_2$). It contains a suitable antimicrobial agent, and may contain suitable substances to increase its viscosity.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Extract 10 mL of Ophthalmic Solution with 25 mL of chloroform, pass the chloroform extract through dry, folded filter paper, and evaporate the filtrate to dryness.
  Acceptance criteria: The residue so obtained meets the requirements.
- **B.** The UV absorption spectrum of the solution used for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Tropicamide RS, concomitantly measured.

ASSAY

- **PROCEDURE**
  Standard solution: 30 µg/mL of USP Tropicamide RS in dilute sulfuric acid (1 in 6)
  Sample solution: Transfer a volume of Ophthalmic Solution, nominally equivalent to 30 mg of tropicamide, to a 100-mL volumetric flask, and add water to volume. Transfer 10.0 mL of this solution to a separator, add 2 mL of sodium carbonate solution (1 in 10), extract with four 20-mL portions of chloroform, and combine the extracts in a second separator. Wash the combined extracts with a 25-mL portion of pH 6.5 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*), and transfer to another separator. Wash the aqueous layer with 10 mL of chloroform, and add it to the extracts. Extract the chloroform solution with four 20-mL portions of dilute sulfuric acid (1 in 6), combine the acid extracts in a 100-mL volumetric flask, and add the dilute acid to volume.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: At the wavelength of maximum absorbance at about 253 nm
  Cell: 1 cm
  Blank: Dilute sulfuric acid (1 in 6)
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*

  Calculate the percentage of the labeled amount of tropicamide ($C_{17}H_{20}N_2O_2$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Tropicamide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tropicamide in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

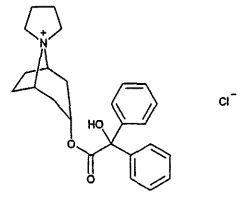
SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **pH** ⟨791⟩**:** 4.0–5.8

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tropicamide RS

Trospium Chloride



$C_{25}H_{30}ClNO_3$ 427.96
Spiro [8-azoniabicyclo[3.2.1]octane-8,1′-pyrrolidinium], 3-[(hydroxydiphenylacetyl)oxy]-, chloride, (1*R*,3*r*,5*S*);
(1*R*,3*r*,5*S*)-3-[(Hydroxydiphenylacetyl)oxy]spiro[8-azoniabicyclo[3.2.1]octane-8,1′-pyrrolidinium] chloride [10405-02-4].

DEFINITION

Trospium Chloride contains NLT 98.0% and NMT 102.0% of trospium chloride ($C_{25}H_{30}ClNO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile, triethylamine, phosphoric acid, and water (300:1:3:700)
  System suitability solution: 0.01 mg/mL of USP Trospium Chloride RS and 0.003 mg/mL each of USP Trospium Chloride Related Compound A RS and USP Trospium Chloride Related Compound B RS in *Mobile phase*

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Standard solution: 0.6 mg/mL of USP Trospium Chloride RS in *Mobile phase*
Sample solution: 0.6 mg/mL of Trospium Chloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1. (Alternatively, a 4.6-mm × 25-cm column that contains 5-µm packing L7 may be used.)
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3 between trospium chloride related compound B and trospium, *System suitability solution*
Relative standard deviation: NMT 0.85% for six replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trospium chloride ($C_{25}H_{30}ClNO_3$) in the portion of Trospium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Trospium Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Trospium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩
Sample: 1 g
Acceptance criteria: NMT 0.1%

• **Organic Impurities**
Mobile phase: Proceed as directed in the *Assay*.
Standard solution: 0.01 mg/mL of USP Trospium Chloride RS and 0.003 mg/mL each of USP Trospium Chloride Related Compound A RS and USP Trospium Chloride Related Compound B RS in *Mobile phase*
Sample solution: 3.0 mg/mL of Trospium Chloride in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except for the following parameters.
Injection volume: 20 µL
Run time: NLT 3 times the retention time of trospium
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3 between trospium chloride related compound B and trospium
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trospium chloride related compound A and trospium chloride related compound B in the portion of Trospium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trospium chloride related compound A or trospium chloride related compound B from the *Sample solution*
r_S = peak response of trospium chloride related compound A or trospium chloride related compound B from the *Standard solution*
C_S = concentration of trospium chloride related compound A or trospium chloride related compound B in the *Standard solution* (mg/mL)
C_U = concentration of Trospium Chloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Trospium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of trospium chloride related compound B from the *Standard solution*
C_S = concentration of USP Trospium Chloride Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Trospium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting level for impurities is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Trospium chloride related compound B[a]	0.7–0.8	0.15
Trospium	1.0	—
Benzilic acid (trospium chloride related compound A)	1.9–2.8	0.15
Any other individual impurity	—	0.10
Total impurities	—	0.5

[a] (1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]octan-3-yl hydroxydiphenylacetate.

• **Limit of Trospium Chloride Related Compound C**
Mobile phase: Acetonitrile, anhydrous formic acid, and water (20: 0.2: 80)
Diluent: Water
Standard solution: 0.1 mg/mL of USP Trospium Chloride Related Compound C RS in *Diluent*, using sonication to dissolve
Sample solution: 100.0 mg/mL of Trospium Chloride in *Diluent*, using sonication to dissolve. [Note—The solution is stable for 12 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0% for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trospium chloride related compound C in the portion of Trospium Chloride taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trospium chloride related compound C from the *Sample solution*
r_S = peak response of trospium chloride related compound C from the *Standard solution*
C_S = concentration of USP Trospium Chloride Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Trospium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry the sample at 105° to constant weight.
  Acceptance criteria: NMT 0.5%
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL of Trospium Chloride in carbon dioxide-free water
  Acceptance criteria: 5.0–7.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Protect from light. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Trospium Chloride RS
  USP Trospium Chloride Related Compound A RS
  Benzilic acid.
  $C_{14}H_{12}O_3$ 228.24
  USP Trospium Chloride Related Compound B RS
  Nortropane benzilate;
  (1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]octan-3-yl hydroxydiphenylacetate.
  $C_{21}H_{23}NO_3$ 337.41
  USP Trospium Chloride Related Compound C RS
  Azoniaspironortropanol chloride;
  (1*R*,3*r*,5*S*)-3-Hydroxyspiro[8-azoniabicyclo[3.2.1]octane-8,1′-pyrrolidinium] chloride, or (1*R*,3*r*,5*S*)-3-Hydroxyspiro[bicyclo[3.2.1]octane-8,1′-pyrrolidin]-1′-ium chloride.
  $C_{11}H_{20}ClNO$ 217.74

Trospium Chloride Tablets

DEFINITION

Trospium Chloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of trospium chloride ($C_{25}H_{30}ClNO_3$).

IDENTIFICATION

- **A.** The retention time and UV spectrum of the major peak of the *Sample solution* correspond to those of the major peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile, triethylamine, phosphoric acid, and water (350:1:3:650)
  Standard solution: 0.1 mg/mL of USP Trospium Chloride RS in *Mobile phase*
  Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 100 mg of trospium chloride, to a 100-mL volumetric flask. Add 75 mL of *Mobile phase*, and sonicate for about 30 min. Dilute with *Mobile phase* to volume. Further dilute with *Mobile phase* to obtain a solution having a nominal concentration of 0.1 mg/mL of trospium chloride. Pass through a suitable filter of 0.45-µm pore size, and use the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 215 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.0 mL/min
  Column temperature: 40°
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of trospium chloride ($C_{25}H_{30}ClNO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of trospium chloride from the *Sample solution*
r_S = peak response of trospium chloride from the *Standard solution*
C_S = concentration of USP Trospium Chloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of trospium chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: 0.1 N hydrochloric acid; 1000 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Mobile phase: Acetonitrile, triethylamine, phosphoric acid, and water (350:1:3:650)
  Standard stock solution: 0.5 mg/mL of USP Trospium Chloride RS in *Mobile phase*
  Standard solution: 0.02 mg/mL in *Medium* from the *Standard stock solution*
  Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 215 nm
  Column: 4.6-mm x 25-cm; 5-µm packing L1
  Flow rate: 1.0 mL/min
  Column temperature: 40°
  Injection volume: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of trospium chloride ($C_{25}H_{30}ClNO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

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C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (Q) of the labeled amount of trospium chloride is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 1000 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: Acetonitrile, triethylamine, phosphoric acid, and water (300:1:3:700)
Standard stock solution: 0.2 mg/mL of USP Trospium Chloride RS in *Medium*
Standard solution: 0.02 mg/mL in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Columns
Guard: 4.0-mm × 4-cm; 5-µm packing L7
Analytical: 4.0-mm × 12.5-cm; 4-µm packing L7
Flow rate: 1.0 mL/min
Column temperature: 40°
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of trospium chloride ($C_{25}H_{30}ClNO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: NLT 75% (Q) of the labeled amount of trospium chloride is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase: Acetonitrile, triethylamine, phosphoric acid, and water (300:1:3:700)
System suitability solution: 0.01 mg/mL of USP Trospium Chloride RS, 0.003 mg/mL of USP Trospium Chloride Related Compound A RS, and 0.015 mg/mL of USP Trospium Chloride Related Compound B RS in *Mobile phase*
Standard solution: 0.003 mg/mL of USP Trospium Chloride RS in *Mobile phase*
Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 150 mg of trospium chloride, to a 50-mL volumetric flask. Add 30 mL of *Mobile phase*, and sonicate for about 30 min. Dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Column temperature: 40°
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3 between trospium chloride related compound B and trospium, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of trospium chloride from the *Standard solution*
C_S = concentration of USP Trospium Chloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of trospium chloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nortropane benzilate (trospium chloride related compound B)	0.7	1.0	0.2
Trospium	1.0	—	—
Benzilic acid (trospium chloride related compound A)	1.9	1.7	0.2
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, protected from light. Store at controlled room temperature.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP Reference Standards** ⟨11⟩
USP Trospium Chloride RS
USP Trospium Chloride Related Compound A RS
Benzilic acid.
$C_{14}H_{12}O_3$ 228.24
[76-93-7.].
USP Trospium Chloride Related Compound B RS
Nortropane benzilate; (1*R*,3*r*,5*S*)-8-azabicyclo [3.2.1] octan-3-yl hydroxydiphenylacetate.
$C_{21}H_{23}NO_3$ 337.41

USP Monographs

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Crystallized Trypsin

» Crystallized Trypsin is a proteolytic enzyme crystallized from an extract of the pancreas of healthy bovine or porcine animals, or both. When assayed as directed herein, it contains not less than 2500 USP Trypsin Units in each mg, calculated on the dried basis, and not less than 90.0 percent and not more than 110.0 percent of the labeled potency.
[NOTE—Determine the suitability of the substrates and check the adjustment of the spectrophotometer by performing the *Assay* using USP Crystallized Trypsin Reference Standard.]

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Trypsin Crystallized RS
Solubility test—An amount, equivalent to 500,000 USP Trypsin Units, is soluble in 10 mL of water and in 10 mL of saline TS.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* species.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.
Residue on ignition ⟨281⟩: not more than 2.5%.

Limit of chymotrypsin—
0.067 M Phosphate buffer , pH 7.0—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of dibasic sodium phosphate solution. Adjust dropwise, if necessary, with dibasic sodium phosphate solution to a pH of 7.0.
Substrate solution—Dissolve 23.7 mg of N-acetyl-L-tyrosine ethyl ester, suitable for use in determining chymotrypsin, in about 50 mL of *0.067 M Phosphate buffer, pH 7.0* with warming. When cool, dilute with additional pH 7.0 buffer to 100 mL. (*Substrate solution* may be stored in the frozen state and used after thawing; it is important, however, to freeze immediately after preparation.)
Crystallized Trypsin solution—Dissolve a sufficient quantity of Crystallized Trypsin, accurately weighed, in 0.0010 N hydrochloric acid to obtain a solution containing 650 USP Trypsin Units per mL.
Procedure—Conduct the test in a suitable spectrophotometer equipped to maintain a temperature of 25 ± 0.1° in the cell compartment. Determine the temperature in the reaction cell before and after the measurement of absorbance to ensure that the temperature does not change by more than 0.5°. Pipet 200 µL of 0.0010 N hydrochloric acid and 3.0 mL of the *Substrate solution* into a 1-cm cell. Place this cell in the spectrophotometer, and adjust the instrument so that the absorbance reads 0.200 at 237 nm. Pipet 200 µL of *Crystallized Trypsin solution* into another 1-cm cell, add 3.0 mL of the *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—This order of addition is to be followed.] At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-second intervals for not less than 5 minutes. Repeat the procedure on the same dilution at least once. Absolute absorbance values are of less importance than the constancy of the rate of change of absorbance. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. The duplicate run at the same dilution should match the first run in rate of absorbance change. Determine the average absorbance change per minute, using only the values within the 3-minute portion of the curve where the rate of absorbance is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075 per minute under the conditions specified in this test. Calculate the number of USP Chymotrypsin Units per mg of Crystallized Trypsin taken by the formula:

$$(A_2 - A_1)/(0.0075TW)$$

in which A_2 is the absorbance straight-line initial reading, A_1 is the absorbance straight-line final reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of Crystallized Trypsin in the volume of solution used in determining the absorbance. Not more than 50 USP Chymotrypsin Units per 2500 USP Trypsin Units is found, indicating the presence of not more than approximately 5% of chymotrypsin.

Assay—
0.067 M Phosphate buffer , pH 7.6—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 13 mL of the monobasic potassium phosphate solution with 87 mL of the anhydrous dibasic sodium phosphate solution.
Substrate solution—Dissolve 85.7 mg of N-benzoyl-L-arginine ethyl ester hydrochloride, suitable for use in assaying Crystallized Trypsin (see NOTE), in water to make 100 mL. Dilute 10 mL of this solution with *0.067 M Phosphate buffer, pH 7.6* to 100 mL. Determine the absorbance of this solution, in a 1-cm cell, at 253 nm, in a suitable spectrophotometer equipped with thermospacers to maintain a temperature of 25 ± 0.1°, using water as the blank. By the addition of *0.067 M Phosphate buffer, pH 7.6*, or of the *Substrate solution* before dilution, adjust the absorbance so that it measures not less than 0.575 and not more than 0.585. Use this *Substrate solution* within 2 hours.
Crystallized Trypsin solution—Dissolve a sufficient quantity of Crystallized Trypsin, accurately weighed, in 0.0010 N hydrochloric acid to obtain a solution containing about 50 to 60 USP Trypsin Units per mL.
Procedure—Pipet 200 µL of 0.0010 N hydrochloric acid and 3.0 mL of the *Substrate solution* into a 1-cm cell. Place this cell in a spectrophotometer, and adjust the instrument so that the absorbance reads 0.050 at 253 nm. Pipet 200 µL of *Crystallized Trypsin solution*, containing 10 to 12 USP Trypsin Units, into another 1-cm cell, add 3.0 mL of *Substrate solution*, and place the cell in the spectrophotometer. At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-second intervals for 5 minutes. Repeat the procedure on the same dilution at least once. Plot a curve of absorbance against time, and use only those values that form a straight line to determine the activity of the Crystallized Trypsin. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. One USP Trypsin Unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified in this *Assay*. Calculate the number of USP Trypsin Units per mg taken by the formula:

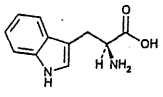
$$(A_1 - A_2)/(0.003TW)$$

in which A_1 is the absorbance straight-line final reading, A_2 is the absorbance straight-line initial reading, T is the elapsed

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time, in minutes, between the initial and final readings, and *W* is the weight, in mg, of Crystallized Trypsin in the volume of solution used in determining the absorbances.

Tryptophan



$C_{11}H_{12}N_2O_2$ 204.23
L-Tryptophan [73-22-3].

DEFINITION
Tryptophan contains NLT 98.5% and NMT 101.5% of $C_{11}H_{12}N_2O_2$, as L-tryptophan, calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Sample solution: Place 200 mg of Tryptophan in a 125-mL flask. Dissolve in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid.
Analysis: Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.
Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Chloride and Sulfate,** *Chloride* ⟨221⟩: A 0.73-g portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.05%). [NOTE—Gently heat the sample preparation to dissolve, if necessary.]
• **Chloride and Sulfate,** *Sulfate* ⟨221⟩: A 0.33-g portion shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.03%). [NOTE—Gently heat the sample preparation to dissolve, if necessary.]
• **Iron** ⟨241⟩: NMT 30 ppm
ORGANIC IMPURITIES
• **Procedure 1**
Solution A: Trifluoroacetic acid in water (1 mL/L)
Solution B: Trifluoroacetic acid in an acetonitrile and water solution (80:20) (1 mL/L trifluoroacetic acid solution)
Standard solution: 1.0 mg/L each of USP Tryptophan Related Compound A RS and USP Tryptophan Related Compound B RS in water
Sample solution: 10.0 mg/mL of tryptophan in water
System suitability solution: 1.0 mg/L of USP Tryptophan Related Compound B RS in water
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
37	35	65
42	0	100
47	0	100
50	95	5
60	95	5

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirement
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unspecified impurity in the portion of Tryptophan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each unspecified impurity in the *Sample solution*
r_S = peak area of tryptophan related compound B in the *Standard solution*
C_S = concentration of USP Tryptophan Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of Tryptophan in the *Sample solution* (µg/mL)

Acceptance criteria
Total impurities 1: NMT 0.01% of the total impurities eluting prior to the tryptophan peak
Total impurities 2: NMT 0.03% of the total impurities eluting after the tryptophan peak. [NOTE—Exclude the peak for tryptophan related compound B.]
Tryptophan related compound A: If a peak for tryptophan related compound A is observed in the *Sample solution*, then perform the test for *Procedure 2: Limit of Tryptophan Related Compound A*, below.
• **Procedure 2: Limit of Tryptophan Related Compound A**
Solution A: 18 mM monobasic sodium phosphate, filtered and degassed (pH 2.5), and acetonitrile (9:1)
Solution B: 10 mM monobasic sodium phosphate, filtered and degassed (pH 2.5), and acetonitrile (1:1)
Solution C: Acetonitrile in water (7:3)
Standard solution: 0.1 mg/L of USP Tryptophan Related Compound A RS in water
Sample solution: 10.0 mg/mL of Tryptophan in water
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
30	44	56	0
30.1	0	0	100
45	0	0	100

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Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
45.1	100	0	0
60	100	0	0

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 216 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirement
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tryptophan related compound A in the portion of Tryptophan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

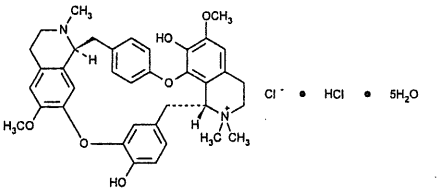
r_U = peak area of tryptophan related compound A in the *Sample solution*
r_S = peak area of tryptophan related compound A in the *Standard solution*
C_S = concentration of USP Tryptophan Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Tryptophan in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 10 ppm

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩**:** −29.4° to −32.8°
  Sample solution: 10 mg/mL, in water. [NOTE—Heat gently to dissolve, if necessary.]
- **pH** ⟨791⟩**:** 5.5–7.0, in a solution (1 in 100)
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Tryptophan RS
  USP Tryptophan Related Compound A RS
  3,3′-[Ethylidenebis(1*H*-indole-1,3-diyl)]bis[2*S*)-2-aminopropanoic]acid.
  $C_{24}H_{26}N_4O_4$ 432.49
  USP Tryptophan Related Compound B RS
  2-Acetamido-3-(1*H*-indol-3-yl)propanoic acid.
  $C_{13}H_{14}N_2O_3$ 246.3

Tubocurarine Chloride



$C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$ 771.72
Tubocuraranium, 7′,12′-dihydroxy-6,6′-dimethoxy-2,2′,2′-trimethylchloride, hydrochloride, pentahydrate.
(+)-Tubocurarine chloride hydrochloride pentahydrate [6989-98-6].
Anhydrous 681.66
[57-94-3].

» Tubocurarine Chloride contains not less than 95.0 percent and not more than 105.0 percent of $C_{37}H_{41}ClN_2O_6 \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Tubocurarine Chloride RS
Clarity of alcohol solution—A solution of 100 mg in 10 mL of alcohol is clear.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*.

C: A solution (1 in 100) responds to the tests for *Chloride* ⟨191⟩.

Specific rotation ⟨781S⟩: between +210° and +224°.
Test solution: 10 mg per mL, in water, allowed to stand for 3 hours.
Water Determination, *Method I* ⟨921⟩: not more than 12.0%.
Residue on ignition ⟨281⟩: not more than 0.25%.
Related compounds—In the chromatogram obtained from the *Assay preparation* in the *Assay*, the sum of the responses of any peaks detected, other than the peak due to tubocurarine, is not more than 5.0% of the total of all peak responses.
Chloride content—Dissolve about 300 mg, accurately weighed, in 5 mL of water, warming slightly to effect solution. Add 5 mL of glacial acetic acid and 50 mL of methanol, and cool to room temperature. Add 1 drop of eosin Y TS, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Not less than 9.9% and not more than 10.7% of Cl is found, calculated on the anhydrous basis.

Assay—
Mobile phase—Mix 3 volumes of acetonitrile and 2 volumes of methanol, and allow the mixture to attain room temperature. To 270 mL of this solution in a 1-liter graduated cylinder add 20.0 mL of 25% tetramethylammonium hydroxide solution in methanol, and add water to make 1 L. Adjust with phosphoric acid to a pH of 4.0, filter, and degas.

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Standard preparation—Dissolve an accurately weighed quantity of USP Tubocurarine Chloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Transfer 30 mg of Tubocurarine Chloride, accurately weighed, to a 100-mL volumetric flask. Dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

System suitability preparation—Dissolve suitable quantities of tubocurarine chloride and phenol in *Mobile phase* to obtain a solution containing about 0.30 mg and 0.50 mg per mL, respectively.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector, and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the two major peaks is not less than 2.0, and the tailing factor, *T*, for tubocurarine chloride is not more than 2.0. The relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%. The relative retention times are about 0.50 and 1.0 for tubocurarine chloride and phenol, respectively.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{37}H_{41}ClN_2O_6 \cdot HCl$ in the portion of Tubocurarine Chloride taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Tubocurarine Chloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Tubocurarine Chloride Injection

» Tubocurarine Chloride Injection is a sterile solution of Tubocurarine Chloride in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers.

USP Reference standards ⟨11⟩—
USP Tubocurarine Chloride RS

Identification—

A: It responds to *Identification* test *C* under *Tubocurarine Chloride*.

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*.

Angular rotation ⟨781⟩: between +0.32° and +0.48° for each mg of tubocurarine chloride per mL claimed on the label, determined in a suitable polarimeter tube and the observed reading being multiplied by the factor 200/*L*, in which *L* is the length, in mm, of the tube.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 10.0 USP Endotoxin Units per mg of tubocurarine chloride.

pH ⟨791⟩: between 2.5 and 5.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Tubocurarine Chloride*.

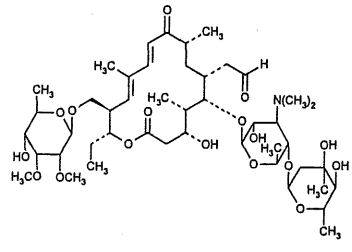
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 15 mg of tubocurarine chloride, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$ in each mL of the Injection taken by the formula:

$$50C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Tubocurarine Chloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Tylosin



$C_{46}H_{77}NO_{17}$ 916.10

(10*E*,12*E*)-(3*R*,4*S*,5*S*,6*R*,8*R*,14*S*,15*R*)-14-[(6-deoxy-2,3-di-*O*-methyl-β-D-allopyranosyl)oxymethyl]-5-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-ribo-hexopyranosyl)-3-dimethylamino-β-D-glucopyranosyl]oxy]-6-formylmethyl-3-hydroxy-4,8,12-trimethyl-9-oxoheptadeca-10,12-dien-15-olide.

Tylosin A [1401-69-0].

» Tylosin is the macrolide antibiotic substance, or the mixture of such substances, produced by the growth of *Streptomyces fradiae*, or by any other means. Its potency is not less than 900 µg of tylosin per mg, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light, moisture, and excessive heat.

Labeling—Label it to indicate that it is for use in animals only.

USP Reference standards ⟨11⟩—
USP Tylosin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020).

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Acid solution—Transfer about 50 mg of Tylosin, accurately weighed, to a 100-mL volumetric flask, add 10 mL of 2 N hydrochloric acid, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. Absorptivity at 290 nm is 22.5 ± 2.5, calculated on the dried basis.
Alkaline solution—To 10.0 mL of the final *Acid solution* add 1.0 mL of 2 N sodium hydroxide, and heat on a water bath for 20 minutes. Cool to room temperature: an absorption maximum is observed at about 332 nm.

B: The retention time of the major peak for tylosin A in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Content of tylosins*.

Loss on drying ⟨731⟩—Dry about 1 g, accurately weighed, in vacuum at a pressure of not more than 5 mm of mercury at 60° for 3 hours: it loses not more than 5% of its weight.

Residue on ignition ⟨281⟩: not more than 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Limit of tyramine—Transfer 100 mg of it to a 25-mL volumetric flask, add 5.0 mL of 0.03 M phosphoric acid, and swirl to dissolve *(Test solution)*. Transfer 5.0 mL of a solution containing 70 µg of tyramine per mL in 0.03 M phosphoric acid to a 25-mL volumetric flask *(Standard solution)*. Transfer 5 mL of 0.03 M phosphoric acid to a 25-mL volumetric flask to provide the blank. Concurrently add to each flask 1.0 mL of a mixture of pyridine and 2.0 mL of filtered ninhydrin solution (1 in 25). Cover the flasks lightly with glass or aluminum foil caps, and heat in a water bath at 85° for not less than 20 minutes. Cool rapidly to room temperature, dilute with water to volume, and mix. Promptly determine the absorbances of the solutions from the *Test solution* and the *Standard solution* at the wavelength of maximum absorbance at about 570 nm, using the solution from the blank to zero the instrument. The absorbance of the solution from the *Test solution* is not greater than that of the solution from the *Standard solution* (0.35% of tyramine). In a valid test the solution from the *Standard solution* exhibits a dark blue color.

Content of tylosins—

Mobile phase—Prepare a mixture of filtered 2 M sodium perchlorate, previously adjusted with 1 N hydrochloric acid to a pH of 2.5 ± 0.1, and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Transfer about 30 mg of USP Tylosin RS, accurately weighed, to a 100-mL volumetric flask, add 10 mL of methanol, and swirl to dissolve. Dilute with water to volume, and mix.

Test solution—Transfer about 30 mg of Tylosin, accurately weighed, to a 100-mL volumetric flask, add 10 mL of methanol, and swirl to dissolve. Dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector, a 4.6-mm × 20-cm column that contains 5-µm packing L1. The flow rate is about 0.7 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the tylosin D peak and the tylosin A peak is not less than 2, the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms over a period of time 1.5 times the elution time of the main tylosin A peak, and measure the peak areas for all the peaks. The relative retention times are about 0.5 for tylosin C, 0.7 for tylosin B, 0.9 for tylosin D, and 1.0 for tylosin A. Calculate the percentages of tylosin A, tylosin B, tylosin C, and tylosin D in the Tylosin taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the area of the tylosin A peak, the tylosin B peak, tylosin C peak, or the tylosin D peak, as appropriate, in the chromatogram obtained from the *Test solution*, and r_s is the sum of the areas of all of the peaks in the chromatogram obtained from the *Test solution:* the content of tylosin A is not less than 80% and the sum of the contents of tylosin A, tylosin B, tylosin C, and tylosin D is not less than 95%.

Assay—Proceed as directed for Tylosin under *Antibiotics—Microbial Assays* ⟨81⟩. Prepare the *Test Dilution* as follows. Transfer about 250 mg of Tylosin, accurately weighed, to a 500-mL volumetric flask, add 50 mL of methanol, and swirl to dissolve. Dilute with *Buffer B.3* to volume, and mix. Transfer 4.0 mL of this solution to a second 500-mL volumetric flask, dilute with a mixture of *Buffer B.3* and methanol (1:1), and mix. This solution contains about 4 µg of tylosin per mL.

Tylosin Granulated

» Tylosin Granulated contains tylosin phosphate mixed with suitable carriers and inactive ingredients. It contains not less than 80.0 percent and not more than 120.0 percent of the labeled amount of tylosin.

Packaging and storage—Preserve in well-closed, polyethylene-lined or polypropylene-lined containers, protected from moisture and excessive heat.
Labeling—Label it to indicate that it is for animal use only. Label it also to indicate that it is for manufacturing, processing, or repackaging.
USP Reference standards ⟨11⟩—
USP Tylosin RS

Identification—

A: The chromatogram of the *Test solution*, obtained as directed in the test for *Content of tylosins*, exhibits a major peak for tylosin A, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard solution* obtained as directed in the test for *Content of tylosins*.

B: Transfer 2 g of Tylosin Granulated to a test tube, add 10 mL of water, and shake for 5 minutes. Filter the resulting suspension, and if necessary adjust the pH of the filtrate to a pH between 6 and 8 with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid. This solution responds to the tests for *Phosphate* ⟨191⟩.

Loss on drying ⟨731⟩—Dry about 1 g of it, accurately weighed, in vacuum at a pressure of not more than 5 mm of mercury at 60° for 5 hours: it loses not more than 12.0% of its weight.

Powder fineness ⟨811⟩: not less than 99% passes a No. 20 sieve, and not more than 10% passes a No. 80 sieve.

Content of tylosins—

Mobile phase , *Standard solution*, and *Chromatographic system*—Proceed as directed in the test for *Content of tylosins* under *Tylosin*.

pH 7.0 buffer—Dissolve 13.6 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 12 N sodium hydroxide to a pH of 7.0.

Test solution—Transfer about 1.4 g of Tylosin Granulated, accurately weighed, to a 250-mL volumetric flask, add

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100 mL of *pH 7.0 buffer*, and shake by mechanical means for about 30 minutes. Dilute with water to volume, mix, and filter. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, dilute with water to volume, and mix. Filter a portion of this solution through a filter having a porosity of 0.5 µm or finer, and use the filtrate as the *Test solution*.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solutions* and the *Test solution* into the chromatograph, record the chromatograms over a period of time that is about twice the elution time of the main tylosin A peak, and measure the areas for all the major peaks: the relative retention times are about 0.5 for tylosin C, 0.7 for tylosin B, 0.9 for tylosin D, and 1.0 for tylosin A. Calculate the percentages of tylosin A, tylosin B, tylosin C, and tylosin D in the Tylosin taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the area of the tylosin A peak, tylosin B peak, tylosin C peak, or tylosin D peak, as appropriate, in the chromatogram obtained from the *Test solution*, and r_s is the sum of the areas of all of the peaks in the chromatogram obtained from the *Test solution:* the content of tylosin A is not less than 80%, and the sum of the contents of tylosin A, tylosin B, tylosin C, and tylosin D is not less than 95%.

Assay—Proceed as directed for Tylosin under *Antibiotics—Microbial Assays* ⟨81⟩. Prepare the *Test Dilution* as follows. Transfer about 2 g of Tylosin Granulated, accurately weighed, to a suitable container, add 200.0 mL of a mixture of *Buffer B.3* and methanol (1:1), seal to prevent evaporation, and shake by mechanical means for about 60 minutes. Filter the suspension so obtained, discarding the first 5 mL of the filtrate. Dilute an accurately measured portion of the filtrate quantitatively and stepwise with a mixture of *Buffer B.3* and methanol (1:1) to obtain a *Test Dilution* having an estimated concentration of about 4 µg of tylosin per mL.

Tylosin Injection

» Tylosin Injection is a sterile solution of tylosin in a suitable vehicle. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of tylosin.

Packaging and storage—Preserve in single-dose or multiple-dose containers, Type I or Type II amber glass, and store at a temperature not to exceed 22°.

Labeling—Label to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Tylosin RS

Identification—The retention time of the major peak for tylosin A in the chromatogram of the *Test solution* corresponds to the major peak for tylosin A in the chromatogram of the *Standard solution,* as obtained in the test for *Content of tylosins.*

Bacterial Endotoxins Test ⟨85⟩—It contains no more than 0.28 USP Endotoxin Unit per mg of tylosin.

Sterility Tests ⟨71⟩ : meets the requirements.

pH ⟨791⟩: between 8.0 and 9.5.

Particulate Matter in Injections ⟨788⟩—Use the procedure in *Method 2—Microscopic Particle Count Test:* not more than 50 particles per mL that are equal to or greater than 10 µm in effective spherical diameter are found, and not more than 5 particles per mL that are equal to or greater than 25 µm in effective spherical diameter are found.

Content of tylosins—

Sodium perchlorate solution—Prepare a 184 g per L solution.

Mobile phase—Prepare a mixture of *Sodium perchlorate solution* and acetonitrile (3:2). Adjust with 1 N hydrochloric acid to a pH of 2.5 ± 0.1, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of methanol and water (1:9).

Standard solution—Dissolve an accurately weighed quantity of USP Tylosin RS in *Diluent* to obtain a solution having a known concentration of about 0.3 mg per mL.

Test solution—Dilute an accurately measured volume of Injection with *Diluent* to obtain a solution having a known concentration of about 0.25 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 20-cm column that contains 5-µm packing L1. The flow rate is about 0.7 mL per minute. The column temperature is maintained at 25°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the order of elution is tylosin C, tylosin B, tylosin D, and tylosin A, with relative retention times of about 0.5, 0.6, 0.8, and 1.0 minutes, respectively; the resolution, *R,* of the peaks for tylosin D and tylosin A is not less than 2.8; and the tailing factor for the tylosin A peak is not more than 1.5.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms over a period equivalent to 1.5 times the elution time of the main tylosin A peak, and measure all the peak areas. Calculate the percentages of tylosin A, tylosin B, tylosin C, and tylosin D in the portion of Injection taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the area of the tylosin A peak, the tylosin B peak, the tylosin C peak, or the tylosin D peak, as appropriate, in the chromatogram obtained from the *Test solution;* and r_s is the sum of the areas of all the peaks in the chromatogram obtained from the *Test solution:* the content of tylosin A is not less than 75%; and the sum of the contents of tylosin A, tylosin B, tylosin C, and tylosin D is not less than 85%.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Proceed as directed for *Tylosin* under *Antibiotics—Microbial Assays* ⟨81⟩. Prepare the *Test Dilution* as follows. Transfer an accurately measured volume of Injection, equivalent to about 250 mg of tylosin, to a 500-mL volumetric flask, add 50 mL of methanol, and swirl to dissolve. Dilute with *Buffer B.3*to volume, and mix. Transfer 4.0 mL of this solution to a second 500-mL volumetric flask, dilute with a mixture of *Buffer B.3* and methanol (1:1), and mix. This solution contains about 4 µg of tylosin per mL.

Tylosin Tartrate

(10*E*,12*E*)-(3*R*,4*S*,5*S*,6*R*,8*R*,14*S*,15*R*)-14-[(6-deoxy-2,3-di-*O*-methyl-B-D-allopyranosyl)oxymethyl]-5-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-dimethylamino-B-D-glucopyranosyl]oxy]-6-formylmethyl-3-hydroxy-4,8,12-trimethyl-9-oxoheptadeca-10,12-dien-15-olide.

Tylosin A (Tylosin) 916.10
[1401-69-0].

USP Monographs

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» Tylosin Tartrate is a tartrate of a mixture of macrolide antibiotic substances, or the mixture of such substances, produced by the growth of *Streptomyces fradiae,* or by any other means. Its potency is not less than 800 µg of tylosin per mg, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light, moisture, and excessive heat. Store at 25°, excursions permitted between 15° and 30°.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Tylosin RS
USP Tylosin Tartrate RS

Change to read:

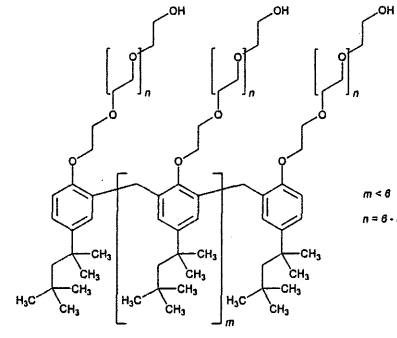
Identification—
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)
B: The retention time of the major peak for tylosin A in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution,* as obtained in the test for *Content of tylosins.*
C: It meets the requirements of the test for *Tartrate* ⟨191⟩.
pH ⟨791⟩**:** between 5.0 and 7.2 in a solution prepared by dissolving 0.25 g in 10 mL of carbon dioxide-free water.
Loss on drying ⟨731⟩—Dry about 1 g, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 4.5% of its weight.
Residue on ignition ⟨281⟩**:** not more than 2.5%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.
Limit of tyramine—In a 25-mL volumetric flask, dissolve 50.0 mg of tylosin in 5.0 mL of a 3.4 g per L solution of phosphoric acid. Add 1.0 mL of pyridine and 2.0 mL of a saturated solution of ninhydrin (about 40 g per L). Close the flask with aluminum foil, and heat in a water bath at 85° for 30 minutes. Cool the solution rapidly to room temperature, and dilute with water to volume. Mix, and measure immediately the absorbance (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) of the solution at 570 nm against a blank solution prepared in a similar manner. The absorbance is not greater than that of a standard prepared at the same time and in the same manner using 5.0 mL of a 35 mg per L solution of tyramine in a 3.4 g per L solution of phosphoric acid. If intended for use in the manufacture of parenteral dosage forms, the absorbance is not greater than that of a standard prepared at the same time and in the same manner using 5.0 mL of a 15 mg per L solution of tyramine in a 3.4 g per L solution of phosphoric acid.

Content of tylosins—
Mobile phase—Prepare a mixture of filtered 200 g per L of sodium perchlorate, previously adjusted with 1 N hydrochloric acid to a pH of 2.5 ± 0.1, and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP Tylosin RS in a mixture of acetonitrile and water (1:1) to obtain a solution having a known concentration of about 0.2 mg per mL. [NOTE—Prepare the *Standard solution* immediately before use.]
Test solution—Dissolve an accurately weighed quantity of Tylosin in a mixture of acetonitrile and water (1:1) to obtain a solution having a known concentration of about 0.2 mg per mL. [NOTE—Prepare the *Test solution* immediately before use.]
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 290-nm detector and a 4.6-mm × 20-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute and the column temperature is maintained at 35°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the order of elution is tylosin C, tylosin B, tylosin D, and tylosin A with relative retention times of about 0.5, 0.6, 0.8, and 1.0 minutes, respectively; the resolution of the peaks representing tylosin D and tylosin A is not less than 2.0; the tailing factors are not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms over a period of time equivalent to 1.5 times the elution time of the main tylosin A peak, and measure the peak areas for all the peaks. Calculate the percentages of tylosin A, tylosin B, tylosin C, and tylosin D in the Tylosin taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the area of the tylosin A peak, the tylosin B peak, the tylosin C peak, or the tylosin D peak, as appropriate, in the chromatogram obtained from the *Test solution;* and r_s is the sum of the areas of all the peaks in the chromatogram obtained from the *Test solution:* the content of tylosin A is not less than 80%; and the sum of the contents of tylosin A, tylosin B, tylosin C, and tylosin D is not less than 95%.
Assay—Proceed as directed for Tylosin under *Antibiotics—Microbial Assays* ⟨81⟩. Prepare the *Test Dilution* as follows. Transfer an accurately weighed quantity of Tylosin Tartrate, equivalent to about 250 mg of tylosin, to a 500-mL volumetric flask, add 50 mL of methanol, and swirl to dissolve. Dilute with *Buffer B.3* to volume, and mix. Transfer 4.0 mL of this solution to a second 500-mL volumetric flask, dilute with a mixture of *Buffer B.3* and methanol (1:1), and mix. This solution contains about 4 µg of tylosin per mL.

Tyloxapol



Phenol, 4-(1,1,3,3-tetramethylbutyl)-, polymer with formaldehyde and oxirane;
p-(1,1,3,3-Tetramethylbutyl)phenol polymer with ethylene oxide and formaldehyde [25301-02-4].

DEFINITION
Tyloxapol is a nonionic liquid polymer of the alkyl aryl polyether alcohol type. [NOTE—Precautions should be exercised to prevent contact of Tyloxapol with metals.]

USP Monographs

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F** ▲ (CN 1-May-2020)
Analysis: On the undried specimen
Acceptance criteria: Meets the requirements

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 1.0%

• **LIMIT OF ANIONIC DETERGENTS**
Standard solution: Transfer 50 mL of water and 1 mL of a solution of sodium lauryl sulfate containing 150 µg/mL to a 125-mL separator.
Sample solution: Mix 20 mL of a solution containing 10 mg/mL of Tyloxapol with 30 mL of water in a 125-mL separator.
Analysis: To both separators add 2 drops of 3 N hydrochloric acid, 1 drop of methylene blue solution (1 in 25), and 25 mL of chloroform. Shake both separators gently for 2 min, allow to stand for 10 min, and transfer the chloroform layers to individual separators. Wash the chloroform extracts with separate 25-mL portions of water, transfer the chloroform solutions to matched 50-mL color-comparison tubes, and view downward over a white surface.
Acceptance criteria: The chloroform solution from the *Sample solution* is not darker than that from the *Standard solution*, corresponding to NMT 0.075% of anionic detergents (as sodium lauryl sulfate).

• **LIMIT OF ETHYLENE OXIDE**
Standard solution
[**CAUTION**—Ethylene oxide is toxic and flammable. Prepare in a well-ventilated hood, using great care.]
Transfer 25 mL of dimethylformamide to a 50-mL volumetric flask and weigh. Add 0.5 mL of ethylene oxide. Reweigh to obtain the weight of ethylene oxide by difference. Dilute with dimethylformamide to volume. Dilute a portion of this solution with dimethylformamide to obtain a solution having a concentration of 10 µg/g of ethylene oxide.
Sample solution: Transfer 1 g of Tyloxapol into a glass-stoppered, 5-mL graduated cylinder. Dilute with dimethylformamide to 2.0 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 1.8-m × 2-mm glass column containing 5% phase G16 on support S12
Temperatures
Injection port: 200°
Detector: 250°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold at Final Temperature (min)
50	0	50	3
50	25	200	5

Carrier gas: Helium
Flow rate: 25 mL/min
Injection volume: 3 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10%
Analysis
Samples: *Standard solution and Sample solution*
Calculate the quantity of ethylene oxide in the portion of Tyloxapol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U)$$

r_U = peak response of ethylene oxide from the *Sample solution*
r_S = peak response of ethylene oxide from the *Standard solution*
C_S = concentration of ethylene oxide in the *Standard solution* (µg/mL)
C_U = concentration of Tyloxapol taken in the *Sample solution* (g/mL)

Acceptance criteria: NMT 10 µg/g

• **LIMIT OF FORMALDEHYDE**
Diluent: Isopropyl alcohol and water (4 in 10)
Standard solution: Transfer 750 µL of a solution containing 27 µg/mL of formaldehyde to a 25-mL volumetric flask containing 5 mL of *Diluent*.
Sample solution: Prepare a solution containing 200 mg/mL of Tyloxapol in *Diluent*. Transfer 500 µL of this solution to a 25-mL volumetric flask containing 5 mL of *Diluent*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Analytical wavelength: 520 nm
Cell: 1 cm
Analysis
Samples: *Standard solution, Sample solution,* and blank
To the *Standard solution*, the *Sample solution*, and a blank (prepared by placing 5 mL of *Diluent* in a 25-mL volumetric flask) add 500 µL of phenylhydrazine hydrochloride solution (7.5 in 100). Mix, and allow to stand for 10 ± 1 min. Add 300 µL of potassium ferricyanide solution (50 mg/mL) to each flask, mix, and allow to stand for 5 min ± 30 s. Then add 2.0 mL of 2.5 N sodium hydroxide to each. Mix, and allow to stand for 4 ± 1 min. Dilute each flask with *Diluent* to volume, mix, and read the absorbances after 10 ± 3 min.
Acceptance criteria: NMT 0.0075%; the absorbance of the *Sample solution* does not exceed that of the *Standard solution*.

• **FREE PHENOL**
Sample solution: 10 mg/mL
Analysis: To 10 mL of *Sample solution* add 1 mL of bromine TS, and mix.
Acceptance criteria: No cloudiness or precipitation is observed immediately.

• **ABSENCE OF CATIONIC DETERGENTS**
Sample solution: 10 mg/mL
Analysis: Place 10 mL of the *Sample solution* in a glass-stoppered, 50-mL graduated cylinder, and make distinctly alkaline to litmus with sodium carbonate TS (about 1 mL). Add 4 mL of aqueous bromophenol blue solution (0.4 mg/mL), mix, and add 10 mL of a 1-in-10 solvent mixture of ethylene dichloride in toluene. Shake gently, and allow the layers to separate.
Acceptance criteria: No blue color is observed in the organic solvent layer.

SPECIFIC TESTS

• **CLOUD POINT**
Sample solution: Transfer 1.0 g of Tyloxapol, previously mixed, to a 150-mL beaker. Add 100.0 mL of water, and mix until dissolved. Warm the solution while mixing.

USP Monographs

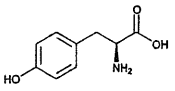
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Analysis: Transient turbidity may be observed as the solution is warmed. Determine the temperature at which the mixture becomes completely turbid.
Acceptance criteria: The cloud point is between 92° and 97°.

• **pH** ⟨791⟩
Sample solution: 50 mg/mL
Acceptance criteria: 4.0–7.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Tyloxapol RS

Tyrosine



$C_9H_{11}NO_3$ 181.19
L-Tyrosine [60-18-4].

DEFINITION
Tyrosine contains NLT 98.5% and NMT 101.5% of L-tyrosine ($C_9H_{11}NO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **Procedure**
Sample: 180 mg of Tyrosine
Blank: Mix 6 mL of formic acid and 50 mL of glacial acetic acid.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis: Dissolve the *Sample* in 6 mL of formic acid and 50 mL of glacial acetic acid, and titrate with the *Titrant*. Perform the *Blank* determination.
Calculate the percentage of tyrosine ($C_9H_{11}NO_3$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = volume of *Titrant* consumed by the *Sample* (mL)
V_B = volume of *Titrant* consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 181.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.4%
• **Chloride and Sulfate,** *Chloride* ⟨221⟩
Standard solution: 0.20 mL of 0.020 N hydrochloric acid
Sample: 0.35 g of Tyrosine. [Note—If necessary, dissolve the *Sample* by heating to near boiling and adding 1 mL of nitric acid.]
Acceptance criteria: NMT 0.04%
• **Chloride and Sulfate,** *Sulfate* ⟨221⟩
Standard solution: 0.50 mL of 0.020 N sulfuric acid
Sample: 1.2 g of Tyrosine. [Note—If necessary, dissolve the *Sample* by adding 6 mL of diluted hydrochloric acid.]
Acceptance criteria: NMT 0.04%
• **Iron** ⟨241⟩: NMT 30 ppm. [Note—If necessary, use 2 mL of hydrochloric acid to dissolve the *Sample*.]
• **Related Compounds**
Diluted ammonia solution: Dilute 16 mL of concentrated ammonia with water to 100 mL.
System suitability solution: Dissolve 10 mg of USP L-Tyrosine RS and 10 mg of USP L-Phenylalanine RS in 1 mL of *Diluted ammonia solution*, and dilute with water to 25.0 mL.
Standard solution: In a suitable flask, dissolve a quantity of USP L-Tyrosine RS in 1 mL of *Diluted ammonia solution*. Dilute with water to volume to obtain a solution of 0.05 mg/mL. [Note—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]
Sample solution: Transfer 0.1 g of Tyrosine into a 10-mL volumetric flask, add sufficient *Diluted ammonia solution* to dissolve, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Isopropyl alcohol and ammonium hydroxide (7:3)
Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
System suitability
Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.
Analysis
Samples: *System suitability solution*, *Standard solution*, and *Sample solution*
Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
Any individual impurity: NMT 0.5%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Sample solution: 50 mg/mL in 1 N hydrochloric acid
Acceptance criteria: −9.8° to −11.2°
• **Loss on Drying** ⟨731⟩: Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP L-Phenylalanine RS
USP L-Tyrosine RS

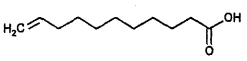
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Tyrothricin

» Tyrothricin is an antibacterial substance produced by the growth of *Bacillus brevis* Dubos (Fam. *Bacteriaceae*). It consists principally of gramicidin and tyrocidine, the tyrocidine usually being present as the hydrochloride. It contains not less than 900 µg and not more than 1400 µg of tyrothricin per mg.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Gramicidin RS
Identification—Add about 5 mg to 5 mL of *p*-dimethylaminobenzaldehyde TS, and shake for 2 minutes. Add 2 drops of sodium nitrite solution (1 in 150) and 5 mL of water, and mix: a blue color is produced.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.
Assay—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* ⟨81⟩, using a suitable, accurately weighed portion of Tyrothricin dissolved quantitatively in alcohol to yield a stock solution of convenient concentration. Dilute an accurately measured volume of this solution quantitatively and stepwise with alcohol to obtain a *Test Dilution* having a concentration of tyrothricin assumed to be 5.0 times the median dose level of the gramicidin Standard. Multiply the result of the gramicidin assay by 5.0 to obtain the amount of tyrothricin, in µg, in each mL of the *Test Dilution*.

Undecylenic Acid



$C_{11}H_{20}O_2$ 184.28
10-Undecenoic acid.
10-Undecenoic acid [112-38-9].

» Undecylenic Acid contains not less than 97.0 percent and not more than 100.5 percent of $C_{11}H_{20}O_2$.

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—
A: To 1 mL add potassium permanganate TS, dropwise: the permanganate color is discharged.
B: Place 3 mL of it and 3 mL of freshly distilled aniline in a tall test tube, and heat for 10 minutes at a rate such that the ring of condensate remains just below the mouth of the tube. Cool, add 10 mL of alcohol and 10 mL of ether, and transfer to a separator. Wash the ether solution with four 20-mL portions of water, and discard the water washings. Heat on a steam bath until the odor of ether no longer is perceptible, then add a few mg of activated carbon, mix, and filter. Evaporate the filtrate nearly to dryness, and recrystallize the residue from 70 percent alcohol: the anilide so obtained melts between 66° and 67.5°.

Specific gravity ⟨841⟩: between 0.910 and 0.913.
Congealing range ⟨651⟩: not lower than 21°.
Refractive index ⟨831⟩: between 1.447 and 1.448.
Residue on ignition ⟨281⟩: not more than 0.15%.
Water-soluble acids—Shake 5 mL with 5 mL of water, and filter the water layer through a filter paper previously moistened with water. Add 1 drop of methyl orange TS, and titrate with 0.01 N sodium hydroxide VS: not more than 1.0 mL of 0.010 N sodium hydroxide is required to match the color produced by 1 drop of methyl orange TS in 5 mL of water.
Iodine value ⟨401⟩: between 131 and 138.
Assay—Dissolve about 750 mg of Undecylenic Acid, accurately weighed, in 50 mL of alcohol, add 3 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to the first pink color that persists for not less than 30 seconds. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 18.43 mg of $C_{11}H_{20}O_2$.

Compound Undecylenic Acid Ointment

» Compound Undecylenic Acid Ointment contains undecylenic acid, calcium undecylenate, copper undecylenate, or zinc undecylenate, individually or in any combination, in a suitable ointment base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of total undecylenic acid ($C_{11}H_{20}O_2$).

Packaging and storage—Preserve in tight containers, and avoid prolonged exposure to temperatures exceeding 30°.
USP Reference standards ⟨11⟩—
USP Undecylenic Acid RS

Assay for zinc undecylenate—
Standard preparations—Prepare a solution of freshly ignited zinc oxide in dilute hydrochloric acid (1 in 60) to obtain the equivalent of 1.0 mg of zinc per mL. Dilute quantitatively with water to obtain separate solutions containing the equivalent of 15 and 30 µg of zinc per mL.
Assay preparation—Transfer about 1.0 g of Ointment, accurately weighed, to a 100-mL beaker. Add 25 mL of dilute hydrochloric acid (1 in 20), swirl, and heat carefully until the mixture is liquefied. Cool, and transfer the mixture to a 250-mL separator. Complete the transfer of the waxy residue by thoroughly rinsing the beaker with 50 mL of water and two 50-mL portions of chloroform and adding the rinsings to the separator. Equilibrate the mixture, and transfer the chloroform extract to a 500-mL separator. Extract the aqueous phase with another 100-mL portion of chloroform, combine the second chloroform extract with the main extract in the 500-mL separator, and transfer the aqueous phase to a 200-mL volumetric flask. Wash the combined chloroform extracts with three 25-mL portions of water, add the aqueous washings to the 200-mL volumetric flask, dilute with water to volume, and mix to obtain a specimen stock solution. [NOTE—Retain the chloroform extract for the *Assay for undecylenic acid*.] Transfer 15.0 mL or other suitable volume (see *Procedure*) of this specimen stock solution to a 100-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Aspirate each *Standard preparation* and the *Assay preparation* into the flame of a suitable atomic absorption spectrophotometer, and determine the absorbances of the solutions at 214 nm. Typically, an

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acetylene–air mixture is adjusted to obtain a blue flame about 7 mm in height with a suitable burner that is rotated to a position perpendicular to the light path. [NOTE—If the absorbance of the *Assay preparation* is outside the central 70% of the range between the absorbances of the *Standard preparations*, discard the *Assay preparation* and prepare another by diluting the specimen stock solution quantitatively as necessary to obtain a suitable absorbance.] Calculate the percentage of zinc undecylenate in the Ointment taken by the formula:

$$(M_r/A_r)(0.2/W)C_{S2}+\frac{(C_{S1}-C_{S2})(A_U-A_{S2})}{(A_{S1}-A_{S2})}$$

in which M_r is the molecular weight of zinc undecylenate, 431.94; A_r is the atomic weight of zinc, 65.39; W is the weight, in g, of Ointment taken; A_U, A_{S1}, and A_{S2} are the absorbances of the *Assay preparation* and the high- and low-concentration *Standard preparations*, respectively; and C_{S1} and C_{S2} are the concentrations, in µg per mL, of the high- and low-concentration *Standard preparations*, respectively.

Assay for undecylenic acid—

Internal standard solution—Prepare a solution in chloroform containing 10 mg of tridecanoic acid in each mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Undecylenic Acid RS in chloroform to obtain a solution having a known concentration of about 3.8 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 3.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix.

Assay preparation—Pass the chloroform extract prepared from the Ointment as directed in *Assay for zinc undecylenate* through phase-separating filter paper into a 250-mL volumetric flask. Rinse the separator with three 15-mL portions of chloroform, passing the rinsings through the filter and combining them with the main chloroform solution, add chloroform to volume, and mix. Transfer 20.0 mL of this solution to a 50-mL volumetric flask, add 3.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix.

Chromatographic system—Under typical conditions, the gas chromatograph is equipped with a flame-ionization detector and contains a 2-mm × 1.8-m glass column packed with 3% liquid phase G1 on 100- to 200-mesh support S1A. The column is maintained at a temperature of about 165°. Dry helium is used as the carrier gas at a flow rate of about 30 mL per minute.

System suitability—Chromatograph five injections of the silylated *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R (see *Chromatography* ⟨621⟩), is not less than 3.0.

Procedure—Transfer 1.0-mL portions of the *Standard preparation* and the *Assay preparation* to separate, stoppered test tubes. To each tube add 50 µL of bis(trimethylsilyl)tri fluoroacetamide, insert the stopper, mix, and allow to stand for 30 minutes. Inject a suitable portion (2 to 5 µL) of the *Standard preparation* into a suitable gas chromatograph, and record the chromatogram so as to obtain not less than 50% of maximum recorder response. Similarly inject a suitable portion of the *Assay preparation*, and record the chromatogram. Measure the peak responses for the first (undecylenic acid) and second (tridecanoic acid) peaks of the chromatograms. [NOTE—Relative retention times are, approximately, 0.43 for undecylenic acid and 1.0 for tridecanoic acid.] Calculate the percentage of total undecylenic acid in the Ointment taken by the formula:

$$62.5(C / W)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Undecylenic Acid RS in the *Standard preparation*; W is the weight, in g, of Ointment taken; and R_U and R_S are the ratios of the peak responses of undecylenic acid to those of tridecanoic acid from the *Assay preparation* and the *Standard preparation*, respectively. The difference between the total undecylenic acid and the undecylenic acid equivalent to the determined zinc undecylenate (the weight of zinc undecylenate multiplied by 0.8533 gives the equivalent of undecylenic acid), both expressed as a percentage of the Ointment, gives the percentage of free undecylenic acid in the Ointment, gives the percentage of free undecylenic acid in the Ointment.

Urea



CH_4N_2O 60.06

Urea;
Carbamide [57-13-6].

DEFINITION

Urea contains NLT 98.0% and NMT 102.0% of urea (CH_4N_2O).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Transfer 1 mL of formic acid to a 1-L volumetric flask and dilute with water to volume.
Solution B: 100% Acetonitrile, passed through a membrane filter of 0.2-µm pore size
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	2.5	97.5
7.0	10.0	90.0
7.01	2.5	97.5
15.0	2.5	97.5

Diluent: Acetonitrile and water (90:10)
System suitability solution: 0.01 mg/mL of USP Urea Related Compound A RS and 10 mg/mL of USP Urea RS in *Diluent*
Standard solution: 5 mg/mL of USP Urea RS in *Diluent*
Sample solution: 5 mg/mL of Urea in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 nm
Column: 15-cm × 4.6-mm; 2.7-µm packing L86
Column temperature: 30°
Flow rate: 1 mL/min

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Injection volume: 2 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for urea related compound A and urea are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between urea related compound A and urea
Relative standard deviation: NMT 1.0% for urea
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of urea (CH_4N_2O) in the portion of Urea taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Urea RS in the *Standard solution* (mg/mL)
C_U = concentration of Urea in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.01 mg/mL of USP Urea Related Compound A RS and 10 mg/mL of USP Urea RS in *Diluent*
Standard solution: 0.01 mg/mL of USP Urea RS and 0.01 mg/mL of USP Urea Related Compound A RS in *Diluent*
Sample solution: 10 mg/mL of Urea in *Diluent*
System suitability
Sample: *System suitability solution*
[NOTE—For the relative retention times, see *Table 2*.]
Suitability requirements
Resolution: NLT 1.5 between urea and urea related compound A
Relative standard deviation: NMT 1.0% for urea
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of urea related compound A in the portion of Urea taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of urea related compound A from the *Sample solution*
r_S = peak response of urea related compound A from the *Standard solution*
C_S = concentration of USP Urea Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Urea in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Urea taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of urea from the *Standard solution*
C_S = concentration of USP Urea RS in the *Standard solution*
C_U = concentration of Urea in the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Urea related compound A	0.9	0.1
Urea	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	2.0

SPECIFIC TESTS
• **ALCOHOL-INSOLUBLE MATTER**
Sample solution: 100 mg/mL of Urea dissolved in warm alcohol
Analysis: If any insoluble residue remains, pass the *Sample solution* through a tared filter, wash the residue and the filter with 20 mL of warm alcohol per 50 mL of *Sample solution*, and dry at 105° for 1 h.
Acceptance criteria: NMT 2 mg (0.04%)
• **STERILITY TESTS** ⟨71⟩**:** Where the label states that Urea is sterile, it meets the requirements.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Urea must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.003 USP Endotoxin Units/mg of urea.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Urea RS
USP Urea Related Compound A RS
Dicarbonimidic diamide.
$C_2H_5N_3O_2$ 103.08

Urea Compounded Irrigation

DEFINITION
Urea Compounded Irrigation contains NLT 90.0% and NMT 110.0% of the labeled amount of urea (CH_4N_2O).

Prepare Urea Compounded Irrigation 200 mg/mL (20%) as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Urea	10 g
Sodium Chloride Irrigation (0.9%), a sufficient quantity to make	50 mL

Dissolve the *Urea* in *Sodium Chloride Irrigation*. Pass through a sterilizing filter of 0.22-µm pore size into a sterile single-dose plastic bottle. [NOTE—Sterilize through filtration. Urea is not heat stable.]

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ASSAY

• **PROCEDURE**

Mobile phase: Water

Standard solution: 0.5 mg/mL of USP Urea RS in water

Sample solution: Transfer 0.25 mL of Irrigation into a 100-mL volumetric flask, and add water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 192 nm

Column: 4.6-mm × 25-cm; 5-µm packing L96

Temperatures

Autosampler: 4°

Column: 25°

Flow rate: 1.0 mL/min

Injection volume: 8 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for urea is about 3.3 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of urea (CH_4N_2O) in the portion of Irrigation taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of urea from the *Sample solution*

r_S = peak response of urea from the *Standard solution*

C_S = concentration of USP Urea RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of urea in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 6.5–9.0

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** It meets the requirements.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.003 USP Endotoxin Units/mg

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in sterile, single-dose plastic bottles. Store at controlled room temperature or in a refrigerator.

Change to read:

• **BEYOND-USE DATE:** ▲In the absence of passing a sterility test and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 60 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator.▲ (CN 1-May-2020)

• **LABELING:** Label it to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Urea RS

Urea for Injection

» Urea for Injection is Urea suitable for parenteral use.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

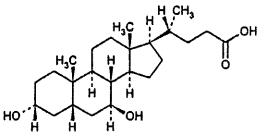
Completeness of solution ⟨641⟩—A 1.0-g portion dissolves in 10 mL of carbon dioxide-free water to yield a clear solution.

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.003 USP Endotoxin Unit per mg of urea.

Other requirements—It responds to the *Identification* tests and meets the requirements for *Melting range, Residue on ignition, Alcohol-insoluble matter, Chloride, Sulfate*, and *Assay* under *Urea*. It meets also the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩, and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Ursodiol



$C_{24}H_{40}O_4$ 392.57

Cholan-24-oic acid, 3,7-dihydroxy-, (3α,5β,7β)-.

3α,7β-Dihydroxy-5β-cholan-24-oic acid [128-13-2].

» Ursodiol contains not less than 98.5 percent and not more than 101.5 percent of $C_{24}H_{40}O_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Ursodiol RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Melting range ⟨741⟩**:** between 200° and 205°.

Specific rotation ⟨781S⟩**:** between 57° and 62°.

Test solution: 20 mg per mL, in alcohol.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Related compounds—

Adsorbent: 0.25-mm layer of chromatographic silica gel.

Solvent—Prepare a mixture of acetone and water (9:1).

Standard solution 1—Prepare a solution of chenodiol in *Solvent* containing 600 µg per mL.

Standard solution 2—Prepare a solution of lithocholic acid in *Solvent* containing 20 µg per mL.

Test solution—Prepare a solution of Ursodiol in *Solvent* containing 40 mg per mL.

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Diluted test solution—Quantitatively dilute 1 mL of the *Test solution* with *Solvent* to obtain a solution having a concentration of 40 µg per mL.

Developing solvent system: a mixture of chloroform, glacial acetic acid, and water (85: 15: 0.5)

Spray reagent: phosphomolybdic acid TS.

Procedure—Separately apply 10 µL each of *Standard solution 1, Standard solution 2,* the *Test solution,* and the *Diluted test solution* to a thin-layer chromatographic plate (see *Thin Layer Chromatography* under *Chromatography* ⟨621⟩), and proceed as directed in the chapter, allowing the solvent front to move about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and air-dry the plate. Spray the plate with phosphomolybdic acid TS, dry at 105° for 5 minutes, and examine the plate: any secondary spot in the chromatogram of the *Test solution* having the same R_F value as the principal spot from *Standard solution 1* is not greater in size or intensity than that obtained from *Standard solution 1:* not more than 1.5% of chenodiol is found. No secondary spot observed in the chromatogram of the *Test solution* having the same R_F value as the principal spot from *Standard solution 2* is greater in size or intensity than that obtained from *Standard solution 2:* not more than 0.05% of lithocholic acid is found. No other secondary spot observed in the chromatogram of the *Test solution* is greater in size or intensity than the principal spot obtained from the *Diluted test solution:* not more than 0.1% of any other impurity is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (55:45). Adjust with 0.6 M phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve an accurately weighed quantity of epiandrosterone in methanol to obtain a solution having a concentration of about 4 mg per mL. Dilute a portion of this solution quantitatively with *Mobile phase* to obtain a solution having a concentration of about 0.8 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Ursodiol RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 4 mg per mL. Transfer this solution to a suitable container, and dilute with *Mobile phase* to give a solution having a known concentration of about 0.8 mg of ursodiol per mL. Transfer equal volumes of this solution and the *Internal standard solution* to a suitable container, and mix.

Assay preparation—Transfer about 100 mg of Ursodiol, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with methanol to volume. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer equal volumes of this solution and the *Internal standard solution* to a suitable container, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a differential refractive index detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Both the detector temperature and the column temperature are maintained at 40°. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.74 for ursodiol and 1.0 for epiandrosterone; the resolution, *R,* between ursodiol and epiandrosterone is not less than 3.8 (If the resolution specification is not met, increase the water content of the *Mobile phase*); and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{40}O_4$ in the portion of Ursodiol taken by the formula:

$$250C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ursodiol RS in the *Standard preparation;* and R_U and R_S are the ratios of the ursodiol peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ursodiol Capsules

» Ursodiol Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ursodiol ($C_{24}H_{40}O_4$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Ursodiol RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay.*

Dissolution ⟨711⟩—

Medium: 0.05 M pH 8.4 phosphate buffer, prepared by mixing 250 mL of 0.2 M monobasic potassium phosphate, 280 mL of 0.2 M potassium hydroxide, and 5 mL of 2% sodium lauryl sulfate solution. Adjust with 0.2 M potassium hydroxide to a pH of 8.4, and dilute with water to 1000 mL; 1000 mL.

Apparatus 2: 75 rpm.

Time: 30 minutes.

Determine the amount of ursodiol ($C_{24}H_{40}O_4$) dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and 0.075 M monobasic potassium phosphate (50:50). Adjust with 85% phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Ursodiol RS, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration equivalent to that expected in the solution under test.

Test solution—Use a filtered portion of the solution under test.

Chromatographic system—The liquid chromatograph is equipped with a refractive index detector, a guard column that contains packing L1, and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute, and the column and detector temperatures are maintained at 40°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor of the ursodiol peak is not more than 1.7; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{24}H_{40}O_4$ dissolved by the formula:

USP Monographs

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$$100{,}000(r_U/r_S)(C/W)$$

in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively; C is the concentration, in mg per mL, of USP Ursodiol RS in the *Standard solution;* and W is the labeled amount, in mg, of ursodiol in each Capsule.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{24}H_{40}O_4$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements for *Weight Variation.*

Assay—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Ursodiol.*

Assay preparation—Accurately weigh the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of ursodiol, to a 50-mL volumetric flask. Add about 40 mL of methanol, and sonicate for about 15 minutes. Cool the mixture to room temperature, dilute with methanol to volume, and centrifuge a portion of this mixture. Transfer 5.0 mL of the clear supernatant to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Transfer equal amounts of this solution and the *Internal standard solution* to a suitable container, mix, and filter.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ursodiol ($C_{24}H_{40}O_4$) in the portion of Capsules taken by the formula:

$$200(C_S/C_U)(R_U/R_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of ursodiol in the *Standard preparation* and the *Assay preparation,* respectively; and R_U and R_S are the ratios of the ursodiol peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ursodiol Compounded Oral Suspension

DEFINITION

Ursodiol Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ursodiol ($C_{24}H_{40}O_4$).

Prepare Ursodiol Compounded Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Ursodiol tablets[a] equivalent to	5 g of ursodiol
Vehicle: a 1:1 mixture of Ora-Sweet,[b] (sugar-free) and Ora-Plus[b] a sufficient quantity to make	100 mL

[a] Urso 250-mg tablets, Axcan Pharma U.S. Inc., Birmingham, AL.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Ursodiol tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an ursodiol liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and 0.01 M dihydrogen potassium phosphate buffer (75:25). Adjust with dilute phosphoric acid to a pH of 5.25. Filter, and degas.

Standard solution: 1.25 mg/mL of USP Ursodiol RS in methanol

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 1.25 mg/mL of ursodiol from Oral Suspension and methanol, and centrifuge.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 201 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for ursodiol is about 7 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ursodiol ($C_{24}H_{40}O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ursodiol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ursodiol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 4.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or controlled room temperature

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ursodiol RS

Ursodiol Tablets

DEFINITION

Ursodiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ursodiol ($C_{24}H_{40}O_4$).

IDENTIFICATION

• **THIN-LAYER CHROMATOGRAPHY**

Standard solution: 1 mg/mL of USP Ursodiol RS in methanol

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Sample solution: Transfer a quantity of finely powdered Tablets, equivalent to about 25 mg of ursodiol, to a conical flask. Add 25.0 mL of methanol, and mix for 20 min. Centrifuge this solution for 10 min at 4000 rpm, and use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture activated for at least 4 h at 105°
Application volume: 25 µL
Developing solvent system: Chloroform, acetone, and acetic acid (7:2:1)
Spray reagent: Dissolve 2.5 g of phosphomolybdic acid in 50 mL of glacial acetic acid. Add 2.5 mL of concentrated sulfuric acid, and mix well.

Analysis: Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Allow the chromatogram to develop until the solvent front has moved about three-fourths of the length of the plate. Spray the plate lightly with *Spray reagent*. Dry the plate by heating at 105° for about 7 min.

Acceptance criteria: The principal indigo-colored spot of the *Sample solution* corresponds in color and in R_F value to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, water, and phosphoric acid (77:23:0.6)

Internal standard solution: 3.75 mg/mL of propylparaben in *Mobile phase*

Standard solution: 3.75 mg/mL of USP Ursodiol RS in *Internal standard solution*

Sample solution: Weigh and finely powder 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 37.5 mg of ursodiol, to a glass-stoppered conical flask. Add 10.0 mL of *Internal standard solution*, and shake by mechanical means for 15 min. Sonicate at 40° for an additional 15 min, and filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractive index
Detector temperature: 40°
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for propylparaben and ursodiol are 0.73 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between ursodiol and propylparaben
Column efficiency: NLT 1600 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{40}O_4$ in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of peak responses from the *Sample solution*
R_S = ratio of peak responses from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Simulated intestinal fluid TS, prepared without pancreatin and adjusted with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid to a pH of 8.0; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Mobile phase: Methanol, water, and phosphoric acid (77:23:0.6)
Sample solution: Pass a portion of the solution under test through a suitable filter.
Standard solution: USP Ursodiol RS in *Medium*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractive index
Detector temperature: 40°
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1600 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{40}O_4$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Standard solution A: 20 µg/mL of USP Ursodiol RS in methanol
Standard solution B: 10 µg/mL of lithocholic acid in methanol
Standard solution C: 300 µg/mL of chenodeoxycholic acid in methanol
Sample solution: Transfer a quantity of finely powdered Tablets, equivalent to about 250 mg of ursodiol, to a conical flask. Add 25.0 mL of methanol, and mix for 20 min. Centrifuge this solution for 10 min at 4000 rpm, and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, activated for at least 4 h at 105°
Application volume: 25 µL of *Standard solutions A*, *B*, and *C*; 50 µL of the *Sample solution*
Developing solvent system: Chloroform, acetone, and acetic acid (7:2:1)
Spray reagent: Dissolve 2.5 g of phosphomolybdic acid in 50 mL of glacial acetic acid. Add 2.5 mL of concentrated sulfuric acid, and mix well.

Analysis: Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Spray the plate lightly with *Spray reagent*. Dry the plate by heating at 105° for about 7 min.

Acceptance criteria: The spot due to lithocholic acid from the *Sample solution*, if present, is not greater in size and intensity

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than that from *Standard solution B* (0.05%). The spot due to chenodeoxycholic acid from the *Sample solution*, if present, is not greater in size and intensity than that from *Standard solution C* (1.5%). No other unidentified spot in the *Sample solution* is greater in size and intensity than the spot from *Standard solution A* (0.1%).

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at a temperature between 20° and 25°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ursodiol RS

Vaccinia Immune Globulin

» Vaccinia Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic solution of globulins derived from the blood plasma of adult human donors who have been immunized with vaccinia virus (Smallpox Vaccine). It is standardized for viral neutralizing activity in eggs or tissue culture with the U.S. Reference Vaccinia Immune Globulin and a specified vaccinia virus. It contains not less than 15 g and not more than 18 g of protein per 100 mL, not less than 90.0 percent of which is gamma globulin. It contains 0.3 M glycine as a stabilizing agent, and contains a suitable antimicrobial agent.

Packaging and storage—Preserve at a temperature between 2° and 8°.
Expiration date—The expiration date is not later than 3 years after date of issue.
Labeling—Label it to state that it is not intended for intravenous injection.

Valacyclovir Compounded Oral Suspension

DEFINITION
Valacyclovir Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$).
Prepare Valacyclovir Compounded Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Valacyclovir tablets[a] equivalent to	5 g of valacyclovir
Vehicle: a 1:1 mixture of Ora-Sweet[b] (regular or sugar-free) and Ora-Plus[b], a sufficient quantity to make	100 mL

[a] Valtrex 500-mg tablets, Glaxo Wellcome Inc., Research Triangle Park, NC.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Valacyclovir tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a valacyclovir liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and 5.0 mM sodium acetate (1:99). Adjust with 6 N hydrochloric acid to a pH of 3.0. Filter and degas.
Diluent: 5.0 mM sodium acetate, adjusted with 6 N hydrochloric acid to a pH of 3.0
Standard solution: Dissolve an appropriately weighed amount of USP Valacyclovir Hydrochloride RS in *Diluent* to be equivalent to 0.2 mg/mL of valacyclovir.
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.2 mg/mL of valacyclovir from Oral Suspension and *Diluent*. Pass through a nylon filter of 0.22-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 3.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for valacyclovir hydrochloride is about 14 min.]
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.2–4.3

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant glass containers. Store in a refrigerator.
- **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored in a refrigerator
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Valacyclovir Hydrochloride RS

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Valacyclovir Tablets

DEFINITION

Valacyclovir Tablets contain an amount of Valacyclovir Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meet the requirements

ASSAY

- **PROCEDURE**

Diluent: 0.1% (v/v) phosphoric acid in water
Mobile phase: Methanol and *Diluent* (5:95)
Standard solution: 0.1 mg/mL of USP Valacyclovir Hydrochloride RS in *Diluent.* [NOTE—USP Valacyclovir Hydrochloride RS contains a detectable quantity of D-valacyclovir.]
Sample solution: Transfer NLT 5 Tablets into a suitable volumetric flask, and add 0.1 M hydrochloric acid (approximately 80% of the volume of the flask). Mechanically shake the sample until the Tablets disintegrate into a fine suspension (60 min), and sonicate for 10 min. Cool to ambient temperature, dilute with 0.1 M hydrochloric acid to volume, and mix to obtain a solution having a concentration of 2.5 mg/mL. Dilute a portion of the sample with *Diluent* to obtain a nominal concentration of 0.1 mg/mL of valacyclovir, and mix. Pass a portion of this solution through a membrane filter of 0.45-µm or finer pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 15-cm; 5-µm packing L66
Column temperature: 10°
Flow rate: 0.75 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.3 between the D-valacyclovir and valacyclovir peaks
Tailing factor: NMT 2.0 for the valacyclovir peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Diluent: Prepare as directed in the *Assay.*
Mobile phase: Acetonitrile and *Diluent* (5:95)
Standard solution: Prepare a solution in *Diluent* containing USP Valacyclovir Hydrochloride RS equivalent to 0.044 mg/mL of valacyclovir free base.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Diluent* to obtain a final concentration of about 0.044 mg/mL of valacyclovir free base considering complete dissolution of the Tablet label claim.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 5-cm, 5-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times D \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium,* 900 mL
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*

Tolerances: NLT 75% (*Q*) of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Analytical wavelength: 252 nm
Cell: 0.02 cm
Blank: *Medium*
Standard solution
For Tablets labeled to contain 500 mg: 0.6 mg/mL of USP Valacyclovir Hydrochloride RS in *Medium.* A small volume of methanol, not exceeding 5% of the final volume, may be used to help solubilize valacyclovir.
For Tablets labeled to contain 1000 mg: 1.2 mg/mL of USP Valacyclovir Hydrochloride RS in *Medium.* A small volume of methanol, not exceeding 5% of the final volume, may be used to help solubilize valacyclovir.

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Sample solution: Pass a portion of the solution under test through a filter of 0.45-µm pore size. Discard the first 3 mL of sample filtrate.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times D \times 100$$

r_U = absorbance of the *Sample solution*
r_S = absorbance of the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

[NOTE—All of the concentrations are expressed as valacyclovir free base.]

Diluent: Prepare as directed in the *Assay*.

Mobile phase: Acetonitrile and *Diluent* (5:95)

Standard solution: Prepare a solution of USP Valacyclovir Hydrochloride RS, equivalent to 0.04 mg/mL of valacyclovir, in *Diluent*.

Sample solution: Transfer 1 Tablet into a suitable volumetric flask. Add *Diluent* (approximately 60% of the volume of the flask), and mechanically shake the samples until the Tablet disintegrates into a fine suspension, and sonicate for 10 min. Cool, dilute with *Diluent* to volume, and mix. Dilute a portion of each sample with *Diluent* to obtain a nominal concentration of 0.04 mg/mL of valacyclovir. Pass a portion of each sample through a membrane filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system and **System suitability:** Proceed as directed in *Dissolution*, *Test 1*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of D-valacyclovir and acyclovir in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of D-valacyclovir or acyclovir from the *Sample solution*
r_S = peak response of USP Valacyclovir Hydrochloride RS from the *Standard solution*
C_S = concentration of valacyclovir hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80
F = relative response factor (see *Table 1*)

Acceptance criteria

Individual impurities: See *Table 1*.

Table 1

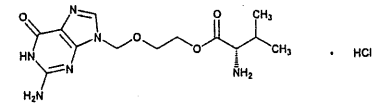
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
D-Valacyclovir[a]	0.82	1.0	—
Acyclovir[b]	0.56	1.4	2.5

[a] D-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy] ethyl ester, monohydrochloride. [NOTE—This is a process impurity.]
[b] 2-Amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one (acyclovir).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Valacyclovir Hydrochloride RS

Valacyclovir Hydrochloride



$C_{13}H_{20}N_6O_4 \cdot HCl$ 360.80

L-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl) methoxy] ethyl ester, monohydrochloride;
L-Valine, ester with 9-[(2-hydroxyethoxy)methyl]guanine, monohydrochloride [124832-27-5].

DEFINITION

Valacyclovir Hydrochloride contains NLT 95.0% and NMT 102.0% of $C_{13}H_{20}N_6O_4 \cdot HCl$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

USP Monographs

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• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: 50 mg/mL in water
Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**
Mobile phase: Methanol, water, and perchloric acid (1:19:0.1)
Standard solution: 0.5 mg/mL of USP Valacyclovir Hydrochloride RS in 0.05 M hydrochloric acid. [NOTE—USP Valacyclovir Hydrochloride RS contains a detectable quantity of D-valacyclovir.]
Sample solution: 0.5 mg/mL of Valacyclovir Hydrochloride in 0.05 M hydrochloric acid
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 15-cm; 5-µm packing L66
Column temperature: 10°
Flow rate: 0.75 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between valacyclovir hydrochloride and D-valacyclovir
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of valacyclovir hydrochloride ($C_{13}H_{20}N_6O_4 \cdot HCl$) in the portion of Valacyclovir Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valacyclovir from the *Sample solution*
r_S = peak response of valacyclovir from the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Valacyclovir Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1% on a 2-g sample

• **LIMIT OF PALLADIUM** (if present)
(See *Plasma Spectrochemistry* ⟨730⟩.)
Diluent: Water and nitric acid (99.8:0.2)
Blank solution: *Diluent*
Standard solutions: Dilute with *Diluent* any commercially available standard stock solution of 1 mg/mL of palladium to prepare the following solutions: 0.03 µg/mL, 0.19 µg/mL, 0.30 µg/mL, 0.38 µg/mL, 0.75 µg/mL, and 1.13 µg/mL of palladium.
Sample solution: 30 mg/mL of Valacyclovir Hydrochloride in *Diluent*
Analytical wavelength: 340.458 nm
Spectrophotometric system: Use a suitable standard inductively coupled plasma–optical emission spectrophotometric system, and construct a calibration curve.
System suitability
Samples: *Blank solution* and *Standard solutions*
Suitability requirements
Relative standard deviation: NMT 10.0%, *Standard solutions*
Correlation coefficient: NLT 0.995, *Blank solution* and *Standard solutions*
Analysis
Samples: *Blank solution* and *Sample solution*
Calculate the concentration of palladium using the calibration curve corrected for the emission response of the *Blank solution* and sample weight. Calculate the amount of palladium in the Valacyclovir Hydrochloride taken to prepare the *Sample solution*.
Acceptance criteria: NMT 10 ppm

• **ORGANIC IMPURITIES, PROCEDURE 1** (for related compounds E, F, and G)
Developing solvent: Methylene chloride, methanol, tetrahydrofuran, and ammonia solution (54:34:12:3)
Standard stock solution: Transfer 5 mg each of USP Valacyclovir Related Compound D RS and USP Valacyclovir Related Compound G RS, 10 mg of USP Valacyclovir Related Compound E RS, and 8.4 mg of USP Valacyclovir Related Compound F RS into a 10-mL volumetric flask. Add 2 mL of water with swirling, followed by 6 mL of alcohol, and sonicate for 20 min. Allow to cool, and dilute with alcohol to volume.
Standard solutions: Transfer 1.0 and 0.5 mL of the *Standard stock solution* into two separate 10-mL volumetric flasks. Dilute the solution in both flasks with alcohol to volume.
Sample solution: Transfer 250 mg of Valacyclovir Hydrochloride into a 5-mL volumetric flask. Add 2 mL of water, and sonicate for 20 min to dissolve. Add alcohol to about 95% volume of the flask. Cool, and dilute with alcohol to volume. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: TLC
Detector: UV, long and short wavelength
Plate: TLC plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Prewash the plate with methanol.
Developing distance: NLT 7 cm from the origin
Application size: 4 µL
Analysis
Samples: *Standard solutions* and *Sample solution*
Develop the plate to the specified distance. Remove the plate from the solvent chamber, and allow to dry. Examine the plate under short-wavelength UV light, and visually estimate the valacyclovir related compounds E and G in the sample using the appropriate standard spots. The chromatograms obtained with the *Standard solutions* each show three clearly separated spots due to valacyclovir related compounds D, E, and G. Spray the plate with 0.01% fluorescamine in ethylene dichloride, and examine the sprayed plate under long-wavelength UV light to estimate the level of valacyclovir related compound F in the sample using the appropriate standard spot. The relative R_F values and limits for each impurity are provided in *Table 1*.
Acceptance criteria: See *Table 1*.

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Table 1

Name	Relative R_F Value	Acceptance Criteria, NMT (%)
Valacyclovir hydrochloride	1	—
Valacyclovir related compound D[a]	1.1	—
Valacyclovir related compound E[b]	1.3	0.2
Valacyclovir related compound F[c]	1.8	0.1
Valacyclovir related compound G[d]	1.9	0.05

[a] This impurity is quantitated using *Procedure 2*.
[b] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-[(benzyloxy) carbonyl]-L-valinate.
[c] 2-Hydroxyethyl-L-valinate.
[d] *N*,*N*-Dimethylpyridin-4-amine.

• **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: 0.3% w/w trifluoroacetic acid solution in water
Solution B: 0.3% w/w trifluoroacetic acid solution in methanol
Diluent: Alcohol and water (1:4)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
35	60	40
35.01	90	10
45	90	10

System suitability solution: 0.4 mg/mL of USP Valacyclovir Hydrochloride RS, 0.8 µg/mL of USP Valacyclovir Related Compound C RS, and 1.6 µg/mL of USP Acyclovir Related Compound A RS in *Diluent*
Sample solution: 0.4 mg/mL of Valacyclovir Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 15°
Flow rate: 0.8 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
Resolution: NLT 1.5 between valacyclovir and valacyclovir related compound C, and NLT 1.5 between valacyclovir related compound C and acyclovir related compound A
Tailing factor: NMT 1.5 for the valacyclovir hydrochloride peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Valacyclovir Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any impurity in the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Guanine (near solvent front)[a, b]	0.31	—
Acyclovir[a, c]	0.42	—
Acyclovir alaninate[d]	0.54	0.2
Valacyclovir	1.00	—
Valacyclovir related compound C[e]	1.06	0.3
Acyclovir related compound A[a, f]	1.09	—
Valacyclovir related compound D[g]	1.17	0.5
Acyclovir isoleucinate[h]	1.30	0.2
N-Formyl valacyclovir[i]	1.61	0.8
Guaninyl valacyclovir[j]	1.66	0.2
Bis valacyclovir[k]	2.0	0.3
Any unspecified impurity	—	0.1

[a] This impurity is quantitated by the *Procedure 3* method.
[b] 2-Amino-1*H*-purin-6(9*H*)-one (guanine).
[c] 9-[(2-Hydroxyethoxy)methyl]guanine (acyclovir).
[d] 9-[(2-Hydroxyethoxy)methyl]guanine L-alaninate.
[e] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-methyl-L-valinate.
[f] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate.
[g] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-ethyl-L-valinate.
[h] 9-[(2-Hydroxyethoxy)methyl]guanine L-isoleucinate.
[i] 9-[(2-Hydroxyethoxy)methyl]guanine *N*-formyl-L-valinate.
[j] [*N*²-(Guanine-*N*²-yl)methyl]-9-[(2-hydroxyethoxy)methyl]guanine L-valinate.
[k] 2,2′-[Methylenebis[imino(6-oxo-1,6-dihydro-9*H*-purine-9,2-diyl)methyleneoxy]]diethyl di(L-valinate).

• **ORGANIC IMPURITIES, PROCEDURE 3**

Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Valacyclovir Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of guanine plus acyclovir or acyclovir acetate or D-valacyclovir from the *Sample solution*
r_S = peak response of valacyclovir from the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Valacyclovir Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guanine and acyclovir[a, b]	0.18	1.51	2.0

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Table 4 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Acyclovir related compound A[c]	0.42	1.12	0.2
D-Valacyclovir[d]	0.55	1.0	3.0
Valacyclovir	1.0	—	—

[a] 2-Amino-1*H*-purin-6(9*H*)-one (guanine).
[b] 9-[(2-Hydroxyethoxy)methyl]guanine (acyclovir).
[c] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate.
[d] D-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy] ethyl ester, monohydrochloride.

Total organic impurities: NMT 5.0% for the sum of all impurities from *Organic Impurities, Procedures 1*, *2*, and *3*

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** For the anhydrous form: NMT 2.0% (200 mg of sample); if labeled as the hydrous form: 5.0%–11.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature below 30°.
- **LABELING:** Where it is the hydrous form, the label so indicates.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Acyclovir Related Compound A RS
[NOTE—USP Acyclovir Related Compound A AS is equivalent.]
2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl acetate.
$C_{10}H_{13}N_5O_4$ 267.24

USP Valacyclovir Hydrochloride RS

USP Valacyclovir Related Compound C RS
2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-methyl-L-valinate hydrochloride.
$C_{14}H_{22}N_6O_4 \cdot HCl$ 374.82

USP Valacyclovir Related Compound D RS
2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9yl)methoxy] ethyl *N*-ethyl-L-valinate.
$C_{15}H_{24}N_6O_4$ 352.39

USP Valacyclovir Related Compound E RS
2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-benzyloxy)carbonyl]-L-valinate.
$C_{21}H_{26}N_6O_6$ 458.47

USP Valacyclovir Related Compound F RS
2-Hydroxyethyl valinate para-toluenesulfonate salt.
$C_7H_{15}NO_3 \cdot C_7H_8O_3S$ 333.40

USP Valacyclovir Related Compound G RS
N,*N*-Dimethylpyridin-4-amine.
$C_7H_{10}N_2$ 122.17

Valganciclovir Tablets

DEFINITION

Valganciclovir Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of valganciclovir ($C_{14}H_{22}N_6O_5$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 10 µg/mL in 0.001 M hydrochloric acid
Wavelength range: 200–350 nm
Medium: 0.001 M hydrochloric acid
Acceptance criteria: Meet the requirements
- **B.** The retention time of the diastereomeric peaks of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dilute 2.5 mL of triethylamine with water to 1000 mL, and adjust with trifluoroacetic acid to a pH of 3.0 ± 0.05.
Mobile phase: Methanol and *Buffer* (7:93)
Diluent: 1 mM hydrochloric acid
System suitability solution: 0.1 µg/mL of USP Ganciclovir Mono-N-methyl Valinate RS and 78 µg/mL of USP Valganciclovir Hydrochloride RS in *Diluent*
Standard solution: 0.09 mg/mL of USP Valganciclovir Hydrochloride RS in *Diluent*. Pass a portion of the solution through a filter of 0.45-µm or finer pore size, and use the filtrate, discarding the initial 2 mL.
Sample stock solution: Nominally 5 mg/mL of valganciclovir hydrochloride prepared as follows. Transfer 5 Tablets into a 500-mL volumetric flask, add about 300 mL of *Diluent*, and shake well until the Tablets are fully disintegrated. Dilute with *Diluent* to volume, mix, and allow the solution to settle.
Sample solution: Nominally 0.075 mg/mL of valganciclovir hydrochloride prepared as follows. Transfer 3.0 mL of the supernatant from the *Sample stock solution* into a 200-mL volumetric flask, and dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.45-µm or finer pore size, and use the filtrate, discarding the initial 2 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; packing L11
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the second diastereomeric valganciclovir peak and the first ganciclovir mono-*N*-methyl valinate peak, *System suitability solution*
Column efficiency: NLT 3000 theoretical plates for the second diastereomeric valganciclovir peak, *Standard solution*
Tailing factor: NMT 3 for the second diastereomeric valganciclovir peak, *Standard solution*
Relative standard deviation: NMT 2.0% for the total areas of the two valganciclovir peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valganciclovir ($C_{14}H_{22}N_6O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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r_U = sum of the peak responses of the valganciclovir diastereomers from the *Sample solution*
r_S = sum of the peak responses of the valganciclovir diastereomers from the *Standard solution*
C_S = concentration of USP Valganciclovir Hydrochloride RS in the *Standard solution*, corrected for water content (mg/mL)
C_U = nominal concentration of valganciclovir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of valganciclovir, 354.36
M_{r2} = molecular weight of valganciclovir hydrochloride, 390.82

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 30 min
Standard stock solution: 5 mg/mL of valganciclovir in *Medium*. Expose the USP Valganciclovir Hydrochloride RS to ambient conditions overnight, and determine the water content before use.
Standard solution: 0.5 mg/mL of valganciclovir in *Medium* from the *Standard stock solution*. Pass a portion of this solution through a polyethylene filter of 10-µm pore size, discarding the first 2 mL of the filtrate.
Sample solution: Pass 10 mL of the solution under test through a polyethylene filter of 10-µm pore size, discarding the first 2 mL of the filtrate. Suitably dilute with *Medium*, if necessary, in comparison with the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Absorption maximum at about 254 nm
Cell length: 0.02 cm quartz cell
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valganciclovir ($C_{14}H_{22}N_6O_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Valganciclovir Hydrochloride RS in the *Standard solution*, corrected for water content (mg/mL)
L = label claim of valganciclovir (mg/Tablet)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of valganciclovir, 354.36
M_{r2} = molecular weight of valganciclovir hydrochloride, 390.82
D = dilution factor

Tolerances: NLT 80% (Q) of the labeled amount of valganciclovir ($C_{14}H_{22}N_6O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity
Mobile phase, Diluent, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Transfer 1 Tablet to a 100-mL volumetric flask, add about 80 mL of *Diluent*, and sonicate until the Tablet is fully disintegrated. Dilute with *Diluent* to volume, mix, and allow the solution to settle. Pipet 3.0 mL of the top portion of the resulting solution into a 200-mL volumetric flask, and dilute with *Diluent* to volume. Pass a portion of the solution through a filter of 0.45-µm or finer pore size, and use the filtrate, discarding the initial 2 mL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of valganciclovir ($C_{14}H_{22}N_6O_5$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = sum of the peak responses of the valganciclovir diastereomers from the *Sample solution*
r_S = sum of the peak responses of the valganciclovir diastereomers from the *Standard solution*
C_S = concentration of USP Valganciclovir Hydrochloride RS in the *Standard solution*, corrected for water content (mg/mL)
C_U = nominal concentration of valganciclovir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of valganciclovir, 354.36
M_{r2} = molecular weight of valganciclovir hydrochloride, 390.82

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Nominally 0.45 mg/mL of valganciclovir prepared as follows. Transfer an equivalent to 450 mg of valganciclovir from finely powdered Tablets (NLT 20) to a 1000-mL volumetric flask. Add about 800 mL of *Diluent*, and sonicate until the solid sample is fully disintegrated. Dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.45-µm or finer pore size, and use the filtrate, discarding the initial 2 mL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ganciclovir and guanine in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of ganciclovir or guanine from the *Sample solution*
r_S = sum of the peak responses of the valganciclovir diastereomers from the *Standard solution*
C_S = concentration of USP Valganciclovir Hydrochloride RS in the *Standard solution*, corrected for water content (mg/mL)
C_U = nominal concentration of valganciclovir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of valganciclovir, 354.36
M_{r2} = molecular weight of valganciclovir hydrochloride, 390.82
F = relative response factor (see *Table 1*)

Calculate the percentage of each individual unidentified and identified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

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Acceptance criteria: See *Table 1*.

Table 1

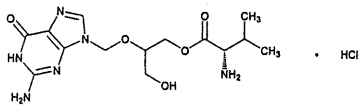
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guanine	0.51	1.9	1.0[a]
Ganciclovir	0.66	1.4	2.0[a]
Valganciclovir 1	1.00	—	—
Valganciclovir 2	1.07	—	—
Ganciclovir mono-*N*-methyl valinate 1	1.21	—	—[b]
Ganciclovir mono-*N*-methyl valinate 2	1.30	—	—[b]
Methoxymethylguanine	1.45	—	—[b]
Isovalganciclovir 1	1.55	—	—[b]
Isovalganciclovir 2	1.61	—	—[b]
Ganciclovir divalinate	2.13	—	—[b]
Monoacetoxyganciclovir	2.31	—	—[b]
Isomonochloroganciclovir	2.52	—	—[b]
Homologue 1	2.69	—	—[b]
Homologue 2	2.77	—	—[b]
Each individual unidentified degradation product	—	—	0.2
Total individual unidentified degradation products	—	—	0.5
Total degradation products	—	—	3.5

[a] Degradant.
[b] Impurity.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ganciclovir Mono-N-methyl Valinate RS
  2-(*RS*)-[(Guanin-9-yl)methoxy]-3-hydroxypropyl *N*-methyl-L-valinate.
  $C_{15}H_{24}N_6O_5$ 368.39
  USP Valganciclovir Hydrochloride RS

Valganciclovir Hydrochloride



$C_{14}H_{22}N_6O_5 \cdot HCl$ 390.82

L-Valine, ester with 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine, monohydrochloride.

L-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy]-3-hydroxypropyl ester, monohydrochloride [175865-59-5].

» Valganciclovir Hydrochloride contains not less than 97.0 percent and not more than 102.0 percent of $C_{14}H_{22}N_6O_5 \cdot HCl$, calculated on the anhydrous and solvent-free basis.

Packaging and storage—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Valganciclovir Hydrochloride RS
USP Methoxymethylguanine RS
USP d-Valganciclovir RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: methanol.

C: A solution in water (1 in 20) meets the requirements of the tests for *Chloride* ⟨191⟩.

Water Determination, *Method I* ⟨921⟩**:** not more than 8.0%, a 100-mg specimen being used.

Residue on ignition ⟨281⟩**:** not more than 0.10%, a 1-g specimen being used.

Limit of isopropyl alcohol—

Internal standard solution—Transfer 100 µL of 1,4-dioxane to a 100-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

Standard stock solution—Transfer 1.0 mL of isopropyl alcohol and 0.1 mL of toluene to a 100-mL volumetric flask, dilute with dimethylformamide to volume, and mix. [NOTE—Toluene is used to verify the system suitability.]

Standard solution—Transfer 2.0 mL of the *Internal standard solution* to a vial. Add 100 µL of *Standard stock solution* to the *Internal standard solution,* and mix.

System suitability solution—Use the *Standard solution.*

Test solution—Transfer between 90 mg to 100 mg of Valganciclovir Hydrochloride, accurately weighed, to a vial. Add 2.0 mL, accurately measured, of *Internal standard solution,* and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m capillary column coated with a 3.0-µm phase G43. The carrier gas is helium, flowing at a rate of about 10.5 mL per minute, and the split ratio is (1:15). The chromatograph is programmed as follows. Initially the column temperature is maintained at 40° for 10 minutes, and then the temperature is increased at a rate of 25° per minute to 240°. [NOTE—Condition the column at 240° after each injection for approximately 15 minutes.] The injection port temperature is maintained at about 250°, and the detector temperature is maintained at about 300°. Chromatograph the *System suitability solution* as directed for *Procedure:* the resolution, *R,* between 1,4-dioxane and toluene is not less than 8; the column efficiency, using the 1,4-dioxane peak, is not less than 6000 theoretical plates; and the relative standard deviation of the response area ratios of the isopropyl alcohol peak to the 1,4-dioxane peak for replicate injections is not more than 15%.

Procedure—Separately inject equal volumes (about 0.5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentages of isopropyl alcohol in

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the portion of Valganciclovir Hydrochloride taken by the formula:

$$100(VC/W)(R_U/R_S)$$

in which V is the volume, in mL, of the *Test solution;* C is the concentration, in mg per mL, of isopropyl alcohol in the *Standard solution;* W is the weight, in mg, of Valganciclovir Hydrochloride taken; and R_U and R_S are the peak area ratios of isopropyl alcohol to the internal standard obtained from the *Test solution* and the *Standard solution,* respectively: not more than 1.0% of isopropyl alcohol is found.

Related compounds—

TEST 1—

Solution A—Use *0.1 M Monobasic ammonium phosphate,* prepare as directed in the *Assay.*

Solution B—Use methanol.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system* (see *Table 1*). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare as directed in the *Assay.*

Test solution—Use the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The chromatograph is programmed as shown in *Table 1.*

Table 1

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–5	92	8	isocratic
5–15	92→80	8→20	linear gradient
15–30	80→30	20→70	linear gradient

[NOTE—Equilibrate the column with starting *Mobile phase* for at least 15 minutes between injections.] The column temperature is maintained at 25°, and the flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the first peak of valganciclovir and the methoxymethylguanine peak is not less than 1.0, and the resolution, *R,* between the two peaks for valganciclovir (*R* and *S* esters of L-valine) is not less than 3.0; the column efficiency determined using the second peak of valganciclovir is not less than 8000 theoretical plates; and the tailing factor for the second peak of valganciclovir is not more than 1.4. [NOTE—The typical retention time for the second peak of valganciclovir is between 5 and 8.5 minutes.]

Procedure—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. The approximate relative retention times for each individual impurity are listed in *Table 3.* Calculate the percentage of each impurity in the portion of Valganciclovir Hydrochloride taken by the formula:

$$100(r_i/F_i)/(\Sigma r_i/F_i)$$

in which the r_i is the area response for each impurity, and F_i is the relative response factor for each individual component listed in *Table 3.* The impurities meet the requirements as specified in *Table 3.*

TEST 2—

Solution A—Dilute 2.5 mL of triethylamine with water to 1000 mL, and adjust with trifluoroacetic acid to a pH of 3.0 ± 0.05. Pass this solution through a filter having a 0.45-µm porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Solution B—Use methanol.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system* (see *Table 2*). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare as directed in the *Assay.*

Test solution—Use the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The chromatograph is programmed as follows in *Table 2.*

Table 2

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–10	93	7	isocratic
10–20	93→70	7→30	linear gradient

[NOTE—Equilibrate the column with starting *Mobile phase* for at least 15 minutes between injections.] The column temperature is maintained at 30°, and the flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the two peaks for valganciclovir (*R* and *S* esters of L-valine) is not less than 1.3; the column efficiency determined using the second peak of valganciclovir is not less than 8000 theoretical plates; and the tailing factor for the second peak of valganciclovir is not more than 1.2. [NOTE—The typical retention time for the second peak of valganciclovir is between 6 and 9 minutes.]

Procedure—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of ganciclovir mono-*N*-methyl valinate impurity in the portion of Valganciclovir Hydrochloride taken by the formula:

$$100(r_i/F_i)/\Sigma(r_i/F_i)$$

in which r_i is the sum of the area responses of ganciclovir mono-*N*-methyl valinate impurity (diastereomers); and F_i is the relative response factor for this impurity and valganciclovir as given in *Table 3.* The impurity limits meet the requirements specified in *Table 3.*

Diastereomer ratio—Using the chromatogram for *Test 1* in the test for *Related compounds,* calculate the percentage of valganciclovir (*R* and *S* esters of L-valine) by the following formulas:

$$100[r_A/(r_A + r_B)]$$

$$100[r_B/(r_A + r_B)]$$

in which r_A and r_B are the peak responses for valganciclovir (*R* and *S* esters of L-valine), respectively. The diastereomer ratio is (45:55) to (55:45).

Enantiomeric purity of valganciclovir—

Mobile phase—Dissolve 16.2 g of perchloric acid in 1000 mL of water, and mix. Pass this solution through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Transfer about 5 mg of USP Valganciclovir Hydrochloride RS and 0.5 mg of USP d-Valganciclovir RS to a 25-mL volumetric flask, dissolve in and dilute with 0.001 N hydrochloric acid to volume, and mix.

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Table 3 (Valganciclovir Hydrochloride)

Component	Common Name	Test Number	Relative Retention Time	Relative Response Factor	Maximum Limit (%)
Valganciclovir	Valganciclovir	1, 2	1.00	1.00	—
A[a]	Ganciclovir	1	0.42	1.4	1.5
B[a]	Guanine	1	0.28	1.9	0.25
C[a]	Methoxymethylguanine	1	0.81	1.0	0.3
D[a, b]	Isovalganciclovir	1	1.26	1.0	0.5
E[a]	Monoacetoxyganciclovir	1	1.36	1.3	0.15
F (other identified impurity)	Bis-valine easter of ganciclovir	1	1.61	0.71	0.1
G[a, b]	Homologue of valganciclovir	1	1.66	1.0	0.25
H	—	1	1.47	1.3	0.1
I	—	1	1.52	1.4	0.1
J (other identified impurity)	Ganciclovir monopropionate	1	2.09	1.1	0.15
K[a]	Valganciclovir dimer (stereoisomer A)	1	2.49	1.0	0.1
L[a]	Valganciclovir dimer (stereoisomer B)	1	2.52	1.0	0.1
M[a]	Valganciclovir dimer (stereoisomer C)	1	2.54	1.0	0.1
N[a, b]	Ganciclovir mono-*N*-methyl valinate	2	1.2	1.0	0.3
Other identified impurity	—	1	—	1.0	0.1 individual; 0.25 total
Unidentified impurity	—	1 and 2	—	1.0	0.1 individual; 0.25 total
Total of all impurities	—	1 and 2	—	—	3.0

[a] Specified impurity.
[b] Reported as the sum of diastereomers.

Test solution—Transfer about 10 mg of Valganciclovir Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with 0.001 N hydrochloric acid to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 15-cm column that contains packing L66. The column temperature is maintained at ambient temperature, and the flow rate is about 0.8 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the second peak of the D-valine ester pair and the first peak of the valganciclovir pair is not less than 3.5; and the column efficiency determined using the second peak of valganciclovir is not less than 1800 theoretical plates.

Procedure—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the enantiomeric purity, in percent, by the following formula:

$$100[r_S/(r_S + r_{IM})]$$

in which r_S is the sum of the peak responses of valganciclovir (*R* and *S* esters of L-valine) and r_{IM} is the sum of the peak responses of the enantiomeric impurities (*R* and *S* esters of D-valine), respectively. The enantiomeric purity is not less than 97.0%.

Assay—

0.10 M Monobasic ammonium phosphate solution—Dissolve 11.5 g of monobasic ammonium phosphate in about 900 mL of water, adjust with phosphoric acid (85%) to a pH of 2.8 ± 0.2, dilute with water to obtain 1000 mL of solution, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *0.10 M Monobasic ammonium phosphate* and methanol (92:8). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Transfer about 10 mg of USP Valganciclovir Hydrochloride RS and 0.5 mg of USP Methoxymethylguanine RS to a 50-mL volumetric flask, dissolve in and dilute with 0.001 N hydrochloric acid to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Valganciclovir Hydrochloride RS in 0.001 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Dissolve an accurately weighed quantity of Valganciclovir Hydrochloride in 0.001 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a concentration of about 0.2 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm ×15-cm column that contains packing L1. The

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column temperature is maintained at 25°, and the flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* meets the system suitability requirements as specified for *Test 1* in the test for *Related compounds.* Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation of the correction factor (C_F) for replicate injections is not more than 1.0%. [NOTE—C_F is calculated as directed below in the *Procedure.*]

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of valganciclovir hydrochloride ($C_{14}H_{22}N_6O_5 \cdot HCl$) in the portion of Valganciclovir Hydrochloride taken by the formula:

$$100[(r_U/W_U)(C_F)(100)]$$

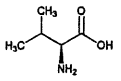
in which r_U is the peak response (sum of two peaks for valganciclovir diastereomers) obtained from the *Assay preparation;* W_U is the weight, in mg, of Valganciclovir Hydrochloride in the *Assay preparation;* and C_F is the correction factor.

The C_F is calculated using the following formula:

$$(W_S/R_S)/100$$

in which W_S is the weight, in mg, of USP Valganciclovir Hydrochloride RS in the *Standard preparation;* and R_S is the area response (sum of two peaks for valganciclovir diastereomers) obtained from the *Standard preparation.*

Valine



$C_5H_{11}NO_2$ 117.15

L-Valine [72-18-4].

DEFINITION

Valine contains NLT 98.5% and NMT 101.5% of L-valine ($C_5H_{11}NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy:* **197K▲** (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample: 110 mg of Valine

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with *Titrant.* Perform the blank determination.

Calculate the percentage of valine ($C_5H_{11}NO_2$) in the portion of Valine taken:

$$\text{Result} = \{[(V_S - V_B) \times N_A \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N_A = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 117.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **CHLORIDE AND SULFATE ⟨221⟩,** *Chloride*

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of Valine

Acceptance criteria: NMT 0.05%

- **CHLORIDE AND SULFATE ⟨221⟩,** *Sulfate*

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Valine

Acceptance criteria: NMT 0.03%

- **IRON ⟨241⟩:** NMT 30 ppm
- **RELATED COMPOUNDS**

System suitability solution: 0.4 mg/mL each of USP L-Valine RS and USP L-Phenylalanine RS in 0.1 N hydrochloric acid

Standard solution: 0.05 mg/mL of USP L-Valine RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of the *Sample solution.*]

Sample solution: 10 mg/mL of Valine in 2 N hydrochloric acid

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Sample: *System suitability solution*

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *System suitability solution, Standard solution,* and *Sample solution*

After air-drying the plate, spray with *Spray reagent,* and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution.*

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **OPTICAL ROTATION ⟨781S⟩,** *Procedures, Specific Rotation*

Sample solution: 80 mg/mL in 6 N hydrochloric acid

Acceptance criteria: +26.6° to +28.8°

- **PH ⟨791⟩**

Sample solution: 50 mg/mL

Acceptance criteria: 5.5–7.0

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 105° for 3 h.

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Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Phenylalanine RS
  USP L-Valine RS

Valproate Sodium Injection

DEFINITION
Valproate Sodium Injection is a sterile aqueous solution of sodium valproate, formed from the interaction of Valproic Acid and Sodium Hydroxide, in Water for Injection, and one or more suitable buffering or sequestering agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid ($C_8H_{16}O_2$). It contains no antimicrobial agents.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ (USP 1-Dec-2019)

Change to read:

- **C.** ▲ (USP 1-Dec-2019) **IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 3.5 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Diluent: Acetonitrile and water (45:55)
System suitability solution: 0.5 mg/mL of USP Valproic Acid RS and 50 µg/mL of USP Valproic Acid Related Compound B RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Valproic Acid RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of valproic acid in water from a suitable volume of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm. ▲ (USP 1-Dec-2019)
Column: 4.6-mm × 15.0-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.6 times the retention time of valproic acid ▲ (USP 1-Dec-2019)
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for valproic acid related compound B and valproic acid are 0.90 and 1.0, respectively.]

Suitability requirements
Resolution: NLT 2.0 between valproic acid related compound B and valproic acid, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

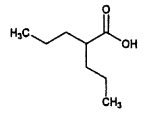
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valproic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 23 USP Endotoxin Units/mL of Injection
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume parenterals
- **pH** ⟨791⟩: 7.0–9.0
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, preferably of Type I glass. Store at controlled room temperature.
- **LABELING:** It states the name and quantity of any buffering or sequestering agent used. It states that it is intended for use by intravenous infusion only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Valproic Acid RS
  USP Valproic Acid Related Compound B RS
  (2*RS*)-2-(1-Methylethyl)pentanoic acid.
  $C_8H_{16}O_2$ 144.21

Valproic Acid



$C_8H_{16}O_2$ 144.21
Pentanoic acid, 2-propyl-;
Propylvaleric acid [99-66-1].

DEFINITION
Valproic Acid contains NLT 98.0% and NMT 102.0% of valproic acid ($C_8H_{16}O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F** ▲ (CN 1-May-2020)

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• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 3.5 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Diluent: Acetonitrile and water (45:55)

System suitability solution: 50 µg/mL of USP Valproic Acid Related Compound B RS and 0.5 mg/mL of USP Valproic Acid RS in *Diluent*

Standard solution: 0.5 mg/mL of USP Valproic Acid RS in *Diluent*

Sample solution: 0.5 mg/mL of Valproic Acid in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15.0-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between valproic acid related compound B and valproic acid, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of valproic acid ($C_8H_{16}O_2$) in the portion of Valproic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Valproic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**

System suitability solution: 0.1 µL/mL of USP Valproic Acid Related Compound A RS and 1.0 µL/mL each of butyric acid and valeric acid in Valproic Acid

Sample solution: Valproic Acid

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 60-m; coated with a 0.3-µm film of phase G25

Temperatures

Injection port: 240°

Detector: 260°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
145	0	145	48
145	5	190	—

Carrier gas: Helium

Flow rate: 150 mL/min

Injection volume: 0.5 µL

Injection type: Split flow ratio, 100:1

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for butyric acid, valeric acid, valproic acid, and valproic acid related compound A are 0.38, 0.52, 1.0, and 1.64, respectively.]

Suitability requirements

Resolution: NLT 23.0 between butyric acid and valeric acid

Column efficiency: NLT 100,000 theoretical plates for valeric acid

Tailing factor: NMT 1.5 for valeric acid

Retention time: The related compound A peak must elute between 41 and 50 min.

Peak area: The related compound A peak area must be NLT 0.01% relative to the valproic acid peak area.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Valproic Acid ($C_8H_{16}O_2$) taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of the responses for all the peaks

Acceptance criteria

Individual impurities: NMT 0.1%

Total impurities: NMT 0.3%

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight glass, stainless steel, or polyethylene (HDPE) containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Valproic Acid RS

USP Valproic Acid Related Compound A RS

2-Allylpent-4-enoic acid;

Also known as Diallylacetic acid.

$C_8H_{12}O_2$ 140.18

USP Valproic Acid Related Compound B RS

2-Isopropylpentanoic acid;

Also known as (2*RS*)-2-(1-Methylethyl)pentanoic acid.

$C_8H_{16}O_2$ 144.21

Valproic Acid Capsules

DEFINITION

Valproic Acid Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid ($C_8H_{16}O_2$).

USP Monographs

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IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 3.5 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Diluent: Acetonitrile and water (45:55)
System suitability solution: 0.5 mg/mL of USP Valproic Acid RS and 50 µg/mL of USP Valproic Acid Related Compound B RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Valproic Acid RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of valproic acid from the contents of the Capsules in *Diluent*, prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules to an appropriate volumetric flask, and dilute with *Diluent* to volume. Sonicate the resulting solution for 5 min. Alternatively, stir the resulting solution for 1 h. Centrifuge a portion of the solution for about 10 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm. For *Identification* test *B*, use a diode array detector in the wavelength range of 200–300 nm.
Column: 4.6-mm × 15.0-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for valproic acid related compound B and valproic acid are 0.90 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between valproic acid related compound B and valproic acid, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valproic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 5 mg/mL of sodium lauryl sulfate in simulated intestinal fluid TS (prepared without the enzyme and with monobasic sodium phosphate instead of monobasic potassium phosphate), adjusted with 5 M sodium hydroxide to a pH of 7.5; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Buffer, Mobile phase, Diluent, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Standard stock solution: 1.5 mg/mL of USP Valproic Acid RS in *Diluent*, prepared as follows. Transfer NLT 150 mg of USP Valproic Acid RS to a suitable volumetric flask. Add 10% of the flask volume of *Diluent* and dissolve the valproic acid. Dilute with *Diluent* to volume.
Standard solution: 0.3 mg/mL of USP Valproic Acid RS from the *Standard stock solution* and *Medium*
Sample solution: Pass a portion of the solution through suitable filter of 0.45-µm pore size and use the filtrate.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 85% (*Q*) of valproic acid ($C_8H_{16}O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Valproic Acid RS
USP Valproic Acid Related Compound B RS
(2*RS*)-2-(1-Methylethyl)pentanoic acid.
$C_8H_{16}O_2$ 144.21

Valproic Acid Oral Solution

DEFINITION

Valproic Acid Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid ($C_8H_{16}O_2$). It is prepared with the aid of Sodium Hydroxide.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B.** ▲The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 3.5 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Diluent: Acetonitrile and water (45:55)

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System suitability solution: 0.5 mg/mL of USP Valproic Acid RS and 50 µg/mL of USP Valproic Acid Related Compound B RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Valproic Acid RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of valproic acid in *Diluent* from a suitable volume of Oral Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
Column: 4.6-mm × 15.0-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
▲**Run time:** NLT 2 times the retention of valproic acid▲ (USP 1-May-2019)
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for valproic acid related compound B and valproic acid are 0.90 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between valproic acid related compound B and valproic acid, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

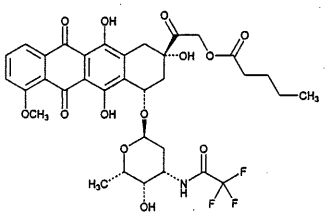
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valproic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 7.0–8.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Valproic Acid RS
USP Valproic Acid Related Compound B RS
(2*RS*)-2-(1-Methylethyl)pentanoic acid.
$C_8H_{16}O_2$ 144.21

Valrubicin



$C_{34}H_{36}F_3NO_{13}$ 723.64

(2*S*-*cis*)-2-[1,2,3,4,6,11-Hexahydro-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-4-[[2,3,6-trideoxy-3-[(trifluoroacetyl)amino]-α-L-*lyxo*-hexopyranosyl]oxy]-2-naphthacenyl]-2-oxoethyl pentanoate;
(8*S*,10*S*)-8-Glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-10-[[2,3,6-trideoxy-3-(2,2,2-trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]oxy]-5,12-naphthacenedione 8²-valerate [56124-62-0].

DEFINITION
Valrubicin contains NLT 98.0% and NMT 102.0% of $C_{34}H_{36}F_3NO_{13}$, calculated on the anhydrous and solvent-free basis.
[CAUTION—Great care should be taken to prevent inhaling particles of Valrubicin and exposing the skin to it.]

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 3.4 mg/mL of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.1
Mobile phase: Acetonitrile and *Solution A* (3:2)
Standard solution: 0.5 mg/mL of USP Valrubicin RS in acetonitrile
Sample solution: 0.5 mg/mL of Valrubicin in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 5-cm; 1.8-µm packing L1
Flow rate: 1.5 mL/min
Column temperature: 40°
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{34}H_{36}F_3NO_{13}$ in the portion of Valrubicin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valrubicin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Valrubicin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.2%
ORGANIC IMPURITIES
• **Procedure**
Solution A: Use *Solution A* as directed in the *Assay*.
Solution B: Acetonitrile

USP Monographs

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Mobile phase: See the gradient table below. [NOTE—Analysis time is 55 min. The steps that follow are for column washing and re-equilibration.]

Time (min)	Solution A (%)	Solution B (%)
0	75	25
45	40	60
55	40	60
58	20	80
68	20	80
70	75	25
80	75	25

Solution C: Acetonitrile and water (1:1)
Stock standard solution: 2 mg/mL of USP Valrubicin RS in acetonitrile and water (1:1). [NOTE—Dissolve first in acetonitrile, using 50% of the final volume and dilute with water to volume.]
Standard solution: 0.01 mg/mL of valrubicin in *Solution C*
System suitability solution: 2 mg/mL of USP Valrubicin Resolution Mixture RS in acetonitrile and water (1:1). [NOTE—Dissolve first in acetonitrile, using 50% of the final volume and dilute with water to volume.]
Sample solution: 2 mg/mL of Valrubicin in acetonitrile and water (1:1). [NOTE—Dissolve first in acetonitrile, using 50% of the final volume and dilute with water to volume.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 254 nm
Column: 4.6-mm × 5-cm; 1.8-µm packing L1
Flow rate: 1.5 mL/min
Temperature
Column: 40°
Autosampler: 4°
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—Identify the peaks by the relative retention times listed in *Impurity Table 1*.]
Suitability requirements
Resolution: NLT 2.0 between doxorubicinone and daunorubicin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Valrubicin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of valrubicin from the *Standard solution*
C_S = concentration of USP Valrubicin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Valrubicin in the *Sample solution* (mg/mL)
F = relative response factor as listed in *Impurity Table 1*

Acceptance criteria
[NOTE—Reporting level is 0.05%.]
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Doxorubicin	0.05	1.0	0.15
Doxorubicinone[a]	0.10	0.63	0.15
Daunorubicin	0.12	0.86	0.15
Daunorubicin bromoketal[b]	0.42	1.4	0.15
Doxorubicin valerate[c]	0.48	1.3	0.15
Doxorubicinone valerate[d]	0.76	0.80	0.15
Valrubicin	1.0	—	—
Dianhydrovalrubicin[e]	1.2	0.47	0.15
Any unspecified impurity	—	1.0	0.10

[a] (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[b] (8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-(2-bromo-1,1-dimethoxyethyl)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[c] 2-{(2*S*,4*S*)-4-[(3-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl}-2-oxoethyl pentanoate.
[d] 2-Oxo-2-[(2*S*,4*S*)-2,4,5,12-tetrahydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl]ethyl pentanoate.
[e] 2-(5,12-Dihydroxy-7-methoxy-6,11-dioxo-6,11-dihydrotetracen-2-yl)-2-oxoethyl pentanoate.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Valrubicin RS
USP Valrubicin Resolution Mixture RS
This Reference Standard is a mixture of the following with their chemical names:
Doxorubicin: (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione.
Doxorubicin aglycone: (8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydro tetracene-5,12-dione.
Daunorubicin: (8*S*,10*S*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
Daunorubicin bromoketal: (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-(2-bromo-1,1-dimethoxyethyl)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
Doxorubicin valerate: 2-{(2*S*,4*S*)-4-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl}-2-oxoethyl pentanoate.
Doxorubicin aglycone valerate: 2-oxo-2-[(2*S*,4*S*)-2,4,5,12-tetrahydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl]ethyl pentanoate.
Valrubicin: (8*S*,10*S*)-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-10-[[2,3,6-trideoxy-3-(2,2,2-trifluoroacetamido)-α-L-*lyxo*-hexopy ranosyl] oxy]-5,12-naphthacenedione 8²-valerate.

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Dianhydrovalrubicin: 2-(5,12-dihydroxy-7-methoxy-6,11-dioxo-6,11-dihydrotetracen-2-yl)-2-oxoethyl pentanoate.

Valrubicin Intravesical Solution

DEFINITION

Valrubicin Intravesical Solution is a sterile solution of Valrubicin in a suitable vehicle. It contains NLT 95.0% and NMT 105.0% of the labeled amount of valrubicin ($C_{34}H_{36}F_3NO_{13}$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: Use the *Standard solution*, prepared as directed in the *Assay*, suitably diluted with methanol.

Sample solution: Use the *Sample solution*, prepared as directed in the *Assay*, suitably diluted with methanol.

Solution A: 0.1 M ammonium formate. Adjust with formic acid to a pH of 4.0.

Developing solvent system: Acetonitrile and *Solution A* (45:55)

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.1 M ammonium formate. Adjust with formic acid to a pH of 4.0.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Standard solution: 0.2 mg/mL of USP Valrubicin RS in methanol

Sample solution: Equivalent to 0.2 mg/mL of valrubicin, from Intravesical Solution in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 mm

Column: 5-mm × 10-cm; 4-µm packing L1, and a guard column

Flow rate: 2.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of $C_{34}H_{36}F_3NO_{13}$ in the Intravesical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Valrubicin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of valrubicin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

ORGANIC IMPURITIES

- **Procedure**

Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sample solution: 0.2 mg/mL of valrubicin in methanol

Analysis

Sample: *Sample solution*

Measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Intravesical Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area for each impurity

r_T = sum of the peak areas of all the peaks

[NOTE—Do not consider any peaks due to solvent or excipients.]

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 3.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Valrubicin impurity 1	0.11	0.5
Valrubicin impurity 2	0.16	0.8
Valrubicin impurity 3	0.51	0.8
Valrubicin impurity 4	0.71	0.8
Any unspecified impurity	—	0.5

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.14 USP Endotoxin Unit/mg of valrubicin
- **pH** ⟨791⟩

Sample solution: 1 in 15 solution in 0.9% sodium chloride solution

Acceptance criteria: 4.0–7.0

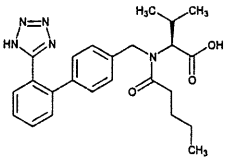
- **OTHER REQUIREMENTS:** Meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store in a refrigerator.
- **LABELING:** Label it to indicate that it is not intended for intravenous or intramuscular injection but is to be used for intravesical instillation.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Valrubicin RS

Valsartan



$C_{24}H_{29}N_5O_3$ 435.52

L-Valine, *N*-(1-oxopentyl)-*N*-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-;

N-[*p*-(*o*-1*H*-Tetrazol-5-ylphenyl)benzyl]-*N*-valeryl-L-valine [137862-53-4].

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DEFINITION

Valsartan contains NLT 98.0% and NMT 102.0% of valsartan ($C_{24}H_{29}N_5O_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, glacial acetic acid, and water (500:1:500)
Standard solution: 0.5 mg/mL of USP Valsartan RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Valsartan in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 273 nm
Column: 3.0-mm × 12.5-cm; 5-µm packing L1
Flow rate: 0.4 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of valsartan ($C_{24}H_{29}N_5O_3$) in the portion of Valsartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
C_U = concentration of Valsartan in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **PROCEDURE 1: LIMIT OF VALSARTAN RELATED COMPOUND A**

Mobile phase: *n*-Hexane, 2-propanol, and trifluoroacetic acid (850:150:1)
System suitability solution: 0.04 mg/mL each of USP Valsartan Related Compound A RS and USP Valsartan RS in *Mobile phase*
Standard solution: 0.01 mg/mL of USP Valsartan Related Compound A RS in *Mobile phase*
Sample solution: 1 mg/mL of Valsartan in *Mobile phase*. Sonicate for 5 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L40
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between valsartan related compound A and valsartan
Relative standard deviation: NMT 5% for valsartan related compound A peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of valsartan related compound A in the portion of Valsartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valsartan related compound A from the *Sample solution*
r_S = peak response of valsartan related compound A from the *Standard solution*
C_S = concentration of USP Valsartan Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Valsartan in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0% of valsartan related compound A.

- **PROCEDURE 2: LIMIT OF VALSARTAN RELATED COMPOUND B, VALSARTAN RELATED COMPOUND C, AND OTHER RELATED COMPOUNDS**

Mobile phase: Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL each of USP Valsartan RS, USP Valsartan Related Compound B RS, and USP Valsartan Related Compound C RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Valsartan in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except for the following.
Detector: UV 225 nm
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.8 between valsartan related compound B and valsartan
Relative standard deviation: NMT 10.0% for valsartan related compound B and NMT 2.0% for valsartan
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of valsartan related compound B and valsartan related compound C in the portion of Valsartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valsartan related compound B or valsartan related compound C from the *Sample solution*
r_S = peak response of valsartan related compound B or valsartan related compound C from the *Standard solution*
C_S = concentration of USP Valsartan Related Compound B RS or USP Valsartan Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Valsartan in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Valsartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other impurity from the *Sample solution*

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r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
C_U = concentration of Valsartan in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria, NMT (%)
Valsartan related compound B[a]	0.2
Valsartan related compound C[b]	0.1
Any other individual impurity[c]	0.1
Total impurities[c]	0.3

[a] *N*-Butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-L-valine.
[b] *N*-Valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-L-valine benzyl ester.
[c] Excluding valsartan related compound A.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%
- **ABSORBANCE**
  Analytical wavelength: 420 nm
  Sample solution: A 1-in-20 solution of valsartan in methanol
  Acceptance criteria: The absorbance divided by the path length is NMT 0.02.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature. Protect from moisture and heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Valsartan RS
  USP Valsartan Related Compound A RS
  N-Valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-D-valine.
  $C_{24}H_{29}N_5O_3$ 435.52
  USP Valsartan Related Compound B RS
  N-Butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-L-valine.
  $C_{23}H_{27}N_5O_3$ 421.49
  USP Valsartan Related Compound C RS
  N-Valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-L-valine benzyl ester.
  $C_{31}H_{35}N_5O_3$ 525.64

Valsartan Tablets

DEFINITION
Valsartan Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, water, and glacial acetic acid (50: 50: 0.1)
  Diluent: Acetonitrile and water (50:50)
  System suitability solution: 2 µg/mL of USP Valsartan Related Compound B RS and 20 µg/mL of USP Valsartan RS in *Diluent*
  Standard solution: 0.20 mg/mL of USP Valsartan RS in *Diluent*
  Sample stock solution: Place NLT 20 Tablets in a suitable volumetric flask, and initially add water (10% of the volume of the flask). Stir or shake until the Tablets disintegrate (about 5 min). Add acetonitrile (about 80% of the volume of the flask). Stir or shake for 30 min, and sonicate for 10 min. Cool, and dilute with acetonitrile to volume, mix, and centrifuge a portion of the suspension.
  Sample solution: Nominally 0.2 mg/mL of valsartan from the *Sample stock solution* in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 4.6-mm × 25-cm; 10-µm packing L1
  Column temperature: 30°
  Flow rate: 1.0 mL/min
  Injection size: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 1.5 between valsartan related compound B and valsartan, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valsartan from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valsartan the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: pH 6.8 phosphate buffer prepared as follows. Dissolve 6.805 g of monobasic potassium phosphate and 0.896 g of sodium hydroxide in and dilute with water to 1000 mL. Adjust with 0.2 M sodium hydroxide or 1 M phosphoric acid as required to a pH of 6.8; 1000 mL degassed.
  Apparatus 2: 50 rpm
  Time: 30 min
  Standard solution: (*L*/1000) mg/mL of USP Valsartan RS in *Medium*, where *L* is the label claim, in mg/Tablet. [NOTE—Dilute with *Medium* as needed.]
  Sample solution: Pass a portion of the solution under test through a suitable filter.
  Analysis
  Analytical wavelength: UV 250 nm
  Blank: *Medium*
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

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$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.067 M phosphate buffer prepared as follows. Dissolve 91.2 g of monobasic potassium phosphate and 12 g of sodium hydroxide in 10 L of water. Adjust with 1 N sodium hydroxide or 1 N orthophosphoric acid to a pH of 6.8; 1000 mL.
Apparatus 2: 50 rpm
Time: 30 min
Standard stock solution: 0.4 mg/mL of USP Valsartan RS prepared as follows. Transfer an appropriate quantity of USP Valsartan RS into a suitable volumetric flask, and add methanol to about 5% of the volume of the flask. Sonicate to dissolve. Dilute with *Medium* to volume.
Standard solution: 0.02 mg/mL of USP Valsartan RS in *Medium* from *Standard stock solution*
Sample solution: Withdraw 10 mL of the solution under test and pass through a suitable filter. Dilute a portion of the solution with *Medium* to the concentration similar to that in the *Standard solution*.
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 250 nm
Cell: 1.0 cm
Blank: *Medium*
System suitability
Sample: *Standard solution*
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
D = dilution factor
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.4 µg/mL of USP Valsartan RS in *Diluent*
Sensitivity solution: 0.1 µg/mL of USP Valsartan RS in *Diluent*, from the *Standard solution*
System suitability
Samples: *Standard solution*, *Sensitivity solution*, and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between valsartan related compound B and valsartan, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of valsartan in the *Sample solution* (µg/mL)

Acceptance criteria
Each individual impurity: NMT 0.2%
Total impurities: NMT 0.4%. [NOTE—Calculate the total impurities from the sum of all individual impurity peaks. Disregard any peak due to valsartan related compound B and any peaks ≤0.05%.]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** When more than one test for *Dissolution* is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Valsartan RS
USP Valsartan Related Compound B RS
N-Butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl] methyl}-L-valine.
$C_{23}H_{27}N_5O_3$ 421.49

Valsartan and Hydrochlorothiazide Tablets

DEFINITION

Valsartan and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: To an amount of ground Tablets, equivalent in weight to a single Tablet, add 2.0 mL of acetone, sonicate for 15 min, and centrifuge.
Application volume: 2 µL
Developing solvent system: Ethyl acetate, dehydrated alcohol, and 3.6 M of ammonium hydroxide (8:2:1)
Analysis: Proceed as directed in the chapter, except develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent system* for 15 min before use. Allow the chromatogram to develop until the solvent front has moved at least 7 cm. After removing the

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plate and marking the solvent front, dry the plate under a current of warm air.

Acceptance criteria: The R_F values of the principal spots from the *Sample solution* correspond to those from the *Standard solution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Acetonitrile and water (1:1)

Solution A: Acetonitrile, water, and trifluoroacetic acid (10:90:0.1)

Solution B: Acetonitrile, water, and trifluoroacetic acid (90:10:0.1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
25	10	90
27	90	10
40	90	10

Standard solution: Transfer 12.5 mg of USP Hydrochlorothiazide RS to a 200-mL volumetric flask, and add 12.5*J* mg of USP Valsartan RS, *J* being the ratio of the labeled amount, in mg, of valsartan to the labeled amount, in mg, of hydrochlorothiazide per Tablet. Add 100 mL of *Diluent*, sonicate for 15 min, dilute with *Diluent* to volume, and mix. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. Dilute with *Diluent* to obtain a solution having a concentration of 0.2 mg/mL of USP Valsartan RS in *Diluent*.

Sample stock solution: To the nominal equivalent of 62.5 mg of hydrochlorothiazide from a number of Tablets add 5 mL of water, and allow to stand for 5 min. Then add 100 mL of *Diluent*, sonicate for 15 min, and shake for 30 min. Dilute with *Diluent* to 250 mL, and centrifuge a portion of this solution at 3000 rpm. Dilute 25.0 mL of the clear supernatant with *Diluent* to 200.0 mL.

Sample solution: Nominally 0.2 mg/mL of valsartan from *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 3.0-mm × 12.5-cm; 5-µm packing L1

Flow rate: 0.4 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Determine the percentage of the labeled amounts of valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved by using one of the following procedures.

Spectrophotometric procedure

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Analytical wavelength: 250 nm for valsartan and 272 nm for hydrochlorothiazide

Cell path length: 0.2-cm quartz

Standard solution: USP Hydrochlorothiazide RS and USP Valsartan RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable glass fiber filter of 1-µm pore size. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Analysis

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

$$\text{Result} = [(AT_2 \times D) - (AT_1 \times E)/(C \times D) - (B \times E)] \times 12{,}500$$

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = [(AT_1 \times C) - (AT_2 \times B)/(D \times C) - (E \times B)] \times 80{,}000$$

AT_1 = absorbance of the *Sample solution* at 272 nm

C = $A1\%V_{250}$, absorptivity (1%, 0.2 cm, 250 nm) of valsartan in *Medium*

AT_2 = absorbance of the *Sample solution* at 250 nm

B = $A1\%V_{272}$, absorptivity (1%, 0.2 cm, 272 nm) of valsartan in *Medium*

D = $A1\%H_{272}$, absorptivity (1%, 0.2 cm, 272 nm) of hydrochlorothiazide in *Medium*

E = $A1\%H_{250}$, absorptivity (1%, 0.2 cm, 250 nm) of hydrochlorothiazide in *Medium*

Chromatographic procedure

Diluent: Water and acetonitrile (1:1)

Solution A: 0.2 M ammonium acetate (15.4 g/L of ammonium acetate in water), adjusted with glacial acetic acid to a pH of 5.6

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	88	12
4	65	35
7	88	12
8	88	12

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System suitability solution: 80 µg/mL of USP Valsartan RS, 60 µg/mL of USP Hydrochlorothiazide RS, 30 µg/mL of USP Benzothiadiazine Related Compound A RS, and 200 µg/mL of USP Valsartan Related Compound B RS in *Diluent*. Transfer 25 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume.

Standard solution: Transfer about 12.5 mg of USP Hydrochlorothiazide RS to a 200-mL volumetric flask. Add about 12.5*J* mg of USP Valsartan RS, with *J* being the ratio of the labeled amount (mg) of valsartan to the labeled amount (mg) of hydrochlorothiazide per Tablet. Dilute with *Diluent* to volume. Transfer 10 mL of this solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.

Sample solution: For Tablets labeled to contain 12.5 mg of hydrochlorothiazide, pass a portion of the solution under test through a suitable filter.

For Tablets labeled to contain 25 mg of hydrochlorothiazide, pass a portion of the solution under test through a suitable filter, and dilute with *Medium* (1:1).

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L11

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between valsartan and valsartan related compound B; NLT 2.0 between hydrochlorothiazide and benzothiadiazine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0% for both valsartan and hydrochlorothiazide, *Standard solution*

Analysis

Calculate the percentage of the labeled amounts of valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response of valsartan or hydrochlorothiazide from the *Sample solution*

r_S = peak response of valsartan or hydrochlorothiazide from the *Standard solution*

C_S = concentration of valsartan or hydrochlorothiazide in the *Standard solution* (mg/mL)

L = label claim for valsartan or hydrochlorothiazide (mg/Tablet)

D = dilution factor of the *Sample solution*, if applicable

V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of both valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 1: 100 rpm

Time: 30 min

Buffer: Mix 1 mL of trifluoroacetic acid and 1 L of water.

Mobile phase: Acetonitrile and *Buffer* (450:550)

Valsartan standard stock solution: 3.2 mg/mL of USP Valsartan RS in *Medium* prepared as follows. To a suitable amount of the USP Valsartan RS in a suitable volumetric flask add methanol to fill 20% of the total volume. Sonicate to dissolve, and add *Medium* to fill 25% of the total volume. Sonicate for 5 min, and dilute with *Medium* to volume.

Hydrochlorothiazide standard stock solution: 0.5 mg/mL of USP Hydrochlorothiazide RS prepared as follows. To a suitable amount of the USP Hydrochlorothiazide RS in a suitable volumetric flask add methanol to fill 10% of the total volume. Sonicate to dissolve, and add *Medium* to fill 60% of the total volume. Sonicate for 5 min, and dilute with *Medium* to volume.

Standard solution: Prepare solutions of concentrations listed in *Table 3* from *Valsartan standard stock solution* and *Hydrochlorothiazide standard stock solution* in *Medium*. Pass through a suitable filter of 0.45-µm pore size, and discard the first few mL of the filtrate.

Table 3

Tablet Strength of Valsartan/ Hydrochlorothiazide (mg/mg)	Concentration of Valsartan (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
320/25	0.32	0.025
320/12.5	0.32	0.0125
160/25	0.16	0.025
160/12.5	0.16	0.0125
80/12.5	0.08	0.0125

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Sample cooler temperature: 20°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times of valsartan and hydrochlorothiazide are 1.0 and 0.25, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for both valsartan and hydrochlorothiazide peaks

Relative standard deviation: NMT 2.0% for both valsartan and hydrochlorothiazide peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of valsartan or hydrochlorothiazide from the *Sample solution*

r_S = peak response of valsartan or hydrochlorothiazide from the *Standard solution*

C_S = concentration of valsartan or hydrochlorothiazide in the *Standard solution* (mg/mL)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

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Tolerances: NLT 80% (*Q*) of the labeled amount of both valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Diluent, Solution A, Solution B, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sample solution: Transfer 1 Tablet to a 200-mL volumetric flask, add 5 mL of water, and allow to stand for 5 min. Add 100 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume, mix, and centrifuge a portion of this solution at 3000 rpm. Dilute a volume of the clear supernatant with *Diluent* to obtain a solution having a nominal concentration of 0.2 mg/mL of valsartan.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of valsartan ($C_{24}H_{29}N_5O_3$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.03 mg/mL of USP Benzothiadiazine Related Compound A RS, 0.06 mg/mL of USP Hydrochlorothiazide RS, 0.08 mg/mL of USP Valsartan RS, and 0.2 mg/mL of USP Valsartan Related Compound B RS in *Diluent*

System suitability solution: Dilute 5.0 mL of the *Standard stock solution* with *Diluent* to 100 mL.

Standard solution: Dilute 10.0 mL of the *System suitability solution* in 100.0 mL of *Diluent*.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.4 between valsartan related compound B and valsartan; NLT 1.4 between benzothiadiazine related compound A and hydrochlorothiazide, *System suitability solution*

Relative standard deviation: NMT 10.0% for the valsartan and hydrochlorothiazide peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Disregard the peak, if any, with a retention time of 22 min.

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A from the *Standard solution*
C_S = concentration of benzothiadiazine related compound A in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Calculate the percentage of each other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each other impurity from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of valsartan in the *Standard solution* (mg/mL)
C_U = nominal concentration of valsartan (for calculating other impurities) in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0% of benzothiadiazine related compound A; NMT 0.2% of any other impurity, excluding valsartan related compound A; NMT 1.3% of total impurities, excluding valsartan related compound A. [NOTE—Valsartan related compound A is the enantiomer of valsartan and coelutes with valsartan in this test.]

ADDITIONAL REQUIREMENTS

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only when *Test 1* is not used.

• **PACKAGING AND STORAGE:** Preserve in tight containers, and protect from moisture and heat. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73

USP Hydrochlorothiazide RS

USP Valsartan RS

USP Valsartan Related Compound B RS
N-Butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl] methyl}-L-valine.
$C_{23}H_{27}N_5O_3$ 421.49

Vancomycin

$C_{66}H_{75}Cl_2N_9O_{24}$ 1449.25

Vancomycin
(*S*$_a$)-(3*S*,6*R*,7*R*,22*R*,23*S*,26*S*,36*R*,38a*R*)-44-[[2-*O*-(3-Amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-3-(carbamoylmethyl)-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-7,22,28,30,32-pentahydroxy-6-[(2*R*)-4-methyl-2-(methylamino)valeramido]-2,5,24,38,39-pentaoxo-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*,16*H*-[1,6,9]oxadiazacyclohexadecino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-26-carboxylic acid;

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[3*S*-[3*R**,6*S**(*S**),7*S**,22*S**,23*R**,26*R**,36*S**,38a*S**]]-3-(2-Amino-2-oxoethyl)-44-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetra-decahydro-7,22,28,30,32-pentahydroxy-6-[[4-methyl-2-(methylamino)-1-oxopentyl]amino]-2,5,24,38,39-pentaoxo-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*, 16*H*-[1,6,9]oxadiazacyclohexadecino[4,5-*m*][10,2,16]-benzoxadiazacyclotetraine-26-carboxylic acid [1404-90-6].

DEFINITION

Vancomycin has a potency equivalent to NLT 950 µg/mg of $C_{66}H_{75}Cl_2N_9O_{24}$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: It does not meet the requirements of the test.

ASSAY

- **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨81⟩

Sample solution: Transfer 100 mg of Vancomycin to a 100-mL volumetric flask. Add 50 mL of water and 1 mL of 0.1 N hydrochloric acid, and swirl to dissolve, using sonication if necessary. Dilute with water to volume. Dilute a volume of this solution with *Buffer B.4* to yield a *Test Dilution* having a concentration assumed to be equal to that of the median dose of the standard.

Analysis: Proceed as directed for Vancomycin in the chapter.

Acceptance criteria: NLT 950 µg/mg of vancomycin on the anhydrous basis

SPECIFIC TESTS

- **COMPOSITION OF VANCOMYCIN**

Buffer: Triethylamine and water (1:500). Adjust with phosphoric acid to a pH of 3.2.

Solution A: Acetonitrile, tetrahydrofuran, and *Buffer* (7:1:92)

Solution B: Acetonitrile, tetrahydrofuran, and *Buffer* (29:1:70)

Mobile phase: See *Table 1*. Make adjustments if necessary, changing the acetonitrile proportion in *Solution A* to obtain a retention time of 7.5–10.5 min for the main vancomycin peak.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	100	0
20	0	100
22	0	100
23	100	0
30	100	0

System suitability solution: 0.5 mg/mL of USP Vancomycin Hydrochloride RS in water. Heat at 65° for 48 h, and allow to cool.

Sample stock solution: Transfer 250 mg of Vancomycin to a 25-mL volumetric flask. Add 5 mL of *Solution A*, then add 0.1 N hydrochloric acid dropwise with swirling until dissolution is achieved. Dilute with *Solution A* to volume.

Sample solution: Dilute 2.0 mL of the *Sample stock solution* to 50 mL with *Solution A*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The elution order is compound 1, vancomycin B, and compound 2. Compound 2 elutes 3–6 min after the start of the period when the percentage of *Solution B* is increasing from 0% to 100%.]

Suitability requirements

Resolution: NLT 3.0 between compound 1 and vancomycin B

Column efficiency: NLT 1500 theoretical plates for the vancomycin B peak

Analysis

Samples: *Sample stock solution* and *Sample solution*

Where baseline separation is not achieved, peak areas are defined by vertical lines extended from the valleys between peaks to the baseline. The main component peak may include a fronting shoulder, which is attributed to monodechlorovancomycin. This shoulder should not be integrated separately.

Correct any peak observed in the chromatograms obtained from the *Sample stock solution* and *Sample solution* by subtracting the area response of any peak observed in the chromatogram of *Solution A* at the corresponding retention time.

Calculate the percentage of vancomycin B in the portion of Vancomycin taken:

$$\text{Result} = \{(D \times r_B)/[(D \times r_B) + r_A]\} \times 100$$

D = dilution factor, *Sample stock solution* to *Sample solution*, 25

r_B = corrected peak area response of the main peak from the *Sample solution*

r_A = sum of the corrected peak area responses of all the peaks, other than the main peak, from the *Sample stock solution*

Calculate the percentage of any individual peak, other than the main peak, in the portion of Vancomycin taken:

$$\text{Result} = \{r_i/[(D \times r_B) + r_A]\} \times 100$$

r_i = corrected peak area response of any individual peak, other than the main peak, from the *Sample stock solution*

D = dilution factor, *Sample stock solution* to *Sample solution*, 25

r_B = corrected peak area response of the main peak from the *Sample solution*

r_A = sum of the corrected peak area responses of all the peaks, other than the main peak, from the *Sample stock solution*

Acceptance criteria: NLT 92% of vancomycin B; NMT 3% of any individual peak other than the main peak

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 20%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

USP Monographs

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• **USP REFERENCE STANDARDS** ⟨11⟩
USP Vancomycin Hydrochloride RS

Vancomycin Injection

Change to read:

DEFINITION
Vancomycin Injection is a sterile ▲ (RB 1-Mar-2019) solution of Vancomycin Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 115.0% of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$). It contains ▲one or more suitable excipients.▲ (RB 1-Mar-2019)

IDENTIFICATION
• **A.** The retention time of the main vancomycin peak of the *Sample stock solution*, obtained as directed in *Composition of Vancomycin*, corresponds to that of a similarly prepared solution of USP Vancomycin Hydrochloride RS.

ASSAY

Change to read:

• **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨81⟩
Sample solution: Allow a container of Injection to thaw, ▲if applicable,▲ (RB 1-Mar-2019) and mix the solution. Dilute a portion of this solution with Buffer B.4 to yield a test dilution having a concentration assumed to be equal to that of the median dose of the standard.
Analysis: Proceed as directed for vancomycin in the chapter.
Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

Change to read:

• **COMPOSITION OF VANCOMYCIN**
Buffer: Triethylamine and water (1:500). Adjust with phosphoric acid to a pH of 3.2.
Solution A: Acetonitrile, tetrahydrofuran, and *Buffer* (7:1:92)
Solution B: Acetonitrile, tetrahydrofuran, and *Buffer* (29:1:70)
Mobile phase: See *Table 1*. Make adjustments if necessary, changing the acetonitrile proportion in *Solution A* to obtain a retention time of 7.5–10.5 min for the main vancomycin peak.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	100	0
20	0	100
22	0	100
23	100	0
30	100	0

System suitability solution: Allow a container of Injection to thaw, ▲if applicable,▲ (RB 1-Mar-2019) and mix the solution. Dilute a portion of the Injection with water to obtain a solution containing 0.5 mg/mL of vancomycin. Heat at 65° for 24 h, and allow to cool.

Sample stock solution: Allow a container of Injection to thaw, ▲if applicable,▲ (RB 1-Mar-2019) and mix the solution.
Sample solution: Equivalent to 0.4 mg/mL of vancomycin hydrochloride from the *Sample stock solution* in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
[NOTE—The elution order is compound 1, vancomycin B, and compound 2. Compound 2 elutes 3–6 min after the start of the period, when the percentage of *Solution B* is increasing from 0% to 100%.]
Resolution: NLT 3.0 between compound 1 and vancomycin B
Column efficiency: NLT 1500 theoretical plates for the vancomycin B peak
Analysis
Samples: *Sample stock solution* and *Sample solution*
Where baseline separation is not achieved, peak areas are defined by vertical lines extended from the valleys between peaks to the baseline. The main component peak may include a fronting shoulder, which is attributed to monodechlorovancomycin. This shoulder should not be integrated separately.
Correct any peak observed in the chromatograms from *Sample stock solution* and *Sample solution* by subtracting the area response of any peak observed in the chromatogram of *Solution A* at the corresponding retention time.
Calculate the percentage of vancomycin B in the portion of Injection taken:

$$\text{Result} = \{(D \times r_B)/[(D \times r_B) + r_A]\} \times 100$$

D = dilution factor, *Sample stock solution* to *Sample solution*
r_B = corrected peak area response of the main peak from the *Sample solution*
r_A = sum of the corrected peak area responses of all the peaks, other than the main peak, from the *Sample stock solution*

Calculate the percentage of any individual peak, other than the main peak, in the portion of Injection taken:

$$\text{Result} = \{r_i/[(D \times r_B) + r_A]\} \times 100$$

r_i = corrected peak area response of any individual peak, other than the main peak, from the *Sample stock solution*
D = dilution factor, *Sample stock solution* to *Sample solution*
r_B = corrected peak area response of the main peak from the *Sample solution*
r_A = sum of the corrected peak area responses of all the peaks, other than the main peak, from the *Sample stock solution*

Acceptance criteria: NLT 88% of vancomycin B; NMT 4% of any individual peak other than the main peak

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Change to read:

- **pH** ⟨791⟩: ▲4.5–5.5 for products stored at room temperature; 3.0–5.0 for products maintained in a frozen state▲ (RB 1-Mar-2019)
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 0.33 USP Endotoxin Units/mg of vancomycin

Change to read:

- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections ▲or large-volume injections, whichever is applicable▲ (RB 1-Mar-2019)
- **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements, except use water instead of diluting *Fluid A*
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

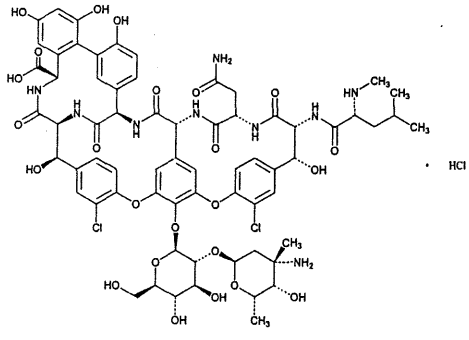
Change to read:

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. ▲Store as directed by product label.▲ (RB 1-Mar-2019)

Change to read:

- **Labeling:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. ▲For the product stored in a frozen state,▲ (RB 1-Mar-2019) the label states that it is to be thawed just before use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP Reference Standards** ⟨11⟩
  USP Vancomycin Hydrochloride RS

Vancomycin Hydrochloride



$C_{66}H_{75}Cl_2N_9O_{24} \cdot HCl$ 1485.71

Vancomycin, monohydrochloride;
Vancomycin monohydrochloride;
(S_a)-(3*S*,6*R*,7*R*,22*R*,23*S*,26*S*,36*R*,38a*R*)-44-[[2-*O*-(3-Amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-3-(carbamoylmethyl)-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-7,22,28,30,32-pentahydroxy-6-[(2*R*)-4-methyl-2-(methylamino)]valeramido]-2,5,24,38,39-pentaoxo-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*,16*H*-[1,6,9]oxadiazacyclohexadecino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-26-carboxylic acid, monohydrochloride;
[3*S*-[3*R**,6*S**(*S**),7*S**,22*S**,23*R**,26*R**,36*S**,38a*S**]]-3-(2-Amino-2-oxoethyl)-44-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-7,22,28,30,32-pentahydroxy-6-[[4-methyl-2-(methylamino)-1-oxopentyl]amino]-2,5,24,38,39-pentaoxo-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*,16*H*-[1,6,9]oxadiazacyclohexadecino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-26-carboxylic acid, monohydrochloride [1404-93-9].

DEFINITION

Vancomycin Hydrochloride is the hydrochloride salt of a kind of vancomycin, a substance produced by the growth of *Streptomyces orientalis* (Fam. Streptomycetaceae), or a mixture of two or more such salts. It has a potency equivalent to NLT 900 µg/mg of vancomycin, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

▲• **Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **Procedure:** Proceed with Vancomycin Hydrochloride as directed under *Antibiotics—Microbial Assays* ⟨81⟩.
  Acceptance criteria: NLT 900 µg/mg on the anhydrous basis

IMPURITIES

Organic Impurities

- **Procedure: Limit of Monodechlorovancomycin**
  [Note—The *System suitability solution*, *Standard solution*, and *Sample solution* should be refrigerated immediately after preparation and during analysis. The solutions are stable for 4 days when refrigerated.]

Mobile phase: Dissolve 2.2 g of 1-heptanesulfonic acid sodium salt in about 500 mL of water, add 125 mL of acetonitrile and 10 mL of acetic acid, and dilute with water to 1 L.

Rinse solution: 10% acetonitrile in water. [Note—Use to rinse needle and column.]

System suitability solution: Equivalent to 1 mg/mL of vancomycin B from USP Vancomycin B with Monodechlorovancomycin RS in water

Standard solution: Equivalent to 50 µg/mL of vancomycin B, from *System suitability solution*

Sample solution: 1 mg/mL of Vancomycin Hydrochloride in water

Blank: Water

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6 mm × 25 cm; packing L1
Column temperature: 60°
Autosampler temperature: 5°

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Flow rate: 1.5 mL/min
Injection volume: 50 µL
[NOTE—This procedure is sensitive to temperature changes. Sufficient tubing should be placed in the column oven to ensure that the samples have reached 60° before separation.]
System suitability
Samples: *System suitability solution*, *Standard solution*, *Sample solution*, and *Blank*
[NOTE—*Blank* and *Standard solution* run times are about 90 min. *System suitability solution* and *Sample solution* run times are about 120 min.]
[NOTE—The relative retention times for vancomycin B and monodechlorovancomycin are 1.0 and 1.1, respectively.]
Suitability requirements
Selectivity: The chromatogram for the *Blank* does not contain peaks that interfere with vancomycin B or monodechlorovancomycin.
Retention times: ±3.0% between monodechlorovancomycin in the *Sample solution* and the mean of the monodechlorovancomycin peaks in the *Standard solution*
Resolution: NLT 1.5 between vancomycin B and monodechlorovancomycin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of monodechlorovancomycin in the portion of Vancomycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak area of monodechlorovancomycin in the *Sample solution*
r_S = average peak area of the vancomycin B peak in the *Standard solution*
C_S = concentration of USP Vancomycin B with Monodechlorovancomycin RS in the *Standard solution* (mg/mL)
C_U = concentration of Vancomycin Hydrochloride in the *Sample solution* (mg/mL)
P = potency of vancomycin B in USP Vancomycin B with Monodechlorovancomycin RS (mg/mg)

Acceptance criteria: NMT 4.7% of monodechlorovancomycin is found.

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Vancomycin Hydrochloride is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*, except to dissolve the specimen in water, instead of in *Fluid A*.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Vancomycin Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.33 USP Endotoxin Unit/mg of vancomycin.
- **pH** ⟨791⟩**:** 2.5–4.5, 50 mg/mL in water
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 5.0%
- **COMPOSITION OF VANCOMYCIN**

Solution A: Triethylamine and water (1:500). Adjust with phosphoric acid to a pH of 3.2.
Solution B: Acetonitrile, tetrahydrofuran, and *Solution A* (7:1:92)
Solution C: Acetonitrile, tetrahydrofuran, and *Solution A* (29:1:70)

Mobile phase: See the gradient table below. [NOTE—Make adjustments, if necessary, changing the acetonitrile proportion in *Solution B* to obtain a retention time of 7.5–10.5 min for the main vancomycin peak.]

Time (min)	Solution B (%)	Solution C (%)
0	100	0
12	100	0
20	0	100
22	0	100
23	100	0
30	100	0

System suitability solution: 0.5 mg/mL of USP Vancomycin Hydrochloride RS in water. Heat at 65° for 48 h, and allow to cool.
Sample solution 1: 10 mg/mL of Vancomycin Hydrochloride in *Solution B*
Sample solution 2: 0.4 mg/mL of Vancomycin Hydrochloride, from *Sample solution 1* in *Solution B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The elution order is compound 1, vancomycin B, and compound 2. Compound 2 elutes 3–6 min after the start of the period when the percentage of *Solution C* increases from 0%–100%.]
Suitability requirements
Resolution: NLT 3.0 between compound 1 and vancomycin B
Column efficiency: NLT 1500 theoretical plates, calculated from the vancomycin B peak
Analysis
Samples: *Sample solution 1* and *Sample solution 2*
[NOTE—Where baseline separation is not achieved, peak areas are defined by vertical lines extended from the valleys between the peaks to the baseline. The main component peak may include a fronting shoulder, which is attributed to monodechlorovancomycin. This shoulder should not be integrated separately.]
[NOTE—Correct any peak observed in the chromatograms obtained from *Sample solution 1* and *Sample solution 2* by subtracting the area response of any peak observed in the chromatogram of *Solution B* at the corresponding retention time.]
Measure the area responses for all of the peaks. Calculate the percentage of vancomycin B in the portion of Vancomycin Hydrochloride taken:

$$\text{Result} = [(D \times r_B)/((D \times r_B) + r_A)] \times 100$$

D = dilution factor, *Sample solution 1* to *Sample solution 2*, 25
r_B = corrected area response of the main peak of *Sample solution 2*
r_A = sum of the corrected area responses of all the peaks, other than the main peak, from *Sample solution 1*

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Calculate the percentage of each other peak in the portion of Vancomycin Hydrochloride taken:

$$\text{Result} = \{r_I/[(D \times r_B) + r_A]\} \times 100$$

r_I = corrected area response of any individual peak, other than the main peak of *Sample solution 1*
D = dilution factor, *Sample solution 1* to *Sample solution 2*, 25
r_B = corrected area response of the main peak of *Sample solution 2*
r_A = sum of the corrected area responses of all the peaks, other than the main peak from *Sample solution 1*

Acceptance criteria: NLT 85.0% of vancomycin B; NMT 5.0% of any peak other than the main peak

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Vancomycin Hydrochloride RS
  USP Vancomycin B with Monodechlorovancomycin RS

Vancomycin Hydrochloride Capsules

DEFINITION
Vancomycin Hydrochloride Capsules contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$).

IDENTIFICATION

Change to read:

- **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Disperse the contents of 1 Capsule in about 20 mL of chloroform. Pass the liquid through a suitable filter. Rinse the filter and residue with chloroform. Dry the residue in a vacuum at 60° for 1 h.

ASSAY
- **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨81⟩

Analysis: Proceed as directed, using NLT 5 Capsules blended at high speed in a glass blender jar for 3–5 min with a sufficient volume of *Buffer B.4* to yield a stock solution having a convenient concentration of vancomycin. Dilute a volume of this stock solution with *Buffer B.4* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Sample solution: Sample per *Dissolution* ⟨711⟩
Analysis: Determine the amount of vancomycin dissolved by assaying a filtered portion of the *Sample solution* as directed for vancomycin under *Antibiotics—Microbial Assays* ⟨81⟩.
Tolerances: NLT 85% (Q) of the labeled amount of vancomycin is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 8.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Vancomycin Hydrochloride RS

Vancomycin Hydrochloride for Injection

DEFINITION
Vancomycin Hydrochloride for Injection is a sterile dry mixture of Vancomycin Hydrochloride and may contain a suitable stabilizing agent. It contains NLT 90.0% and NMT 115.0% of the labeled amount of vancomycin.

IDENTIFICATION

Change to read:

- **▲• SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨81⟩

Sample solution 1 (where it is represented as being in a single-dose container): Constitute a container of Vancomycin Hydrochloride for Injection in water corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe. Dilute to an equivalent of 1 mg/mL of vancomycin with water.
Sample solution 2 (where it is packaged for dispensing): Dissolve the contents of 1 container of Vancomycin Hydrochloride for Injection in water, and dilute with water to obtain a solution having a concentration of 1 mg/mL of vancomycin.
Sample solution 3 (where the label states the quantity of vancomycin in a given volume of constituted solution): Constitute a container of Vancomycin Hydrochloride for Injection in water corresponding to the volume of diluent specified in the labeling. Dilute a portion to obtain a final concentration equivalent to 1 mg/mL of vancomycin in water.
Analysis: Proceed as directed in *Antibiotics—Microbial Assays* ⟨81⟩. [NOTE—Use a measured volume of the appropriate *Sample solution*, diluted quantitatively with *Buffer B.4* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.]
Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements under small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.33 USP Endotoxin Unit/mg of vancomycin
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*, except to dissolve the specimen in water instead of in *Fluid A*.

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- **pH** ⟨791⟩: 2.5–4.5, 50 mg/mL in water
- **Water Determination,** *Method I* ⟨921⟩: NMT 5.0%
- **Injections and Implanted Drug Products** ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*: Meets the requirements at the time of use.
- **Content of Vancomycin**

Solution A: Triethylamine and water (1:500). Adjust with phosphoric acid to a pH of 3.2.
Solution B: Acetonitrile, tetrahydrofuran, and *Solution A* (7:1:92)
Solution C: Acetonitrile, tetrahydrofuran, and *Solution A* (29:1:70)
Mobile phase: See the gradient table below.
[Note—Make adjustments if necessary, changing the acetonitrile proportion in *Solution B* to obtain a retention time of 7.5–10.5 min for the main vancomycin peak.]

Time (min)	Solution B (%)	Solution C (%)
0	100	0
12	100	0
20	0	100
22	0	100
23	100	0
30	100	0

System suitability solution: 0.5 mg/mL of USP Vancomycin Hydrochloride RS. Heat at 65° for 48 h, and allow to cool.
Sample solution 1: Equivalent to 10 mg/mL of vancomycin from Vancomycin Hydrochloride for Injection in *Solution B*
Sample solution 2: 0.4 mg/mL of vancomycin from *Sample solution 1* in *Solution B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[Note—The elution order is compound 1, vancomycin B, and compound 2. Compound 2 elutes 3–6 min after the start of the period when the percentage of *Solution C* increases from 0% to 100%.]
Suitability requirements
Resolution: NLT 3.0 between compound 1 and vancomycin B
Column efficiency: NLT 1500 theoretical plates, calculated from the vancomycin B peak
Analysis
Samples: *Sample solution 1* and *Sample solution 2*
[Note—Where baseline separation is not achieved, peak areas are defined by vertical lines extended from the valleys between peaks to the baseline. The main component peak may include a fronting shoulder, which is attributed to monodechlorovancomycin. This shoulder should not be integrated separately.]
[Note—Correct any peak observed in the chromatograms obtained from *Sample solution 1* and *Sample solution 2* by subtracting the area response of any peak observed in the chromatogram of *Solution B* at the corresponding elution time.]
Measure the area responses for all of the peaks.

Calculate the percentage of vancomycin B in the specimen tested:

$$\text{Result} = [D \times r_B/((D \times r_B) + r_A)] \times 100$$

D = dilution factor, *Sample solution 1* to *Sample solution 2*, 25
r_B = corrected area response of the main peak from *Sample solution 2*
r_A = sum of the corrected area responses of all the peaks, other than the main peak, from *Sample solution 1*

Calculate the percentage of each other peak taken:

$$\text{Result} = \{r_I/[(D \times r_B) + r_A]\} \times 100$$

r_I = corrected area response of any individual peak, other than the main peak, from *Sample solution 1*
D = dilution factor, *Sample solution 1* to *Sample solution 2*, 25

Acceptance criteria: NLT 80.0% of vancomycin B; NMT 9.0% of any peak other than the main peak

- **Other Requirements:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
- **Labeling:** Meets the requirements under *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*
- **USP Reference Standards** ⟨11⟩
USP Vancomycin Hydrochloride RS

Add the following:

▲Vancomycin Hydrochloride Compounded Oral Solution

DEFINITION

Vancomycin Hydrochloride Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$).

Prepare Vancomycin Hydrochloride Compounded Oral Solution 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Vancomycin (as Vancomycin Hydrochloride)	5 g (calculate)[a]
SyrSpend SF PH4 (Liquid Cherry Flavored),[b] a sufficient quantity to make	100 mL

[a] Calculate the amount of vancomycin hydrochloride needed based on the potency equivalent (activity) of vancomycin.
[b] Fagron Inc., St. Paul, MN.

Calculate the amount of *Vancomycin Hydrochloride* needed based on the potency equivalent (activity) of vancomycin. Pour the *Vancomycin Hydrochloride* into a suitable container. Wet the powder with a small amount of *SyrSpend SF PH4 (Liquid Cherry Flavored)* and triturate to make a smooth paste. Add the *SyrSpend SF PH4 (Liquid Cherry Flavored)* to make the contents pourable. Transfer the contents stepwise and

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quantitatively to a calibrated container using the remainder of the *SyrSpend SF PH4 (Liquid Cherry Flavored)*. Add sufficient *SyrSpend SF PH4 (Liquid Cherry Flavored)* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: 50 mM ammonium acetate adjusted with ammonium hydroxide to a pH of 8.0

Solution B: Methanol and water (35:65)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
20	30	70
25	0	100
25.01	50	50

Standard solution: 0.5 mg/mL of vancomycin B prepared from USP Vancomycin B RS in water

Sample solution: Transfer 1 mL of Oral Solution to a 100-mL volumetric flask and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 2.1-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 0.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for vancomycin B is about 14.8 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of vancomycin from the *Sample solution*

r_S = peak response of vancomycin B from the *Standard solution*

C_S = concentration of vancomycin B in the *Standard solution* (mg/mL)

C_U = nominal concentration of vancomycin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 2.5–3.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant plastic containers. Store in a refrigerator.

• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored in a refrigerator.

• **LABELING:** Label it to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Vancomycin B RS▲ (USP 1-May-2020)

Vancomycin Hydrochloride for Oral Solution

DEFINITION

Vancomycin Hydrochloride for Oral Solution contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$).

ASSAY

• **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨81⟩

Sample solution: Dissolve the contents of 1 container of Vancomycin Hydrochloride for Oral Solution in water as directed in the labeling. Dilute a portion of this solution with *Buffer B.4* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the standard.

Analysis: Proceed as directed for vancomycin in the chapter.

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For powder packaged in single-unit containers: Meets the requirements

• **DELIVERABLE VOLUME** ⟨698⟩

For powder packaged in multiple-unit containers: Meets the requirements

SPECIFIC TESTS

• **pH** ⟨791⟩: 2.5–4.5, for a solution constituted as directed in the labeling

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 5.0%

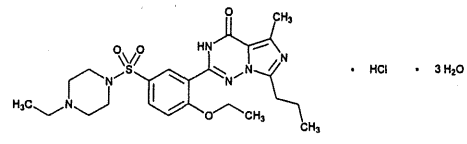
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Vancomycin Hydrochloride RS

Vardenafil Hydrochloride



$C_{23}H_{32}N_6O_4S \cdot HCl \cdot 3H_2O$ 579.11

Piperazine, 1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-*f*][1,2,4]triazin-2-yl)-4-ethoxyphenyl] sulfonyl]-4-ethyl-, monohydrochloride, trihydrate-;

2-[2-Ethoxy-5-(4-ethyl-piperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3*H*-imidazo[5,1-*f*][1,2,4]triazin-4-one, hydrochloride, trihydrate [330808-88-3].

Anhydrous 525.06

[224785-91-5].

DEFINITION

Vardenafil Hydrochloride contains NLT 98.0% and NMT 102.0% of vardenafil hydrochloride ($C_{23}H_{32}N_6O_4S \cdot HCl$), calculated on the anhydrous basis.

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IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General, *Chloride* ⟨191⟩:** Meets the requirements

ASSAY

- **Procedure**

Protect all solutions containing vardenafil from light.

Buffer: Dissolve 1.3 g of monobasic potassium phosphate and 0.7 g of dibasic sodium phosphate dihydrate in 1 L of water.

Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	80	20
2	80	20
22	25	75
27	25	75

System suitability solution: 0.5 mg/mL of USP Vardenafil System Suitability RS prepared as follows. Transfer an appropriate quantity of the Reference Standard to a volumetric flask. Add 20% of the flask volume of acetonitrile and dilute with *Buffer* to volume.

Standard solution: 0.15 mg/mL of USP Vardenafil Hydrochloride RS prepared as follows. Transfer an appropriate quantity of USP Vardenafil Hydrochloride RS to a volumetric flask. Add 20% of the flask volume of acetonitrile and dilute with *Buffer* to volume.

Sample solution: 0.15 mg/mL of Vardenafil Hydrochloride prepared as follows. Transfer an appropriate quantity of Vardenafil Hydrochloride to a volumetric flask. Add 20% of the flask volume of acetonitrile and dilute with *Buffer* to volume.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 3-mm × 25-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 0.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between vardenafil and 7-methyl vardenafil, *System suitability solution*

Relative standard deviation: NMT 0.85%, *Standard solution*

Tailing factor: 0.8–1.5, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of vardenafil hydrochloride ($C_{23}H_{32}N_6O_4S \cdot HCl$) in the portion of Vardenafil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of vardenafil from the *Sample solution*

r_S = peak response of vardenafil from the *Standard solution*

C_S = concentration of USP Vardenafil Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Vardenafil Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Organic Impurities**

Protect all solutions containing vardenafil from light.

Buffer, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Diluent: Acetonitrile and *Buffer* (20:80)

Standard stock solution: 0.5 mg/mL of USP Vardenafil Hydrochloride RS prepared as follows. Transfer an appropriate quantity of the Reference Standard to a volumetric flask. Add 20% of the flask volume of acetonitrile and dilute with *Buffer* to volume.

Standard solution: 0.5 µg/mL of USP Vardenafil Hydrochloride RS in *Diluent* from the *Standard stock solution*

Sample solution: 0.5 mg/mL of Vardenafil Hydrochloride prepared as follows. Transfer an appropriate quantity of Vardenafil Hydrochloride to a volumetric flask. Add 20% of the flask volume of acetonitrile and dilute with *Buffer* to volume.

System suitability

[Note—See *Table 2* for the relative retention times.]

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between vardenafil and 7-methyl vardenafil, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Vardenafil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of vardenafil from the *Standard solution*

C_S = concentration of USP Vardenafil Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Vardenafil Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
7-Methyl vardenafil[a]	0.83	0.15
Vardenafil	1.0	—

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Table 2 *(continued)*

Name		Relative Retention Time	Acceptance Criteria, NMT (%)
Unspecified impurities	Vardenafil acid[b]	0.48	0.10 each
	Vardenafil dimer[c]	1.2	
	Any unspecified individual impurity	—	
Total impurities		—	0.3

[a] 2-{2-Ethoxy-5-[(4-ethylpiperazin-1-yl)sulfonyl]phenyl}-5,7-dimethylimidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-one.
[b] 4-Ethoxy-3-(5-methyl-4-oxo-7-propyl-3,4-dihydroimidazo [5,1-*f*][1,2,4] triazin-2-yl)benzenesulfonic acid.
[c] 2,2′-[(Piperazine-1,4-disulfonyl)bis(6-ethoxy-3,1-phenylene)]bis[5-methyl-7-propylimidazo[5,1-*f*][1,2,4]-triazin-4(3*H*)-one.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 8.8%–10.5%

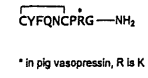
ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store in well-closed containers at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Vardenafil Hydrochloride RS
  USP Vardenafil System Suitability RS
  This mixture contains Vardenafil Hydrochloride and approximately 1% of 7-methyl vardenafil (2-{2-ethoxy-5-[(4-ethylpiperazin-1-yl)sulfonyl]phenyl}-5,7-dimethylimidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-one).
  $C_{21}H_{28}N_6O_4S$ 460.55

Varicella-Zoster Immune Globulin

» Varicella-Zoster Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile 15 percent to 18 percent solution of pH 7.0 containing the globulin fraction of human plasma consisting of not less than 99 percent of immunoglobulin G with traces of immunoglobulin A and immunoglobulin M, in 0.3 M glycine as a stabilizer and 1:10,000 thimerosal as a preservative. It is derived from adult human plasma selected for high titers of varicella-zoster antibodies. Each unit of blood or plasma has been found nonreactive for hepatitis B surface antigen by a suitable method. The proteins of the plasma pools are fractionated by the cold ethanol precipitation method. The content of specific antibody is not less than 125 units, deliverable from a vial containing not more than 2.5 mL solution. The unit is defined as equivalent to 0.01 mL of a Varicella-Zoster Immune Globulin lot found effective in clinical trials and used as a reference for potency determinations, based on a fluorescent-antibody membrane antigen (FAMA) method for antibody titration.

Packaging and storage—Preserve at a temperature between 2° and 8°.
Expiration date—The expiration date is not later than 2 years after date of issue from manufacturer's cold storage.
Labeling—Label it to state that it is to be administered by intramuscular injection, in the recommended dose based on body weight.

Vasopressin



$C_{46}H_{65}N_{15}O_{12}S_2$ 1084.24
Vasopressin, 8-L-arginine [113-79-1].

DEFINITION
Vasopressin is a polypeptide hormone having the properties of causing the contraction of vascular and other smooth muscles, and of antidiuresis. It is prepared by chemical synthesis. It contains NLT 95.0% and NMT 105.0% of vasopressin ($C_{46}H_{65}N_{15}O_{12}S_2$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION
- **A.** The retention time of the vasopressin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. MASS SPECTRAL ANALYSIS**
  Infusion solution: Acetonitrile, water, and trifluoroacetic acid (80: 20: 0.08)
  Standard solution: 1 mg/mL of USP Vasopressin RS in water
  Sample solution: 1 mg/mL of Vasopressin in water.
  [NOTE—The final concentrations of the *Standard solution* and the *Sample solution* can be adjusted, depending on the sensitivity of the mass spectrometer used in the testing.]
  Instrumental conditions
  (See *Mass Spectrometry* ⟨736⟩.)
  Mode: LC/MS spectrometer
  Interface/detection: Infusion system connected to an electrospray interface (positive ion)
  Flow rate: 0.3 mL/min
  Injection size: 10 µL
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Acceptance criteria: Should contain peaks with mass-to-charge ratios of 1084 and 543

ASSAY
- **PROCEDURE**
  Mobile phase: Dissolve 6.6 g of dibasic ammonium phosphate in 950 mL of water. Adjust with concentrated phosphoric acid to a pH of 3.0. Dilute with water to 1000 mL. To 870 mL of this solution add 130 mL of acetonitrile, and mix. Filter under vacuum through a nylon membrane of 0.45-µm pore size. [NOTE—The retention time of the vasopressin peak is very sensitive to small changes in acetonitrile concentration in the *Mobile phase*.]
  System suitability solution: Dissolve suitable quantities of USP Lypressin RS and USP Vasopressin RS in 0.25% glacial acetic acid to obtain a solution having a known concentration of about 25 µg/mL of each substance.
  Standard solution: Dissolve the entire contents of a vial of USP Vasopressin RS in a known volume of 0.25% glacial acetic acid. [NOTE—The solution may be diluted as necessary to a working concentration range for the *Assay*.]

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Sample solution: Transfer about 10 mg of Vasopressin to a 25-mL volumetric flask. Dissolve in 0.25% glacial acetic acid, and dilute with the same solvent to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40 ± 1°
Flow rate: 1.0 mL/min
Injection size: 20 µL. [NOTE—The column is allowed to equilibrate for 1 h before making the first injection.]
System suitability
Samples: *System suitability solution* and *Standard solution*.
[NOTE—Inject into an equilibrated liquid chromatograph, allowing about 60 min for complete elution.]
[NOTE—The retention time of the vasopressin peak is between 6 and 9 min.]
Suitability requirements
Resolution: NLT 1.1 between the vasopressin and lypressin peaks
Relative standard deviation: NMT 2.0% for the vasopressin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of vasopressin ($C_{46}H_{65}N_{15}O_{12}S_2$) in the portion of Vasopressin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Vasopressin RS in the *Standard solution* (mg/mL)
C_U = concentration of Vasopressin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the anhydrous, acetic acid-free basis

IMPURITIES
- **ORDINARY IMPURITIES:** The sum of the responses of impurities from the *Sample solution* in the *Assay* is NMT 5% of the area of the vasopressin peak.

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total bacterial count is NMT 2×10^2 cfu/g. For products of animal origin, it also meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 8.0%
- **ACETIC ACID IN PEPTIDES** ⟨503⟩**:** NMT 15.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of Type I glass, and store in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Lypressin RS
USP Vasopressin RS

Vasopressin Injection

» Vasopressin Injection is a sterile solution of Vasopressin in a suitable diluent. Each mL of Vasopressin Injection possesses an activity of not less than 90.0 percent and not more than 110.0 percent of that stated on the label in USP Vasopressin Units. It may contain a suitable preservative.

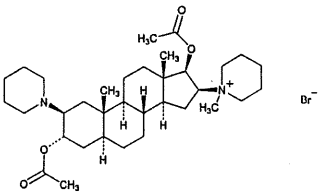
Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Do not freeze.
Labeling—Label it to indicate its origin (animal or synthetic). Label it also to state the potency in USP Vasopressin Units per mL.
USP Reference standards ⟨11⟩—
USP Vasopressin RS
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 17.0 Endotoxin Units per USP Vasopressin Unit.
pH ⟨791⟩: between 2.5 and 4.5.
Particulate Matter in Injections ⟨788⟩ —It meets the requirements under small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase, System suitability solution, Chromatographic system, and *Procedure*—Proceed as directed in the *Assay* under *Vasopressin*.
Diluent—Dissolve 5.0 g of chlorobutanol in 5.0 mL of glacialacetic acid, add 5.0 g of alcohol, 1.1 g of sodium acetate, and 1000mL of water, and mix.
Standard preparation—Dissolve the entire contents of a vial ofUSP Vasopressin RS in a known volume of *Diluent*. [NOTE—The solution may be diluted as necessary to a workingconcentration range for the *Assay*.]
Assay preparation—Pipet 2.0 mL of Injection into a 25-mL volumetric flask, dilute with 0.25% glacial acetic acid to volume, and mix.
Procedure—Calculate the potency, in USP Vasopressin Units per mL, by the formula:

$$C(r_U/r_S)$$

in which C is the concentration, in USP Vasopressin Units per mL, of the *Standard preparation;* and r_U and r_S are the mean values of the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively. [NOTE—The runtime should be long enough to allow for the elution of the chlorobutanol peak (approximately 60 minutes).]

Vecuronium Bromide



$C_{34}H_{57}BrN_2O_4$ 637.73
Piperidinium, 1-[(2β,3α,5α,16β,17β)-3,17-bis(acetyloxy)- 2-(1-piperidinyl) androstan-16-yl]-1-methyl-, bromide;
1-(3α,17β-Dihydroxy-2β-piperidino-5α-androstan-16β,5α-yl)-1-methylpiperidinium bromide, diacetate [50700-72-6].

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DEFINITION

Vecuronium Bromide contains NLT 98.0% and NMT 102.0% of $C_{34}H_{57}BrN_2O_4$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K.▲** (CN 1-May-2020) Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Pipet 1.0 mL of 1 N hydrochloric acid into a 1000-mL volumetric flask, and dilute with acetonitrile to volume.

Solution A: Dissolve 8.0 g of sodium perchlorate in 6.0 mL of water, and dilute with acetonitrile to 1 L.

Solution B: Dissolve 1.6 g of ammonium chloride in 8 mL of ammonium hydroxide, and dilute with methanol to 1 L. [NOTE—Avoid excessive degassing to prevent the loss of ammonium hydroxide.]

Mobile phase: *Solution A* and *Solution B* (60:40)

Standard solution: 0.5 mg/mL of USP Vecuronium Bromide RS in *Diluent*

Sample solution: 0.5 mg/mL of Vecuronium Bromide in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection size: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000 theoretical plates
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of vecuronium bromide ($C_{34}H_{57}BrN_2O_4$) in the portion of Vecuronium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Vecuronium Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Vecuronium Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Cation suppressor regeneration solution: 0.02 M tetrabutylammonium hydroxide

Mobile phase
[NOTE—Filter components before combining. Avoid evaporation of tetrahydrofuran during degassing.]
Combine methanol, water, and hydrochloric acid (250:1500:1). Leave at room temperature for a few min. Add 45 mL of tetrahydrofuran, and then dilute with water to 2 L.
[NOTE—This applies to all of the solution preparations. The addition, with sonication, of a small amount of acetonitrile (NMT 0.5 mL per 25 mg) to the weighed quantity of the samples may be used to aid in dissolution. Shaking and sonication may also be used after the addition of the required amount of 2.5 mM hydrochloric acid.]

System suitability solution: 5 µg/mL each of USP Vecuronium Bromide RS, USP Pancuronium Bromide RS, USP Vecuronium Bromide Related Compound A RS, USP Vecuronium Bromide Related Compound B RS, USP Vecuronium Bromide Related Compound C RS, and USP Vecuronium Bromide Related Compound F RS in 2.5 mM hydrochloric acid

Standard solution: 0.005 mg/mL of USP Vecuronium Bromide RS in 2.5 mM hydrochloric acid

Sample solution: 1 mg/mL of Vecuronium Bromide prepared as follows. To 25 mg of Vecuronium Bromide in a 25-mL volumetric flask add 0.5 mL of acetonitrile, sonicate, and rapidly dilute with 2.5 mM hydrochloric acid.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with 4-mm cation suppressor
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Flow rate (for cation suppressor): 2 mL/min
Injection size: 25 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The system may need equilibration for 4 h. Rinse and store the column in acetonitrile and water (1:1).]
Suitability requirements
Peak-to-valley ratio: NLT 2.0 between the vecuronium bromide related compound F peak and the height of the trough between the vecuronium bromide related compound F and pancuronium peaks, *System suitability solution*
Relative standard deviation: NMT 10.0% for three replicate injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Vecuronium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area response for each impurity from the *Sample solution*
r_S = peak area response for USP Vecuronium Bromide RS from the *Standard solution*
C_S = concentration of the USP Vecuronium Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Vecuronium Bromide in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pancuronium bromide	0.5	1.1	0.5

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Vecuronium bromide related compound F[a]	0.6	1.3	0.5
Vecuronium bromide related compound C[b]	0.9	1.4	0.5
Vecuronium bromide	1.0	—	—
Vecuronium bromide related compound A[c]	1.8	0.4	0.3
Vecuronium bromide related compound B[d]	2.2	1.0	0.5
Any other individual unidentified impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] 3-Deacetyl vecuronium bromide; piperidinium, 1-[(2β,3α,5α,16β,17β)-17-acetyloxy-3-hydroxy-2-(1-piperidinyl) androstan-16-yl]-1-methyl bromide.
[b] 3,17-bis Deacetyl vecuronium bromide; piperidinium, 1-[(2β,3α,5α,16β,17β)-3,17-dihydroxy-2-(1-piperidinyl) androstan-16-yl]-1-methyl bromide.
[c] Dipiperidino diol diacetate; 3α,17β-diacetyl-oxy-2β,16β-bispiperidinyl-5α-androstan.
[d] 17-Deacetyl vecuronium bromide; piperidinium, 1-[(2β,3α,5α,16β,17β)-3-acetyloxy-17-hydroxy-2-(1-piperidinyl) androstan-16-yl]-1-methyl bromide.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL, in dehydrated alcohol
  Acceptance criteria: −16° to −20° at 20°
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 10 USP Endotoxin Units/mg of vecuronium bromide
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 2 h: it loses NMT 2.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Pancuronium Bromide RS
  3α,17β-dihydroxy-2β,16β-dipiperidinyl-5α-androstane, 3,17-diacetate, dimethobromide.
  $C_{35}H_{60}Br_2N_2O_4$ 732.67
  USP Vecuronium Bromide RS
  USP Vecuronium Bromide Related Compound A RS
  3α,17β-diacetyl-oxy-2β,16β-bispiperidinyl-5α-androstan.
  $C_{33}H_{54}N_2O_4$ 542.79
  USP Vecuronium Bromide Related Compound B RS
  Piperidinium, 1-[(2β,3α,5α,16β,17β)-3-acetyloxy-17-hydroxy-2-(1-piperidinyl) androstan-16-yl]-1-methyl bromide.
  $C_{32}H_{55}BrN_2O_3$ 595.69
  USP Vecuronium Bromide Related Compound C RS
  Piperidinium, 1-[(2β,3α,5α,16β,17β)-3,17-dihydroxy-2-(1-piperidinyl) androstan-16-yl]-1-methyl bromide.
  $C_{30}H_{53}BrN_2O_2$ 553.66
  USP Vecuronium Bromide Related Compound F RS
  Piperidinium, 1-[(2β,3α,5α,16β,17β)-17-acetyloxy-3-hydroxy-2-(1-piperidinyl) androstan-16-yl]-1-methyl bromide.
  $C_{32}H_{55}BrN_2O_3$ 595.69

Venlafaxine Tablets

DEFINITION

Venlafaxine Tablets contain an amount of venlafaxine hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of venlafaxine free base ($C_{17}H_{27}NO_2$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 3.4 g of potassium dihydrogen phosphate in 700 mL of water. Add 5 mL of triethylamine, and adjust with phosphoric acid to a pH of 3.0.
  Diluent: Methanol and 0.1 N hydrochloric acid (80:20)
  Mobile phase: Acetonitrile and *Buffer* (30:70)
  Standard solution: 0.3 mg/mL of venlafaxine free base from a suitable quantity of USP Venlafaxine Hydrochloride RS in *Diluent*
  Sample solution: Nominally 0.3 mg/mL of venlafaxine (from NLT 20 finely powdered Tablets) in *Diluent*. Sonicate for 30 min. Centrifuge and pass a portion of the supernatant through a suitable membrane filter of 0.45-µm pore size. [NOTE—A centrifuge speed of 4000 rpm for 10 min may be suitable.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 275 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.0 mL/min
  Injection volume: 10 µL
  Run time: 5 times the retention time of the venlafaxine peak
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of venlafaxine free base ($C_{17}H_{27}NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of venlafaxine free base in the *Standard solution* (mg/mL)
C_U = nominal concentration of venlafaxine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of venlafaxine free base ($C_{17}H_{27}NO_2$)

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: Water, deaerated; 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min

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Standard stock solution: 0.84 mg/mL of venlafaxine free base from a suitable quantity of USP Venlafaxine Hydrochloride RS in methanol
Standard solution: (*L*/1000) mg/mL of venlafaxine free base in *Medium*, where *L* is the label claim in mg/Tablet, from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Detector: UV 275 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of venlafaxine free base ($C_{17}H_{27}NO_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of venlafaxine free base in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of venlafaxine free base ($C_{17}H_{27}NO_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: Prepare as directed in the *Assay*.
Solution A: Acetonitrile and *Buffer* (20:80)
Solution B: Acetonitrile and *Buffer* (35:65)
Diluent: Acetonitrile and *Buffer* (30:70)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
25	0	100
30	0	100
32	100	0
45	100	0

System suitability solution: 0.0018 mg/mL of USP Venlafaxine Related Compound A RS and 0.6 mg/mL of USP Venlafaxine Hydrochloride RS in *Diluent*
Standard stock solution: 0.6 mg/mL of USP Venlafaxine Hydrochloride RS in *Diluent*
Standard solution: 0.0018 mg/mL of USP Venlafaxine Hydrochloride RS in *Diluent* from the *Standard stock solution*
Sample solution: Nominally 0.6 mg/mL of venlafaxine (from NLT 20 finely powdered Tablets) in *Diluent*. Centrifuge and pass a portion through a suitable membrane filter of 0.45-µm pore size. [NOTE—Sonicate, if necessary. A centrifuge speed of 4000 rpm for 10 min may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between venlafaxine related compound A and venlafaxine, *System suitability solution*
Tailing factor: NMT 1.8, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*
r_S = peak response of venlafaxine from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of venlafaxine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Descyclohexanol venlafaxine[a]	0.4	1.3	0.20
Venlafaxine related compound A[b]	0.9	—	—
Venlafaxine	1.0	—	—
Any unspecified degradation product	—	1.0	0.20
Total degradation products	—	—	1.0

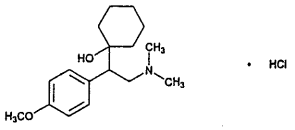
[a] 2-(4-Methoxyphenyl)-*N*,*N*-dimethylethylamine.
[b] Process impurity, included for system suitability and identification only. Controlled in the drug substance and is not to be reported or included in the total degradation products for the drug product.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Venlafaxine Hydrochloride RS
USP Venlafaxine Related Compound A RS
1-(1-(4-Methoxyphenyl)-2-(methylamino)ethyl) cyclohexanol hydrochloride.
$C_{16}H_{25}NO_2 \cdot HCl$ 299.84

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Venlafaxine Hydrochloride



$C_{17}H_{27}NO_2 \cdot HCl$ 313.86

Cyclohexanol, 1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl]-, hydrochloride;
(±)-1-[α-[(Dimethylamino)methyl]-*p*-methoxybenzyl] cyclohexanol hydrochloride [99300-78-4].

DEFINITION

Venlafaxine Hydrochloride contains NLT 98.0% and NMT 102.0% of venlafaxine hydrochloride ($C_{17}H_{27}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  [NOTE—If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the Reference Standard separately in methanol or 2-propanol, evaporate to dryness, and record the new spectra of the residues.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: Phosphoric acid and water (1:10)
Buffer: 3.4 g of monobasic potassium phosphate in 700 mL of water. Adjust with *Solution A* to a pH of 3.0.
Diluent: Acetonitrile and water (50:50)
Mobile phase: Acetonitrile and *Buffer* (30:70)
Standard solution: 0.04 mg/mL of USP Venlafaxine Hydrochloride RS in *Diluent*
Sample solution: 0.04 mg/mL of Venlafaxine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30 ± 2°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of the venlafaxine peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of venlafaxine hydrochloride ($C_{17}H_{27}NO_2 \cdot HCl$) in the portion of Venlafaxine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Venlafaxine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
System suitability solution: 0.5 mg/mL of USP Venlafaxine Hydrochloride RS and 1.5 µg/mL of USP Venlafaxine Related Compound A RS in *Mobile phase*
Standard solution: 0.001 mg/mL of USP Venlafaxine Hydrochloride RS in *Mobile phase*
Sample solution: 1 mg/mL of Venlafaxine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30 ± 2°
Flow rate: 0.7 mL/min
Injection volume: 10 µL
Run time: 7 times the retention time of the venlafaxine peak
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between venlafaxine and venlafaxine related compound A
Relative standard deviation: NMT 2.0% for the venlafaxine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Venlafaxine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of venlafaxine from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Venlafaxine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity peak (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Descyclohexanol venlafaxine[a]	0.6	1.3	0.15
Didesmethyl venlafaxine[b]	0.8	1.0	0.15
Venlafaxine related compound A	0.9	1.0	0.15

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Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Venlafaxine	1.0	—	—
Deoxy venlafaxine[c]	3.1	0.75	0.15
Any individual unknown impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 2-(4-Methoxyphenyl)-*N*,*N*-dimethylethylamine.
[b] 1-[2-Amino-1-(4-methoxyphenyl)ethyl]cyclohexanol.
[c] 2-Cyclohexyl-2-(4-methoxyphenyl)-*N*,*N*-dimethylethylamine.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at 105° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Venlafaxine Hydrochloride RS
  USP Venlafaxine Related Compound A RS
  1-(1-(4-Methoxyphenyl)-2-(methylamino)ethyl) cyclohexanol hydrochloride.
  $C_{16}H_{25}NO_2 \cdot HCl$ 299.84

Venlafaxine Hydrochloride Extended-Release Capsules

DEFINITION
Venlafaxine Hydrochloride Extended-Release Capsules contain an amount of Venlafaxine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Wavelength range: 250–310 nm
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, triethylamine, and water (250:4:750). Adjust with phosphoric acid to a pH of 3.5.
  Standard solution: 0.25 mg/mL of USP Venlafaxine Hydrochloride RS in *Mobile phase*
  Sample stock solution: Nominally 1.0 mg/mL of venlafaxine (from the contents of NLT 10 Capsules) prepared as follows. Transfer a weighed quantity of Capsule contents to a suitable volumetric flask. Add 8% of the flask volume of acetonitrile, and shake for 40 min. Add 50% of flask volume of *Mobile phase*, and shake for an additional 20 min. Dilute with *Mobile phase* to volume. Pass a portion through a suitable filter of 0.45-µm pore size.
  Sample solution: 0.25 mg/mL of venlafaxine (using the filtrate from the *Sample stock solution*) in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 226 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  Run time: 1.5 times the retention time of venlafaxine
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 1.5%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of venlafaxine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: Water; 900 mL
  Apparatus 1: 100 rpm
  Times: 3, 6, 16, and 24 h
  Mobile phase: Acetonitrile, triethylamine, and water (450:4:550). Adjust with phosphoric acid to a pH of 3.5.
  Standard stock solution: 0.1 mg/mL of USP Venlafaxine Hydrochloride RS in water
  Standard solution: 0.05 mg/mL of USP Venlafaxine Hydrochloride RS in acetonitrile, from the *Standard stock solution*
  Sample stock solution: Pass a portion of the solution under test through a suitable filter.
  Sample solution: *Sample stock solution* and acetonitrile (50:50)
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 274 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 60 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times D \times (M_{r1}/M_{r2})$$

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r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, 2
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - (2 \times V_S))] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V - (3 \times V_S))] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 1*.

Table 1

Time Point, *i*	Time (h)	Amount Dissolved
1	3	NMT 40%
2	6	35%–60%
3	16	60%–85%
4	24	NLT 75%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 4, 8, 12, and 20 h
Capsule correction solution: Dissolve 6 empty Capsule shells in 900 mL of water.
Blank: Dilute 150 mL of *Capsule correction solution* with water to 900 mL.
Standard solution: (*L*/900) mg/mL of USP Venlafaxine Hydrochloride RS, where *L* is the label claim, in mg/Capsule, prepared as follows. To a weighed amount of the Standard equivalent to the sample claim, add *Capsule correction solution* to fill 17% of final flask volume. Dilute with water to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Detector: 274 nm
Analysis
Samples: *Standard solution* and *Sample solution*

[NOTE—If necessary, the volume of *Medium* may be corrected for volumes removed from any previous sample time points.]
Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - (2 \times V_S))] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_i = \{[C_i \times (V - ([i - 1] \times V_S))] + [(C_{i-1} + C_{i-2} + \ldots + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 2*.

Table 2

Time Point, *i*	Time (h)	Amount Dissolved
1	2	10%–30%
2	4	33%–53%
3	8	58%–78%
4	12	68%–88%
5	20	NLT 80%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Times: 4, 8, and 16 h
Buffer: Dissolve 1.4 g of monobasic potassium phosphate in 1 L of water. Add 5 mL of triethylamine, and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard stock solution: 0.9 mg/mL of USP Venlafaxine Hydrochloride RS in *Medium*
Standard solution: (*L*/750) mg/mL of USP Venlafaxine Hydrochloride RS in *Medium* from the *Standard stock*

USP Monographs

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solution, where *L* is the label claim, in mg/Capsule. Pass a portion through a suitable filter of 0.45-µm pore size.

Sample solution: At the end of the specified time interval, withdraw a known volume of the solution from the dissolution vessel, and replace with an equal volume of fresh *Medium*. Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Column temperature: 30°
Injection volume: 10 µL
Run time: 2 times the retention time of venlafaxine

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times V] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 3*.

Table 3

Time Point, *i*	Time (h)	Amount Dissolved
1	4	35%–55%
2	8	65%–90%
3	16	NLT 85%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 4, 8, 12, and 20 h
Solution A: Dilute 10 mL of phosphoric acid with water to 100 mL.
Buffer: 11.4 g/L of ammonium dihydrogen phosphate in water
Mobile phase: Acetonitrile and *Buffer* (35:65). Adjust with *Solution A* to a pH of 4.4.
Standard stock solution: 0.24 mg/mL of USP Venlafaxine Hydrochloride RS in *Medium*. Sonication may be used to aid in dissolution.
Standard solution: See *Table 4* for the concentration of USP Venlafaxine Hydrochloride RS in *Medium* from the *Standard stock solution*. Using a glass syringe, pass a portion through a suitable filter of 0.45-µm pore size.

Table 4

Label Claim (*L*)	Standard Solution (mg/mL)
37.5	0.05
75	0.1
150	0.1

Sample solution: At the end of the specified time interval, withdraw a known volume of the solution from the dissolution vessel, and replace with an equal volume of fresh *Medium*. For Capsules that are labeled to contain 150 mg of venlafaxine, dilute this solution with an equal volume of *Medium*. Using a glass syringe, pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of venlafaxine

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times D \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, 2 for Capsules labeled to contain 150 mg of venlafaxine; 1 for Capsules labeled to contain 37.5 or 75 mg of venlafaxine
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

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Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times V] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{[C_5 \times V] + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 5*.

Table 5

Time Point, *i*	Time (h)	Amount Dissolved
1	2	10%–30%
2	4	35%–55%
3	8	60%–80%
4	12	NLT 70%
5	20	NLT 85%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 5, 8, and 20 h
Buffer: 11.4 g/L of monobasic ammonium phosphate in water. Adjust with dilute phosphoric acid (1 in 10) or dilute ammonia solution (1 in 10) to a pH of 4.4.
Mobile phase: Acetonitrile and *Buffer* (25.5: 74.5)
Standard solution: (*L*/900) mg/mL of USP Venlafaxine Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Capsule
Sample solution: At the end of the specified time interval, withdraw a known volume of the solution from the dissolution vessel, and replace with an equal volume of fresh *Medium*. Pass a portion of the withdrawn sample through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 1.5 times the retention time of venlafaxine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times V] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 6*.

Table 6

Time Point, *i*	Time (h)	Amount Dissolved
1	2	NMT 20%
2	5	35%–55%
3	8	60%–80%
4	20	NLT 80%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: Water; 900 mL, deaerated
Apparatus 1: 100 rpm
Times: 2, 4, 8, 12, and 24 h
Buffer: 10 mL/L of triethylamine in water adjusted with phosphoric acid to a pH of 3.0
Mobile phase: Acetonitrile and *Buffer* (20:80)
Standard solution: (*L*/900) mg/mL of venlafaxine from USP Venlafaxine Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Capsule
Sample solution: Centrifuge a portion of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 2.5 mL/min

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Injection volume: 20 µL
Run time: 1.5 times the retention time of venlafaxine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - (2 \times V_S))] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V - (3 \times V_S))] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{[C_5 \times (V - (4 \times V_S))] + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 7*.

Table 7

Time Point, *i*	Time (h)	Amount Dissolved
1	2	NMT 30%
2	4	40%–60%
3	8	60%–80%
4	12	70%–90%
5	24	NLT 85%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 4, 8, 12, and 20 h
Buffer: 1.7 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid (1 in 10) to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (80:20)
Standard solution: (*L*/900) mg/mL of USP Venlafaxine Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Capsule
Sample solution: At the end of the specified time interval, withdraw a known volume of the solution from the dissolution vessel, and replace with an equal volume of fresh *Medium*. Pass a portion of the withdrawn sample through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of venlafaxine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times V] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{[C_5 \times V] + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 8*.

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Table 8

Time Point, *i*	Time (h)	Amount Dissolved
1	2	NMT 10%
2	4	NMT 30%
3	8	40%–70%
4	12	60%–90%
5	20	NLT 80%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Times: 1, 6, 16, and 24 h

Diluent: Acetonitrile and water (30:70)

Buffer: Dissolve 8.9 g of dibasic sodium phosphate dihydrate and 2.5 g of sodium 1-octanesulfonate in 1 L of water. Adjust with 10% phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (32:68)

Standard stock solution: 0.9 mg/mL of USP Venlafaxine Hydrochloride RS prepared as follows. Dissolve the weighed amount of the Standard first in acetonitrile using 20% of flask volume. Sonicate to dissolve, and dilute with *Diluent* to volume.

Standard solution: (*L*/900) mg/mL of USP Venlafaxine Hydrochloride RS from *Standard stock solution* in *Diluent*, where *L* is the label claim, in mg/Capsule

Sample solution: At the end of the specified time interval, withdraw a known volume of the solution from the dissolution vessel, and replace with an equal volume of fresh *Medium*. Pass a portion of the withdrawn sample through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: 1.7 times the retention time of venlafaxine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of venlafaxine, 277.40

M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times V] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 900 mL

V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)

L = label claim (mg/Capsule)

Tolerances: See *Table 9*.

Table 9

Time Point, *i*	Time (h)	Amount Dissolved
1	1	NMT 25%
2	6	50%–70%
3	16	70%–95%
4	24	NLT 80%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

Medium: Water; 900 mL, degassed

Apparatus 1: 100 rpm

Times: 2, 4, 8, 12, and 20 h

Buffer: Dissolve 3.4 g of monobasic potassium phosphate in 700 mL of water. Add 5 mL of triethylamine. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (30:70)

Standard stock solution: 1.6 mg/mL of USP Venlafaxine Hydrochloride RS prepared as follows. Dissolve a weighed amount of the Standard first in methanol using 20% of flask volume. Sonicate to dissolve, and dilute with water to volume.

Standard solution: (*L*/900) mg/mL of USP Venlafaxine Hydrochloride RS from the *Standard stock solution* in *Medium*, where *L* is the label claim, in mg/Capsule

Sample solution: At the end of the specified time interval, withdraw a known volume of the solution from the dissolution vessel, and replace it with an equal volume of fresh *Medium*. Pass a portion of the withdrawn sample through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 2 times the retention time of venlafaxine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

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Calculate the concentration, C_i, of venlafaxine ($C_{17}H_{27}NO_2$) in *Medium* (mg/mL) after time point *(i)*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at each time point *(i)*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{[C_5 \times V] + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of venlafaxine in *Medium* in the portion of sample withdrawn at the specified time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium* (mL)
L = label claim (mg/Capsule)

Tolerances: See *Table 10*.

Table 10

Time Point, *i*	Time (h)	Amount Dissolved
1	2	NMT 25%
2	4	30%–50%
3	8	55%–80%
4	12	65%–90%
5	20	NLT 80%

The percentages of the labeled amount of venlafaxine ($C_{17}H_{27}NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, and Sample solution: Proceed as directed in the *Assay*.
System suitability solution: 0.25 µg/mL of USP Venlafaxine Related Compound A RS in the *Standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 4 times the retention time of venlafaxine
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for venlafaxine related compound A and venlafaxine are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between venlafaxine related compound A and venlafaxine
Tailing factor: NMT 2.0 for venlafaxine
Relative standard deviation: NMT 5.0% for venlafaxine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

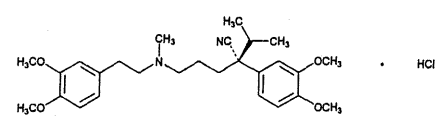
r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of venlafaxine from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of venlafaxine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Acceptance criteria
Individual impurities: NMT 0.2%
Total impurities: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Venlafaxine Hydrochloride RS
USP Venlafaxine Related Compound A RS
1-(1-(4-Methoxyphenyl)-2-(methylamino)ethyl) cyclohexanol hydrochloride.
$C_{16}H_{25}NO_2 \cdot HCl$ 299.84

Verapamil Hydrochloride



$C_{27}H_{38}N_2O_4 \cdot HCl$ 491.06
Benzeneacetonitrile, α-[3-[[2-(3,4-dimethoxyphenyl)ethyl] methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)-, monohydrochloride, (±)-;
(±)-5-[(3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile monohydrochloride [152-11-4].

DEFINITION
Verapamil Hydrochloride contains NLT 98.0% and NMT 102.0% of Verapamil Hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$), calculated on the dried basis.

USP Monographs

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IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak for verapamil in the *Sample solution* corresponds to that of *Standard solution B*, as obtained in the test for *Organic Impurities*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 1.23 g/L of sodium acetate containing about 33 mL/L of glacial acetic acid
Mobile phase: Acetonitrile, 2-aminoheptane, and *Buffer* (300:5:700)
Standard solution: 1 mg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*
Sample solution: 1 mg/mL of Verapamil Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.9 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of verapamil
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Verapamil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of verapamil from the *Sample solution*
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Verapamil Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1.9 mg/mL of USP Verapamil Hydrochloride RS and 1.5 mg/mL of USP Verapamil Related Compound B RS in *Mobile phase*
Standard solution A: 5.6 µg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*
Standard solution B: 9.4 µg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*
Sample solution: 1.9 mg/mL of Verapamil Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 12.5- to 15-cm; packing L1
Flow rate: 0.9 mL/min
Injection volume: 10 µL
Run time: NLT 4 times the retention time of verapamil
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for verapamil related compound B and verapamil are 0.88 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the verapamil related compound B and verapamil peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution*, *Standard solution A*, and *Standard solution B*
Acceptance criteria
Individual impurities: Any single peak response is not greater than that of the verapamil peak response from *Standard solution A* (0.3%).
Total impurities: The sum of the peak responses, other than that of verapamil, from the *Sample solution* is not greater than the verapamil peak response from *Standard solution B* (0.5%).

SPECIFIC TESTS

- **pH ⟨791⟩**

Sample solution: 50 mg/mL. Prepare with gentle heating.
Acceptance criteria: 4.5–6.5

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Verapamil Hydrochloride RS
USP Verapamil Related Compound B RS
Benzeneacetonitrile, α-[2-[[2-(3,4-dimethoxyphenyl)-ethyl]methylamino]ethyl]-3,4-dimethoxy-α-(1-methylethyl)-, monohydrochloride.
$C_{26}H_{36}N_2O_4 \cdot HCl$ 477.05

Verapamil Hydrochloride Extended-Release Capsules

DEFINITION

Verapamil Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.01 N sodium acetate in water containing 33 mL/L of glacial acetic acid

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Mobile phase: Acetonitrile, 2-aminoheptane, and *Solution A* (30: 0.5: 70)
System suitability solution: 0.12 mg/mL of USP Verapamil Hydrochloride RS and 0.1 mg/mL of USP Verapamil Related Compound B RS in *Mobile phase*
Standard solution: 0.12 mg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*
Sample stock solution: Nominally 1.2 mg/mL of verapamil hydrochloride prepared as follows. Transfer an equivalent to 240 mg of verapamil hydrochloride, from the pool of Capsule contents (NLT 20), to a 200-mL volumetric flask. Add about 150 mL of *Mobile phase* (prewarm the *Mobile phase* to 45°). While sonicating, stir for 1 h, cool to room temperature, dilute with *Mobile phase* to volume, and mix. Centrifuge a portion for 20 min, and use the supernatant.
Sample solution: Nominally 0.12 mg/mL of verapamil hydrochloride in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm. For *Identification B*, use a diode array detector in the range of 220–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of verapamil
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for verapamil related compound B and verapamil are 0.84 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between verapamil and verapamil related compound B, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of verapamil from the *Sample solution*
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 100 rpm. Use a wire helix sinker as necessary.
Times: 2, 4, 8, and 24 h
Solution A, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*, except for the *Injection volume*.
Injection volume: 30 µL
System suitability solution: 0.25 mg/mL of USP Verapamil Hydrochloride RS and 0.2 mg/mL of USP Verapamil Related Compound B RS in *Medium*
Standard solution: 0.267 mg/mL of USP Verapamil Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between verapamil and verapamil related compound B, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at each time point (*i*):

$$Q_2 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_4 = (Q_2 \times V_{S1}/V) + [(r_U/r_S) \times (C_S/L) \times (V - V_{S1}) \times 100]$$

$$Q_8 = (Q_2 \times V_{S1}/V) + \{Q_4 \times V_{S2}/[V - (V_{S1} + V_{S2})]\} + \{(r_U/r_S) \times (C_S/L) \times [V - (V_{S1} + V_{S2})] \times 100\}$$

$$Q_{24} = (Q_2 \times V_{S1}/V) + \{Q_4 \times V_{S2}/[V - (V_{S1} + V_{S2})]\} + \{Q_8 \times V_{S3}/[V - (V_{S1} + V_{S2} + V_{S3})]\} + \{(r_U/r_S) \times (C_S/L) \times [V - (V_{S1} + V_{S2} + V_{S3})] \times 100\}$$

r_U = peak response of verapamil from the *Sample solution*
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution*
L = label claim (mg/Capsule)
V = initial volume of *Medium*, 900 mL
V_{Si} = volume of *Medium* taken at each time point (mL)

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
2	10–25
4	15–40
8	40–65
24	NLT 80

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Apparatus 2, Times, Solution A, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in *Test 1*.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
2	NMT 25

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Table 2 *(continued)*

Time (h)	Amount Dissolved (%)
4	15–40
8	40–65
24	NLT 80

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Mobile phase, and **Sample stock solution:** Prepare as directed in the *Assay*.

System suitability solution: 6 µg/mL of USP Verapamil Hydrochloride RS and 4.8 µg/mL of USP Verapamil Related Compound B RS in *Mobile phase*

Standard solution: 6 µg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*

Sample solution: Use the *Sample stock solution*.

Chromatographic system: Proceed as directed in the *Assay*, except for the *Injection volume* and *Run time*.

Injection volume: 50 µL

Run time: NLT 5 times the retention time of verapamil

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 3* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between verapamil and verapamil related compound B, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of verapamil from the *Standard solution*

C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Verapamil related compound B[a]	0.84	—
Verapamil	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] For resolution measurement only. Do not include it in the total.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Verapamil Hydrochloride RS

USP Verapamil Related Compound B RS

Benzeneacetonitrile, α-[2-[[2-(3,4-dimethoxyphenyl)-ethyl]methylamino]ethyl]-3,4-dimethoxy-α-(1-methylethyl)-, monohydrochloride.

$C_{26}H_{36}N_2O_4 \cdot HCl$ 477.05

Verapamil Hydrochloride Injection

DEFINITION

Verapamil Hydrochloride Injection is a sterile solution of Verapamil Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A.** ▲The UV spectrum of the major peak of the *Diluted sample solution* corresponds to that of the *Diluted standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

ASSAY

Change to read:

- **PROCEDURE**

Solution A: 0.015 N sodium acetate solution containing 33 mL of glacial acetic acid per liter

Mobile phase: Acetonitrile, *Solution A*, and 2-aminoheptane (30: 70: 0.5)

System suitability solution: 1.9 mg/mL of USP Verapamil Hydrochloride RS and 1.5 mg/mL of USP Verapamil Related Compound B RS in *Mobile phase*

Standard solution: 2.5 mg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*

▲**Diluted standard solution:** 0.5 mg/mL of USP Verapamil Hydrochloride RS from the *Standard solution* in *Mobile phase*▲ (USP 1-May-2019)

Sample solution: Nominally 2.5 mg/mL of verapamil hydrochloride from a volume of Injection in *Mobile phase*

▲**Diluted sample solution:** Nominally 0.5 mg/mL of verapamil hydrochloride from the *Sample solution* in *Mobile phase*▲ (USP 1-May-2019)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)

Column: 4.6-mm × ▲▲ (USP 1-May-2019) 15-cm; ▲5-µm▲ (USP 1-May-2019) packing L1

Flow rate: 0.9 mL/min

Injection volume: 10 µL

Run time: NLT 4 times the retention time of verapamil

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System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for verapamil related compound B and verapamil are about 0.88 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between verapamil related compound B and verapamil, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution*, ▲*Diluted standard solution*,▲ (USP 1-May-2019) *Sample solution*, ▲and *Diluted sample solution*
[NOTE—The *Diluted standard solution* and *Diluted sample solution* are used for *Identification A*.▲ (USP 1-May-2019)]
Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of verapamil from the *Sample solution*
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Solution A, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:**
Proceed as directed in the *Assay*.
Standard solution: 2.5 mg/mL of USP Verapamil Hydrochloride RS, and 7.5 µg/mL each of USP Verapamil Related Compound A RS, USP Verapamil Related Compound E RS, and USP Verapamil Related Compound F RS in *Mobile phase*
System suitability
▲Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between verapamil related compound B and verapamil, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 15 for verapamil related compound F, *Standard solution*▲ (USP 1-May-2019)
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each ▲specified degradation product▲ (USP 1-May-2019) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ▲verapamil related compound A, E, or F▲ (USP 1-May-2019) from the *Sample solution*
r_S = peak response of ▲verapamil related compound A, E, or F▲ (USP 1-May-2019) from the *Standard solution*
C_S = concentration of ▲USP Verapamil Related Compound A RS, USP Verapamil Related Compound E RS, or USP Verapamil Related Compound F RS▲ (USP 1-May-2019) in the *Standard solution* (mg/mL) ▲▲ (USP 1-May-2019)
C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (mg/mL)

▲Calculate the percentage of any unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (mg/mL)▲ (USP 1-May-2019)

Acceptance criteria: See *Table 1*.

▲Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Verapamil related compound F	0.4	0.3
Verapamil related compound A	0.5	0.3
Verapamil related compound E	0.7	0.3
Verapamil related compound B[a]	0.88	—
Verapamil	1.0	—
Any unspecified degradation product	—	0.2
Total degradation products	—	1.0

[a] For resolution measurement only. It is not to be reported and not to be included in the total degradation products.▲ (USP 1-May-2019)

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.0–6.5
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 16.7 USP Endotoxin Units/mg of verapamil hydrochloride
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, protected from light. ▲Store at controlled room temperature.▲ (USP 1-May-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Verapamil Hydrochloride RS

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USP Verapamil Related Compound A RS
▲2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-(methylamino) pentanenitrile hydrochloride. ▲ (USP 1-May-2019)
$C_{17}H_{26}N_2O_2 \cdot HCl$ 326.87
USP Verapamil Related Compound B RS
▲4-[(3,4-Dimethoxyphenethyl)(methyl)amino]-2-(3,4-dimethoxyphenyl)-2-isopropylbutanenitrile hydrochloride. ▲ (USP 1-May-2019)
$C_{26}H_{36}N_2O_4 \cdot HCl$ ▲477.04▲ (USP 1-May-2019)
USP Verapamil Related Compound E RS
3,4-Dimethoxybenzaldehyde.
▲$C_9H_{10}O_3$ 166.17▲ (USP 1-May-2019)
USP Verapamil Related Compound F RS
(3,4-Dimethoxyphenyl)methanol.
▲$C_9H_{12}O_3$ 168.19▲ (USP 1-May-2019)

Verapamil Hydrochloride Compounded Oral Solution

DEFINITION

Verapamil Hydrochloride Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

Prepare Verapamil Hydrochloride Compounded Oral Solution 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Verapamil Hydrochloride powder	5 g
Vehicle for Oral Solution (regular or sugar-free), *NF*, a sufficient quantity to make	100 mL

Add *Verapamil Hydrochloride powder* and about 40 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring the preparation to final volume, and mix well.

ASSAY

• **PROCEDURE**

Solution A: 0.01 N sodium acetate solution containing 33 mL/L of acetic acid

Mobile phase: Acetonitrile, 2-aminoheptane, and *Solution A* (50: 0.5: 50). Filter, and degas.

Standard solution: 500 µg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*

Sample solution: Agitate containers of Oral Solution for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for verapamil hydrochloride is about 4.8 min.]

Suitability requirements
Relative standard deviation: NMT 0.7% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
• **LABELING:** Label to indicate the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Verapamil Hydrochloride RS

Verapamil Hydrochloride Compounded Oral Suspension

DEFINITION

Verapamil Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

Prepare Verapamil Hydrochloride Compounded Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Verapamil Hydrochloride	5 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

If using tablets, comminute to a fine powder using a suitable mortar, or add *Verapamil Hydrochloride* powder. Add about 40 mL of the *Vehicle* in small portions, and mix to obtain a uniform paste. Transfer the mortar contents, stepwise and quantitatively, to a calibrated bottle. Add the *Vehicle* in portions to rinse the mortar, add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Solution A: 0.01 N sodium acetate solution containing 33 mL/L of acetic acid

Mobile phase: Acetonitrile, 2-aminoheptane, and *Solution A* (50: 0.5: 50). Filter, and degas.

Standard solution: 500 µg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*

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Sample solution: Agitate containers of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for verapamil hydrochloride is about 4.8 min.]
Suitability requirements
Relative standard deviation: NMT 0.7% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Verapamil Hydrochloride RS

Verapamil Hydrochloride Tablets

» Verapamil Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Verapamil Hydrochloride RS
USP Verapamil Related Compound A RS
3,4-Dimethoxy-α-[3-(methylamino)propyl]-α-(1-methylethyl)-benzeneacetonitrile monohydrochloride.
$C_{17}H_{26}N_2O_2 \cdot HCl$ 326.87
USP Verapamil Related Compound B RS
Benzeneacetonitrile, α-[2-[[2-(3,4-dimethoxyphenyl)-ethyl]methylamino]ethyl]-3,4-dimethoxy-α-(1-methylethyl)-, monohydrochloride.
$C_{26}H_{36}N_2O_4 \cdot HCl$ 477.05
USP Verapamil Related Compound E RS
3,4-Dimethoxybenzaldehyde.
USP Verapamil Related Compound F RS
(3,4-Dimethoxyphenyl)methanol.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—
Test specimen—Transfer a portion of finely powdered Tablets, equivalent to about 25 mg of verapamil hydrochloride, to a separator. Add 25 mL of water, and shake by mechanical means for 30 minutes. Add 1 mL of 1 N sodium hydroxide, and extract with 25 mL of chloroform, shaking by mechanical means for 10 minutes. Pass the chloroform extract through a filter containing anhydrous sodium sulfate. Triturate the chloroform extract with 400 mg of potassium bromide and evaporate to dryness. Dry at 105° for 2 hours.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ dissolved from the difference between UV absorbances at the wavelengths of maximum absorbance at about 278 nm and 300 nm using filtered portions of the solution under test, suitably diluted with *Medium* if necessary, in comparison with a Standard solution having a known concentration of USP Verapamil Hydrochloride RS in the same *Medium*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Transfer 1 Tablet to a 100-mL volumetric flask, add 50 mL of 0.01 N hydrochloric acid, and heat on a steam bath for 50 minutes. Sonicate the heated solution for about 10 minutes, cool, dilute with 0.01 N hydrochloric acid to volume, mix, and filter. Dilute an accurately measured portion of the filtrate quantitatively with 0.01 N hydrochloric acid to obtain a *Test preparation* containing about 48 µg of verapamil hydrochloride per mL. Dissolve an accurately weighed quantity of USP Verapamil Hydrochloride RS in 0.01 N hydrochloric acid to obtain a *Standard preparation* having a known concentration of about 48 µg per mL. Concomitantly determine the absorbances of the *Test preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 278 nm and the absorbance of the *Test preparation* at 300 nm, with a suitable spectrophotometer using 0.01 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{27}H_{38}N_2O_4 \cdot HCl$ in the Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

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in which T is the labeled quantity, in mg, of verapamil hydrochloride in the Tablet; C is the concentration, in µg per mL, of USP Verapamil Hydrochloride RS in the *Standard preparation*; D is the concentration, in µg per mL, of verapamil hydrochloride in the *Test preparation*, on the basis of the labeled quantity per Tablet and the extent of dilution; A_U is the difference between absorbances at 278 nm and 300 nm of the *Test preparation*; and A_S is the absorbance of the *Standard preparation* at 278 nm.

Related compounds—

Aqueous solvent mixture, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve accurately weighed quantities of USP Verapamil Hydrochloride RS, USP Verapamil Related Compound A RS, USP Verapamil Related Compound E RS, and USP Verapamil Related Compound F RS in *Mobile phase* to obtain a solution having known concentrations of about 1.6 mg of USP Verapamil Hydrochloride RS per mL and 0.0048 mg each of USP Verapamil Related Compound A RS, USP Verapamil Related Compound E RS, and USP Verapamil Related Compound F RS per mL.

Test solution—Use the *Assay preparation*.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than four times the retention time for verapamil. Record the chromatograms, and measure all of the peak responses. [NOTE—The retention times are about 0.4 for verapamil related compound F, 0.5 for verapamil related compound A, 0.7 for verapamil related compound E, and 1.0 for verapamil.] Calculate the quantity, in mg, of each individual impurity in the portion of Tablets taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of verapamil related compound A, verapamil related compound E, or verapamil related compound F in the *Standard solution* [NOTE—For calculating any other unspecified impurity, C is the concentration, in mg per mL, of USP Verapamil Hydrochloride RS in the *Standard solution*.] ; and r_U and r_S are the peak responses of the appropriate impurity obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.3% of any specified impurity is found; and the sum of all impurities is not more than 1.0%.

Assay—

Aqueous solvent mixture—Prepare a 0.015 N sodium acetate solution containing about 33 mL of glacial acetic acid per L.

Mobile phase—Prepare a filtered and degassed mixture of *Aqueous solvent mixture*, acetonitrile, and 2-aminoheptane (70: 30: 0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Verapamil Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 1.6 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of verapamil hydrochloride, to a stoppered centrifuge tube, and add 25 mL of *Mobile phase*. Shake by mechanical means for 15 minutes, centrifuge, and if necessary filter the supernatant.

System suitability solution—Dissolve suitable quantities of USP Verapamil Hydrochloride RS and USP Verapamil Related Compound B RS in *Mobile phase* to obtain a solution having known concentrations of about 1.9 mg per mL and 1.5 mg per mL, respectively.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 12.5- to 15-cm column that contains packing L1. The flow rate is about 0.9 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.88 for verapamil related compound B and 1.0 for verapamil; the resolution, R, between the verapamil related compound B and verapamil peaks is not less than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and allow the *Assay preparation* to elute for not less than four times the retention time for verapamil. Record the chromatograms, and measure the responses for all of the major peaks. Calculate the quantity, in mg, of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Tablets taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Verapamil Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Verapamil Hydrochloride Extended-Release Tablets

DEFINITION

Verapamil Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Diluted sample solution* corresponds to that of the *Diluted standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: To 0.82 g of sodium acetate, add 33 mL of glacial acetic acid and dilute with water to 1 L.

Mobile phase: Acetonitrile, 2-aminoheptane, and *Buffer* (60:1:140)

System suitability solution: 2.5 mg/mL of USP Verapamil Hydrochloride RS and 2.0 mg/mL of USP Verapamil Related Compound B RS in *Mobile phase*

Standard solution: 1.2 mg/mL of USP Verapamil Hydrochloride RS in *Mobile phase*

Diluted standard solution: 0.6 mg/mL of USP Verapamil Hydrochloride RS in *Mobile phase* from the *Standard solution*

Sample solution: Nominally 1.2 mg/mL of verapamil hydrochloride from Tablets prepared as follows. Transfer an amount equivalent to 240 mg of verapamil hydrochloride, from NLT 20 powdered Tablets, to a 200-mL volumetric flask, and add about 160 mL of *Mobile phase*. Sonicate for 15 min, stir for 15 min, dilute with *Mobile phase* to volume, and mix. Centrifuge a portion for 20 min, and use the supernatant.

Diluted sample solution: Nominally 0.6 mg/mL of verapamil hydrochloride in *Mobile phase* from the *Sample solution*

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 3 times the retention time of verapamil
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for verapamil related compound B and verapamil are 0.85 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between verapamil and verapamil related compound B, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution*, *Diluted standard solution*, *Sample solution*, and *Diluted sample solution*
[NOTE—The *Diluted standard solution* and *Diluted sample solution* are used for *Identification B*.]
Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of verapamil from the *Sample solution*
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of verapamil hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*. Proceed as directed in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.
Acid stage medium: Using 900 mL of simulated gastric fluid TS (without enzyme), conduct this stage of the test for 1 h.
Buffer stage medium: Using 900 mL of simulated intestinal fluid TS (without enzyme), conduct this stage of the test for 7 h.
Apparatus 2: 50 rpm
Times
Acid stage: 1 h
Buffer stage: 2, 3.5, 5, and 8 h
Standard solution: USP Verapamil Hydrochloride RS in 0.01 N hydrochloric acid
Sample solution: Pass portions of the solution under test through a suitable filter. Dilute with medium as necessary.
Blank solution: 0.01 N hydrochloric acid
Analysis: Wrap each Tablet in a wire helix to prevent the Tablets from floating. After 1 h in the *Acid stage medium*, withdraw a specimen for analysis, and carefully transfer the dosage form, including the wire helix, to a vessel containing the *Buffer stage medium*, which has been previously warmed to 37 ± 0.5°. At each time interval, pass a portion of the solution under test through a suitable glass microfiber filter paper. Dilute, if necessary, the filtered portions of the solutions under test with water at the 1-h interval and with 0.1 N hydrochloric acid at the 2-, 3.5-, 5-, and 8-h intervals. Determine the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved.
[NOTE—Use only filters that have been shown not to absorb verapamil.]
Detector: UV 278 nm
Tolerances: See *Table 1* and *Table 2*.

Table 1. For Products Labeled to Contain 180 or 240 mg

Time (h)	Amount Dissolved (%)
1	7–15
2	16–30
3.5	31–50
5	51–75
8	NLT 85

Table 2. For Products Labeled to Contain 120 mg

Time (h)	Amount Dissolved (%)
1	10–21
2	18–33
3.5	35–60
5	50–82
8	NLT 85

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. Proceed as directed for *Test 1*, except that in the *Analysis*, the Tablet is not required to be wrapped in a wire helix.
Tolerances: See *Table 3*, *Table 4*, and *Table 5*.

Table 3. For Products Labeled to Contain 240 mg

Time (h)	Amount Dissolved (%)
1	8–20
2	15–35
3.5	35–65
5	55–85
8	NLT 80

Table 4. For Products Labeled to Contain 180 mg

Time (h)	Amount Dissolved (%)
1	10–25
2	20–40
3.5	40–75
8	NLT 80

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Table 5. For Products Labeled to Contain 120 mg

Time (h)	Amount Dissolved (%)
1	10–25
2	20–40
3.5	35–70
5	55–85
8	NLT 80

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*. Proceed as directed for *Test 1*.

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved (%)
1	8–20
2	15–35
3.5	27–57
5	45–75
8	NLT 80

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: Simulated intestinal fluid TS (without enzyme); 50 mL

Apparatus 7: 20 cycles/min (see *Drug Release* ⟨724⟩)

Detector: UV 278 nm

Standard solution: USP Verapamil Hydrochloride RS in *Medium*

Analysis: Scrape about 2 mm × 2 mm of the coating from the side edge of the Tablet under test. Glue the system to a plastic rod sample holder at the area where the color has been removed. Attach each plastic sample holder to an arm of the apparatus, which reciprocates at an amplitude of about 2 cm and 15–30 cycles/min. The Tablet is continuously immersed in tubes containing 50 mL of *Medium* at 37°. At the end of each specified test interval, the systems are transferred to the next row of new test tubes containing 50 mL of fresh *Medium*. Remove the tubes after the last test interval, and allow them to cool to room temperature. Add 2.0 mL of 1.0 M phosphoric acid to each tube, and dilute with water to 50 mL. Stir and mix each tube thoroughly. Determine the percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) for the filtered portions of the solution under test, suitably diluted with *Medium*.

Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved (%)
3	NMT 10
6	20–50
9	52.5–82.5
14	NLT 85

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Phosphate buffer: Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water. Add 190 mL of 0.2 N sodium hydroxide in 400 mL of water, adjust with 0.2 N sodium hydroxide to a pH of 7.5 ± 0.1, and dilute with water to 1000 mL.

Medium: *Phosphate buffer*; 900 mL

Apparatus 2: 50 rpm

Detector: UV 278 nm

Standard solution: USP Verapamil Hydrochloride RS in *Medium*

Sample solution: Pass portions of the solution under test through a suitable filter. Dilute with *Medium* as necessary.

Analysis: Determine the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved.

Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved (%)
1	2–12
2	10–25
4	25–50
8	NLT 80

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Acid stage medium, Buffer stage medium, Apparatus 2, Times, and **Detector:** Proceed as directed for *Test 1*.

Standard solution: 0.04 mg/mL of USP Verapamil Hydrochloride RS in 0.01 N hydrochloric acid

Sample solution: Pass portions of the solution under test through a suitable filter. Dilute with 0.1 N hydrochloric acid to prepare a sample of concentration similar to that of the *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed for *Test 1*.

Calculate the concentration (C_i) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved in medium (mg/mL) at each time point (i):

$$C_i = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of verapamil from the *Sample solution*

A_S = absorbance of verapamil from the *Standard solution*

C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)

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D = dilution factor

Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved ($Q_i$), at each time point ($i$):

$$Result_1 = (C_1 \times V) \times (1/L) \times 100$$

$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_5 = (\{C_5 \times [V - (4 \times V_S)]\} + [(C_4 + C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of verapamil hydrochloride in the portion of the sample withdrawn at time point i (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point from the medium in the *Buffer stage* (mL)

Tolerances: See *Table 9*.

Table 9

Time Point (i)	Time (h)	Amount Dissolved (%)		
		Tablet Strength—240 mg	Tablet Strength—180 mg	Tablet Strength—120 mg
1	1	10–25	10–25	15–30
2	2	25–45	27–47	35–55
3	3.5	50–75	55–80	60–85
4	5	70–90	NLT 75	NLT 80
5	8	NLT 85	NLT 85	NLT 85

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Acid stage medium: Simulated gastric fluid TS (without enzyme); 900 mL
Buffer stage medium: Simulated intestinal fluid TS (without enzyme); 900 mL
Apparatus 2: 50 rpm, with a sinker (see *Dissolution* ⟨711⟩, *Figure 2a*)
Times
Acid stage : 1 h
Buffer stage : 2, 3.5, 5, and 8 h
Diluent: 0.01 N hydrochloric acid
Dilute phosphoric acid: Dilute 5.0 mL of phosphoric acid with water to 50 mL.
Buffer: 1.74 g/L of dibasic potassium phosphate in water. Adjust to a pH of 7.5 using *Dilute phosphoric acid*.
Mobile phase: Acetonitrile and *Buffer* (650:350)
Standard solution: Prepare the corresponding USP Verapamil Hydrochloride RS solutions in *Diluent* as directed in *Table 10*. Pass through a suitable membrane filter of 0.45-µm pore size.

Table 10

Tablet Strength (mg)	Concentration of USP Verapamil Hydrochloride RS (µg/mL)
240	270
120	135
180	203

Sample solutions: Pass a portion of the solution under test at each time point through a suitable filter of 1-µm pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 1.4 times the retention time of verapamil
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solutions*
After 1 h in the *Acid stage medium*, withdraw 10 mL of the solution under test. Carefully transfer the dosage form including the sinker to a vessel containing the *Buffer stage medium*, previously warmed to 37 ± 0.5°.
Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved in the *Acid stage medium*:

$$Result = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of verapamil from the *Sample solutions*
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Acid stage medium*, 900 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) in the sample withdrawn from the vessel at each *Buffer stage* time point (i):

$$Result_i = (r_U/r_S) \times C_S$$

r_U = peak response of verapamil from the *Sample solution* at each time point (i)
r_S = peak response of verapamil from the *Standard solution*
C_S = concentration of USP Verapamil Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at each time point (i):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$

USP Monographs

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$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of verapamil hydrochloride in the *Sample solution* at the specified time point *i* (mg/mL)
V = volume of *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 11*.

Table 11

Time Point (*i*)	Time (h)	Amount Dissolved (%)		
		Tablet Strength—240 mg	Tablet Strength—180 mg	Tablet Strength—120 mg
1	1	7–20	7–20	10–21
2	2	15–30	15–30	18–33
3	3.5	31–60	31–60	30–55
4	5	55–85	55–85	50–82
5	8	NLT 85	NLT 85	NLT 85

The percentages of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$Result = (r_U/r_T) \times 100$$

r_U = peak response for each unspecified degradation product from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 12*.

Table 12

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Verapamil related compound B[a]	0.85	—
Verapamil	1.0	—
Any unspecified degradation product	—	0.5
Total degradation products	—	1.0

[a] For resolution measurement only. Do not include it in the total.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **Labeling:** The labeling indicates the *Dissolution* test with which the product complies.

• **USP Reference Standards** ⟨11⟩

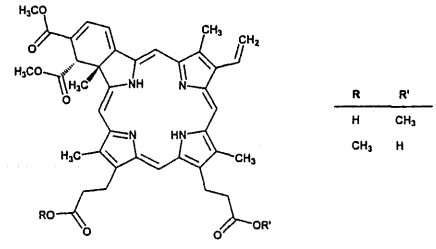
USP Verapamil Hydrochloride RS

USP Verapamil Related Compound B RS

Benzeneacetonitrile, 4-[(3,4-Dimethoxyphenethyl)(methyl)amino]-2-(3,4-dimethoxyphenyl)-2-isopropylbutanenitrile hydrochloride.

$C_{26}H_{36}N_2O_4 \cdot HCl$ 477.04

Verteporfin



$C_{41}H_{42}N_4O_8$ 718.79

23*H*,25*H*-Benzo[*b*]porphine-9,13-dipropanoic acid, 18-ethenyl-4,4a-dihydro-3,4-bis(methoxycarbonyl)-4a,8,14,19-tetramethyl-, monomethyl ester, *trans*-; (±)-*trans*-3,4-Dicarboxy-4,4a-dihydro-4a,8,14,19-tetramethyl-18-vinyl-23*H*,25*H*-benzo[*b*]porphine-9,13-dipropanoic acid, 3,4,9-trimethyl ester mixture with (±)-*trans*-3,4-dicarboxy-4,4a-dihydro-4a,8,14,19-tetramethyl-18-vinyl-23*H*,25*H*-benzo[*b*]porphine-9,13-dipropionic acid, 3,4,13-trimethyl ester [129497-78-5].

DEFINITION

Verteporfin contains NLT 94.0% and NMT 102.0% of verteporfin ($C_{41}H_{42}N_4O_8$), calculated on the anhydrous basis.

[**Caution**—Verteporfin is a light-activated drug used in photodynamic therapy. Care should be taken to avoid contact with eyes and skin.]

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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ASSAY

• **PROCEDURE**

Solution A: Acetonitrile, 1% (w/v) aqueous ammonium sulfate, glacial acetic acid, and 3.6 M sulfuric acid (10: 10: 1: 0.027)

Solution B: Tetrahydrofuran, 1% (w/v) aqueous ammonium sulfate, glacial acetic acid, and 3.6 M sulfuric acid (10: 10: 1: 0.034)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
60	80	20
90	60	40
91	60	40
120	30	70
121	30	70
125	0	100
137	0	100
140	80	20
150	80	20

Diluent: Acetonitrile and tetrahydrofuran (1:1)

Standard solution: 0.25 mg/mL of USP Verteporfin RS prepared as follows. Transfer USP Verteporfin RS to a suitable volumetric flask, add *Diluent* equivalent to 60% of the flask volume and dissolve, then dilute with water to volume. Protect this solution from light.

Sample solution: Equivalent to 0.25 mg/mL of Verteporfin prepared as follows. Transfer 25 mg of Verteporfin to a 100-mL volumetric flask, add 60 mL of *Diluent* and dissolve, then dilute with water to volume. Protect this solution from light.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Vis 410 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between the two verteporfin regioisomer peaks

Tailing factor: NMT 1.3

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of verteporfin ($C_{41}H_{42}N_4O_8$) in the portion of Verteporfin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of two verteporfin regioisomer peak responses from the *Sample solution*

r_S = sum of two verteporfin regioisomer peak responses from the *Standard solution*

C_S = concentration of USP Verteporfin RS in the *Standard solution* (mg/mL)

C_U = concentration of Verteporfin in the *Sample solution* (mg/mL)

Calculate the ratio for the two verteporfin regioisomer peaks from the *Sample solution*.

Acceptance criteria

Assay: 94.0%–102.0% on the anhydrous basis

Peak ratio: 0.9–1.1

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**

Procedure

Solution A, Solution B, Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.25 µg/mL of USP Verteporfin RS in water from the *Standard solution*

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Resolution: NLT 2.5 between the two verteporfin peaks, *Standard solution*

Tailing factor: NMT 1.3, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Verteporfin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Impurity	0.56	0.6
Verteporfin (first regioisomer peak)	1.0	—
Any other individual impurity	—	0.8
Total impurities	—	4.0

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^2 cfu/g.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Unit/mg of verteporfin

• **WATER DETERMINATION,** *Method Ic* ⟨921⟩: NMT 1.4%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a freezer.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Verteporfin RS

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Verteporfin for Injection

DEFINITION
Verteporfin for Injection is a sterile mixture of verteporfin and lipids. It contains NLT 91.0% and NMT 110.0% of the labeled amount of verteporfin ($C_{41}H_{42}N_4O_8$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
  Sample solution: 10 µg/mL in methanol
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the two major peaks of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, tetrahydrofuran, 1% (w/v) ammonium sulfate solution, and acetic acid (11:9:20:2), adjusted with 3.6 M sulfuric acid to a pH of 3.0
  Diluent 1: Acetonitrile and tetrahydrofuran (1:1)
  Diluent 2: Acetonitrile, tetrahydrofuran, and water (3:3:4)
  Standard solution: 0.1 mg/mL of USP Verteporfin RS prepared as follows. Prepare 0.167 mg/mL of USP Verteporfin RS in *Diluent 1*, then dilute this solution with water. Protect the solution from light.
  Sample solution: Reconstitute 1 vial of Verteporfin for Injection in deionized water to obtain 2 mg/mL of verteporfin. Transfer the contents to a 200-mL volumetric flask, rinsing the vial with *Diluent 2*. Dilute with *Diluent 2* to volume. Protect the solution from light.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV-Vis 410 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 30°
  Flow rate: 1.4 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 2.5 between the two verteporfin isomer peaks
  Tailing factor: NMT 1.3
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of verteporfin ($C_{41}H_{42}N_4O_8$) in the portion of Verteporfin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of two verteporfin isomer peak responses from the *Sample solution*
r_S = sum of two verteporfin isomer peak responses from the *Standard solution*
C_S = concentration of USP Verteporfin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of verteporfin in the *Sample solution* (mg/mL)

Acceptance criteria: 91.0%–110.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

IMPURITIES
- **LIMIT OF VERTEPORFIN RELATED COMPOUND A**
  Mobile phase, Sample solution, Chromatographic system and System suitability: Proceed as directed in the *Assay*.
  Diluent: Acetonitrile and tetrahydrofuran (1:1)
  Standard stock solution: 0.167 mg/mL of USP Verteporfin RS and 6.67 µg/mL of USP Verteporfin Related Compound A RS in *Diluent*
  Standard solution: 0.1 mg/mL of USP Verteporfin RS and 4 µg/mL of USP Verteporfin Related Compound A RS prepared as follows. Dissolve 3 parts of the *Standard stock solution* with 2 parts of water. Protect the solution from light.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV-Vis 410 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 30°
  Flow rate: 1.4 mL/min
  Injection volume: Equal volumes for *Standard solution* and *Sample solution*
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 2.5 between the two verteporfin isomer peaks
  Tailing factor: NMT 1.3
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Standard solution*
  Calculate the percentage of verteporfin related compound A in the portion of Verteporfin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of verteporfin related compound A from the *Sample solution*
r_S = peak response of verteporfin related compound A from the *Standard solution*
C_S = concentration of USP Verteporfin Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of verteporfin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 4.0%

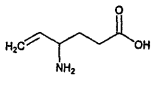
SPECIFIC TESTS
- **PYROGEN TEST ⟨151⟩**
  Test dose: 4 mg/kg
  Acceptance criteria: Meets the requirements
- **STERILITY TESTS ⟨71⟩:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 3.0%, use a mixture of methanol and formamide (7:3) as the solvent.
- **PARTICULATE MATTER IN INJECTIONS ⟨788⟩:** Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, and store at controlled room temperature, protected from light.

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- **LABELING:** The label states that it is to be protected from light after constitution, indicates that it is intended for intravenous use only, and includes the name and amount of diluent for constitution.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Verteporfin RS
  USP Verteporfin Related Compound A RS
  trans-(±)-18-Ethenyl-4,4a-dihydro-3,4-bis(methoxycarbonyl)-4a,8,14,19-tetramethyl- 23*H*, 25*H*-benzo[*b*]porphine-9,13-dipropionic acid.
  $C_{40}H_{40}N_4O_8$ 704.77

Vigabatrin



$C_6H_{11}NO_2$ 129.16
5-Hexenoic acid, 4-amino-;
4-Amino-5-hexenoic acid [68506-86-5].

DEFINITION
Vigabatrin contains NLT 98.0% and NMT 102.0% of vigabatrin ($C_6H_{11}NO_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197M▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: 3.4 g/L of monobasic potassium phosphate
  Mobile phase: Acetonitrile, methanol, and *Buffer* (4:40:1000). Adjust with phosphoric acid to a pH of 2.8.
  Standard solution: 2.0 mg/mL of USP Vigabatrin RS in *Mobile phase*
  System suitability solution: 12 µg/mL of USP Vigabatrin Related Compound A RS in the *Standard solution*
  Sample solution: 2.0 mg/mL of Vigabatrin in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25.0-cm; 10-µm packing L9
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  Run time: NLT 1.8 times the retention time of vigabatrin
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for vigabatrin related compound A and vigabatrin are about 0.5 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between vigabatrin related compound A and vigabatrin
  Relative standard deviation: NMT 1.0% for the vigabatrin peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of vigabatrin ($C_6H_{11}NO_2$) in the portion of Vigabatrin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Vigabatrin RS in the *Standard solution* (mg/mL)
C_U = concentration of Vigabatrin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **LIMIT OF γ-AMINOBUTYRIC ACID**
  Solution A: 1.6 g/L of 9-fluorenylmethyl chloroformate (FMOC) in acetone
  Buffer A: 4.1 g/L of anhydrous sodium acetate. Adjust with glacial acetic acid to a pH of 4.2.
  Buffer B: 31 g/L of boric acid. Adjust with 0.5 g/mL of sodium hydroxide to a pH of 7.7.
  Mobile phase: Acetonitrile and *Buffer A* (32:68)
  System suitability stock solution: 2.0 mg/mL of USP Vigabatrin RS and 4 µg/mL of USP γ-Aminobutyric Acid RS in water
  System suitability solution: Transfer 1.0 mL of *System suitability stock solution* to a suitable container. Pipet 2 mL of *Buffer B* into the container, and mix. Pipet 3 mL of *Solution A* into the container, mix, and allow to stand for 5 min. Pipet 3 mL of ethyl acetate into the container, shake for a few seconds, and allow the layers to separate. Use the lower layer within 8 h of preparation.
  Standard stock solution: 4 µg/mL of USP γ-Aminobutyric Acid RS in water
  Standard solution: Transfer 1.0 mL of *Standard stock solution* to a suitable container. Pipet 2 mL of *Buffer B* into the container, and mix. Pipet 3 mL of *Solution A* into the container, mix, and allow to stand for 5 min. Pipet 3 mL of ethyl acetate into the container, shake for a few seconds, and allow the layers to separate. Use the lower layer within 8 h of preparation.
  Sample stock solution: 2.0 mg/mL of Vigabatrin in water
  Sample solution: Transfer 1.0 mL of *Sample stock solution* to a suitable container. Pipet 2 mL of *Buffer B* into the container, and mix. Pipet 3 mL of *Solution A* into the container, mix, and allow to stand for 5 min. Pipet 3 mL of ethyl acetate into the container, shake for a few seconds, and allow the layers to separate. Use the lower layer within 8 h of preparation.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 263 nm
  Column: 4.6-mm × 15.0-cm; 5-µm packing L11
  Flow rate: 1 mL/min
  Injection volume: 25 µL
  Run time: NLT 1.5 times the retention time of FMOC-vigabatrin
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for (9-fluorenyl) methanol (FMOC-OH), FMOC-γ-aminobutyric acid, and FMOC-vigabatrin are about 0.5, 0.6, and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between FMOC-OH and FMOC-γ-aminobutyric acid, *System suitability solution*
  Relative standard deviation: NMT 2.0% for the FMOC-γ-aminobutyric acid peak, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of γ-aminobutyric acid in the portion of Vigabatrin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of FMOC-γ-aminobutyric acid from the *Sample solution*
r_S = peak response of FMOC-γ-aminobutyric acid from the *Standard solution*
C_S = concentration of USP γ-Aminobutyric Acid RS in the *Standard stock solution* (mg/mL)
C_U = concentration of Vigabatrin in the *Sample stock solution* (mg/mL)

Acceptance criteria: NMT 0.2% of γ-aminobutyric acid

• **ORGANIC IMPURITIES**

Buffer: 58.5 g/L of monobasic sodium phosphate in dilute phosphoric acid (23 in 1000)
Mobile phase: Acetonitrile, *Buffer*, and water (25:25:950)
System suitability solution: 6 µg/mL of USP Vigabatrin Related Compound A RS, 8 µg/mL of USP Vigabatrin Related Compound B RS, 8 µg/mL of USP Vigabatrin Related Compound E RS, and 4.0 mg/mL of USP Vigabatrin RS in *Mobile phase*
Standard solution: 6 µg/mL of USP Vigabatrin Related Compound A RS, 8 µg/mL of USP Vigabatrin Related Compound B RS, and 8 µg/mL of USP Vigabatrin Related Compound E RS in *Mobile phase*
Sample solution: 4.0 mg/mL of Vigabatrin in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Columns: *Column A* and *Column B* are coupled in series before the *Detector*.
Column A: 4.6-mm × 25.0-cm; 5-µm packing L15
Column B: 4.6-mm × 25.0-cm; 10-µm packing L9
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: NLT 2.2 times the retention time of vigabatrin
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between vigabatrin related compound A and vigabatrin
Tailing factor: NMT 2.0 each for vigabatrin related compound A and vigabatrin related compound E
Relative standard deviation: NMT 5.0% for vigabatrin related compound E
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of vigabatrin related compound A, vigabatrin related compound B, or vigabatrin related compound E in the portion of Vigabatrin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of vigabatrin related compound A, vigabatrin related compound B, or vigabatrin related compound E from the *Sample solution*
r_S = peak response of vigabatrin related compound A, vigabatrin related compound B, or vigabatrin related compound E from the *Standard solution*
C_S = concentration of USP Vigabatrin Related Compound A RS, USP Vigabatrin Related Compound B RS, or USP Vigabatrin Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Vigabatrin in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Vigabatrin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of vigabatrin related compound E from the *Standard solution*
C_S = concentration of USP Vigabatrin Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Vigabatrin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Vigabatrin related compound E	0.5	0.1
Vigabatrin related compound A	0.9	0.1
Vigabatrin	1.0	—
Vigabatrin related compound B	1.4	0.1
Any other individual impurity	—	0.1
Total impurities[a]	—	0.5

[a] Sum of all impurities found in the tests for *Organic Impurities* and *Limit of γ-Aminobutyric Acid*.

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP γ-Aminobutyric Acid RS
4-Aminobutyric acid.
$C_4H_9NO_2$ 103.12
USP Vigabatrin RS
USP Vigabatrin Related Compound A RS
5-Vinylpyrrolidin-2-one.
C_6H_9NO 111.14
USP Vigabatrin Related Compound B RS
(*E*)-2-(2-Aminoethyl)but-2-enoic acid hydrochloride.
$C_6H_{11}NO_2 \cdot HCl$ 165.62
USP Vigabatrin Related Compound E RS
2-(2-Aminobut-3-enyl)malonic acid.
$C_7H_{11}NO_4$ 173.17

Vigabatrin for Oral Solution

DEFINITION

Vigabatrin for Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of vigabatrin ($C_6H_{11}NO_2$).

USP Monographs

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Combine an appropriate number of Vigabatrin for Oral Solution packets to prepare a 50 mg/mL solution of vigabatrin in water. Pass a portion through a suitable filter, and prepare a 2-mg/mL solution by mixing a suitable portion of the filtrate with acetone. Evaporate the solution to dryness in a stream of nitrogen. Prepare a potassium bromide (KBr) pellet using a suitable amount of the residue. Alternatively, the *Sample* may be prepared by directly mixing an amount of the contents of NLT 2 packets of Vigabatrin for Oral Solution equivalent to about 3 mg of vigabatrin with about 200 mg of potassium bromide.

Acceptance criteria: The spectrum of the *Sample* corresponds to that of the spectrum of USP Vigabatrin RS prepared in a similar manner.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 3.4 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile, methanol, and *Buffer* (4:40:1000). Adjust with phosphoric acid to a pH of 2.8.

System suitability solution: 2 mg/mL of USP Vigabatrin RS and 12 µg/mL of USP Vigabatrin Related Compound A RS in *Mobile phase*

Standard solution: 2.0 mg/mL of USP Vigabatrin RS in *Mobile phase*

Sample solution: Nominally 2.0 mg/mL of vigabatrin from the contents of NLT 10 Vigabatrin for Oral Solution packets prepared as follows. Combine the contents from the packets, and transfer a suitable amount of the powder equivalent to NLT 200 mg of vigabatrin to a suitable volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 10-µm packing L9

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for vigabatrin related compound A and vigabatrin are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between vigabatrin related compound A and vigabatrin peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vigabatrin ($C_6H_{11}NO_2$) in the portion of Vigabatrin for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of vigabatrin from the *Sample solution*

r_S = peak response of vigabatrin from the *Standard solution*

C_S = concentration of USP Vigabatrin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of vigabatrin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: 1.5 g/L of ammonium acetate in water

Mobile phase: Acetonitrile and *Buffer* (5:95)

System suitability solution: 0.1 mg/mL each of USP Vigabatrin RS, USP Vigabatrin Related Compound A RS, USP Vigabatrin Related Compound B RS, and USP Povidone RS in *Mobile phase*

Sensitivity solution: 0.01 mg/mL of USP Vigabatrin Related Compound A RS in *Mobile phase*

Standard solution: 0.07 mg/mL of USP Vigabatrin Related Compound A RS in *Mobile phase*

Sample solution: Nominally 22 mg/mL of vigabatrin prepared as follows. Transfer a suitable amount of powder from the combined contents of NLT 10 Vigabatrin for Oral Solution packets, equivalent to NLT 220 mg of vigabatrin, to a suitable volumetric flask. Add *Mobile phase* to 80% of the volume of the flask. Sonication may be used to aid in dissolution. Allow the resulting solution to cool to room temperature. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Run time: 12 times the retention time of the vigabatrin peak

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between vigabatrin related compound B and povidone, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Vigabatrin for Oral Soution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of vigabatrin related compound A from the *Standard solution*

C_S = concentration of USP Vigabatrin Related Compound A in the *Standard solution*

C_U = nominal concentration of vigabatrin in the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

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Table 1

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)
Vigabatrin	0.12	—	—
Vigabatrin related compound B[b]	0.13	—	—
Povidone[c]	0.25	—	—
Vigabatrin related compound A	1.0	1.0	0.15
Any individual unspecified degradation product	—	0.026	0.15
Total impurities	—	—	0.5

[a] RRF relative to vigabatrin related compound A.
[b] Included for peak identification only. Not to be included in *Total impurities*.
[c] Povidone is due to excipient. Included for identification only. Not to be included in *Total impurities*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Povidone RS
  USP Vigabatrin RS
  USP Vigabatrin Related Compound A RS
  5-Vinylpyrrolidin-2-one.
  C_6H_9NO 111.14
  USP Vigabatrin Related Compound B RS
  (*E*)-2-(2-Aminoethyl)but-2-enoic acid hydrochloride.
  $C_6H_{11}NO_2 \cdot HCl$ 165.62

Vigabatrin Tablets

DEFINITION

Vigabatrin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of vigabatrin ($C_6H_{11}NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Grind an appropriate number of Tablets to prepare a 50-mg/mL solution of vigabatrin in water. Pass a portion of the solution through a suitable filter, and prepare a 2-mg/mL solution by mixing a suitable portion of the filtrate with acetone. Evaporate the solution to dryness in a stream of nitrogen. Prepare a potassium bromide pellet using a suitable amount of the residue. Alternatively, the *Sample* may be prepared by directly mixing an amount of finely ground Tablets (NLT 2) equivalent to about of 3 mg of vigabatrin with about 200 mg of potassium bromide.
  Acceptance criteria: The IR spectrum of the *Sample* is consistent with a similarly prepared pellet of USP Vigabatrin RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 3.4 g/L of monobasic potassium phosphate in water
  Mobile phase: Acetonitrile, methanol, and *Buffer* (4:40:1000). Adjust with phosphoric acid to a pH of 2.8.
  System suitability solution: 1.0 mg/mL of USP Vigabatrin RS and 12 µg/mL of USP Vigabatrin Related Compound A RS in *Mobile phase*
  Standard solution: 1.0 mg/mL of USP Vigabatrin RS in *Mobile phase*
  Sample stock solution: Nominally 5.0 mg/mL of vigabatrin from Tablets (NLT 10) prepared as follows. Transfer a suitable number of Tablets to a suitable volumetric flask. Add *Mobile phase* to about 80% of the flask volume, and stir for 1 h to give a uniform dispersion of fine particulate. Dilute with *Mobile phase* to volume, and pass a portion of the solution through a suitable filter of 0.45-µm pore size.
  Sample solution: Nominally 1.0 mg/mL of vigabatrin from the *Sample stock solution* and *Mobile phase*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25-cm; 10-µm packing L9
  Flow rate: 1.5 mL/min
  Injection volume: 50 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for vigabatrin related compound A and vigabatrin are about 0.7 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between vigabatrin related compound A and vigabatrin, *System suitability solution*
  Tailing factor: NMT 2.0, *Standard solution*
  Relative standard deviation: NMT 1.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of vigabatrin ($C_6H_{11}NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of vigabatrin from the *Sample solution*
r_S = peak response of vigabatrin from the *Standard solution*
C_S = concentration of USP Vigabatrin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of vigabatrin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Mobile phase: Dissolve 6 g of monobasic sodium phosphate in 800 mL of water. Add 100 mL of acetonitrile, and dilute with water to 1 L. Adjust with phosphoric acid to a pH of 2.3.
  System suitability solution: 0.6 mg/mL of USP Vigabatrin RS and 6 µg/mL of USP Vigabatrin Related Compound A RS in *Mobile phase*
  Standard solution: *L*/900 mg/mL of USP Vigabatrin RS in water
  Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 10-µm packing L9
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for vigabatrin related compound A and vigabatrin are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between vigabatrin related compound A and vigabatrin, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of vigabatrin ($C_6H_{11}NO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of vigabatrin from the *Sample solution*
r_S = peak response of vigabatrin from the *Standard solution*
C_S = concentration of USP Vigabatrin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*

Tolerances: NLT 75% (Q) of the labeled amount of vigabatrin ($C_6H_{11}NO_2$) is dissolved in 30 min.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: 1.5 g/L of ammonium acetate in water
Mobile phase: Acetonitrile and *Buffer* (5:95)
System suitability solution: 0.1 mg/mL each of USP Vigabatrin RS, USP Vigabatrin Related Compound A RS, USP Vigabatrin Related Compound B RS, and USP Povidone RS in *Mobile phase*
Sensitivity solution: 0.01 mg/mL of USP Vigabatrin Related Compound A RS in *Mobile phase*
Standard solution: 0.07 mg/mL of USP Vigabatrin Related Compound A RS in *Mobile phase*
Sample solution: Nominally 22 mg/mL of vigabatrin prepared as follows. Transfer a suitable amount of finely powdered Tablets (NLT 10) to a suitable volumetric flask. Add *Mobile phase* to 80% of the flask volume. Sonication may be used to aid in dissolution. Allow the resulting solution to cool to room temperature, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Run time: 12 times the retention time of the vigabatrin peak
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between vigabatrin related compound B and povidone, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of vigabatrin related compound A from the *Standard solution*
C_S = concentration of USP Vigabatrin Related Compound A in the *Standard solution*
C_U = nominal concentration of vigabatrin in the *Sample solution*
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)
Vigabatrin	0.12	—	—
Vigabatrin related compound B[b]	0.13	—	—
Povidone[c]	0.25	—	—
N-Carboxymethyl vinylpyrrolidinone[d]	0.38	2.1	0.15
Vigabatrin related compound A	1.0	1.0	0.3
N-3-Oxocarboxypentyl vinylpyrrolidinone[e]	1.28	1.0	0.15
Any individual unspecified degradation product	—	0.026	0.15
Total impurities	—	—	1.0

[a] RRF relative to vigabatrin related compound A.
[b] Included for peak identification only. Not to be included in *Total impurities* as it is controlled in the drug substance.
[c] Povidone is due to excipient. Included for identification only. Not to be included in *Total impurities*.
[d] 2-(2-Oxo-5-vinylpyrrolidin-1-yl)acetic acid.
[e] 4-Oxo-6-(2-oxo-5-vinylpyrrolidin-1-yl) hexanoic acid.

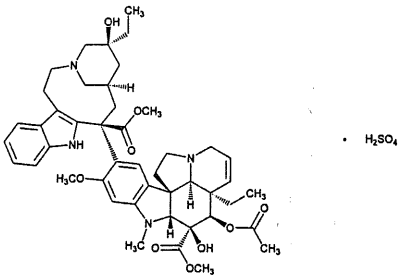
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Povidone RS
USP Vigabatrin RS
USP Vigabatrin Related Compound A RS
5-Vinylpyrrolidin-2-one.
C_6H_9NO 111.14
USP Vigabatrin Related Compound B RS
(*E*)-2-(2-Aminoethyl)but-2-enoic acid hydrochloride.
$C_6H_{11}NO_2 \cdot HCl$ 165.62

USP Monographs

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Vinblastine Sulfate



$C_{46}H_{58}N_4O_9 \cdot H_2SO_4$ 909.05
Vincaleukoblastine, sulfate (1:1) (salt);
Vincaleukoblastine sulfate (1:1) (salt) [143-67-9].

DEFINITION
Vinblastine Sulfate contains NLT 96.0% and NMT 102.0% of $C_{46}H_{58}N_4O_9 \cdot H_2SO_4$, corrections being applied for loss in weight.
[CAUTION—Handle vinblastine sulfate with great care, because it is a potent cytotoxic agent.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
  Analysis: The sample specimen and Reference Standard are previously dried under vacuum at 60° for 16 h.
  Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sulfate*
  Sample: 10 mg/mL in water
  Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: Diethylamine and water (14:986). Adjust with phosphoric acid to a pH of 7.5.
  Solution B: Acetonitrile and methanol (20:80)
  Mobile phase: *Solution A* and *Solution B* (38:62)
  Standard solution: 0.4 mg/mL of USP Vinblastine Sulfate RS in water
  System suitability solution: 0.4 mg/mL each of vincristine sulfate and vinblastine sulfate in water prepared as follows. Transfer USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS to a suitable volumetric flask, and dissolve in the *Standard solution*. [NOTE—No loss on drying determination is needed for USP Vincristine Sulfate RS.]
  Sample solution: 0.4 mg/mL of Vinblastine Sulfate in water. Equilibrate a portion of Vinblastine Sulfate for 30 min in ambient humidity. Using another portion of the equilibrated specimen, determine the loss on drying.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 262 nm
  Columns
  Precolumn: Packed with porous silica gel; installed between the pump and the injector
  Analytical: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 2 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 4.0 between vincristine and vinblastine, *System suitability solution*
  Relative standard deviation: NMT 0.73%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$) in the portion of Vinblastine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Vinblastine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Vinblastine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0%, corrections being applied for loss in weight

IMPURITIES
- **ORGANIC IMPURITIES**
  Mobile phase, Standard solution, System suitability solution, and **System suitability:** Proceed as directed in the *Assay*.
  Sample solution A: Use the *Sample solution* prepared in the *Assay*.
  Sample solution B: 16 µg/mL of Vinblastine Sulfate in water from *Sample solution A*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 262 nm
  Columns
  Precolumn: Packed with porous silica gel; installed between the pump and the injector
  Analytical: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 2 mL/min
  Injection volume: 200 µL (20 µL for *System suitability*)
  Analysis
  Samples: *Sample solution A* and *Sample solution B*
  Calculate the percentage of each individual impurity in the portion of Vinblastine Sulfate taken:

$$\text{Result} = [r_{UA}/(\Sigma r_{UA} + 25r_{UB})] \times 100$$

r_{UA} = peak response for each individual impurity appearing after the solvent peak from *Sample solution A*
r_{UB} = peak response for vinblastine from *Sample solution B*

Calculate the percentage of total impurities:

$$\text{Result} = [\Sigma r_{UA}/(\Sigma r_{UA} + 25r_{UB})] \times 100$$

r_{UA} = peak response for each impurity appearing after the solvent peak from *Sample solution A*
r_{UB} = peak response for vinblastine from *Sample solution B*

Acceptance criteria
Individual impurities: NMT 1.0%
Total impurities: NMT 3.0%

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SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample: 1.5 mg/mL in water
  Acceptance criteria: 3.5–5.0
- **Loss on Drying**
  (See *Thermal Analysis* ⟨891⟩.)
  [Note—In this procedure, perform weighings rapidly with minimum exposure of the substances to air.]
  Sample: 10 mg
  Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument. Heat the *Sample* at the rate of 5°/min between ambient temperature and 200° in an atmosphere of nitrogen at a flow rate of 40 mL/min. From the thermogram, determine the accumulated loss in weight between ambient temperature and a point on the plateau before decomposition is indicated (about 160°).
  Acceptance criteria: It loses NMT 15.0% of its weight.
- **Sterility Tests** ⟨71⟩: Where the label states that Vinblastine Sulfate is sterile, it meets the requirements.
- **Bacterial Endotoxins Test** ⟨85⟩: Where the label states that Vinblastine Sulfate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 10.0 USP Endotoxin Units/mg of vinblastine sulfate.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store in a freezer.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP Reference Standards** ⟨11⟩
  USP Vinblastine Sulfate RS
  USP Vincristine Sulfate RS
  [Note—No loss on drying determination is needed.]
  USP Vincristine Sulfate (Assay) RS

Vinblastine Sulfate for Injection

DEFINITION

Vinblastine Sulfate for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$).

[**Caution**—Handle Vinblastine Sulfate for Injection with great care because it is a potent cytotoxic agent.]

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Use material previously dried in a vacuum at 60° for 16 h.
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General** ⟨191⟩, *Sulfate*
  Sample solution: 100 mg/mL in water
  Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**
  Solution A: Diethylamine and water (14:986). Adjust with phosphoric acid to a pH of 7.5.
  Solution B: Acetonitrile and methanol (20:80)
  Mobile phase: *Solution A* and *Solution B* (38:62)
  Standard solution: 0.4 mg/mL of USP Vinblastine Sulfate RS in water
  System suitability solution: 0.4 mg/mL each of vincristine sulfate and vinblastine sulfate in water prepared as follows. Transfer USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS to a suitable volumetric flask, and dissolve in *Standard solution*.
  Sample stock solution: Pipet a suitable volume of water into each of five containers of Vinblastine Sulfate for Injection to obtain a solution in each having a nominal concentration of 1 mg/mL of vinblastine sulfate. Insert the stopper, shake to mix, and combine the solutions from the five containers.
  Sample solution: Nominally equivalent to 0.4 mg/mL of vinblastine sulfate in water, from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 262 nm
  Columns
  Precolumn: Packed with porous silica gel; installed between the pump and the injector
  Analytical: 4.6-mm × 15-cm; packing L1
  Flow rate: 2 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 4.0 between vincristine and vinblastine, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$) in the portion of Vinblastine Sulfate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Vinblastine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of vinblastine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** ⟨905⟩
  Procedure for content uniformity
  Buffer: Dissolve 13.61 g of sodium acetate in 900 mL of water in a 1000-mL volumetric flask. Adjust with glacial acetic acid to a pH of 5.0 while stirring, and dilute with water to volume.
  Standard solution: 40 µg/mL of USP Vinblastine Sulfate RS in *Buffer*
  Sample solution: Nominally equivalent to 40–50 µg/mL of vinblastine sulfate by dissolving the contents of one container of Vinblastine Sulfate for Injection in *Buffer*
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV
  Analytical wavelength: 269 nm
  Cell: 1 cm
  Blank: *Buffer*
  Analysis
  Samples: *Standard solution* and *Sample solution*

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Concomitantly determine the absorbances of the *Sample solution* and the *Standard solution*.
Calculate the percentage of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$) in the portion of Vinblastine Sulfate for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Vinblastine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of vinblastine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and System suitability: Prepare as directed in the *Assay*.
Sample solution A: Use the *Sample solution*, prepared as directed in the *Assay*.
Sample solution B: 16 µg/mL of vinblastine sulfate in water, from *Sample solution A*
Chromatographic system: Proceed as directed in the *Assay*, except use an injection volume of 200 µL.
Analysis
Samples: *Sample solution A* and *Sample solution B*
Calculate the percentage of each impurity in the portion of Vinblastine Sulfate for Injection taken:

$$\text{Result} = [r_U/(\Sigma r_U + 25r_S)] \times 100$$

r_U = peak response of each impurity appearing after the solvent peak from *Sample solution A*
r_S = peak response of vinblastine from *Sample solution B*

Calculate the percentage of total impurities:

$$\text{Result} = [\Sigma r_U/(\Sigma r_U + 25r_S)] \times 100$$

r_U = peak response of each impurity appearing after the solvent peak from *Sample solution A*
r_S = peak response of vinblastine from *Sample solution B*

Acceptance criteria
Individual impurities: NMT 2.0%
Total impurities: NMT 5.0%

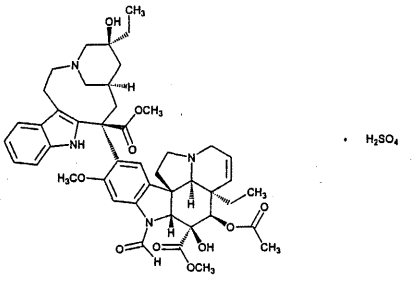
SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 10.0 USP Endotoxin Units/mg of vinblastine sulfate.
• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions*: At the time of use, it meets the requirements.
• **OTHER REQUIREMENTS:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, and store in a refrigerator.
• **LABELING:** The label states: "For Intravenous Use Only—Fatal If Given By Other Routes." When dispensed, the container or syringe (holding the individual dose prepared for administration to the patient) must be enclosed in an overwrap bearing the statement: "Do Not Remove Covering Until Moment of Injection. For Intravenous Use Only—Fatal If Given By Other Routes."
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Vinblastine Sulfate RS
USP Vincristine Sulfate RS
[NOTE—No loss on drying determination is needed for USP Vincristine Sulfate RS.]
USP Vincristine Sulfate (Assay) RS

Vincristine Sulfate



$C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$ 923.04
Vincaleukoblastine, 22-oxo-, sulfate (1:1) (salt); Leurocristine sulfate (1:1) (salt) [2068-78-2].

DEFINITION

Vincristine Sulfate contains NLT 95.0% and NMT 105.0% of vincristine sulfate ($C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$), calculated on the dried basis.
[**CAUTION**—Handle vincristine sulfate with great care because it is a potent cytotoxic agent.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
Standard: Prepare as directed in the chapter using either USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS. If USP Vincristine Sulfate (Assay) RS is used, dissolve a portion of it in a mixture of methylene chloride and methanol (3:1). Transfer the supernatant to an open container, and evaporate it at room temperature under nitrogen.
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sulfate*
Sample solution: 100 mg/mL
Acceptance criteria: Meets the requirements
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Solution A: Diethylamine and water (1:59). Adjust with phosphoric acid to a pH of 7.5.
Mobile phase: Methanol and *Solution A* (70:30)
Standard solution: 1.2 mg/mL of USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS in water
System suitability solution: 1 mg/mL of USP Vinblastine Sulfate RS in the *Standard solution*. [NOTE—No loss on

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drying determination is needed for USP Vinblastine Sulfate RS.]

Sample solution: 1.2 mg/mL of Vincristine Sulfate in water. Equilibrate a portion of Vincristine Sulfate for 30 min in ambient humidity. Using another portion of the equilibrated specimen, determine the loss on drying.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 297 nm

Columns

Precolumn: Porous silica gel packing

Guard: 2- to 5-cm; packing L1

Analytical: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between vincristine sulfate and vinblastine sulfate, *System suitability solution*. [NOTE—For a particular column, the resolution may be increased by increasing the proportion of water in the *Mobile phase*.]

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of vincristine sulfate ($C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$) in the portion of Vincristine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS in the *Standard solution* (mg/mL)

C_U = concentration of Vincristine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Diethylamine and water (3:197). Adjust with phosphoric acid to a pH of 7.5.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	38	62
12	38	62
27	8	92
29	38	62
34	38	62

Sample solution A: Prepare as directed for the *Sample solution* in the *Assay*.

Sample solution B: 0.04 mg/mL of Vincristine Sulfate from *Sample solution A* in water

Chromatographic system: Proceed as directed in the *Assay*, except for the *Flow rate* and *Injection volume*.

Flow rate: 2 mL/min

Injection volume: 200 µL

Analysis

Samples: *Sample solution A* and *Sample solution B*

Calculate the percentage of each impurity in the portion of Vincristine Sulfate taken:

$$\text{Result} = [r_{UA}/(\Sigma r_{UA} + 30r_{UB})] \times 100$$

r_{UA} = peak response of each impurity appearing after the solvent peak from *Sample solution A*

r_{UB} = peak response of vincristine from *Sample solution B*

Calculate the percentage of total impurities in the portion of Vincristine Sulfate taken:

$$\text{Result} = [\Sigma r_{UA}/(\Sigma r_{UA} + 30r_{UB})] \times 100$$

r_{UA} = peak response of each impurity appearing after the solvent peak from *Sample solution A*

r_{UB} = peak response of vincristine from *Sample solution B*

Acceptance criteria

Individual impurities: NMT 1.0%

Total impurities: NMT 4.0%

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 1 mg/mL

Acceptance criteria: 3.5–4.5

• **LOSS ON DRYING**

(See *Thermal Analysis* ⟨891⟩.)

[NOTE—In this procedure, perform weighings rapidly with minimum exposure of the substances to air.]

Sample: 10 mg

Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument. Heat the *Sample* at the rate of 5°/min between ambient temperature and 200° in an atmosphere of nitrogen at a flow rate of 40 mL/min. From the thermogram, determine the accumulated loss in weight between ambient temperature and a point on the plateau before decomposition is indicated (at about 160°).

Acceptance criteria: NMT 12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a freezer.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Vinblastine Sulfate RS

[NOTE—No loss on drying determination is needed for USP Vinblastine Sulfate RS.]

USP Vincristine Sulfate RS

USP Vincristine Sulfate (Assay) RS

Vincristine Sulfate Injection

DEFINITION

Vincristine Sulfate Injection is a sterile solution of Vincristine Sulfate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of vincristine sulfate ($C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$).

[**CAUTION**—Handle Vincristine Sulfate Injection with great care because it is a potent cytotoxic agent.]

USP Monographs

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IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 10 mg/mL of USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS in dichloromethane and methanol (3:1)

Sample solution: Transfer a volume of Injection, equivalent to 2 mg of vincristine sulfate, to a small centrifuge tube. For each mL of solution add 1 drop of ammonium hydroxide. Add 0.2 mL of dichloromethane. Place the cap on the tube, shake it vigorously for NLT 1 min, and centrifuge for 1 min. Carefully withdraw the dichloromethane layer, and transfer to a small stoppered vial.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Fresh ether, methanol, and methylamine solution (2 in 5) (19:2:1)

Spray reagent: Dissolve 2.0 g of ceric ammonium sulfate in 100 mL of water with heating and stirring, and slowly add 100 mL of phosphoric acid. Filter if necessary.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatographic plate in a methanol prewash tank; for maximum sensitivity, dry it NMT 2 h before use. Score it about 15 cm above the points of application. Apply the *Standard solution* and the *Sample solution* about 2.5 cm from the lower edge of the plate, and dry thoroughly (a current of cool air may be used to help dry the spots). Place the plate in the nonequilibrated developing chamber that contains a paper liner around the back and sides and *Developing solvent system* to a depth of 2 cm. Remove the plate when the solvent moves to the scored line (about 80 min), and discard the solvent system. Dry the plate in a fume hood at room temperature, heat on a metal plate on a steam bath for 15 min, and spray the plate while still hot with *Spray reagent*. Continue heating the plate for 15 min to stabilize the spots.

Acceptance criteria: The R_F value and the color of the principal spot from the *Sample solution* correspond to those from the *Standard solution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Diethylamine and water (1:59). Adjust with phosphoric acid to a pH of 7.5.

Mobile phase: Methanol and *Solution A* (7:3)

Standard solution: 1.2 mg/mL of USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS in water

System suitability solution: 1 mg/mL of USP Vinblastine Sulfate RS in the *Standard solution*

Sample solution: Nominally 1.0 mg/mL from Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 297 nm

Columns

Precolumn: Porous silica gel packing

Guard: 2- to 5-cm; packing L1

Analytical: 4.6-mm × 25-cm; packing L7

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between vincristine sulfate and vinblastine sulfate, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vincristine sulfate ($C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of vincristine sulfate in the *Standard solution* (mg/mL)

C_U = nominal concentration of vincristine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Solution A: Diethylamine and water (3:197). Adjust with phosphoric acid to a pH of 7.5.

Solution B: Methanol

Solution C: Prepare a suitable dilution of any preservative present in the Injection, as identified in the labeling.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	38	62
12	38	62
27	8	92
29	38	62
34	38	62

Sample solution A: 1 mg/mL of vincristine sulfate from Injection in water

Sample solution B: 0.04 mg/mL in water from *Sample solution A*

Chromatographic system: Proceed as directed in the *Assay*, except use a *Flow rate* of 2 mL/min and an *Injection size* of 200 µL.

Analysis

Samples: *Solution C*, *Sample solution A*, and *Sample solution B*

Inject *Solution C* to identify the peaks due to the preservatives. Disregard any peaks at these retention times for the calculations of any other individual impurity and total impurities.

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = [r_{UA}/(\Sigma r_{UA} + 25 r_{UB})] \times 100$$

r_{UA} = peak response of each impurity appearing after the solvent peak from *Sample solution A*

r_{UB} = peak response of vincristine from *Sample solution B*

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Calculate the percentage of total impurities in the portion of Injection taken:

$$\text{Result} = [(\Sigma r_{UA}/(\Sigma r_{UA} + 25 r_{UB})] \times 100$$

The terms in the formula are as defined above.

Acceptance criteria: See *Table 2.*

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Vincristine	1.0	—
N-Desformylvincristine	1.4	3.0
Any other individual impurity	—	2.0
Total impurities	—	6.0

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 62.5 USP Endotoxin Units/mg of vincristine sulfate.
- **PH** ⟨791⟩: 3.5–5.5
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant glass containers, and store in a refrigerator.
- **LABELING:** The label states: "For Intravenous Use Only—Fatal If Given By Other Routes."

  Where labeled as containing more than 2 mg, it must also be labeled as a Pharmacy Bulk Package (see *Injections* ⟨1⟩). The labeling directs that the drug be dispensed only in containers enclosed in an overwrap labeled as directed below. When packaged in a Pharmacy Bulk Package, it is exempt from the requirement in *Injections* ⟨1⟩ that the closure be penetrated only one time after constitution with a suitable sterile transfer device or dispensing set, when it contains a suitable substance or mixture of substances to prevent the growth of microorganisms.

  When dispensed, the container or syringe (holding the individual dose prepared for administration to the patient) must be enclosed in an overwrap bearing the statement: "Do Not Remove Covering Until Moment of Injection. For Intravenous Use Only—Fatal If Given By Other Routes."
- **USP REFERENCE STANDARDS** ⟨11⟩

  USP Vinblastine Sulfate RS

  [NOTE—No loss on drying determination is needed.]

  USP Vincristine Sulfate RS

  USP Vincristine Sulfate (Assay) RS

Vincristine Sulfate for Injection

DEFINITION

Vincristine Sulfate for Injection is a sterile mixture of Vincristine Sulfate with suitable diluents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of vincristine sulfate ($C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$).

[**CAUTION**—Handle Vincristine Sulfate for Injection with great care because it is a potent cytotoxic agent.]

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

  Standard solution: 10 mg/mL of USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS in dichloromethane and methanol (3:1)

  Sample stock solution: 25 mg/mL of vincristine sulfate from Vincristine Sulfate for Injection in water

  Sample solution: 10 mg/mL of vincristine sulfate from *Sample stock solution* in methanol

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

  Mode: TLC

  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

  Application volume: 20 µL

  Developing solvent system: Fresh ether, methanol, and methylamine solution (2 in 5) (19:2:1)

  Spray reagent: Dissolve 2.0 g of ceric ammonium sulfate in 100 mL of water with heating and stirring, and slowly add 100 mL of phosphoric acid. Filter if necessary.

  Analysis: Develop the chromatographic plate in a methanol prewash tank; for maximum sensitivity, dry it NMT 2 h before use. Score it about 15 cm above the points of application. Apply the *Sample solution* and the *Standard solution* at points about 2.5 cm from the lower edge of the plate, and dry thoroughly (a current of cool air may be used to help dry the spots). Place the plate in the nonequilibrated developing chamber that contains a paper liner around the back and sides and *Developing solvent system* to a depth of about 2 cm. Remove the plate when the solvent moves to the scored line (about 80 min), and discard the solvent system. Dry the plate in a fume hood at room temperature, heat on a metal plate on a steam bath for about 15 min, and spray the plate while still hot with *Spray reagent.* Continue heating the plate for 15 min to stabilize the spots.

  Acceptance criteria: The R_F value and the color of the principal spot from the *Sample solution* correspond to those from the *Standard solution.*
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

  Solution A: Diethylamine and water (1:59). Adjust with phosphoric acid to a pH of 7.5.

  Mobile phase: Methanol and *Solution A* (70:30)

  Standard solution: 1.2 mg/mL of USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS in water

  System suitability solution: 1 mg/mL of USP Vinblastine Sulfate RS in the *Standard solution*

  Sample solution: 1.2 mg/mL of vincristine sulfate from Vincristine Sulfate for Injection in water. [NOTE—Shake to mix.]

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *System Suitability.*)

  Mode: LC

  Detector: UV 297 nm

  Columns

  Precolumn: Porous silica gel packing

  Guard: 2- to 5-cm; packing L1

  Analytical: 4.6-mm × 25-cm; packing L7

  Flow rate: 1.5 mL/min

  Injection size: 10 µL

  System suitability

  Samples: *Standard solution* and *System suitability solution*

  Suitability requirements

  Resolution: NLT 4.0 between vincristine sulfate and vinblastine sulfate, *System suitability solution.*

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[NOTE—For a particular column, the resolution may be increased by increasing the proportion of water in the *Mobile phase*.]

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vincristine sulfate ($C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$) in the portion of Vincristine Sulfate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of vincristine sulfate in the *Standard solution* (mg/mL)
C_U = nominal concentration of vincristine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Buffer solution: Dissolve 6.3 g of ammonium formate in about 900 mL of water. Adjust with formic acid to a pH of 5.0 while stirring, and dilute with water to 1000 mL.

Standard solution: 40–50 µg/mL of USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS in *Buffer solution*

Sample solution: Dissolve the contents of one container of Vincristine Sulfate for Injection in a suitable volume of *Buffer solution* to obtain a solution containing 40–50 µg/mL of vincristine sulfate.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Cell: 1 cm

Analytical wavelength: 262 nm

Blank: *Buffer solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in mg, of vincristine sulfate ($C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$) in the container of Vincristine Sulfate for Injection taken:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times F$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Vincristine Sulfate RS in the *Standard solution* (µg/mL)
V = final volume of the *Sample solution*
F = unit conversion factor, 0.001 mg/µg

Acceptance criteria: Meets the requirements for solids

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Diethylamine and water (3:197). Adjust with phosphoric acid to a pH of 7.5.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	38	62
12	38	62
27	8	92
29	38	62
34	38	62

Sample solution A: 1 mg/mL of vincristine sulfate from Vincristine Sulfate for Injection in water

Sample solution B: 0.04 mg/mL of vincristine sulfate in water from *Sample solution A*

Chromatographic system: Proceed as directed in the *Assay*, except use a *Flow rate* of 2 mL/min and an *Injection size* of 200 µL.

Analysis

Samples: *Sample solution A* and *Sample solution B*

Calculate the percentage of each impurity in the portion of Vincristine Sulfate for Injection taken:

$$\text{Result} = [r_{UA}/(\Sigma r_{UA} + 25r_{UB})] \times 100$$

r_{UA} = peak response of each impurity appearing after the solvent peak from *Sample solution A*
r_{UB} = peak response of vincristine from *Sample solution B*

Calculate the percentage of total impurities in the portion of Vincristine Sulfate for Injection taken:

$$\text{Result} = [\Sigma r_{UA}/(\Sigma r_{UA} + 25r_{UB})] \times 100$$

r_{UA} = peak response of each impurity appearing after the solvent peak from *Sample solution A*
r_{UB} = peak response of vincristine from *Sample solution B*

Acceptance criteria

Individual impurities: NMT 2.0%

Total impurities: NMT 5.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 100.0 USP Endotoxin Units/mg of vincristine sulfate.

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

• **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, and store in a refrigerator.

• **LABELING:** The label states, "For Intravenous Use Only—Fatal If Given By Other Routes".

Where labeled as containing more than 2 mg, it must also be labeled as a Pharmacy Bulk Package (see *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*). The labeling directs that the drug be dispensed only in containers enclosed in an overwrap labeled as directed below. When packaged in a Pharmacy Bulk Package, it is exempt from the requirement in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products* that the closure be penetrated only one time after constitution with a suitable sterile transfer device or dispensing set, when it contains a suitable

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substance or mixture of substances to prevent the growth of microorganisms.

When dispensed, the container or syringe (holding the individual dose prepared for administration to the patient) must be enclosed in an overwrap bearing the statement, "Do Not Remove Covering Until Moment of Injection. For Intravenous Use Only—Fatal If Given By Other Routes."

• **USP REFERENCE STANDARDS** ⟨11⟩

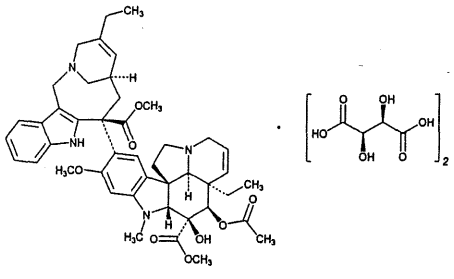
USP Vinblastine Sulfate RS

[NOTE—No loss on drying determination is needed for USP Vinblastine Sulfate RS.]

USP Vincristine Sulfate RS

USP Vincristine Sulfate (Assay) RS

Vinorelbine Tartrate



$C_{45}H_{54}N_4O_8 \cdot 2C_4H_6O_6$ 1079.11

8′-Norvincaleukoblastine,3′,4′-didehydro-4′-deoxy-,[*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:2) (salt); 3′,4′-Didehydro-4′-deoxy-8′-norvincaleukoblastine L-(+)-tartrate (1:2) (salt) [125317-39-7].

DEFINITION

Vinorelbine Tartrate contains NLT 98.0% and NMT 102.0% of $C_{45}H_{54}N_4O_8 \cdot 2C_4H_6O_6$, calculated on the anhydrous basis.

[CAUTION—Vinorelbine Tartrate is cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Dissolve 10 mg in 5 mL of water, add 0.5 mL of 5 N sodium hydroxide, and extract with 5 mL of methylene chloride. Filter the organic extract through anhydrous sodium sulfate, and evaporate the organic extract to a volume of about 0.5 mL.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C.**

Sample solution: Equivalent to 15 mg/mL of tartaric acid in water

Analysis: To 0.1 mL of the *Sample solution* add 0.1 mL of 100 mg/mL of potassium bromide, 0.1 mL of 20 mg/mL of resorcinol, and 3 mL of sulfuric acid. Heat on a hot water bath for 5–10 min until a dark blue color develops. Allow to cool, and pour the solution into water.

Acceptance criteria: The color changes to red (presence of tartrate).

ASSAY

• **PROCEDURE**

Buffer: Dissolve 6.9 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 4.2, and dilute with water to 1000 mL.

Mobile phase: Dissolve 1.22 g of sodium 1-decanesulfonate in 620 mL of methanol, and add 380 mL of *Buffer*.

System suitability solution: Prepare a solution containing 1.4 mg/mL of USP Vinorelbine Tartrate RS and 0.01 mg/mL of USP Vinorelbine Related Compound A RS in water. Expose a portion of this solution in a suitable xenon lamp apparatus capable of supplying a dose of 1600 KJ/m² between 310 and 800 nm at a power of 500 W/m² for 1 h to generate an additional photodegradation product, 3,6-epoxy vinorelbine.

Standard solution: 1.4 mg/mL of USP Vinorelbine Tartrate RS in *Mobile phase*

Sample solution: 1.4 mg/mL of Vinorelbine Tartrate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 267 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between vinorelbine and vinorelbine related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of vinorelbine tartrate ($C_{45}H_{54}N_4O_8 \cdot 2C_4H_6O_6$) in the portion of Vinorelbine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of vinorelbine from the *Sample solution*

r_S = peak response of vinorelbine from the *Standard solution*

C_S = concentration of USP Vinorelbine Tartrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Vinorelbine Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, Sample solution, and System suitability: Proceed as directed in the *Assay*.

Standard stock solution: Use the *Standard solution* as prepared in the *Assay*.

Standard solution: 0.28 µg/mL of vinorelbine tartrate in *Mobile phase*, from *Standard stock solution*

Chromatographic system: Proceed as directed in the *Assay*, except use a run time of NLT three times the retention time of vinorelbine.

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Vinorelbine Tartrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria: See *Table 1*.
[NOTE—Disregard any peaks with an area less than or equal to one-half of the area of the peak for vinorelbine in the *Standard solution*.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3,6-Epoxy vinorelbine[a]	0.8	0.3
Vinorelbine	1.0	—
Vinorelbine related compound A[b]	1.2	0.2
Any unspecified impurity[c]	—	0.2
Total Impurities[d]	—	0.7

[a] 3′,4′,7,8-Tetradehydro-3,4′-dideoxy-3,6-epoxy-6,7-dihydro-8′-norvincaleukoblastine.
[b] 4-*O*-Deacetylvinorelbine.
[c] Any individual impurity or coeluted impurities comprising an individual peak.
[d] Excluding 3,6-epoxy vinorelbine.

SPECIFIC TESTS
- **CLARITY OF SOLUTION**
  Sample solution: Equivalent to 10 mg/mL of anhydrous vinorelbine in water from Vinorelbine Tartrate
  Acceptance criteria: The solution is clear.
- **COLOR OF SOLUTION**
  Sample solution: Equivalent to 10 mg/mL of anhydrous vinorelbine in water from Vinorelbine Tartrate
  Analysis: Determine the absorbance of the *Sample solution* in a 1-cm cell at 420 nm in a suitable spectrophotometer, using water as the blank.
  Acceptance criteria: NMT 0.03
- **pH** ⟨791⟩: 3.3–3.8, in a 10-mg/mL solution
- **WATER DETERMINATION,** *Method Ia* ⟨921⟩: NMT 4.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a freezer.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Vinorelbine Related Compound A RS
  4-*O*-Deacetylvinorelbine tartrate.
  $C_{43}H_{52}N_4O_7 \cdot 2C_4H_6O_6$ 1037.07
  USP Vinorelbine Tartrate RS

Vinorelbine Injection

DEFINITION
Vinorelbine Injection is a sterile solution of Vinorelbine Tartrate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of vinorelbine ($C_{45}H_{54}N_4O_8$).
[CAUTION—Handle Vinorelbine Injection with great care because it is a potent cytotoxic agent.]

IDENTIFICATION
- **A.** The retention time and UV spectrum of the major peak of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  Buffer: Dissolve 6.9 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 4.2, and dilute with water to 1000 mL.
  Mobile phase: Dissolve 1.22 g of sodium 1-decanesulfonate in 620 mL of methanol, and add 380 mL of *Buffer*.
  System suitability solution: Prepare a solution containing 1.4 mg/mL of USP Vinorelbine Tartrate RS and 0.01 mg/mL of USP Vinorelbine Related Compound A RS in water. Expose a portion of this solution in a suitable xenon lamp apparatus capable of supplying a dose of 1600 KJ/m² between 310 and 800 nm at a power of 500 W/m² for 1 h to generate an additional photodegradation product, 3,6-epoxy vinorelbine.
  Standard solution: 0.14 mg/mL of USP Vinorelbine Tartrate RS in water
  Sample solution: Nominally equivalent to 0.1 mg/mL of vinorelbine in water, from Injection
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Diode array
  Column: 3.9-mm × 15-cm; packing L1
  Column temperature: 40°
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for 3,6-epoxy vinorelbine, vinorelbine, and vinorelbine related compound A are 0.8, 1.0, and 1.2, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between vinorelbine and vinorelbine related compound A, *System suitability solution*
  Relative standard deviation: ▲NMT▲ (ERR 1-Nov-2018) 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of vinorelbine ($C_{45}H_{54}N_4O_8$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution* at 267 nm
r_S = peak response from the *Standard solution* at 267 nm
C_S = concentration of USP Vinorelbine Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of vinorelbine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of vinorelbine, 778.93
M_{r2} = molecular weight of vinorelbine tartrate, 1079.11

Acceptance criteria: 90.0%–110.0%

IMPURITIES
- **ORGANIC IMPURITIES**
  Mobile phase and **System suitability solution:** Proceed as directed in the *Assay*.

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Sample solution: Nominally equivalent to 1.0 mg/mL of vinorelbine in *Mobile phase*, from Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 267 nm
Column: 3.9-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between vinorelbine and vinorelbine related compound A
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. Disregard any peaks less than 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3,6-Epoxy vinorelbine[a]	0.8	1.0
Vinorelbine	1.0	—
Vinorelbine related compound A[b]	1.2	0.3
Any unspecified impurity	—	0.2
Total impurities	—	2.0

[a] 3′,4′,7,8-Tetradehydro-3,4′-dideoxy-3,6-epoxy-6,7-dihydro-8′-norvincaleukoblastine.
[b] 4-*O*-Deacetylvinorelbine.

SPECIFIC TESTS
• **CLARITY OF SOLUTION**
Sample solution: 10 mg/mL
Acceptance criteria: The solution is clear.
• **COLOR OF SOLUTION**
Sample solution: 10 mg/mL
Analysis: Determine the absorbance of the *Sample solution* in a 1-cm cell at 420 nm in a suitable spectrophotometer, using water as the blank.
Acceptance criteria: NMT 0.060
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 3.0 USP Endotoxin Units/mg of vinorelbine.
• **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
• **pH** ⟨791⟩: 3.3–3.8
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers as described in *Packaging and Storage Requirements* ⟨659⟩, preferably of Type I glass, protected from light. Store in a refrigerator.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Vinorelbine Related Compound A RS
4-*O*-Deacetylvinorelbine tartrate.
$C_{43}H_{52}N_4O_7 \cdot 2C_4H_6O_6$ 1037.07
USP Vinorelbine Tartrate RS

Vitamin A

DEFINITION
Vitamin A contains a suitable form of retinol ($C_{20}H_{30}O$; vitamin A alcohol) and possesses vitamin A activity equivalent to NLT 95.0% of that declared on the label. It may consist of retinol or esters of retinol formed from edible fatty acids, principally acetic and palmitic acids. It may be diluted with edible oils, or it may be incorporated in solid edible carriers or excipients, and it may contain suitable antimicrobial agents, dispersants, and antioxidants.

IDENTIFICATION
• **A. COLOR REACTION WITH ANTIMONY III**
Sample solution: Vitamin A equivalent to 6 µg/mL of retinol in chloroform
Analysis: To 1 mL of the *Sample solution* add 10 mL of antimony trichloride TS.
Acceptance criteria: A transient blue color appears at once.
• **B. THIN-LAYER CHROMATOGRAPHY**
Standard solution: USP Retinyl Acetate RS and USP Retinyl Palmitate RS in methylene chloride, each equivalent to about 0.2 mg/mL of retinol ($C_{20}H_{30}O$)
Sample solution for the liquid form of Vitamin A: Dissolve a volume, equivalent to about 5 mg of retinol, in methylene chloride to obtain 10.0 mL of solution.
Sample solution for the solid form of Vitamin A: Transfer a quantity, equivalent to about 5 mg of retinol, to a separator, add 75 mL of water, and shake vigorously for 1 min. Extract with 10.0 mL of methylene chloride by shaking for 1 min, and centrifuge to clarify the methylene chloride extract.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume
Standard solution: 15 µL
Sample solution: 10 µL
Developing solvent system: Cyclohexane and ether (4:1)
Developing distance: 10 cm
Spray reagent: Phosphomolybdic acid TS
System suitability
Sample: *Standard solution*
Suitability requirements: The chromatogram shows two main blue-green spots of the corresponding esters. The R_F values are 0.45 ± 0.10 and 0.7 ± 0.1 for retinyl acetate and retinyl palmitate, respectively.
Analysis
Samples: *Standard solution* and the appropriate *Sample solution*
Allow the solvent front to move a distance of 10 cm, remove the plate, and air-dry. Spray the plate with *Spray reagent*.
Acceptance criteria: The appropriate *Sample solution* exhibits a main blue-green spot at an R_F value that corresponds to one of the two main spots from the

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Standard solution and is consistent with the labeled ester. The appropriate *Sample solution* of Vitamin A labeled as containing retinol exhibits the main blue-green spot at an approximate R_F value of 0.1.

ASSAY

• **VITAMIN A ASSAY** ⟨571⟩

Sample: Use a suitable quantity of Vitamin A.

Analysis: Proceed as directed in *Vitamin A Assay* ⟨571⟩, *Assay, Chemical Methods* or *Chromatographic Methods*, depending on the form of Vitamin A under test.

Acceptance criteria: Equivalent to NLT 95.0% of the labeled amount of vitamin A activity

SPECIFIC TESTS

• **ABSORBANCE RATIO**

[NOTE—This test is required only if *Vitamin A Assay* ⟨571⟩, *Assay, Chemical Methods* is used for the *Assay*.]

Sample: Use a suitable quantity of Vitamin A.

Analysis: Proceed as directed in *Vitamin A Assay* ⟨571⟩, *Assay, Chemical Methods, Procedure 1* or *Procedure 2*.

Acceptance criteria

Procedure 1: The ratio of the corrected absorbance [A_{325}] to the observed absorbance A_{325} is NLT 0.85.

Procedure 2: The absorption maximum is between 325 and 327 nm. The absorbance ratios are as follows: A_{300}/A_{326} is NMT 0.60; A_{350}/A_{326} is NMT 0.54; and A_{370}/A_{326} is NMT 0.14.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably under an atmosphere of an inert gas, protected from light.

Change to read:

• **LABELING:** Label it to indicate the form in which the vitamin is present, and to indicate the presence of any antimicrobial agent, dispersant, antioxidant, or other added substance, and to indicate the vitamin A activity in terms of the equivalent amount of retinol, in mg/g. ▲▲ (USP 1-Aug-2019)

Add the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩

USP Retinyl Acetate RS

USP Retinyl Palmitate RS▲ (ERR 1-Jan-2019)

Vitamin A Capsules

DEFINITION

Vitamin A Capsules contain NLT 95.0% and NMT 120.0% of the labeled amount of vitamin A.

IDENTIFICATION

• **A.**

Sample solution: Equivalent to 6 µg/mL of retinol from Capsules in methylene chloride

Analysis: To 1 mL of *Sample solution* add 10 mL of antimony trichloride TS.

Acceptance criteria: A transient blue color appears at once.

Change to read:

• **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 0.5 mg/mL of retinol from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in methylene chloride

Sample solution: Dissolve or extract with methylene chloride the contents of a quantity of Capsules to obtain a solution with a nominal concentration of 0.5 mg/mL of retinol.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL, ▲8-mm band▲ (USP 1-Aug-2019)

Developing solvent system: Cyclohexane and ether (4:1)

Spray reagent: Phosphomolybdic acid TS

Analysis: Proceed as directed in the chapter, using the *Developing solvent system*. Locate the spots on the plate using the *Spray reagent*.

Acceptance criteria: The blue-green spot formed is indicative of the presence of retinol, and its R_F value corresponds to that of the *Standard solution*. (The approximate R_F values of the predominant spots, corresponding to the different forms of retinol, are 0.1 for the alcohol form, 0.45 for the acetate form, and 0.7 for the palmitate form.)

ASSAY

• **VITAMIN A ASSAY** ⟨571⟩

Analysis: Use the appropriate procedure described in *Vitamin A Assay* ⟨571⟩, *Assay, Chromatographic Methods*.

[NOTE—The procedure used is stated in the labeling only if *Procedure 1* is not used.]

Acceptance criteria: 95.0%–120.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Immersion fluid: Use 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution with a pH of 4.5 ± 0.05.

Temperature: 37 ± 2°

Time: 45 min

Acceptance criteria: Meet the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

• **LABELING:** Label the Capsules to indicate the form in which the vitamin is present, and to indicate the vitamin A activity in terms of the equivalent amount of retinol in ▲µg/Capsule. Expression of the amount of vitamin A in terms of units may be added in parentheses after the mass units.[1] ▲ (USP 1-Aug-2019)

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Retinyl Acetate RS

USP Retinyl Palmitate RS

[1] Where articles are labeled in terms of units in addition to the required labeling, the relationship of the USP Units or International Units (IU) to mass is as follows: 1 USP Vitamin A Unit = 0.3 µg of *all-trans*-retinol (vitamin A alcohol) or 0.344 µg of *all-trans*-retinyl acetate (vitamin A acetate) or 0.55 µg of *all-trans*-retinyl palmitate (vitamin A palmitate), and 1 µg of retinol (3.3 USP Vitamin A Units) = 1 retinol equivalent (RE).

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Vitamin A Oral Liquid Preparation

DEFINITION

Vitamin A Oral Liquid Preparation is an emulsion, suspension, or solution that contains retinyl acetate or retinyl palmitate in an amount equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$).

IDENTIFICATION

[NOTE—Use low-actinic glassware.]

• **A.**

Sample solution: Equivalent to 6 µg/mL of retinol from Oral Liquid Preparation in methylene chloride

Analysis: To 1 mL of *Sample solution* add 10 mL of antimony trichloride TS.

Acceptance criteria: A transient blue color appears at once.

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 0.5 mg/mL of retinol from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in methylene chloride

Sample solution: Dissolve or extract with methylene chloride a quantity of Oral Liquid Preparation to obtain a solution with a nominal concentration of 0.5 mg/mL of retinol.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Application volume: 10 µL, 8-mm band

Developing solvent system: Cyclohexane and ether (4:1)

Developing distance: 10 cm

Spray reagent: 0.2 g/mL of phosphomolybdic acid in alcohol. Filter, and use only the clear filtrate.

Analysis: Apply the *Sample solution* at the starting point of the chromatogram, and proceed as directed in the chapter. Allow the solvent front to move 10 cm, remove the plate, and air-dry. Spray with *Spray reagent*.

Acceptance criteria: The blue-green spot formed is indicative of the presence of retinol, and its R_F value corresponds to that of the *Standard solution*. The approximate R_F values of the predominant spots, corresponding to the different forms of retinol, are 0.1 for the alcohol form, 0.45 for the acetate form, and 0.7 for the palmitate form.

ASSAY

• **VITAMIN A**

[NOTE—Use low-actinic glassware.]

Mobile phase: *n*-Hexane

Standard solution 1: 13 µg/mL of retinol from USP Retinyl Acetate RS in *n*-hexane

Standard solution 2: 13 µg/mL of retinol from USP Retinyl Palmitate RS in *n*-hexane

System suitability solution: Mix equal volumes of *Standard solution 1* and *Standard solution 2*.

Sample solution

For Oral Liquid Preparation in oil vehicles packaged in single-unit containers: Deliver the contents of NLT 30 single-unit containers, following the directions for use as stated in the labeling. Weigh directly the individual contents delivered from each single-unit container, and calculate the average. [NOTE—Do not weigh the contents delivered by difference between full containers and empty containers. Capsules intended as single-unit containers are not rinsed after expulsion of the contents.] Mix the contents to obtain a homogeneous sample. Transfer an amount of the composite to a suitable volumetric flask. Dissolve with hexane, and dilute with hexane quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent of about 13 µg/mL of retinol, based on the labeled amount.

For Oral Liquid Preparation in oil vehicles packaged in multiple-unit containers: Dissolve an accurately measured volume of Oral Liquid Preparation in a suitable volume of hexane, and dilute with hexane, quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent of about 13 µg/mL of retinol, based on the labeled amount.

For Oral Liquid Preparation in aqueous vehicles: Transfer a weighed quantity, or an accurately measured volume of Oral Liquid Preparation, into a separatory funnel, and extract quantitatively with hexane or other suitable solvent. Dilute with hexane, quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent of about 13 µg/mL of retinol, based on the labeled amount.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 15-cm; 3-µm packing L8

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 10 between *all-trans*-retinyl acetate and *all-trans*-retinyl palmitate

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution 1* or *Standard solution 2* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$), in each individual container:

$$\text{Result} = (r_U/r_S) \times (C/W) \times D \times U \times (100/L)$$

r_U = peak response of the corresponding *all-trans*-retinyl ester from the *Sample solution*

r_S = peak response of the *all-trans*-retinyl ester from the appropriate *Standard solution*

C = concentration of retinol ($C_{20}H_{30}O$) in the appropriate *Standard solution* (mg/mL)

W = weight or volume of Oral Liquid Preparation composite taken (mg or mL)

D = dilution factor (dilution volume/aliquot volume)

U = for multiple-unit containers: labeled volume of each dosage unit (mL); for single-unit containers: average mass (mg) of the contents delivered from each individual container, following the directions for use as stated in the labeling

L = label claim of vitamin A as retinol ($C_{20}H_{30}O$) (mg/dosage unit)

Acceptance criteria: 90.0%–120.0% of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$)

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers: Meets the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers

Analysis: Empty the single-unit containers, following the directions for use as stated in the labeling. [NOTE—Do not weigh the contents delivered by difference between full containers and empty containers. Capsules intended for use as single-unit containers are not rinsed after expulsion of the contents.]

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Acceptance criteria: The contents so delivered, and weighed directly, meet the requirements.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. It may be packaged in single-unit containers. It also may be suitable as single-unit containers provided they are packaged in secondary containers. ▲▲ (CN 1-May-2020)
- **LABELING:** The label states that the product is Vitamin A Oral Liquid Preparation. Label the Oral Liquid Preparation to indicate the ester form in which the vitamin is present, and to indicate the amount of vitamin A delivered in each dosage unit in terms of the equivalent amount of retinol in µg/dosage unit. Expression of the amount of vitamin A in terms of units may be added in parentheses after the mass units.[1] Capsules used as single-unit containers may be exempted from the requirements of individual labeling, provided they are packaged in an appropriately labeled secondary container, including directions for use and delivery of each dosage unit of Oral Liquid Preparation. Label the Oral Liquid Preparation packaged in multiple-unit containers to indicate the volume of each dosage unit.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Retinyl Acetate RS
  USP Retinyl Palmitate RS

Vitamin A Tablets

DEFINITION

Vitamin A Tablets contain retinyl acetate or retinyl palmitate in an amount equivalent to NLT 95.0% and NMT 120.0% of the labeled amount of vitamin A as retinol ($C_{20}H_{30}O$).

IDENTIFICATION

[NOTE—Use low-actinic glassware.]

- **A.**

  Sample solution: A solution containing the equivalent of 6 µg/mL of retinol from powdered Tablets in methylene chloride

  Analysis: To 1 mL of *Sample solution* add 10 mL of antimony trichloride TS.

  Acceptance criteria: A transient blue color appears at once.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

  Standard solution: Equivalent to 0.5 mg/mL of retinol from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in methylene chloride

  Sample solution: To a portion of finely powdered Tablets, equivalent to 5 mg of retinol, add 15 mL of water, sonicate, and shake vigorously for 2 min. Extract with 10 mL of methylene chloride by shaking for 2 min. Centrifuge, and use the lower layer of methylene chloride extract.

  Application volume: 10 µL, 8-mm band

  Developing solvent system: Cyclohexane and ether (4:1)

  Spray reagent: Phosphomolybdic acid TS

  Analysis: Proceed as directed in the chapter, using the *Developing solvent system*. Locate the spots on the plate using the *Spray reagent*.

  Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Add the following:

▲[NOTE—In the following assays, where more than one assay method is given for an individual ingredient, the requirements may be met by following any one of the specified methods, with the method used being stated in the labeling only if *Procedure 1* is not used.]▲ (USP 1-May-2020)

Change to read:

- **PROCEDURE** ▲1

  [NOTE—Where vitamin A (retinyl acetate or retinyl palmitate) is specified in the following procedure, use the chemical form present in the formulation and the relevant Reference Standard. ▲ (USP 1-May-2020) Use low-actinic glassware.]

  Mobile phase: *n*-Hexane

  Standard solution 1: 15 µg/mL of retinol from USP Retinyl Acetate RS in *n*-hexane

  Standard solution 2: 15 µg/mL of retinol from USP Retinyl Palmitate RS in *n*-hexane

  System suitability solution: Mix equal volumes of *Standard solution 1* and *Standard solution 2*.

  Sample solution: Finely powder NLT 20 Tablets. To a portion of the powder, equivalent to 5 Tablets, accurately weighed, add 15 mL of water and sonicate for 5 min. Add 15 mL of *n*-hexane, and shake for 15 min on a wrist-action shaker in a water bath maintained at 60°. Add 10 mL of dimethyl sulfoxide, and shake for an additional period of 30 min on a wrist-action shaker in a water bath maintained at 60°. [NOTE—Set up the wrist-action shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge, and transfer the hexane layer to a 100-mL volumetric flask. Repeat the extraction with 3 additional 15-mL portions of *n*-hexane by thoroughly shaking each for 5 min. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Further dilute this solution with *n*-hexane to obtain a solution with a concentration equivalent to 15 µg/mL of retinol.

  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 325 nm
  Column: 4.6-mm × 15-cm; 3-µm packing L8
  Flow rate: 1 mL/min
  Injection volume: 40 µL

  System suitability
  Sample: *System suitability solution*
  Suitability requirements
  Resolution: NLT 10 between *all-trans*-retinyl palmitate and *all-trans*-retinyl acetate
  Relative standard deviation: NMT 3.0%

  Analysis
  Samples: *Standard solution 1* or *Standard solution 2* and *Sample solution*
  Calculate the percentage of the labeled amount of vitamin A activity, as retinol ($C_{20}H_{30}O$), in the portion of Tablets taken:

[1] Where articles are labeled in terms of units in addition to the required labeling, the relationship of the USP Units or International Units (IU) to mass is as follows: 1 USP Vitamin A Unit = 0.3 µg of *all-trans*-retinol (vitamin A alcohol) or 0.344 µg of *all-trans*-retinyl acetate (vitamin A acetate) or 0.55 µg of *all-trans*-retinyl palmitate (vitamin A palmitate), and 1 µg of retinol (3.3 USP Vitamin A Units) = 1 retinol equivalent (RE).

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the *all-trans*-retinyl ester from the *Sample solution*
r_S = peak area of the *all-trans*-retinyl ester from the appropriate *Standard solution*
C_S = concentration of retinol in the appropriate *Standard solution* (µg/mL)
C_U = nominal concentration of vitamin A, as retinol, in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$)

Add the following:

▲• **PROCEDURE 2**

[NOTE—Where vitamin A (retinyl acetate or retinyl palmitate) is specified in the following procedure, use the chemical form present in the formulation and the relevant Reference Standard. Use low-actinic glassware.]

Solution A: Methanol and water (90:10)
Solution B: Methanol and isopropyl alcohol (55:45)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	0	100
13	0	100
13.1	100	0
15	100	0

Vitamin A standard stock solution: 0.2 mg/mL of retinol ($C_{20}H_{30}O$) from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in isopropyl alcohol. Dissolve with the aid of sonication if necessary.
Standard solution: 0.04 mg/mL of retinol in methanol from *Vitamin A standard stock solution*
Sample solution: Finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 4.0 mg of retinol into a 100-mL volumetric flask. Add 10 mL of water and immediately swirl the sample, add 20 mL of dimethyl sulfoxide, and sonicate for 30 min with vigorous intermittent shaking. Cool to room temperature, add 70 mL of isopropyl alcohol, and swirl for 1 min to dissolve the vitamin. Place the sample in a sonicator for 5 min, and then cool the flask to room temperature. Dilute with methanol to volume and mix well. Pass a portion of the solution through a nylon filter of 0.45-µm pore size, and discard the first milliliter of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 325 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for retinyl acetate and NMT 2.0 for retinyl palmitate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of vitamin A activity, as retinol ($C_{20}H_{30}O$), in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the relevant *all-trans*-retinyl ester from *Sample solution 1*
r_S = peak area of the relevant *all-trans*-retinyl ester from the *Standard solution*
C_S = concentration of retinol in the *Standard solution* (mg/mL)
C_U = nominal concentration of vitamin A, as retinol, in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%▲ (USP 1-May-2020)

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

[NOTE—Perform this test under light conditions that minimize photodegradation.]
Medium: 1% (w/v) sodium ascorbate and 1% (w/v) octoxynol 9 in 0.05 M phosphate buffer with a pH of 6.8; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
▲▲ (USP 1-May-2020)
Sample solution: Withdraw a portion of the solution under test, pass through a suitable filter of 0.45-µm pore size, and use the pooled sample as the test specimen.
Standard solution: Dissolve a suitable amount of USP Retinyl Acetate RS or USP Retinyl Palmitate RS in isopropyl alcohol, and dilute with *Medium* to obtain a concentration similar to that of the *Sample solution*. [NOTE—The amount of isopropyl alcohol should be 5%–10%.]
▲▲ (USP 1-May-2020)
Analysis
▲Proceed as directed in *Assay, Procedure 2*, making any necessary modifications.▲ (USP 1-May-2020)
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of retinyl acetate or retinyl palmitate dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V/L) \times 100$$

r_U = peak area of the *all-trans*-retinyl ester from the *Sample solution*
r_S = peak area of the *all-trans*-retinyl ester from the appropriate *Standard solution*
C_S = concentration of retinol in the appropriate *Standard solution* (µg/mL)
V = volume of *Medium*, 900 mL
L = label claim of vitamin A, as retinol (µg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of retinyl acetate or retinyl palmitate is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature. Protect Tablets from light.

USP Monographs

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Change to read:

- **LABELING:** Label it to indicate the chemical form of vitamin A present and to indicate the vitamin A activity in terms of the equivalent amount of retinol ▲in mg/Tablet.▲ (USP 1-May-2020)
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Retinyl Acetate RS
  USP Retinyl Palmitate RS

Vitamin E

DEFINITION

Change to read:

Vitamin E is a form of alpha tocopherol ($C_{29}H_{50}O_2$). It includes the following: ▲*RRR*-▲ (USP 1-Aug-2019)or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol ($C_{29}H_{50}O_2$), ▲*RRR*-▲ (USP 1-Aug-2019)or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate ($C_{31}H_{52}O_3$), and ▲*RRR*-▲ (USP 1-Aug-2019)or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$). It contains NLT 96.0% and NMT 102.0% of $C_{29}H_{50}O_2$, $C_{31}H_{52}O_3$, or $C_{33}H_{54}O_5$.

IDENTIFICATION

Change to read:

- **A.**
  [NOTE—Use low-actinic glassware.]
  Sample solutions
  Alpha tocopherol: 1 mg/mL in dehydrated alcohol
  Alpha tocopheryl acetate: Transfer 220 mg of ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate to a round-bottom, glass-stoppered, 150-mL flask, and dissolve in 25 mL of dehydrated alcohol. Add 20 mL of diluted sulfuric acid in alcohol (1 in 7), and reflux in an all-glass apparatus for 3 h, protected from sunlight. Cool, transfer to a 200-mL volumetric flask, and add diluted sulfuric acid in alcohol (1 in 72) to volume.
  Alpha tocopheryl acid succinate: [**CAUTION**—Wear safety goggles.] Transfer an amount of the sample, equivalent to 200 mg of alpha tocopherol, to a round-bottom, glass-stoppered, 250-mL flask, dissolve in 50 mL of dehydrated alcohol, and reflux for 1 min. While the solution is boiling, add, through the condenser, 1 g of potassium hydroxide pellets, one at a time to avoid overheating.
  Continue refluxing for 20 min and, without cooling, add 2 mL of hydrochloric acid dropwise through the condenser. [NOTE—This technique is essential to prevent oxidative action by air while the sample is in an alkaline medium.]
  Cool, and transfer the contents of the flask to a 500-mL separator, rinsing the flask with 100 mL each of water and ether, and adding the rinsings to the separator. Shake vigorously, allow the layers to separate, and collect each of the two layers in individual separators. Extract the aqueous layer with two 50-mL portions of ether, and add these extracts to the main ether extract. Wash the combined ether extracts with four 100-mL portions of water, then evaporate the ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7–8 mL remain. Complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in diluted sulfuric acid in alcohol (1 in 72), transfer to a 200-mL volumetric flask, and dilute with the alcoholic sulfuric acid to volume.
  Analysis
  Sample: Use the appropriate *Sample solution*.
  To 10 mL of *Sample solution* add 2 mL of nitric acid, with swirling, and heat at about 75° for 15 min.
  Acceptance criteria: A bright red or orange color develops.

Change to read:

- **B. OPTICAL ROTATION** ⟨781⟩
  Sample solutions
  Alpha tocopherol: Dissolve 100 mg of alpha tocopherol in 50 mL of ether.
  Alpha tocopheryl acetate: Transfer a volume of the *Sample solution* for *Alpha tocopheryl acetate* from *Identification A*, equivalent to 100 mg of alpha tocopheryl acetate, to a separator, and add 200 mL of water. Extract with ether, first with 75 mL, then with 25 mL, and combine the ether extracts in another separator.
  Alpha tocopheryl acid succinate: Transfer a volume of *Sample solution* for *Alpha tocopheryl acid succinate* from *Identification A*, equivalent to 100 mg of alpha tocopheryl acid succinate, to a separator, and add 200 mL of water. Extract with ether, first with 75 mL, then with 25 mL, and combine the ether extracts in another separator.
  Analysis
  Sample: Use the appropriate *Sample solution*.
  To the entire volume of a *Sample solution*, as prepared above, add 20 mL of a solution (1 in 10) of potassium ferricyanide in sodium hydroxide solution (1 in 125), and shake for 3 min. Wash the ether solution with four 50-mL portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until 7–8 mL remain, then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 mL of 2,2,4-trimethylpentane, and determine the optical rotation using *c* as the number of grams of total tocopherols, determined in the appropriate *Assay*, in each 100 mL of solution used for the test.
  Acceptance criteria
  ▲*RRR*▲ (USP 1-Aug-2019) **Isomers:** NLT +24°
  ▲*all-rac*▲ (USP 1-Aug-2019) **Forms:** ▲−0.01° to +0.01°▲ (USP 1-Aug-2019)
- **C.** The retention time of the major peak of alpha tocopherol of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **ALPHA TOCOPHEROL**
  [NOTE—Use low-actinic glassware.]
  Internal standard solution: 10 mg/mL of squalane in cyclohexane
  System suitability solution: 0.1 mg/mL each of USP Alpha Tocopherol RS and USP Alpha Tocopheryl Acetate RS in cyclohexane
  Standard solution: 10 mg/mL of USP Alpha Tocopherol RS in *Internal standard solution*
  Sample solution: 10 mg/mL of Vitamin E ▲(*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol) in *Internal standard solution*

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 30-m fused silica capillary; bonded with a 0.25-µm film of phase G2
Temperatures
Injection port: 290°
Column: 280°
Detector: 290°
Carrier gas: Helium
Flow rate: 1 mL/min
Injection type: Split, split ratio 100:1
Injection volume: 1 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between alpha tocopherol and alpha tocopheryl acetate, *System suitability solution*
Relative standard deviation: NMT 2.0% for the peak response ratios of alpha tocopherol to the internal standard from replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol ($C_{29}H_{50}O_2$) in the portion of Vitamin E taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alpha tocopherol to the internal standard from the *Sample solution*
R_S = peak response ratio of alpha tocopherol to the internal standard from the *Standard solution*
C_S = concentration of USP Alpha Tocopherol RS in the *Standard solution* (mg/mL)
C_U = concentration of Vitamin E in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% of ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol ($C_{29}H_{50}O_2$)

Change to read:

• **ALPHA TOCOPHERYL ACETATE**
[NOTE—Use low-actinic glassware.]
Internal standard solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* for *Alpha Tocopherol* except as follows. For the *Standard solution, Sample solution,* and *Relative standard deviation,* substitute alpha tocopheryl acetate for alpha tocopherol, and substitute USP Alpha Tocopheryl Acetate RS for USP Alpha Tocopherol RS.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate ($C_{31}H_{52}O_3$) in the portion of Vitamin E taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alpha tocopheryl acetate to the internal standard from the *Sample solution*
R_S = peak response ratio of alpha tocopheryl acetate to the internal standard from the *Standard solution*
C_S = concentration of USP Alpha Tocopheryl Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Vitamin E in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% of ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate ($C_{31}H_{52}O_3$)

Change to read:

• **ALPHA TOCOPHERYL ACID SUCCINATE**
[NOTE—Use low-actinic glassware.]
Internal standard solution, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* for *Alpha Tocopherol.* For the *Relative standard deviation,* substitute alpha tocopheryl acid succinate for alpha tocopherol.
Standard solution: Transfer 30.0 mg of USP Alpha Tocopheryl Acid Succinate RS into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution,* and mix on a vortex mixer to dissolve.
Sample solution: Transfer 30.0 mg of Vitamin E (▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate) into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimeth oxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution,* and mix on a vortex mixer to dissolve.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$) in the portion of Vitamin E taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alpha tocopheryl acid succinate to the internal standard from the *Sample solution*
R_S = peak response ratio of alpha tocopheryl acid succinate to the internal standard from the *Standard solution*
C_S = concentration of USP Alpha Tocopheryl Acid Succinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Vitamin E in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% of Vitamin E as ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$)

SPECIFIC TESTS

• **ACIDITY**
Diluent: Alcohol and ether (1:1), neutralized to phenolphthalein with 0.1 N sodium hydroxide
Sample: 1.0 g
Analysis: Dissolve the *Sample* in 25 mL of *Diluent,* add 0.5 mL of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide until the solution remains faintly pink after shaking for 30 s.
Acceptance criteria: Alpha tocopheryl acid succinate requires 18.0–19.3 mL of 0.10 N sodium hydroxide; the other forms of Vitamin E require NMT 1.0 mL of 0.10 N sodium hydroxide.

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ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Protect ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol with a blanket of an inert gas, ▲and store at room temperature.▲ (USP 1-Aug-2019)

Change to read:

- **LABELING:** Label Vitamin E to indicate the chemical form and to indicate whether it is the ▲*RRR*▲ (USP 1-Aug-2019) or the ▲*all-rac*▲ (USP 1-Aug-2019) form. The Vitamin E activity may be expressed in ▲mg/g, or,▲ (USP 1-Aug-2019) in terms of the equivalent amount of ▲*RRR*▲ (USP 1-Aug-2019)-alpha tocopherol, in mg/g, based on the following relationship between the former USP Units (equal to the former International Units) and mass.[1]
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alpha Tocopherol RS
  USP Alpha Tocopheryl Acetate RS
  USP Alpha Tocopheryl Acid Succinate RS

Vitamin E Capsules

DEFINITION

Change to read:

Vitamin E Capsules contain Vitamin E or Vitamin E Preparation, where Vitamin E is a form of alpha tocopherol ($C_{29}H_{50}O_2$) that includes ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol ($C_{29}H_{50}O_2$), ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate ($C_{31}H_{52}O_3$), and ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$); and Vitamin E Preparation is a combination of a single form of Vitamin E with one or more inert substances. Vitamin E Capsules contain NLT 95.0% and NMT 120.0% of the labeled amount of vitamin E.

IDENTIFICATION

Change to read:

- **A.**
  [NOTE—Use low-actinic glassware.]

Sample solutions

Alpha tocopherol: Nominally 1 mg/mL in dehydrated alcohol

Alpha tocopheryl acetate: Transfer 220 mg of ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate from the Capsule contents to a round-bottom, glass-stoppered, 150-mL flask, and dissolve in 25 mL of dehydrated alcohol. Add 20 mL of diluted sulfuric acid in alcohol (1 in 7), and reflux in an all-glass apparatus for 3 h, protected from sunlight. Cool, transfer to a 200-mL volumetric flask, and add diluted sulfuric acid in alcohol (1 in 72) to volume.

Alpha tocopheryl acid succinate: [**CAUTION**—Wear safety goggles.] Transfer an amount of Capsule contents, equivalent to 200 mg of alpha tocopherol, to a round-bottom, glass-stoppered, 250-mL flask, dissolve in 50 mL of dehydrated alcohol, and reflux for 1 min. While the solution is boiling, add, through the condenser, 1 g of potassium hydroxide pellets, one at a time to avoid overheating.

Continue refluxing for 20 min and, without cooling, add 2 mL of hydrochloric acid dropwise through the condenser. [NOTE—This technique is essential to prevent oxidative action by air while the sample is in an alkaline medium.]

Cool, and transfer the contents of the flask to a 500-mL separator, rinsing the flask with 100 mL each of water and of ether, and adding the rinsings to the separator. Shake vigorously, allow the layers to separate, and collect each of the two layers in individual separators. Extract the aqueous layer with two 50-mL portions of ether, and add these extracts to the main ether extract. Wash the combined ether extracts with four 100-mL portions of water, then evaporate the ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7–8 mL remain. Complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in diluted sulfuric acid in alcohol (1 in 72), transfer to a 200-mL volumetric flask, and dilute with diluted sulfuric acid in alcohol (1 in 72) to volume.

Analysis

Sample: Use the appropriate *Sample solution*.

To 10 mL of *Sample solution* add 2 mL of nitric acid, with swirling, and heat at about 75° for 15 min.

Acceptance criteria: A bright red or orange color develops.

Change to read:

- **B. OPTICAL ROTATION** ⟨781⟩

Sample solutions

Alpha tocopherol: Dissolve an amount of the sample equivalent to 100 mg of alpha tocopherol in 50 mL of ether.

Alpha tocopheryl acetate: Transfer a volume of the *Sample solution* for *Alpha tocopheryl acetate* from *Identification A*, equivalent to 100 mg of the test article, to a separator, and add 200 mL of water. Extract with ether, first with 75 mL, then with 25 mL, and combine the ether extracts in another separator.

Alpha tocopheryl acid succinate: Transfer a volume of the *Sample solution* for *Alpha tocopheryl acid succinate* from *Identification A*, equivalent to 100 mg of the test article, to a separator, and add 200 mL of water. Extract with ether, first with 75 mL, then with 25 mL, and combine the ether extracts in another separator.

Analysis

Sample: Use the appropriate *Sample solution*.

[1] In terms of ▲former▲ (USP 1-Aug-2019) USP Units, 1 mg of ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol = 1.1 former USP Vitamin E Units; 1 mg of ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate = 1 former USP Vitamin E unit; 1 mg of ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate = 0.89 former USP Vitamin E Units; 1 mg of ▲*RRR*▲ (USP 1-Aug-2019)-alpha tocopherol = 1.49 former USP Vitamin E Units; 1 mg of ▲*RRR*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate = 1.36 former USP Vitamin E Units; and 1 mg of ▲*RRR*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate = 1.21 former USP Vitamin E Units. In terms of ▲*RRR*▲ (USP 1-Aug-2019)-alpha tocopherol equivalents, 1 mg of ▲*RRR*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate = 0.91; 1 mg of ▲*RRR*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate = 0.81; 1 mg of ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol = 0.74; 1 mg of ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate = 0.67; and 1 mg of ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate = 0.60. ▲Note that 1 mg of Institute of Medicine (IOM) alpha tocopherol equivalent = 1 mg of 2 *R*-alpha tocopherol = 1 mg of *RRR*-alpha tocopherol = 2 mg of *all-rac*-alpha tocopherol.▲ (USP 1-Aug-2019)

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To the entire volume of a *Sample solution*, as prepared above, add 20 mL of a solution (1 in 10) of potassium ferricyanide in sodium hydroxide solution (1 in 125), and shake for 3 min. Wash the ether solution with four 50-mL portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until 7–8 mL remain, then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 mL of 2,2,4-trimethylpentane, and determine the optical rotation using *c* as the number of grams of total tocopherols, determined in the appropriate *Assay*, in each 100 mL of solution used for the test.

Acceptance criteria

For Capsules labeled to contain ▲*RRR*▲ (USP 1-Aug-2019) isomers: NLT +24°

For Capsules labeled to contain ▲*all-rac*▲ (USP 1-Aug-2019) forms: ▲−0.01° to +0.01°▲ (USP 1-Aug-2019)

• **C.** The retention time of the major peak of alpha tocopherol of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **ALPHA TOCOPHEROL**

[NOTE—Use low-actinic glassware.]

Internal standard solution: 10 mg/mL of squalane in cyclohexane

System suitability solution: 0.1 mg/mL each of USP Alpha Tocopherol RS and USP Alpha Tocopheryl Acetate RS in cyclohexane

Standard solution: 10 mg/mL of USP Alpha Tocopherol RS in *Internal standard solution*

Sample solution: Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp knife or by other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer the combined Capsule content to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of *n*-hexane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the odor of *n*-hexane is no longer perceptible. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight/Capsule. Dissolve a portion of the combined Capsule contents in *Internal standard solution* to prepare a vitamin E (▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol) solution with a nominal concentration of 10 mg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused silica capillary; bonded with a 0.25-µm film of phase G2

Temperatures

Injection port: 290°

Column: 280°

Detector: 290°

Carrier gas: Helium

Flow rate: 1 mL/min

Injection type: Split, split ratio 100:1

Injection volume: 1 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.5 between alpha tocopherol and alpha tocopheryl acetate, *System suitability solution*

Relative standard deviation: NMT 2.0% for peak response ratios of alpha tocopherol to the internal standard from replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin E (▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol) in the portion of the Capsule contents taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alpha tocopherol to the internal standard from the *Sample solution*

R_S = peak response ratio of alpha tocopherol to the internal standard from the *Standard solution*

C_S = concentration of USP Alpha Tocopherol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of vitamin E as ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin E as ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol ($C_{29}H_{50}O_2$)

Change to read:

• **ALPHA TOCOPHERYL ACETATE:** Proceed as directed in the *Assay* for *Alpha Tocopherol* except as follows. For the *Standard solution* and *Sample solution*, substitute alpha tocopheryl acetate for alpha tocopherol, and substitute USP Alpha Tocopheryl Acetate RS for USP Alpha Tocopherol RS.

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin E as ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acetate ($C_{31}H_{52}O_3$)

Change to read:

• **ALPHA TOCOPHERYL ACID SUCCINATE**

Internal standard solution, System suitability solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in the *Assay* for *Alpha Tocopherol*.

Standard solution: Transfer 30.0 mg of USP Alpha Tocopheryl Acid Succinate RS into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

Sample solution: Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp knife or by other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer the combined Capsule content to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of *n*-hexane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the odor of *n*-hexane is no longer perceptible. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight/Capsule. Transfer a portion of the combined Capsule contents, equivalent to

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30.0 mg of vitamin E ▲(*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate), into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin E as ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$)

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩

Buffer solution: Dissolve 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid in water to obtain 1000 mL of solution having a pH of 4.5 ± 0.05.

Medium: *Buffer solution*

Time: 45 min

Acceptance criteria: Meet the requirements

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature. Protect Capsules containing ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol from light.

Change to read:

- **LABELING:** Label to indicate the chemical form of vitamin E present and to indicate whether the ▲*RRR*▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019) form is present, excluding any different forms that may be introduced as a minor constituent of the vehicle. Designate the quantity of vitamin E present ▲in mg/Capsule.▲ (USP 1-Aug-2019)
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Alpha Tocopherol RS
USP Alpha Tocopheryl Acetate RS
USP Alpha Tocopheryl Acid Succinate RS

Vitamin E Preparation

DEFINITION

Change to read:

Vitamin E Preparation is a combination of a single form of Vitamin E with one or more inert substances. It may be in a liquid or solid form. It contains NLT 95.0% and NMT 120.0% of the labeled amount of vitamin E. Vitamin E Preparation labeled to contain an ▲*all-rac*▲ (USP 1-Aug-2019) form of Vitamin E may contain also a small amount of a ▲*RRR*▲ (USP 1-Aug-2019) form of Vitamin E, occurring as a minor constituent of an added substance.

IDENTIFICATION

- **A.**

[NOTE—Use low-actinic glassware.]

Sample solution: [**CAUTION**—Wear safety goggles.] Transfer an amount of Preparation, equivalent to 200 mg of alpha tocopherol, to a round-bottom, glass-stoppered, 250-mL flask. Dissolve in 50 mL of dehydrated alcohol, and reflux for 1 min. While the solution is boiling, add, through the condenser, 1 g of potassium hydroxide pellets, one at a time to avoid overheating.

Continue refluxing for 20 min and, without cooling, add 2 mL of hydrochloric acid dropwise through the condenser. [NOTE—This technique is essential to prevent oxidative action by air while the sample is in an alkaline medium.]

Cool, and transfer the contents of the flask to a 500-mL separator, rinsing the flask with 100 mL each of water and of ether, and adding the rinsings to the separator. Shake vigorously, allow the layers to separate, and collect each of the two layers in individual separators. Extract the aqueous layer with two 50-mL portions of ether, and add these extracts to the main ether extract. Wash the combined ether extracts with four 100-mL portions of water, then evaporate the ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7–8 mL remain. Complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in dehydrated alcohol, transfer to a 250-mL volumetric flask, and dilute with dehydrated alcohol to volume.

Analysis: To 10 mL of the *Sample solution*, add 2 mL of nitric acid, with swirling, and heat at about 75° for 15 min.

Acceptance criteria: A bright red or orange color develops.

Change to read:

- **B. OPTICAL ROTATION** ⟨781⟩

Sample solution: A volume of the *Sample solution* from *Identification A*, equivalent to 100 mg of Preparation

Analysis: Transfer the *Sample solution* to a separator, and add 200 mL of water. Extract with ether, first with 75 mL, then with 25 mL, and combine the ether extracts in another separator. To the combined ether extracts, add 20 mL of a solution (1 in 10) of potassium ferricyanide in sodium hydroxide solution (1 in 125), and shake for 3 min. Wash the ether solution with four 50-mL portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7–8 mL remain, then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 mL of 2,2,4-trimethylpentane, and determine the optical rotation using *c* as the number of grams of total tocopherols, as determined in the appropriate *Assay*, in each 100 mL of solution used for the test.

Acceptance criteria

For Preparation labeled to contain ▲*RRR*▲ (USP 1-Aug-2019) isomers: NLT +24°

For Preparation labeled to contain ▲*all-rac*▲ (USP 1-Aug-2019) forms: ▲–0.01° to +0.01°▲ (USP 1-Aug-2019)

- **C.** The retention time of the major peak of the *Sample solution* corresponds that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **ALPHA TOCOPHEROL**

[NOTE—Use low-actinic glassware.]

Internal standard solution: 10 mg/mL of squalane in cyclohexane

System suitability solution: 0.1 mg/mL each of USP Alpha Tocopherol RS and USP Alpha Tocopheryl Acetate RS in cyclohexane

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Standard solution: 10 mg/mL of USP Alpha Tocopherol RS in *Internal standard solution*

Sample solution

For the Preparation in liquid form: Dissolve a portion of Preparation in *Internal standard solution* to prepare a vitamin E ▲(*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol) solution with a nominal concentration of 10 mg/mL.

For the Preparation in solid form: Transfer a portion of Preparation, equivalent to 50 mg of alpha tocopherol, into a flask suitable for refluxing. Add 5 mL of water, and heat on a water bath at 60° for 10 min. Add 25 mL of alcohol, and reflux for 30 min. Cool, and transfer to a separator with the aid of 50 mL of water and 50 mL of ether. Shake vigorously, allow the layers to separate, and collect each layer in individual separators. Extract the aqueous layer with two 25-mL portions of ether, combining the extracts with the original ether layer. Wash the combined extract with one 25-mL portion of water, filter the ether solutions through 1 g of anhydrous sodium sulfate, and, with the aid of a stream of nitrogen, evaporate the ether solution on a water bath controlled at a temperature that will not cause the ether solution to boil over. Remove the container from the water bath when 5 mL remains, and complete the evaporation without the application of heat. Dissolve the residue in *Internal standard solution* to prepare a vitamin E ▲(*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol) solution with a nominal concentration of 10 mg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused silica capillary; bonded with a 0.25-µm film of phase G2

Temperatures

Injection port: 290°

Column: 280°

Detector: 290°

Carrier gas: Helium

Flow rate: 1 mL/min

Injection type: Split, split ratio 100:1

Injection volume: 1 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.5 between alpha tocopherol and alpha tocopheryl acetate, *System suitability solution*

Relative standard deviation: NMT 2.0% for the peak response ratios of alpha tocopherol to the internal standard from replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin E ▲(*RRR*-▲ (USP 1-Aug-2019)or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol) in the portion of Preparation taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alpha tocopherol to the internal standard from the *Sample solution*

R_S = peak response ratio of alpha tocopherol to the internal standard from the *Standard solution*

C_S = concentration of USP Alpha Tocopherol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of vitamin E as ▲*RRR*-▲ (USP 1-Aug-2019)or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin E as ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol ($C_{29}H_{50}O_2$)

Change to read:

• **ALPHA TOCOPHERYL ACETATE:** Proceed as directed in the *Assay* for *Alpha Tocopherol* except as follows. For the *Standard solution* and *Sample solution*, substitute alpha tocopheryl acetate for alpha tocopherol, and substitute USP Alpha Tocopheryl Acetate RS for USP Alpha Tocopherol RS.

Acceptance criteria: 95.0%–120.0% ▲of the labeled amount of vitamin E as *RRR*- or *all-rac*-alpha tocopheryl acetate ($C_{31}H_{52}O_3$)▲ (USP 1-Aug-2019)

Change to read:

• **ALPHA TOCOPHERYL ACID SUCCINATE**

Internal standard solution, System suitability solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in the *Assay* for *Alpha Tocopherol*.

Standard solution: Transfer 30.0 mg of USP Alpha Tocopheryl Acid Succinate RS into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

Sample solution

For the Preparation in liquid form: Transfer a portion of Preparation, equivalent to 30.0 mg of vitamin E ▲(*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate), into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

For the Preparation in solid form: Transfer a portion of Preparation, equivalent to 30 mg of vitamin E ▲(*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopheryl acid succinate), into a flask suitable for refluxing. Add 5 mL of water, and heat on a water bath at 60° for 10 min. Add 25 mL of alcohol, and reflux for 30 min. Cool, and transfer to a separator with the aid of 50 mL of water and 50 mL of ether. Shake vigorously, allow the layers to separate, and collect each layer in individual separators. Extract the aqueous layer with two 25-mL portions of ether, combining the extracts with the original ether layer. Wash the combined extract with one 25-mL portion of water, filter the ether solutions through 1 g of anhydrous sodium sulfate, and, with the aid of a stream of nitrogen, evaporate the ether solution on a water bath controlled at a temperature that will not cause the ether solution to boil over. Remove the container from the water bath when 5 mL remains. Quantitatively transfer the remains into a 20-mL vial, and complete the evaporation without the application of heat. Add 2.0 mL of methanol, 1.0 mL of 2,2- dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min.

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Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

Acceptance criteria: 95.0%–120.0% ▲of the labeled amount of vitamin E as *RRR*- or *all-rac*-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$)▲ (USP 1-Aug-2019)

SPECIFIC TESTS

• **ACIDITY** (for liquid forms of Preparation)

Diluent: Alcohol and ether (1:1), neutralized to phenolphthalein with 0.1 N sodium hydroxide

Sample solution: Dissolve 1 g of Preparation in 25 mL of *Diluent*.

Analysis: To 25 mL of the *Sample solution* add 0.5 mL of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide until the solution remains faintly pink after shaking for 30 s.

Acceptance criteria: NMT 1.0 mL of 0.10 N sodium hydroxide is required.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Protect Preparation containing ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019)-alpha tocopherol with a blanket of inert gas.

Change to read:

• **LABELING:** Label it to indicate the chemical form of vitamin E present, and to indicate whether the ▲*RRR*-▲ (USP 1-Aug-2019) or ▲*all-rac*▲ (USP 1-Aug-2019) form is present, excluding any different forms that may be introduced as a minor constituent of the vehicle. ▲Vitamin E content is expressed in mg/g.▲ (USP 1-Aug-2019)

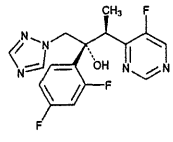
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Alpha Tocopherol RS

USP Alpha Tocopheryl Acetate RS

USP Alpha Tocopheryl Acid Succinate RS

Voriconazole



$C_{16}H_{14}F_3N_5O$ 349.31

4-Pyrimidineethanol, α-(2,4-difluorophenyl)-5-fluoro-β-methyl-α-(1*H*-1,2,4-triazol-1-ylmethyl)-, (α*R*,β*S*)-; (α*R*,β*S*)-α-(2,4-Difluorophenyl)-5-fluoro-β-methyl-α-(1*H*-1,2,4-triazol-1-ylmethyl)-4-pyrimidineethanol [137234-62-9].

DEFINITION

Voriconazole contains NLT 97.5% and NMT 102.0% of Voriconazole ($C_{16}H_{14}F_3N_5O$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** ⟨197K⟩

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of *System suitability solution A*, as obtained in the test for *Voriconazole Related Compound B*.

ASSAY

• **PROCEDURE**

Buffer: 1.9 g/L of ammonium formate in water. Adjust with formic acid to a pH of 4.0.

Mobile phase: Acetonitrile, methanol, and *Buffer* (15:30:55)

Standard solution: 25 µg/mL of USP Voriconazole RS in *Mobile phase*. [NOTE—Sonicate to dissolve, if necessary.]

Sample solution: 25 µg/mL of Voriconazole in *Mobile phase*. [NOTE—Sonicate to dissolve, if necessary.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 256 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 3500 theoretical plates

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of voriconazole ($C_{16}H_{14}F_3N_5O$) in the portion of Voriconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Voriconazole RS in the *Standard solution* (µg/mL)

C_U = concentration of Voriconazole in the *Sample solution* (µg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **VORICONAZOLE RELATED COMPOUNDS C AND D**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.25 µg/mL of USP Voriconazole RS ▲in *Mobile phase*▲ (ERR 1-Jul-2019)

Standard solution: 2.5 µg/mL each of USP Voriconazole RS, USP Voriconazole Related Compound C RS, and USP Voriconazole Related Compound D RS in *Mobile phase*. [NOTE—Sonicate to dissolve, if necessary.]

Sample solution: 500 µg/mL of Voriconazole in *Mobile phase*. [NOTE—Sonicate to dissolve, if necessary.]

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the voriconazole peak, *Standard solution*

Column efficiency: NLT 3500 theoretical plates for the voriconazole peak, *Standard solution*

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Relative standard deviation: NMT 10.0%, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of voriconazole related compound C and voriconazole related compound D in the portion of Voriconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of voriconazole related compound C or voriconazole related compound D from the *Sample solution*

r_S = peak response of voriconazole related compound C or voriconazole related compound D from the *Standard solution*

C_S = concentration of USP Voriconazole Related Compound C RS or USP Voriconazole Related Compound D RS in the *Standard solution* (µg/mL)

C_U = concentration of Voriconazole in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Voriconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of voriconazole from the *Standard solution*

C_S = concentration of USP Voriconazole RS in the *Standard solution* (µg/mL)

C_U = concentration of Voriconazole in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Voriconazole related compound C[a]	0.26	0.2
Voriconazole related compound D[b]	0.61	0.1
Voriconazole	1.0	—
Any unspecified impurity[c]	—	0.1
Total impurities[d]	—	0.5

[a] 1-(2,4-Difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone.

[b] (2*RS*,3*SR*)-2-(2,4-Difluorophenyl)-3-(pyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl) butan-2-ol.

[c] Disregard peaks less than 0.05%.

[d] Include voriconazole related compound B and voriconazole related compound F.

• **VORICONAZOLE RELATED COMPOUND F**

Sodium hydroxide solution: 470 g/L of sodium hydroxide in water

Mobile phase: Methanol, water, and *Sodium hydroxide solution* (500: 1500: 0.175). [NOTE—Minimize the carbonate formation in the *Mobile phase* by degassing methanol and water before mixing.]

Suppressant solution: 12 mM of sulfuric acid in water

Chloride stock solution: 85 µg/mL of sodium chloride in water

Standard stock solution: 250 µg/mL of USP Voriconazole Related Compound F RS. Dissolve in 50% of the final volume with methanol, and dilute with *Mobile phase* to volume.

Standard solution: 5 µg/mL of USP Voriconazole Related Compound F RS from the *Standard stock solution* in a mixture of methanol and *Mobile phase* (50:50)

System suitability solution A: 5 µg/mL of USP Voriconazole Related Compound F RS from the *Standard stock solution* and 1.7 µg/mL of sodium chloride in a mixture of methanol and *Mobile phase* (50:50)

System suitability solution B: 2.5 µg/mL of USP Voriconazole Related Compound F RS from the *Standard solution* in *Mobile phase*

Sample solution: 5 mg/mL of Voriconazole. Dissolve in 50% of the final volume with methanol, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: Ion chromatography/LC

Detector: Conductivity with anion suppressor

Column: 4-mm × 5-cm guard column and 4-mm × 25-cm analytical column; both packing L46

Column temperature: 40°

Flow rate: 1 mL/min

Flow rate (for anion suppressor): 2 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for acetate ion (for information only), voriconazole related compound F, and chloride ion are 0.47, 1.0, and 1.5, respectively.]

Suitability requirements

Resolution: NLT 3.5 between the voriconazole related compound F and chloride peaks, *System suitability solution A*

Tailing factor: NMT 2.0, *System suitability solution B*

Relative standard deviation: NMT 10.0%, *System suitability solution B*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of voriconazole related compound F in the portion of Voriconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of voriconazole related compound F from the *Sample solution*

r_S = peak response of voriconazole related compound F from the *Standard solution*

C_S = concentration of USP Voriconazole Related Compound F RS in the *Standard solution* (µg/mL)

C_U = concentration of Voriconazole in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.1%

• **VORICONAZOLE RELATED COMPOUND B**

Initially dissolve the Standard and sample materials in 4% of the final volume of acetonitrile.

Buffer: 0.8 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 5.0.

Mobile phase: Acetonitrile and *Buffer* (18:82)

System suitability solution A: 500 µg/mL of USP Voriconazole RS and 2.5 µg/mL of USP Voriconazole Related Compound B in *Mobile phase*.

System suitability solution B: 0.25 µg/mL of USP Voriconazole Related Compound B RS in *Mobile phase*

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Standard solution: 2.5 µg/mL of USP Voriconazole Related Compound B RS in *Mobile phase*
Sample solution: 500 µg/mL of Voriconazole in *Mobile phase.*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 256 nm
Column: 4.6-mm × 25-cm; 5-µm packing L45
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution A* and *System suitability solution B*
[NOTE—The relative retention times for voriconazole and voriconazole related compound B are 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 4.0 between the voriconazole and voriconazole related compound B peaks, *System suitability solution A*
Tailing factor: NMT 2.0 for the voriconazole related compound B peak, *System suitability solution A*
Relative standard deviation: NMT 10.0%, *System suitability solution B*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of voriconazole related compound B in the portion of Voriconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of voriconazole related compound B from the *Sample solution*
r_S = peak response of voriconazole related compound B from the *Standard solution*
C_S = concentration of USP Voriconazole Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of Voriconazole in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Voriconazole is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.2 USP Endotoxin Units/mg of voriconazole.
- **STERILITY TESTS** ⟨71⟩: Where the label states that Voriconazole is sterile, it meets the requirements.
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.4%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Voriconazole RS
USP Voriconazole Related Compound B RS
(2*S*,3*R*)-2-(2,4-Difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol.
$C_{16}H_{14}F_3N_5O$ 349.31
USP Voriconazole Related Compound C RS
1-(2,4-Difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl) ethanone.
$C_{10}H_7N_3OF_2$ 223.18
USP Voriconazole Related Compound D RS
(2*RS*,3*SR*)-2-(2,4-Difluorophenyl)-3-(pyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol.
$C_{16}H_{15}F_2N_5O$ 331.32
USP Voriconazole Related Compound F RS
{(1*RS*,4*SR*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]hept-1-yl} methanesulfonic acid.
$C_{10}H_{16}O_4S$ 232.30

Voriconazole Compounded Ophthalmic Solution, Veterinary

DEFINITION

Voriconazole Compounded Ophthalmic Solution, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of voriconazole ($C_{16}H_{14}F_3N_5O$).
Prepare Voriconazole Compounded Ophthalmic Solution, Veterinary 10 mg/mL as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Voriconazole for Injection[a] equivalent to	200 mg
Sodium Chloride Injection (0.9%), a sufficient quantity to make	20 mL

[a] V-Fend 200-mg injection, Pfizer, Inc., Groton, CT.

Reconstitute *Voriconazole for Injection* with *Sodium Chloride Injection (0.9%)* to bring to a final volume of 20 mL. [NOTE—May reconstitute according to package insert to achieve a 10-mg/mL solution.] Shake well until a clear solution is achieved. Aseptically draw up into sterile unit-dose 1-mL syringes.

ASSAY

- **PROCEDURE**

Solution A: 30 mM monobasic sodium phosphate adjusted with phosphoric acid to a pH of 4.0
Mobile phase: Methanol, acetonitrile, and *Solution A* (30:15:55). Pass through a PTFE filter of 0.45-µm pore size.
Standard solution: 0.25 mg/mL of voriconazole prepared from USP Voriconazole RS in *Mobile phase.* Sonicate for about 5 min to dissolve.
Sample solution: Transfer 250 µL of Ophthalmic Solution, Veterinary into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well to dissolve. Protect from light.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 256 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for voriconazole is about 4.2 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of voriconazole ($C_{16}H_{14}F_3N_5O$) in the portion of Ophthalmic Solution, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of voriconazole from the *Sample solution*
r_S = peak response of voriconazole from the *Standard solution*
C_S = concentration of voriconazole in the *Standard solution* (mg/mL)
C_U = nominal concentration of voriconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

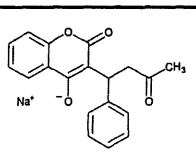
SPECIFIC TESTS
- **pH** ⟨791⟩: 5.7–6.7
- **Sterility Tests** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to be Examined, Membrane Filtration.*
- **Particulate Matter in Ophthalmic Solutions** ⟨789⟩: It meets the requirements.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in sterile unit-dose 1-mL syringes. Store at 2°–8°.

Change to read:

- **Beyond-Use Date:** ▲In the absence of passing a sterility test, the BUDs in *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test is passed, NMT 60 days after the date on which it was compounded when stored at 2°–8°.▲ (CN 1-May-2020)
- **Labeling:** Label it to indicate that it is for veterinary use only. Label it to state that this is a single-dose container intended for use in the eye and to not use if a precipitate is present. Label it with a warning that it is not for injection. Label it to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
USP Voriconazole RS

Warfarin Sodium



$C_{19}H_{15}NaO_4$ 330.31
2*H*-1-Benzopyran-2-one, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-, sodium salt;
3-(α-Acetonylbenzyl)-4-hydroxycoumarin sodium salt;
Sodium 2-oxo-3-(3-oxo-1-phenylbutyl)-2*H*-chromen-4-olate [129-06-6].

DEFINITION
Warfarin Sodium is an amorphous solid or a crystalline clathrate. The crystalline form consists principally of warfarin sodium and isopropyl alcohol in a 2:1 molecular ratio. It contains NLT 8.0% and NMT 8.5% of isopropyl alcohol. Warfarin Sodium contains NLT 97.0% and NMT 102.0% of warfarin sodium ($C_{19}H_{15}NaO_4$), calculated on the anhydrous basis for the amorphous form or on the anhydrous and isopropyl alcohol-free basis for the crystalline form.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Standard: Use USP Warfarin RS.
Sample: Dissolve 100 mg in 25 mL of water, and adjust with hydrochloric acid to a pH of less than 3, using short-range pH indicator paper. Stir the mixture, and allow the precipitate to coagulate. Filter the mixture, wash the precipitate with four, 5-mL portions of water, and dry under vacuum over phosphorus pentoxide for 4 h. Use the warfarin obtained.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **C.**
Methoxyphenylacetic reagent: Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of 10% tetramethylammonium hydroxide aqueous solution, and add 20 mL of absolute alcohol.
Diluted ammonia: Dilute 41 g of ammonium hydroxide with water to 100 mL.
Ammonium carbonate solution: 158 mg/mL of ammonium carbonate in water
Sample solution: Dissolve 30 mg of Warfarin Sodium in 0.5 mL of water.
Analysis: Add 1.5 mL of *Methoxyphenylacetic reagent* to the *Sample solution*, and cool in ice water for 30 min.
Acceptance criteria: A voluminous, white, crystalline precipitate is formed. Place the precipitate in a water bath at 20° and stir for 5 min. The precipitate does not disappear. Add 1 mL of *Diluted ammonia.* The precipitate dissolves completely. Add 1 mL of *Ammonium carbonate solution.* No precipitate is formed.

ASSAY
- **Procedure**
Buffer: Transfer 1.36 g of monobasic potassium phosphate to a 200-mL volumetric flask, and dissolve in 50 mL of water. Add 39.1 mL of 0.2 N sodium hydroxide, and dilute with water to volume. Adjust with sodium hydroxide or phosphoric acid to a pH of 7.4 ± 0.1.
Mobile phase: Methanol, glacial acetic acid, and water (64:1:36)
Standard stock solution: 0.376 mg/mL of USP Warfarin RS prepared as follows. Transfer USP Warfarin RS to a suitable volumetric flask, and dissolve in 0.1 N sodium hydroxide equivalent to 39% of the final volume. Add 0.2 M monobasic potassium phosphate, equivalent to 25% of the final volume, and dilute with water to volume.
Standard solution: Transfer 5 mL of *Standard stock solution* and 15 mL of *Buffer* into a conical flask, and mix.
Sample stock solution: 0.4 mg/mL of Warfarin Sodium prepared as follows. Transfer 100 mg of Warfarin Sodium, accurately weighed, to a 250-mL volumetric flask, and dissolve in 97.8 mL of 0.1 N sodium hydroxide. Add 62.5 mL of 0.2 M monobasic potassium phosphate, and dilute with water to volume.
Sample solution: Transfer 5 mL of *Sample stock solution* and 15 mL of *Buffer* into a conical flask, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 280 nm

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Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1.4 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of warfarin sodium ($C_{19}H_{15}NaO_4$) in the portion of Warfarin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of warfarin from the *Sample solution*
r_S = peak response of warfarin from the *Standard solution*
C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL)
C_U = concentration of Warfarin Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of warfarin sodium, 330.31
M_{r2} = molecular weight of warfarin, 308.33

Acceptance criteria: 97.0%–102.0% on the anhydrous basis for the amorphous form or on the anhydrous and isopropyl alcohol-free basis for the crystalline form

IMPURITIES

• Organic Impurities

Diluent: Methanol and water (25:75)
Mobile phase: Acetonitrile, glacial acetic acid, and water (32:1:68)
Standard stock solution: 0.12 mg/mL each of USP Warfarin RS and USP Warfarin Related Compound A RS prepared as follows. Transfer USP Warfarin RS and USP Warfarin Related Compound A RS to a suitable volumetric flask, and add 0.1 N sodium hydroxide and methanol equivalent to 2% and 25% of the final volume, respectively. Dilute with water to volume.
Standard solution: 2.4 µg/mL each of USP Warfarin RS and USP Warfarin Related Compound A RS in *Diluent*, from *Standard stock solution*
Sample solution: 0.8 mg/mL of Warfarin Sodium in *Diluent*
System suitability solution: 2.4 µg/mL of USP Warfarin Related Compound A RS and 0.8 mg/mL of Warfarin Sodium in *Diluent* prepared as follows. Transfer 0.5 mL of *Standard stock solution* to a 25-mL volumetric flask and dilute with *Sample solution* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 25-cm; packing L10
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of the warfarin peak
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3 between warfarin and warfarin related compound A peaks, *System suitability solution*
Relative standard deviation: NMT 5.0% for the warfarin and warfarin related compound A peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of Alice's ketone (sodium salt of warfarin related compound A) in the portion of Warfarin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of warfarin related compound A from the *Sample solution*
r_S = peak response of warfarin related compound A from the *Standard solution*
C_S = concentration of USP Warfarin Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Warfarin Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of Alice's ketone, 286.30
M_{r2} = molecular weight of warfarin related compound A, 264.32

Calculate the percentage of any other individual impurity in the portion of Warfarin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of warfarin from the *Standard solution*
C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL)
C_U = concentration of Warfarin Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of warfarin sodium, 330.31
M_{r2} = molecular weight of warfarin, 308.33
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.06%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Hydroxycoumarin[a]	0.4	2.0	0.3
Benzalacetone[b]	0.6	2.0	0.3
Warfarin	1.0	—	—
Alice's ketone[c]	1.2	1.0	0.3
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 4-Hydroxy-2*H*-chromen-2-one.
[b] (*E*)-4-Phenylbut-3-en-2-one.
[c] Sodium salt of warfarin related compound A; 3-(o-Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one sodium salt.

SPECIFIC TESTS

• Isopropyl Alcohol Content (Crystalline Clathrate Form)

Internal standard solution: 4.25 mg/mL of *n*-propyl alcohol in water
Standard stock solution: 4.25 mg/mL of isopropyl alcohol in water
Standard solution: Transfer 2.0 mL of the *Standard stock solution* and 2.0 mL of the *Internal standard solution* to a headspace vial, seal, and mix.

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Sample solution: Transfer 100 mg of Warfarin Sodium, 2.0 mL of water, and 2.0 mL of the *Internal standard solution* to a headspace vial, seal, and mix.
Blank solution: Transfer 2.0 mL of water and 2.0 mL of the *Internal standard solution* to a headspace vial, seal, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m; 1.8-µm coating of phase G43
Temperatures
Injector: 140°
Detector: 240°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	20
40	10	240	10

Carrier gas: Nitrogen
Flow rate: 1 mL/min
Injector type: Headspace; split ratio, 80:1
Temperatures
Equilibration: 65°
Needle: 75°
Transfer line: 85°
Headspace carrier pressure: 15 psi
Times
Equilibration: 20 min
Pressurization: 3.0 min
Loop fill: 0.2 min
Injection: 0.1 min
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for isopropyl alcohol and *n*-propyl alcohol are about 0.66 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between isopropyl alcohol and *n*-propyl alcohol
Tailing factor: NMT 1.3 for the isopropyl alcohol peak
Relative standard deviation: NMT 2.0%, peak response ratio of isopropyl alcohol to *n*-propyl alcohol
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank solution*
Calculate the percentage of isopropyl alcohol in the portion of Warfarin Sodium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of isopropyl alcohol to *n*-propyl alcohol from the *Sample solution*
R_S = peak response ratio of isopropyl alcohol to *n*-propyl alcohol from the *Standard solution*
C_S = concentration of isopropyl alcohol in the *Standard solution* (mg/mL)
C_U = concentration of Warfarin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 8.0%–8.5%
• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 7.2–8.3
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 4.5% for the amorphous form; NMT 0.3% for the crystalline clathrate form
• **ABSORBANCE IN ALKALINE SOLUTION**
Sample solution: 125 mg/mL in sodium hydroxide solution (1 in 20). Pass through a membrane filter.
Blank: Sodium hydroxide solution (1 in 20)
Analysis: Determine the absorbance of the solution in a 1-cm cell at 385 nm, with a suitable spectrometer, within 15 min.
Acceptance criteria: NMT 0.1

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **LABELING:** Label it to indicate whether it is the amorphous or the crystalline form.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Warfarin RS
USP Warfarin Related Compound A RS
3-(*o*-Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one.
$C_{18}H_{16}O_2$ 264.32

Warfarin Sodium for Injection

DEFINITION
Warfarin Sodium for Injection is a sterile, freeze-dried mixture of Warfarin Sodium and suitable added substances. It contains NLT 95.0% and NMT 105.0% of the labeled amount of warfarin sodium ($C_{19}H_{15}NaO_4$). It may contain a suitable buffer.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Dissolve 100 mg in 25 mL of water, and adjust with hydrochloric acid to a pH of less than 3, using short-range pH indicator paper. Stir the mixture, and allow the precipitate to coagulate. Filter the mixture. Wash the precipitate with four 5-mL portions of water, and dry under vacuum over phosphorus pentoxide for 4 h. Use the warfarin obtained.
Standard: Use USP Warfarin RS.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: Transfer 1.36 g of monobasic potassium phosphate to a 200-mL volumetric flask, and dissolve in 50 mL of water. Add 39.1 mL of 0.2 N sodium hydroxide, and dilute with water to volume. Adjust with sodium hydroxide or phosphoric acid to a pH of 7.4 ± 0.1.
Mobile phase: Methanol, glacial acetic acid, and water (64:1:36)
Standard stock solution: 0.94 mg/mL of USP Warfarin RS prepared as follows. Transfer USP Warfarin RS to a suitable volumetric flask, and dissolve in 0.1 N sodium hydroxide equivalent to 39% of the final volume. Add 0.2 M monobasic potassium phosphate, equivalent to 25% of the final volume, and dilute with water to volume.
Standard solution: Transfer 5 mL of *Standard stock solution* into a 50-mL volumetric flask, and dilute with *Buffer* to volume.

USP Monographs

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Sample solution: Nominally 0.1 mg/mL of warfarin sodium in *Buffer* prepared as follows. Dissolve the contents of NLT 10 containers of Warfarin Sodium for Injection in *Buffer*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1.4 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of warfarin sodium ($C_{19}H_{15}NaO_4$) in the portion of Warfarin Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of warfarin from the *Sample solution*
r_S = peak response of warfarin from the *Standard solution*
C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of warfarin sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of warfarin sodium, 330.31
M_{r2} = molecular weight of warfarin, 308.33

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements

IMPURITIES

• **Organic Impurities**
Diluent: Methanol and water (25:75)
Mobile phase: Methanol, tetrahydrofuran, glacial acetic acid, and water (53:1:1:47)
Standard stock solution 1: 0.126 mg/mL of USP Warfarin RS prepared as follows. Transfer USP Warfarin RS to a suitable volumetric flask, and add 0.1 N sodium hydroxide and methanol equivalent to 2% and 25% of the final volume, respectively. Sonicate to dissolve. Dilute with water to volume.
Standard stock solution 2: 2.4 µg/mL of USP Warfarin RS in *Diluent* from *Standard stock solution 1*
Standard solution: 1.2 µg/mL of USP Warfarin RS in *Diluent* from *Standard stock solution 1*
System suitability stock solution 1: 0.24 mg/mL of USP Warfarin Related Compound A RS in methanol
System suitability stock solution 2: 2.4 µg/mL of USP Warfarin Related Compound A RS in *Diluent* from *System suitability stock solution 1*
System suitability solution: 1.2 µg/mL of each of USP Warfarin RS and USP Warfarin Related Compound A RS in *Diluent* from *Standard stock solution 2* and *System suitability stock solution 2*
Sample solution: Nominally equivalent to 0.5 mg/mL of warfarin sodium in *Diluent* prepared as follows. Transfer the contents of NLT 10 vials of Warfarin Sodium for Injection into a suitable volumetric flask, dissolve, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L11
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
Run time: NLT 4 times the retention time of warfarin
System suitability
Samples: *Standard solution* and *System suitability solution*
[Note—The relative retention times for warfarin and warfarin related compound A are about 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 2 between warfarin and warfarin related compound A peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of Alice's ketone in the portion of Warfarin Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of warfarin related compound A from the *Sample solution*
r_S = peak response of warfarin from the *Standard solution*
C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL)
C_U = concentration of warfarin sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of warfarin sodium, 330.31
M_{r2} = molecular weight of warfarin, 308.33
F = relative response factor for Alice's ketone, 0.9

[Note—Alice's ketone is a sodium salt of warfarin related compound A, 3-(*o*-hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one sodium salt.]
Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

• **Water Determination,** *Method I* ⟨921⟩**:** NMT 4.5%
• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 7.2–8.3
• **Constituted Solution:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*
• **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 24.0 of USP Endotoxin Units/mg of warfarin sodium
• **Sterility Tests** ⟨71⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in light-resistant containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*. Store at controlled room temperature.
• **Labeling:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*
• **USP Reference Standards** ⟨11⟩
USP Warfarin RS
USP Warfarin Related Compound A RS
3-(*o*-Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one.
$C_{18}H_{16}O_2$ 264.33

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Warfarin Sodium Tablets

DEFINITION
Warfarin Sodium Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of warfarin sodium ($C_{19}H_{15}NaO_4$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Triturate a quantity of finely powdered Tablets, equivalent to about 200 mg of warfarin sodium, with 50 mL of water, centrifuge, and filter the supernatant. Extract with 50 mL of ether, transfer the aqueous layer to a second separator, and discard the ether. Adjust with hydrochloric acid to a pH of less than 3, using short-range pH indicator paper, and extract with 50 mL of chloroform. Transfer the chloroform layer to another separator, extract with 50 mL of sodium hydroxide solution (1 in 250), and discard the chloroform. Transfer the aqueous layer to a beaker, and adjust with hydrochloric acid to a pH of less than 3 (using the pH indicator paper) to precipitate the warfarin. Stir the mixture, and allow the precipitate to coagulate. Filter, and wash the precipitate with four, 5-mL portions of water. If the precipitate is not white or practically white, dissolve it in a minimum volume of sodium hydroxide solution (1 in 250), dilute with water to 50 mL, and repeat the foregoing procedure, beginning with "Extract with 50 mL of ether." Dry the warfarin under vacuum over phosphorus pentoxide for 4 h.

ASSAY
• **PROCEDURE**
Buffer: Transfer 1.36 g of monobasic potassium phosphate to a 200-mL volumetric flask, and dissolve in 50 mL of water. Add 39.1 mL of 0.2 N sodium hydroxide, and dilute with water to volume. Adjust with sodium hydroxide or phosphoric acid to a pH of 7.4 ± 0.1.
Diluent: Acetonitrile and *Buffer* (15:85)
Mobile phase: Methanol, glacial acetic acid, and water (68:1:32)
Standard stock solution: 0.31 mg/mL of USP Warfarin RS prepared as follows. Transfer USP Warfarin RS to a suitable volumetric flask, and dissolve in 0.1 N sodium hydroxide equivalent to 39% of the final volume. Add 0.2 M monobasic potassium phosphate, equivalent to 25% of the final volume, and dilute with water to volume.
Standard solution: Transfer 15.0 mL of *Standard stock solution* into a 50-mL volumetric flask, and dilute with *Diluent* to volume.
Sample solution: Nominally 0.1 mg/mL of warfarin sodium in *Diluent* prepared as follows. Weigh, and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of warfarin sodium, to a 50-mL volumetric flask, and add about 30 mL of *Diluent*. Sonicate for 10 min, and then shake by mechanical means for 60 min. Dilute with *Diluent* to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1.4 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of warfarin sodium ($C_{19}H_{15}NaO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of warfarin from the *Sample solution*
r_S = peak response of warfarin from the *Standard solution*
C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of warfarin sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of warfarin sodium, 330.31
M_{r2} = molecular weight of warfarin, 308.33

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: Prepare as directed in the *Assay*.
Standard solution: USP Warfarin RS in water, having a known concentration of 0.0008*L* mg/mL, *L* being the labeled amount, in mg, of warfarin sodium in the Tablets. [NOTE—Use a small amount of 0.1 N sodium hydroxide to aid in dissolution.]
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system: Proceed as directed in the *Assay* except the *Injection volume*.
Injection volume: 40 µL
System suitability: Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of warfarin sodium ($C_{19}H_{15}NaO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of warfarin from the *Sample solution*
r_S = peak response of warfarin from the *Standard solution*
C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of warfarin sodium, 330.31
M_{r2} = molecular weight of warfarin, 308.33
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of warfarin sodium ($C_{19}H_{15}NaO_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Diluent: Methanol and water (25:75). Alternatively, a mixture of acetonitrile and water (25:75) can be used.
Mobile phase: Methanol, tetrahydrofuran, glacial acetic acid, and water (57:1:1:42)

USP Monographs

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Standard stock solution: 0.126 mg/mL of USP Warfarin RS prepared as follows. Transfer USP Warfarin RS to a suitable volumetric flask, and add 0.1 N sodium hydroxide and methanol equivalent to 2% and 25% of the final volume, respectively. Sonicate, if necessary, to dissolve. Dilute with water to volume.
Standard solution (for 1-mg Tablets): 1.0 µg/mL of USP Warfarin RS in *Diluent* from *Standard stock solution*
Standard solution (for other strengths): 2.0 µg/mL of USP Warfarin RS in *Diluent* from *Standard stock solution*
System suitability stock solution: 0.4 mg/mL of USP Warfarin Related Compound A RS in methanol
System suitability solution: 2.0 µg/mL of each of USP Warfarin RS and USP Warfarin Related Compound A RS in *Diluent* from *Standard stock solution* and *System suitability stock solution*
Sample solution (for 1-mg Tablets): Nominally 0.4 mg/mL of warfarin sodium in *Diluent* prepared as follows. Transfer 10 Tablets into a suitable volumetric flask, dissolve Tablets in *Diluent* by sonicating for 10 min and shaking for 60 min. Dilute with *Diluent* to volume. Pass through a suitable filter, and discard the first few mL.
Sample solution (for other strengths): Nominally 0.8 mg/mL of warfarin sodium in *Diluent* prepared as follows. Transfer 8–10 Tablets into a suitable volumetric flask, dissolve Tablets in *Diluent* by sonicating for 10 min and shaking for 60 min. Dilute with *Diluent* to volume. Pass through a suitable filter, and discard the first few mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L11
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 200 µL
Run time: NLT 4 times the retention time of warfarin
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for warfarin and warfarin related compound A are about 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 2 between warfarin and warfarin related compound A peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of Alice's ketone in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of warfarin related compound A from the *Sample solution*
r_S = peak response of warfarin from the *Standard solution*
C_S = concentration of USP Warfarin RS in the *Standard solution* (mg/mL)
C_U = concentration of warfarin sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of warfarin sodium, 330.31
M_{r2} = molecular weight of warfarin, 308.33
F = relative response factor for Alice's ketone, 0.9

[NOTE—Alice's ketone is a sodium salt of warfarin related compound A, 3-(*o*-hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one sodium salt.]

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Warfarin RS
  USP Warfarin Related Compound A RS
  3-(*o*-Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one.
  $C_{18}H_{16}O_2$ 264.33

Water for Hemodialysis

[NOTE—See *Water for Hemodialysis Applications* ⟨1230⟩ for guidelines on microbial and chemical testing.]

H_2O 18.02

DEFINITION
Water for Hemodialysis is water that complies with the U.S. Environmental Protection Agency National Primary Drinking Water Regulations and that has been subjected to further treatment, using a suitable process, to reduce chemical and microbiological components. It is produced and used onsite under the direction of qualified personnel. It contains no added antimicrobials and is not intended for injection.

SPECIFIC TESTS
- **TOTAL ORGANIC CARBON** ⟨643⟩: Meets the requirements
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 100 cfu/mL. It meets the requirements of the test for absence of *Pseudomonas aeruginosa*.
- **WATER CONDUCTIVITY,** *Bulk Water* ⟨645⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains less than 1 USP Endotoxin Unit/mL.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in unreactive storage containers that are designed to prevent bacterial entry. Store at room temperature.

Delete the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP 1,4-Benzoquinone RS▲ (ERR 1-Nov-2018)

Water for Injection

[NOTE—For microbiological guidance, see general information chapter *Water for Pharmaceutical Purposes* ⟨1231⟩.]

H_2O 18.02

DEFINITION
Water for Injection is water purified by distillation or a purification process that is equivalent or superior to distillation in the removal of chemicals and microorganisms. It is prepared from water complying with the U.S. Environmental Protection Agency National Primary Drinking Water Regulations or with the drinking water regulations of the European Union or of Japan or with the World Health Organization's Guidelines for Drinking Water Quality. It contains no added substance.

USP Monographs

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[NOTE—Water for Injection, whether it is available in bulk or packaged forms, is intended for use in the preparation of parenteral solutions. Where used for the preparation of parenteral solutions subject to final sterilization, use suitable means to minimize microbial growth, or first render the Water for Injection sterile and, thereafter, protect it from microbial contamination. For parenteral solutions that are prepared under aseptic conditions and are not sterilized by appropriate filtration or in the final container, first render the Water for Injection sterile and, thereafter, protect it from microbial contamination. In addition to the *Specific Tests*, Water for Injection that is packaged for commercial use elsewhere meets the additional requirements for *Packaging and Storage* and *Labeling* as indicated under *Additional Requirements*.]

SPECIFIC TESTS

[NOTE—Required for bulk and packaged forms of *Water for Injection*.]

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Less than 0.25 USP Endotoxin Unit/mL
- **WATER CONDUCTIVITY,** *Bulk Water* ⟨645⟩: Meets the requirements
- **TOTAL ORGANIC CARBON** ⟨643⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

[NOTE—Required for packaged forms of *Water for Injection*.]

- **PACKAGING AND STORAGE:** Where packaged, preserve in unreactive storage containers that are designed to prevent microbial entry.
- **LABELING:** Where packaged, label the article to state that it contains no antimicrobial or other substance, and that it is not intended for direct parenteral administration.

Delete the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP 1,4-Benzoquinone RS▲ (ERR 1-Nov-2018)

Bacteriostatic Water for Injection

[NOTE—For microbiological guidance, see general information chapter *Water for Pharmaceutical Purposes* ⟨1231⟩.]

DEFINITION

Bacteriostatic Water for Injection is prepared from Water for Injection that is sterilized and suitably packaged, containing one or more suitable antimicrobial agents.

[NOTE—Use Bacteriostatic Water for Injection with due regard for the compatibility of the antimicrobial agent or agents it contains with the particular medicinal substance that is to be dissolved or diluted.]

SPECIFIC TESTS

- **CALCIUM**
  Sample: 100 mL
  Analysis: Add 2 mL of ammonium oxalate TS to the *Sample*.
  Acceptance criteria: No turbidity is produced.
- **CARBON DIOXIDE**
  Sample: 25 mL
  Analysis: Add 25 mL of calcium hydroxide TS to the *Sample*.
  Acceptance criteria: The mixture remains clear.
- **SULFATE**
  Sample: 100 mL
  Analysis: Add 1 mL of barium chloride TS to the *Sample*.
  Acceptance criteria: No turbidity is produced.
- **PH** ⟨791⟩
  Sample: To 100 mL of Bacteriostatic Water for Injection add 0.3 mL of saturated potassium chloride solution.
  Acceptance criteria: 4.5–7.0
- **ANTIMICROBIAL AGENT(S):** Meets the requirements in *Antimicrobial Effectiveness Testing* ⟨51⟩, and meets the labeled claim for content of the antimicrobial agent(s), as determined by the method set forth in *Antimicrobial Agents—Content* ⟨341⟩
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Less than 0.5 USP Endotoxin Unit/mL

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass, of not larger than 30-mL size.
- **LABELING:** Label it to indicate the name(s) and proportion(s) of the added antimicrobial agent(s). Label it also to include the statement "Not For Use In Newborns", in boldface capital letters on the label immediately under the official name, printed in a contrasting color, preferably red. Alternatively, the statement may be placed prominently elsewhere on the label if the statement is enclosed within a box.

Sterile Water for Inhalation

[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]

H_2O 18.02

DEFINITION

Sterile Water for Inhalation is prepared from Water for Injection that is sterilized and suitably packaged. It contains no added antimicrobial agents. [NOTE—Do not use Sterile Water for Inhalation for parenteral administration or for other sterile compendial dosage forms.]

SPECIFIC TESTS

- **OXIDIZABLE SUBSTANCES**
  Sample: 100 mL
  Analysis: Add 10 mL of 2 N sulfuric acid, and heat to boiling. For Sterile Water for Inhalation in containers having a fill volume of less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.
  Acceptance criteria: The pink color does not completely disappear. Alternatively, perform the test for *Total Organic Carbon* ⟨643⟩, *Sterile Water*.
- **TOTAL ORGANIC CARBON,** *Sterile Water* ⟨643⟩: Meets the requirements. Alternatively, perform the test for *Oxidizable Substances*.
- **WATER CONDUCTIVITY,** *Sterile Water* ⟨645⟩: Meets the requirements
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Less than 0.5 USP Endotoxin Unit/mL

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** Label it to indicate that it is for inhalation therapy only and that it is not for parenteral administration.

Delete the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP 1,4-Benzoquinone RS▲ (ERR 1-Nov-2018)

Sterile Water for Injection

[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]

H_2O 18.02

DEFINITION

Sterile Water for Injection is prepared from Water for Injection that is sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

SPECIFIC TESTS

- **OXIDIZABLE SUBSTANCES**
  Sample: 100 mL
  Analysis: Add 10 mL of 2 N sulfuric acid, and heat to a boil. For Sterile Water for Injection in containers having a fill volume less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.
  Acceptance criteria: The pink color does not completely disappear. Alternatively, perform the test for *Total Organic Carbon* ⟨643⟩, *Sterile Water.*
- **TOTAL ORGANIC CARBON,** *Sterile Water* ⟨643⟩**:** Meets the requirements. Alternatively, perform the test for *Oxidizable Substances.*
- **WATER CONDUCTIVITY,** *Sterile Water* ⟨645⟩**:** Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Less than 0.25 USP Endotoxin Unit/mL

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers of not larger than 1-L size. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** Label it to indicate that no antimicrobial or other substance has been added, and that it is not suitable for intravascular injection without first having been made approximately isotonic by the addition of a suitable solute.

Delete the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP 1,4-Benzoquinone RS▲ (ERR 1-Nov-2018)

Sterile Water for Irrigation

[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]

H_2O 18.02

DEFINITION

Sterile Water for Irrigation is prepared from Water for Injection that is sterilized and suitably packaged. It contains no antimicrobial agent or other added substance.

SPECIFIC TESTS

- **OXIDIZABLE SUBSTANCES**
  Sample: 100 mL
  Analysis: Add 10 mL of 2 N sulfuric acid, and heat to a boil. For Sterile Water for Irrigation in containers having a fill volume less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.
  Acceptance criteria: The pink color does not completely disappear. Alternatively, perform the test for *Total Organic Carbon* ⟨643⟩, *Sterile Water.*
- **TOTAL ORGANIC CARBON,** *Sterile Water* ⟨643⟩**:** Meets the requirements. Alternatively, perform the test for *Oxidizable Substances.*
- **WATER CONDUCTIVITY,** *Sterile Water* ⟨645⟩**:** Meets the requirements
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Less than 0.25 USP Endotoxin Unit/mL

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass. The container may contain a volume of more than 1 L and may be designed to empty rapidly.
- **LABELING:** Label it to indicate that no antimicrobial or other substance has been added. The designations "For irrigation only" and "Not for injection" appear prominently on the label.

Delete the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP 1,4-Benzoquinone RS▲ (ERR 1-Nov-2018)

Purified Water

[NOTE—For microbiological guidance, see general information chapter *Water for Pharmaceutical Purposes* ⟨1231⟩.]

H_2O 18.02

DEFINITION

Purified Water is water obtained by a suitable process. It is prepared from water complying with the U. S. Environmental Protection Agency National Primary Drinking Water Regulations or with the drinking water regulations of the European Union or of Japan, or with the World Health Organization's Guidelines for Drinking Water Quality. It contains no added substance.

[NOTE—Purified Water whether it is available in bulk or packaged forms, is intended for use as an ingredient of official preparations and in tests and assays unless

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otherwise specified (see *General Notices, 8.230. Water*). Where used for sterile dosage forms, other than for parenteral administration, process the article to meet the requirements under *Sterility Tests* ⟨71⟩, or first render the Purified Water sterile and thereafter protect it from microbial contamination. Do not use Purified Water in preparations intended for parenteral administration. For such purposes use Water for Injection, Bacteriostatic Water for Injection, or Sterile Water for Injection. In addition to the *Specific Tests*, Purified Water that is packaged for commercial use elsewhere meets the additional requirements for *Packaging and Storage* and *Labeling* as indicated under *Additional Requirements*.]

SPECIFIC TESTS

[NOTE—Required for bulk and packaged forms of *Purified Water*]

- **TOTAL ORGANIC CARBON** ⟨643⟩: Meets the requirements
- **WATER CONDUCTIVITY,** *Bulk Water* ⟨645⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

[NOTE—Required for packaged forms of Purified Water]

- **PACKAGING AND STORAGE:** Where packaged, preserve in unreactive storage containers that are designed to prevent microbial entry.
- **LABELING:** Where packaged, label it to indicate the method of preparation and that it is not intended for parenteral administration.

Delete the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP 1,4-Benzoquinone RS▲ (ERR 1-Nov-2018)

Sterile Purified Water

[NOTE—For microbiological guidance, see *Water for Pharmaceutical Purposes* ⟨1231⟩.]

H_2O 18.02

DEFINITION

Sterile Purified Water is Purified Water sterilized and suitably packaged. It contains no antimicrobial agents. [NOTE—Do not use Sterile Purified Water in preparations intended for parenteral administration. For such purposes use Water for Injection, Bacteriostatic Water for Injection, or Sterile Water for Injection.]

SPECIFIC TESTS

- **OXIDIZABLE SUBSTANCES**

Sample: 100 mL

Analysis: Add 10 mL of 2 N sulfuric acid, and heat to a boil. For Sterile Purified Water in containers having a fill volume of less than 50 mL, add 0.4 mL of 0.02 M potassium permanganate, and boil for 5 min; where the fill volume is 50 mL or more, add 0.2 mL of 0.02 M potassium permanganate, and boil for 5 min. If a precipitate forms, cool in an ice bath to room temperature, and pass through a sintered-glass filter.

Acceptance criteria: The pink color does not completely disappear. Alternatively, perform the test for *Total Organic Carbon* ⟨643⟩, *Sterile Water*.

- **TOTAL ORGANIC CARBON,** *Sterile Water* ⟨643⟩: Meets the requirements. Alternatively, perform the test for *Oxidizable Substances*.
- **WATER CONDUCTIVITY,** *Sterile Water* ⟨645⟩: Meets the requirements
- **STERILITY TESTS** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in suitable tight containers.
- **LABELING:** Label it to indicate the method for preparation and to indicate that it is not for parenteral administration.

Delete the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP 1,4-Benzoquinone RS▲ (ERR 1-Nov-2018)

Pure Steam

[NOTE—For microbiological guidance, see general information chapter *Water for Pharmaceutical Purposes* ⟨1231⟩.]

H_2O 18.02

DEFINITION

Pure Steam is water that has been heated above 100° and vaporized in a manner that prevents source water entrainment. It is prepared from water complying with the EPA National Primary Drinking Water Regulations, or with drinking water regulations of the European Union or of Japan, or with WHO drinking water guidelines. It contains no added substance. The level of steam saturation or dryness and the amount of noncondensable gases are to be determined by the Pure Steam application.

[NOTE—Pure Steam is intended for use where the steam or its condensate comes in contact with the article or the preparation. Pure Steam quality is difficult to assess in its vapor state; therefore, the attributes of its condensate are used to test its quality. The process used to create and collect the condensate for analysis must not adversely impact these quality attributes.]

SPECIFIC TESTS

- **TOTAL ORGANIC CARBON** ⟨643⟩: The condensate meets the requirement.
- **WATER CONDUCTIVITY,** *Bulk Water* ⟨645⟩: The condensate meets the requirement.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: The condensate contains less than 0.25 USP Endotoxin Unit/mL when used in the production of parenterals.

ADDITIONAL REQUIREMENTS

Delete the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP 1,4-Benzoquinone RS▲ (ERR 1-Nov-2018)

Wheat Bran

DEFINITION

Wheat Bran is the outer fraction of the cereal grain, comprising the pericarp, seed coat (testa), nucellar tissue, and aleurone layer, and is derived from *Triticum aestivum* L., *T. compactum* Host, *T. durum* Desf., and other common einkorn and emmer wheat cultivars. It is obtained by the milling and processing of the whole wheat grain meeting U.S. Standards for Number 1 wheat (7 CFR 810.2201). It contains NLT 36.0% of dietary fiber.

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IDENTIFICATION

• **A. Botanic Characteristics**

Microscopic: Fragments of aleurone and nucellar layers (about 60% of the components) and fragments of seed coat and pericarp (about 40%); aleurone and nucellar tissues composed of a usually single layer of thick-walled, isodiametric, translucent cells having conspicuous protoplasm and a single, inconspicuous layer of thick-walled, nearly transparent cells; inconspicuous seed coat, consisting of two layers of thin-walled cells crossing at roughly right angles to each other; pericarp composed of an inconspicuous endocarp layer of elongated, thick-walled tube cells, a cross layer with cells longer than wide, arranged side-by-side in rows, having thick, highly pitted side and end walls, and epicarp and hypoderm layers with cells longer than wide, arranged alternately in rows and having thick, highly pitted side and end walls; epicarp and hypoderm cells larger than and crossing at right angles to the cells of the cross layer; and a few trichomes also present, with lumens narrower than the thickness of their cell walls and originating from isodiametric-polygonal epicarp cells. If micronized, the original structures are mostly destroyed.

COMPOSITION

• **Content of Total Dietary Fiber**

Buffer: pH 6.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

Protease solution: Dissolve 5 mg of protease in 0.1 mL of *Buffer*.

Samples: Prepare two samples in parallel previously dried in a vacuum oven at 100° for 5 h. Mill the dried Wheat Bran to a coarse powder, and store in a desiccator until use.

Analysis: To correct for any contribution from reagents, perform examinations of reagent blanks, which are treated similarly to the *Samples*. Transfer 1.0 g of each *Sample* into separate 400-mL, tall-form beakers. Add 50 mL of *Buffer*, and adjust the pH, if necessary, to 6.0 ± 0.1. Add 0.2 mL of heat-stable α-amylase solution. Cover the beaker with aluminum foil, place in a boiling water bath for 15 min at 100°, shaking gently every 5 min, and cool to room temperature. Adjust with about 10 mL of 0.275 N sodium hydroxide solution to a pH of 7.5 ± 0.1. Add freshly prepared *Protease solution*, cover the beaker with aluminum foil, and incubate for 30 min at 60° with continuous agitation. Cool, and adjust with 10 mL of 0.325 N hydrochloric acid to a pH of 4.5 ± 0.2. Add 0.3 mL of amyloglucosidase, cover with aluminum foil, and incubate for 20 min at 60°, with continuous agitation. Heat 280 mL of alcohol to 60°, add to the digest, and allow the precipitate to form at room temperature for 60 min. Place 0.5 g of chromatographic siliceous earth in a crucible with fritted disk, dry at 130° to constant weight, and weigh. Wet the chromatographic siliceous earth in the crucible using a stream of 78% alcohol from a washing bottle, and apply suction to evenly distribute the chromatographic siliceous earth over the fritted disk. Maintain suction, and quantitatively transfer the enzyme digest precipitate to the crucible. Wash the residue successively with three 20-mL portions of 78% alcohol, two 10-mL portions of alcohol, and two 10-mL portions of acetone. In some cases, gums may form during filtration, trapping liquid in residue. If so, break the surface film with a spatula to improve filtration. Dry the crucible containing the residue at 105° in an air oven for 16 h, cool in a desiccator, and determine the weight of the residue. Determine the percentage of protein in the first *Sample* as directed in the test for *Limit of Protein*. Incinerate the residue from the second *Sample* as directed in *Articles of Botanical Origin* ⟨561⟩, *Total Ash*.

Calculate the corrected weight, *W*, of the sample residue:

$$W = W_U \times (1 - P_U/100 - A_U/100) - W_B \times (1 - P_B/100 - A_B/100)$$

W_U = average weight of the *Sample* residues
P_U = percentage of protein present in the *Sample*
A_U = percentage of ash found in the *Sample*
W_B = average weight of the blank residues
P_B = percentage of protein found in the test for *Limit of Protein* for the blank
A_B = percentage of ash found in the blank

Calculate the percentage of the total dietary fiber in the portion of Wheat Bran taken:

$$\text{Result} = (W/W_I) \times 100$$

W_I = weight of the *Sample* taken

Correct the final percentage of the total dietary fiber for fat and for water.

Acceptance criteria: NLT 36.0%

CONTAMINANTS

• **Articles of Botanical Origin,** *Limits of Elemental Impurities* ⟨561⟩**:** Meet the requirements

• **Limit of Insect Infestation**

Sample: 50 g of Wheat Bran

Analysis: Prepare a smooth slurry by transferring the *Sample* to a 1-L beaker and adding 500 mL of 1.5 N hydrochloric acid. Add 50 mL of light mineral oil, and carefully heat to boiling on a hot plate. Boil for 10 min to digest, stirring occasionally to prevent scorching. Remove from the hot plate, and stir for 5 min with a magnetic stirrer, increasing the stirring speed until a vortex is formed without visible splashing. Quantitatively transfer the contents of the beaker to a separatory funnel with the aid of hot water. Allow to stand for 30 min, stirring gently with a glass rod several times during the first 10 min. Drain the lower layer to about 2.5 cm from the layer interface. Wash the funnel with hot water, and allow 5 min for the layers to separate. Drain the lower layer, and wash with cold water several times until the lower phase is clear. Filter the contents of the funnel through ruled filter paper with the aid of a Büchner funnel and suction. Thoroughly rinse the separatory funnel with water and a detergent solution, filtering each rinse through the same paper. Examine the ruled filter paper under a microscope at 30× magnification.

Acceptance criteria: NMT 25 insect fragments are seen.

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^4 cfu/g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

SPECIFIC TESTS

• **Limit of Protein**

Sample: 1 g of Wheat Bran

Analysis: Place the *Sample* in a 500-mL Kjeldahl flask, and proceed as directed in *Nitrogen Determination* ⟨461⟩, *Method I*. Multiply the percentage of nitrogen found by 6.31.

Acceptance criteria: NMT 18.5%

• **Limit of Fat**

Sample: 2 g of Wheat Bran, previously dried in a vacuum oven at 100° for 5 h

Analysis: Transfer the *Sample* to an extraction thimble, and mix with an equivalent quantity of dry, clean sand. Place a fat-free cotton or glass wool plug on top of the thimble. Place the thimble in a continuous-extraction apparatus provided with a tared collection flask. Pour 75 mL of solvent

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hexane through the sample into the collection flask. Extract at a condensation rate of 5–6 drops/s for 4 h, then at a rate of 2–3 drops/s for the next 16 h. Detach the collection flask, carefully evaporate the solvent, and dry the collection flask and its contents in a drying oven at 100° for 30 min to constant weight. Calculate the percentage of the extract (crude fat) in the portion of Wheat Bran taken.
Acceptance criteria: NMT 6%

- **ABSENCE OF PEROXIDASE ACTIVITY**

Sample: 1 g of Wheat Bran
Analysis: Transfer the *Sample* to a test tube, and add 50 mL of water. Add, in the order specified, 2 mL of 5.68 mM erythorbic acid, 3 mL of 0.69 mM dichloroindophenol, and 0.1 mL of 1.2% hydrogen peroxide, each freshly prepared. Stopper the test tube tightly, and shake until the *Sample* is dissolved. Place into a water bath at 38° for 5 min.
Acceptance criteria: No color change is observed, indicating the absence of peroxidase activity.

- **WATER DETERMINATION,** *Method III, Procedure for Articles of Botanical Origin* ⟨921⟩**:** NMT 12%
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨561⟩**:** NMT 8%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, secured against insect attack.

Witch Hazel

DEFINITION

Witch Hazel is a clear, colorless distillate prepared from recently cut and partially dried dormant twigs of *Hamamelis virginiana* L.
Prepare Witch Hazel as follows. Macerate a weighed amount of the twigs for about 24 h in about twice their weight of water, and then distill until NLT 800 mL and NMT 850 mL of clear, colorless distillate is obtained from each 1000 g of the twigs taken. Add 150 mL of alcohol to each 850 mL of distillate, and mix thoroughly.

OTHER COMPONENTS

- **ALCOHOL DETERMINATION** ⟨611⟩, *Procedures, Method I—Distillation Method***:** 14.0%–15.0% of ethanol

CONTAMINANTS

- **NONVOLATILE RESIDUE:** Evaporate 100 mL to dryness in a tared dish on a hot plate at 60°. The weight of the residue does not exceed 25 mg (0.025%).
- **LIMIT OF TANNINS**

Solution A: 1000 mL of water adjusted with phosphoric acid to a pH of 2.5 ± 0.1
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
20	0	100

Standard solution: 0.03 mg/mL of tannic acid
Sample solution: Witch Hazel
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: If any peak corresponding to the retention time of tannic acid in the *Standard solution* is present for the *Sample solution*, its peak area is NMT that of the tannic acid in the *Standard solution*.

Delete the following:

▲• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis***:** Meets the requirements▲ (USP 1-May-2019)

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩**:** 0.979–0.983
- **pH** ⟨791⟩**:** 3.0–5.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.

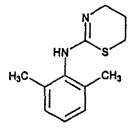
Xenon Xe 133

Xenon, isotope of mass 133.
Xenon, isotope of mass 133 [14932-42-4].

» Xenon Xe 133 is a gas suitable for inhalation in diagnostic studies. Xenon 133 is a radioactive nuclide that may be prepared from the fission of uranium 235. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of ^{133}Xe at the date and time indicated in the labeling.

Packaging and storage—Preserve in single-dose or in multiple-dose vials having leak-proof stoppers, at room temperature.
Other requirements—It meets the requirements for *Labeling*, except for the information specified for *Labeling* under *Injections;* for *Radionuclide identification;* and for *Radionuclidic purity* and *Assay for radioactivity* under *Xenon Xe 133 Injection*, except to determine the radioactivity in MBq (mCi) per container.

Xylazine



$C_{12}H_{16}N_2S$ 220.33
4*H*-1,3-Thiazin-2-amine, *N*-(2,6-dimethylphenyl)-5,6-dihydro-;
5,6-Dihydro-2-(2,6-xylidino)-4*H*-1,3-thiazine [7361-61-7].

DEFINITION

Xylazine contains NLT 98.0% and NMT 102.0% of xylazine ($C_{12}H_{16}N_2S$).

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IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A** or **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 5 µg/mL in 0.1 N hydrochloric acid
Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**
Solution A: Dissolve 3.03 g of sodium 1-heptanesulfonate in 800 mL of water, adjust with 2 N sulfuric acid to a pH of 3.0, and dilute with water to 1000 mL. Pass through a filter of 0.5-µm or finer pore size.
Solution B: Acetonitrile
Diluent: *Solution A* and *Solution B* (50:50)
Mobile phase: See *Table 1*. Return to original conditions, and equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
10	60	40
15	60	40

Standard solution: 0.4 mg/mL of USP Xylazine RS in *Diluent*
Sample solution: 0.4 mg/mL of Xylazine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of xylazine ($C_{12}H_{16}N_2S$) in the portion of Xylazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Xylazine RS in the *Standard solution* (mg/mL)
C_U = concentration of Xylazine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **LIMIT OF 3-AMINO-1-PROPANOL**
Standard solution: 0.5 mg/mL of 3-amino-1-propanol in methanol
Sample solution: 100 mg/mL of Xylazine in methanol. Sonicate to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Alcohol and ammonium hydroxide (80:20)
Spray reagent: 2 mg/mL of ninhydrin in alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram in a saturated chromatographic chamber containing the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and air-dry the plate. Spray the plate with the *Spray reagent*, and immediately heat the plate in an oven at 105°. When the spots are visible, remove the plate from the oven, and allow to cool.
Acceptance criteria: The intensity of the spot for 3-amino-1-propanol from the *Sample solution* is not greater than that of the spot for 3-amino-1-propanol from the *Standard solution* (0.5%).
• **LIMIT OF ACETONE AND ISOPROPYL ALCOHOL**
Proceed as directed in Residual Solvents ⟨467⟩.
Acceptance criteria
Acetone: NMT 0.02%
Isopropyl alcohol: NMT 0.2%
• **ORGANIC IMPURITIES**
Solution A, Solution B, and **Diluent:** Prepare as directed in the *Assay*.
Mobile phase: See *Table 2*. Return to original conditions, and equilibrate the system.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
8	75	25
35	30	70
40	30	70

Standard solution: 0.008 mg/mL of USP Xylazine RS in *Diluent*, prepared by diluting the *Standard solution* from the *Assay*
Sample solution: Transfer 100 mg of Xylazine into a 10-mL volumetric flask, add 5.0 mL of *Solution B*, and swirl to dissolve. Add 4 mL of *Solution A*, and swirl. Dilute with *Solution A* to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; packing L7 (use a guard column)
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of each impurity in the portion of Xylazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of any individual impurity from the *Sample solution* that is not present in the chromatogram of the *Diluent*
r_S = peak area of xylazine from the *Standard solution*
C_S = concentration of USP Xylazine RS in the *Standard solution* (mg/mL)
C_U = concentration of Xylazine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2,6-Dimethylaniline	0.8	1.4	0.5
Xylazine	1.0	—	—
Specified unknown	1.3	2.8	0.5
2,6-Dimethylphenyl isothiocyanate	2.0	2.7	0.5
Any individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	1.0

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at 60° for 4 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** Where it is intended for veterinary use only, the label so states.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Xylazine RS

Xylazine Hydrochloride

$C_{12}H_{16}N_2S \cdot HCl$ 256.79
4*H*-1,3-Thiazin-2-amine, *N*-(2,6-dimethylphenyl)-5,6-dihydro-, monohydrochloride.
5,6-Dihydro-2-(2,6-xylidino)-4*H*-1,3-thiazine hydrochloride [23076-35-9].

» Xylazine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{12}H_{16}N_2S \cdot HCl$.

Packaging and storage—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
Labeling—Where it is intended for veterinary use only, the label so states.
USP Reference standards ⟨11⟩—
USP Xylazine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—
Test solution: 5 mg per mL, in methanol.
Developing solvent system: methanol and ammonium hydroxide (98.5: 1.5).
Procedure—Separately apply 1 µL of the *Test solution* and the *Standard solution.* Allow the applications to dry with the aid of a stream of nitrogen, develop in a saturated chromatographic chamber, and dry the plate in a current of air: the size, intensity, and R_F value of the principal spot obtained from the *Test solution* correspond to those of the principal spot obtained from the *Standard solution.*
Melting range ⟨741⟩: between 164° and 168°.
pH ⟨791⟩: between 4.0 and 6.0, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chromatographic purity—Examine the chromatogram obtained from the *Assay preparation.* Calculate the percentage of impurities in the Xylazine Hydrochloride taken by the formula:

$$100r_S/(r_U + r_S)$$

in which r_S is the sum of the areas of all the impurity peaks observed; and r_U is the area of the xylazine peak: the sum of the impurity responses is not greater than 2.0%.

Assay—
Mobile phase—Dissolve 6.0 g of sodium 1-heptanesulfonate in 2500 mL of water, add 60 mL of glacial acetic acid, dilute with water to 3000 mL, and mix. Prepare a mixture of 2200 mL of this solution and 1800 mL of methanol, and pass through a filter having a 0.5-µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Prepare a solution of USP Xylazine Hydrochloride RS in *Mobile phase* having a known concentration of about 1 mg per mL.
Assay preparation—Transfer about 25 mg of Xylazine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, a 2-mm × 2-cm guard column that contains packing L1, and a 3.9-mm × 30-cm analytical column that contains packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—After daily use, rinse the column with 100 mL of acetonitrile and with 100 mL of methanol, and store the column containing methanol.]
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{12}H_{16}N_2S \cdot HCl$ in the portion of Xylazine Hydrochloride taken by the formula:

$$25C(r_U/r_S)$$

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in which C is the concentration, in mg per mL, of USP Xylazine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the areas of the xylazine peak responses in the chromatograms obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Xylazine Injection

» Xylazine Injection is a sterile solution of Xylazine in Water for Injection prepared with the aid of Hydrochloric Acid or a sterile solution of Xylazine Hydrochloride in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of xylazine ($C_{12}H_{16}N_2S$).

Packaging and storage—Preserve in single-dose or multiple-dose containers.
Labeling—Where it is intended for veterinary use only, the label so states.
USP Reference standards ⟨11⟩—
USP Xylazine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 5 µg per mL.
Medium: 0.1 N hydrochloric acid.

B: Transfer a volume of Injection equivalent to about 50 mg of xylazine to a separator, add 1 mL of sodium carbonate solution (1 in 20), and extract with four 10-mL portions of methylene chloride, combining the methylene chloride extracts in a beaker and evaporating to dryness. Add 10 mL of methanol to the beaker, and swirl to dissolve the residue. The test solution thus obtained responds to *Identification* test *B* under *Xylazine Hydrochloride.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.7 USP Endotoxin Units per mg of xylazine.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 4.5 and 5.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Dissolve 6.0 g of sodium 1-heptanesulfonate in 3000 mL of water, adjust to a pH of 3.0 by dropwise addition of phosphoric acid. Add 1000 mL of acetonitrile, mix, and pass through a filter having a 0.5-µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed portion of USP Xylazine Hydrochloride RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 1.2 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, discarding the first 3 mL of the filtrate. Use the clear filtrate as the *Standard preparation.* This solution contains about 0.12 mg of USP Xylazine Hydrochloride RS per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 200 mg of xylazine, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, discarding the first 3 mL of the filtrate. Use the clear filtrate as the *Assay preparation.*

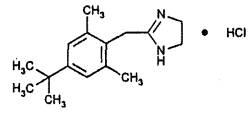
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, a 2-mm × 2-cm guard column that contains packing L2, and a 4.6-mm × 25-cm analytical column that contains packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Assay preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the main xylazine peak and the closest eluting other peak, if any, is not less than 2.5; and the tailing factor for the xylazine peak is not more than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—After daily use, rinse the column with 100 mL of water and with 100 mL of methanol, and store the column containing methanol.]

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of xylazine ($C_{12}H_{16}N_2S$) in each mL of the Injection taken by the formula:

$$(220.34/256.80)(2000C/V)(r_U/r_S)$$

in which 220.34 and 256.80 are the molecular weights of xylazine and xylazine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Xylazine Hydrochloride RS in the *Standard preparation; V* is the volume, in mL, of Injection taken to prepare the *Assay preparation;* and r_U and r_S are the areas of the xylazine peak responses in the chromatograms obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Xylometazoline Hydrochloride



$C_{16}H_{24}N_2 \cdot HCl$ 280.84
1*H*-Imidazole, 2-[[4-(1,1-dimethylethyl)-2,6-dimethylphenyl] methyl]-4,5-dihydro-, monohydrochloride;
2-(4-*tert*-Butyl-2,6-dimethylbenzyl)-2-imidazoline monohydrochloride [1218-35-5].

DEFINITION

Change to read:

Xylometazoline Hydrochloride contains ▲NLT 98.0% and NMT 102.0%▲ (USP 1-Aug-2019) of xylometazoline hydrochloride ($C_{16}H_{24}N_2 \cdot HCl$), calculated on the dried basis.

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IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020), ▲⟨197K⟩, or ⟨197A⟩▲ (USP 1-Aug-2019)

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

Add the following:

▲• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
Sample solution: 5 mg/mL of Xylometazoline Hydrochloride in water
Acceptance criteria: Meets the requirements of the silver nitrate precipitate test▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**
▲**Solution A:** 1.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
20	15	85
35	15	85
37	70	30
45	70	30

System suitability solution: 0.02 mg/mL each of USP Xylometazoline Hydrochloride RS and USP Xylometazoline Related Compound A RS in water
Standard solution: 0.1 mg/mL of USP Xylometazoline Hydrochloride RS in water
Sample solution: 0.1 mg/mL of Xylometazoline Hydrochloride in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times are given in *Table 2*.]
Suitability requirements
Resolution: NLT 2.0 between xylometazoline related compound A and xylometazoline, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of xylometazoline hydrochloride ($C_{16}H_{24}N_2 \cdot HCl$) in the portion of Xylometazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of xylometazoline from the *Sample solution*
r_S = peak response of xylometazoline from the *Standard solution*
C_S = concentration of USP Xylometazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Xylometazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis▲ (USP 1-Aug-2019)

IMPURITIES

• **RESIDUE ON IGNITION** ⟨**281**⟩: NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**
▲**Solution A, Solution B, Mobile phase, System suitability solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.5 µg/mL of USP Xylometazoline Hydrochloride RS in water
Standard solution: 0.002 mg/mL of USP Xylometazoline Hydrochloride RS in water
Sample solution: 1.0 mg/mL of Xylometazoline Hydrochloride in water
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—The relative retention times are given in *Table 2*.]
Suitability requirements
Resolution: NLT 2.0 between xylometazoline related compound A and xylometazoline, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Xylometazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of xylometazoline from the *Standard solution*
C_S = concentration of USP Xylometazoline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Xylometazoline Hydrochloride in the *Sample solution* (mg/mL)

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F = relative response factor for the corresponding impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Xylometazoline related compound A	0.89	0.67	0.2
Xylometazoline	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5 ▲(USP 1-Aug-2019)

SPECIFIC TESTS

Change to read:

- **pH** ⟨791⟩
  ▲Sample solution: 50 mg/mL of Xylometazoline Hydrochloride in water
  Acceptance criteria: 5.0–6.6 ▲(USP 1-Aug-2019)
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 4 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards** ⟨11⟩
  USP Xylometazoline Hydrochloride RS
  ▲USP Xylometazoline Related Compound A RS
  N-(2-Aminoethyl)-2-[4-(*tert*-butyl)-2,6-dimethylphenyl] acetamide.
  $C_{16}H_{26}N_2O$ 262.39 ▲(USP 1-Aug-2019)

Xylometazoline Hydrochloride Nasal Solution

DEFINITION

Xylometazoline Hydrochloride Nasal Solution is an isotonic solution of Xylometazoline Hydrochloride in water. It contains NLT 90.0% and NMT 110.0% of the labeled amount of xylometazoline hydrochloride ($C_{16}H_{24}N_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A.** **▲**The UV spectrum of the xylometazoline peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

- **Procedure**
  ▲Solution A: 1.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
20	15	85
35	15	85
37	70	30
45	70	30

System suitability solution: 0.01 mg/mL each of USP Xylometazoline Hydrochloride RS and USP Xylometazoline Related Compound A RS in water
Standard solution: 0.05 mg/mL of USP Xylometazoline Hydrochloride RS in water
Sample solution: Nominally 0.05 mg/mL of xylometazoline hydrochloride from a suitable volume of Nasal Solution in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. For *Identification A*, use a diode array detector in the range of 210–300 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times are given in *Table 2*.]
Suitability requirements
Resolution: NLT 2.0 between xylometazoline related compound A and xylometazoline, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of xylometazoline hydrochloride ($C_{16}H_{24}N_2 \cdot HCl$) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of xylometazoline from the *Sample solution*
r_S = peak response of xylometazoline from the *Standard solution*
C_S = concentration of USP Xylometazoline Hydrochloride RS in the *Standard solution* (mg/mL)

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C_U = nominal concentration of xylometazoline hydrochloride in the *Sample solution* (mg/mL) ▲(USP 1-Aug-2019)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL of USP Xylometazoline Hydrochloride RS in water

Sensitivity solution: 0.5 µg/mL of USP Xylometazoline Hydrochloride RS from the *Standard solution* in water

Sample solution: Nominally 0.5 mg/mL of xylometazoline hydrochloride from a suitable volume of Nasal Solution in water

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—The relative retention times are given in *Table 2*.]

Suitability requirements

Resolution: NLT 2.0 between xylometazoline related compound A and xylometazoline, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of xylometazoline from the *Standard solution*

C_S = concentration of USP Xylometazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of xylometazoline hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding degradation product (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.10%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Xylometazoline related compound A	0.79	0.67	0.2
Xylometazoline	1.0	—	—
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	0.5 ▲(USP 1-Aug-2019)

SPECIFIC TESTS

• **pH** ⟨791⟩: 5.0–7.5

Add the following:

▲• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/g, and the total combined molds and yeasts count does not exceed 10^1 cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. ▲(USP 1-Aug-2019)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store at controlled room temperature. ▲(USP 1-Aug-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

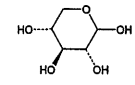
USP Xylometazoline Hydrochloride RS

▲USP Xylometazoline Related Compound A RS

N-(2-Aminoethyl)-2-[4-(*tert*-butyl)-2,6-dimethylphenyl] acetamide.

$C_{16}H_{26}N_2O$ 262.39 ▲(USP 1-Aug-2019)

Xylose



D-Xylopyranose

$C_5H_{10}O_5$ 150.13

D-Xylose;

D-Xylose [acyclic form] [58-86-6].

D-Xylopyranose [7261-26-9].

α-D-Xylopyranose [6763-34-4].

β-D-Xylopyranose [2460-44-8].

DEFINITION

Xylose contains NLT 98.0% and NMT 102.0% of xylose ($C_5H_{10}O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A** ▲(CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (75:25)
System suitability solution: 10 mg/mL of USP Xylose RS and 0.2 mg/mL of USP Fructose RS in *Mobile phase*
Standard solution: 10 mg/mL of USP Xylose RS in *Mobile phase*
Sample solution: 10 mg/mL of Xylose in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 192 nm
Column: 4.6-mm × 15-cm; 5-µm packing L8
Column temperature: 30°
Flow rate: 2 mL/min
Injection volume: 25 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between the xylose and fructose peaks
Relative standard deviation: NMT 2.0% for xylose
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of xylose ($C_5H_{10}O_5$) in the portion of Xylose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Xylose RS in the *Standard solution* (mg/mL)
C_U = concentration of Xylose in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

PERFORMANCE TESTS
• **COLOR OF SOLUTION**
Sample: A freshly prepared 100 mg/mL solution
Acceptance criteria: The solution is clear and colorless.

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.05%
• **IRON** ⟨241⟩
Test preparation: Dissolve 2.0 g in 45 mL of water, and add 2 mL of hydrochloric acid.
Acceptance criteria: NMT 5 µg/g
• **ORGANIC IMPURITIES**
Use the chromatogram of the *Sample solution*, obtained as directed in the *Assay*.
Calculate the percentage of each individual impurity, excluding any solvent peaks, in the portion of Xylose taken:

$$\text{Result} = (r_U/r_T) \times 100$$

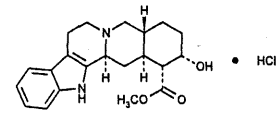
r_U = peak response of each individual impurity
r_T = sum of all of the responses in the chromatogram

Acceptance criteria
Individual impurities: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 100 mg/mL in 0.012 N ammonium hydroxide
Acceptance criteria: +18.2° to +19.4°
• **LOSS ON DRYING** ⟨731⟩
Sample: 2–5 g
Analysis: Dry the *Sample* at a pressure not exceeding 50 mm of mercury at 60° to constant weight, a current of dried air being passed through the oven during the drying period to remove water vapor.
Acceptance criteria: NMT 0.1%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fructose RS
USP Xylose RS

Yohimbine Hydrochloride



$C_{21}H_{26}N_2O_3 \cdot HCl$ 390.90
17α-Hydroxy-20-α-yohimban-16-β-carboxylic acid, methyl ester, hydrochloride [65-19-0].

» Yohimbine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{26}N_2O_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
Labeling—Where it is intended for veterinary use only, it is so labeled.
USP Reference standards ⟨11⟩—
USP Yohimbine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution—Dissolve 10 mg of it in 1 mL of methanol, add 1 drop of ammonium hydroxide, and mix.
Application volume: 1 µL.
Developing solvent system: methylene chloride, methanol, and ammonium hydroxide (90:14:1), in a saturated chamber.
Procedure—Allow the plate to air-dry in a hood. Expose the dry plate for 30 minutes to short-wavelength UV light, then examine under long-wavelength UV light: the size, intensity, and R_F value of the principal spot in the chromatogram obtained from the *Test solution* correspond to those characteristics of the principal spot in the chromatogram obtained from the Standard solution.

Change to read:

C: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: 0.1 N hydrochloric acid in methanol.
D: To 10 mg of it add 3 drops of sulfuric acid. Mix, and add 50 mg of ammonium vanadate: a violet color is produced

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(differentiation from *strychnine,* which produces a red color). Add 1 mL of water: no color change occurs.
Specific rotation ⟨781S⟩: between +100° and +105°.
Test solution: 10 mg per mL, in water, prepared by warming on a steam bath and allowing to cool.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.
Chromatographic purity—Use the chromatogram of the *Assay preparation* obtained as directed in the *Assay.* Calculate the percentage of each impurity in the portion of Yohimbine Hydrochloride taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the response of the individual impurity; and r_s is the sum of all the responses in the chromatogram: not more than 1.0% of any individual impurity is found, and the sum of all the impurities found is not more than 2.0%.

Assay—
Mobile phase—Prepare a mixture of water, dibasic sodium phosphate dihydrate solution (11.88 g per L), and monobasic potassium phosphate solution (9.08 g per L) (355:100:50). Add 4 g of sodium dodecyl sulfate, and mix. Add 285 mL of acetonitrile, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Yohimbine Hydrochloride RS in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.
Assay preparation—Transfer about 50 mg of Yohimbine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with methanol to volume, and mix.
System suitability solution—Quantitatively dilute an accurately measured volume of the *Standard preparation* with methanol to obtain a solution having a concentration of 0.40 µg of USP Yohimbine Hydrochloride RS per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 229-nm detector and a 4-mm × 12.5-cm column that contains 4-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the main yohimbine peak gives a measurable response. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 1%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{26}N_2O_3 \cdot HCl$ in the portion of Yohimbine Hydrochloride taken by the formula:

$$250C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Yohimbine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the yohimbine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Yohimbine Injection

» Yohimbine Injection is a sterile solution of Yohimbine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of yohimbine ($C_{21}H_{26}N_2O_3$).

Packaging and storage—Preserve in single-dose or multiple-dose containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging.* Store at controlled room temperature.
Labeling—Where it is intended for veterinary use only, it is so labeled.
USP Reference standards ⟨11⟩—
USP Yohimbine Hydrochloride RS

Identification, *Thin-Layer Chromatographic Identification Test* ⟨201⟩—
Test solution—Transfer a volume of Injection, equivalent to about 40 mg of yohimbine, to a separator, add 5 mL of a sodium carbonate solution (1 in 20), and extract with four 10-mL portions of chloroform, combining the chloroform extracts in a beaker and evaporating to dryness. Add 20 mL of methanol to the beaker, and swirl to dissolve the residue.
Standard solution—Prepare a solution of USP Yohimbine Hydrochloride RS in methanol containing 2 mg per mL.
Mixed solution: a mixture of the *Test solution* and the *Standard solution* (1:1).
Application volume: 1 µL.
Developing solvent system: methylene chloride, methanol, and ammonium hydroxide (90:14:1), in a saturated chamber.
Procedure—Allow the plate to air-dry in a hood. Expose the dry plate for 30 minutes to short-wavelength UV light, then examine under long-wavelength UV light: the size, intensity, and R_F value of the principal spots in the chromatograms obtained from the *Test solution* and the *Mixed solution* correspond to those characteristics of the principal spot in the chromatogram obtained from the *Standard solution.*
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 45.5 USP Endotoxin Units per mg of yohimbine.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 3.7 and 4.3.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Diluent—Prepare a mixture of acetonitrile, water, and glacial acetic acid (49:49:2).
Mobile phase—Prepare a mixture of water, acetonitrile, and glacial acetic acid (603:377:20) containing 3.5 g of sodium 1-decanesulfonate in each 1000 mL. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 55 mg of USP Yohimbine Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask, add 20 mL of water, warm, and swirl to dissolve. Add 84 mg of anhydrous citric acid, and swirl to dissolve. Allow the solution to cool, adjust with 1 N sodium hydroxide to a pH of 4.0, dilute with water to volume, and mix. Transfer 125.0 µL of this stock solution to a second 25-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.011 mg of USP Yohimbine Hydrochloride RS per mL.
Resolution solution—Prepare a solution in methanol containing about 0.56 mg of methylparaben and 0.06 mg of

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propylparaben per mL. Transfer 200 µL of this solution to a 25-mL volumetric flask, add 125.0 µL of the stock solution used to prepare the *Standard preparation,* dilute with *Diluent* to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 0.25 mg of yohimbine, to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for methylparaben, 0.7 for propylparaben, and 1.0 for yohimbine; and the resolution, *R,* between methylparaben and propylparaben and between propylparaben and yohimbine is not less than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of yohimbine ($C_{21}H_{26}N_2O_3$) in each mL of the Injection taken by the formula:

$$(354.45/390.90)(25{,}000C/V)(r_U/r_S)$$

in which 354.45 and 390.90 are the molecular weights of yohimbine and yohimbine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Yohimbine Hydrochloride RS in the *Standard preparation;* V is the volume, in µL, of Injection taken to prepare the *Assay preparation;* and r_U and r_S are the yohimbine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Yttrium Y 90 Ibritumomab Tiuxetan Injection

» Ibritumomab Tiuxetan is the immunoconjugate resulting from a stable thiourea covalent bond between the monoclonal antibody ibritumomab and the linker-chelator tiuxetan [*N*-[2-bis(carboxymethyl)amino]-3-(*p*-isothiocyanatophenyl) propyl]-[*N*-[2-bis(carboxymethyl)amino]-2-(methyl)ethyl)glycine. This chelate provides a high-affinity, conformationally restricted chelation site for ^{90}Y and ^{111}In. The approximate molecular weight of Ibritumomab Tiuxetan is 148 kD.

Ibritumomab is a murine IgG_1 kappa monoclonal antibody directed against the CD20 antigen, which is found on the surface of normal and malignant B lymphocytes. Ibritumomab is produced in Chinese hamster ovary cells and is composed of two murine gamma 1 heavy chains of 445 amino acids each and two kappa light chains of 213 amino acids each.

Yttrium Y 90 Ibritumomab Tiuxetan Injection is a sterile, nonpyrogenic preparation of the immunoconjugate of ibritumomab and tiuxetan that is labeled with ^{90}Y and is suitable for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{90}Y as the ibritumomab complex, expressed in megabecquerels (or millicuries) per mL at the time indicated in the labeling. It may contain buffers and stabilizers. It contains no antimicrobial agents. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The immunoreactive fraction, as determined by a validated method, is not less than 90 percent.

Packaging and storage—Preserve in single-dose containers, and store in a refrigerator for not more than 8 hours. [NOTE—Translucent protein particles may develop, which are removed by filtration prior to administration using a 0.22-µm low-protein-binding filter.]

Labeling—Label it to include the following in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of Yttrium Y 90 ibritumomab tiuxetan as total MBq (or mCi) and concentration of yttrium ^{90}Y ibritumomab tiuxetan, in MBq (or mCi) per mL, at the time of calibration; the expiration date and time; the storage temperature; and the statement, "Caution—Radioactive Material." The labeling indicates that, in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{90}Y is 64.1 hours.

Radionuclide identification (see *Radioactivity* ⟨821⟩)—

A: The beta radiation of the Injection shows a mass absorption coefficient within 5% of the value found for a known standard of the ^{90}Y when tested under the same counting conditions.

B: The beta-ray spectrum, obtained on an energy calibrated beta spectrometer, is identical to that of the spectrum of ^{90}Y of known purity, showing a maximum beta particle energy (E_{max}) at about 2280 keV. [NOTE—Because of the inherent difficulty in measuring beta radiation, a second comparative test should be performed.]

Bacterial Endotoxins Test ⟨85⟩—The limit of endotoxin content is not more than $175/V$ USP Endotoxin Units per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩**:** between 5.5 and 7.5.

Radiochemical purity—

Adsorbent: 1- × 8-cm instant silica gel strip.

Test solution: the Injection.

Application volume: 10 µL.

Developing solvent system: 0.9% sodium chloride solution.

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩ by ascending chromatography. Determine the distribution of radioactivity on the chromatogram by scanning with a suitable collimated radiochromatogram strip scanner, and determine the percentage of radiochemical purity of the test specimen. Not less than 95% of the ^{90}Y activity is present as a band between the R_f values of 0.0 and 0.1.

Radionuclidic purity (Content of ^{90}Sr in an yttrium Y 90 chloride solution) (see *Radioactivity* ⟨821⟩)—Prepare a strontium/yttrium carrier solution containing 0.34 mg of

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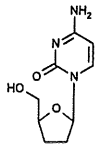
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yttrium chloride ($YCl_3 \cdot 6H_2O$) and 0.30 mg of strontium chloride ($SrCl_2 \cdot 6H_2O$) per mL of 0.1 N hydrochloric acid. Apply about 50 µL of this solution at the origin of a 2- × 19-cm cellulose phosphate chromatographic strip (see *Chromatography* ⟨621⟩), and allow to dry. Apply about 5 µL of the yttrium Y 90 chloride radiolabeling solution at the origin, and develop the chromatogram by ascending chromatography over a period of about 1.25 hours, using 3 N hydrochloric acid as the developing solvent, until the solvent front migrates to the 15-cm mark. Allow to dry. Cut the strip at the 8-cm mark, and place the upper section (solvent front) in a suitable liquid scintillation solvent. Using a suitable counting assembly, determine the radioactivity, in KBq (or µCi) per mL of yttrium Y 90 chloride solution. The total radioactivity of ^{90}Sr is not greater than 740 KBq per 37 GBq (or 20 µCi per Ci) of ^{90}Y at the expiration date as stated on the labeling.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the radioactive component may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the date of manufacture.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the total radioactivity, in MBq (or µCi), of the unshielded Injection, using a calibrated system.

Zalcitabine



$C_9H_{13}N_3O_3$ 211.22

Cytidine, 2′,3′-dideoxy-.

2′,3′-Dideoxycytidine. [7481-89-2].

» Zalcitabine contains not less than 98.0 percent and not more than 102.0 percent of $C_9H_{13}N_3O_3$, calculated on the dried basis.

[**CAUTION**—Great care should be taken to prevent inhaling particles of Zalcitabine and exposing it to the skin.]

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Zalcitabine RS

USP Zalcitabine Related Compound A RS

2′,3′-Didehydro-2′,3′-dideoxycytidine.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

C: Prepare a test solution of it in a mixture of methanol and water (1:1) containing 50 mg per mL. Similarly prepare a Standard solution, using USP Zalcitabine RS. Separately apply 10 µL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a paper-lined chromatographic chamber saturated with a solvent system consisting of the clear lower layer of a mixture of alcohol, dichloromethane, and water (3:2:2), and develop the chromatogram. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to dry. Locate the spots on the plate by examination under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Specific rotation ⟨781S⟩: between +73° and +77°.

Test solution: 7 mg per mL, in water.

Water Determination, *Method Ia* ⟨921⟩: not more than 0.3%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—

Phosphate buffer, Mobile phase, Resolution solution, Standard preparation, and *Assay preparation*—Prepare as directed in the *Assay*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between zalcitabine and zalcitabine related compound A is not less than 2.0, and the tailing factor for zalcitabine is not greater than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2%.

Procedure—Inject a volume (about 20 µL) of the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses of the major peaks. Inject a volume of acetonitrile in water (3 in 100) as a chromatographic blank. Calculate the percentage of each impurity in the portion of zalcitabine taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity, and r_s is the sum of the responses of all of the peaks: not more than 0.3% of any individual impurity is found, and the sum of all impurities is not more than 2.0%.

Ordinary impurities ⟨466⟩—

Test solution: 50 mg per mL, in a mixture of methanol and water (1:1).

Standard solution: a mixture of methanol and water (1:1).

Eluant: the lower layer of a mixture of alcohol, dichloromethane, and water (3:2:2).

Visualization: 1.

Assay—

Phosphate buffer—Dissolve 6.8 g of monobasic potassium phosphate and 8.7 g of dibasic potassium phosphate in 2000 mL of water. Adjust, if necessary, with dilute phosphoric acid or potassium hydroxide solution (1 in 10) to a pH of 6.8 ± 0.05.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (97:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Dissolve USP Zalcitabine RS and USP Zalcitabine Related Compound A RS in a mixture of

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acetonitrile in water (3 in 100), and dilute quantitatively, and stepwise if necessary, with the same solvent to obtain a solution containing about 0.024 mg of each per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Zalcitabine RS in a mixture of acetonitrile and water (3 in 100) to obtain a solution having a known concentration of about 0.5 mg per mL.

Assay preparation—Transfer about 100 mg of Zalcitabine, accurately weighed, to a 200-mL volumetric flask. Dissolve in a mixture of acetonitrile and water (3 in 100), dilute with the same solvent to volume, and mix.

Chromatographic system—The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor for the zalcitabine peak is not greater than 1.5, and the relative standard deviation is not more than 2.0%. Chromatograph the *Resolution solution:* the resolution, *R*, between zalcitabine and zalcitabine related compound A is not less than 2.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}N_3O_3$ in the portion of Zalcitabine taken by the formula:

$$200C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Zalcitabine RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Zalcitabine Tablets

» Zalcitabine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of zalcitabine ($C_9H_{13}N_3O_3$). [**CAUTION**—Great care should be taken to prevent inhaling particles of zalcitabine and exposing it to the skin.]

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Zalcitabine RS
USP Zalcitabine Related Compound A RS
2′,3′-Didehydro-2′,3′-dideoxycytidine.

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 20 minutes.

Phosphate buffer—Transfer 8.7 g of dibasic potassium phosphate to a 1 L volumetric flask, dilute with water to volume, and adjust with phosphoric acid to a pH of 6.8.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer*, methanol, and acetonitrile (96:4:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 10 mg of USP Zalcitabine RS, accurately weighed, to a 250-mL volumetric flask. Add about 200 mL of *Medium*, and sonicate until complete solution is effected. Dilute with *Medium* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 3.9-mm × 15-cm column that contains 10-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as described for *Procedure:* the retention time is about 5 minutes; the tailing factor is not more than 2; and the relative standard deviation is not more than 2%.

Procedure—Separately inject equal volumes (about 150 µL) of the *Standard preparation* and a filtered portion of the solution under test into the chromatograph, record the chromatograms, and measure the responses of the major peaks. Calculate the quantity, in mg, of zalcitabine in the Tablets taken by the formula:

$$900C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Zalcitabine RS in the *Standard preparation*; and r_U and r_S are the zalcitabine peak responses obtained from the solution under test and the *Standard preparation*, respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_9H_{13}N_3O_3$ is dissolved in 20 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Buffer solution—Dissolve 3.4 g of monobasic potassium phosphate in sufficient water to make 1 L. Using a suitable pH meter, adjust with phosphoric acid to a pH of 2.2. [NOTE—If too much phosphoric acid is added, yielding a pH below 2.2, the pH may be adjusted to 2.2 with 0.025 M monobasic potassium phosphate.] Add 1.08 g of sodium 1-octanesulfonic acid, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (85:15).

Diluent—Prepare a mixture of water and acetonitrile (17:3).

Resolution solution—Dissolve weighed quantities of USP Zalcitabine RS and USP Zalcitabine Related Compound A RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with the same solvent to obtain a solution containing about 0.02 mg per mL of zalcitabine and 0.002 mg per mL of zalcitabine related compound A.

Standard preparation—Dissolve an accurately weighed quantity of USP Zalcitabine RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with the same solvent to obtain a solution having a known concentration of about 0.008 mg per mL.

Assay preparation—Transfer 5 Tablets to a volumetric flask suitable to obtain a solution containing about 0.008 mg zalcitabine per mL. Add a volume of *Diluent* that is about six-tenths of the volume of the flask, sonicate for 15 minutes, and shake by mechanical means for 10 minutes. Dilute with *Diluent* to volume, and mix. Filter before use.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector, a precolumn cartridge that contains packing L1, and a 4.6-mm × 25-cm analytical column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the zalcitabine and zalcitabine related compound A peaks is not less than 1.1, and the tailing factor for the zalcitabine peak is not more than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for

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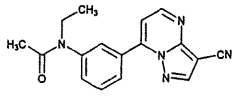
Procedure: the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses of the major peaks. Calculate the quantity, in mg, of $C_9H_{13}N_3O_3$ in the portion of Tablets taken by the formula:

$$CV(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Zalcitabine RS in the *Standard preparation*; V is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation*; and r_U and r_S are the zalcitabine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Zaleplon



$C_{17}H_{15}N_5O$ 305.33

Acetamide,*N*-[3-(3-cyanopyrazolo[1,5-*a*]pyrimidin-7-yl) phenyl]-*N*-ethyl-;
3′-(3-Cyanopyrazolo[1,5-*a*]pyrimidin-7-yl)-*N*-ethylacetanilide [151319-34-5].

DEFINITION

Zaleplon contains NLT 98.0% and NMT 102.0% of zaleplon ($C_{17}H_{15}N_5O$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.3 g/L of ammonium formate in water. Adjust with formic acid to a pH of 4.0.
Mobile phase: Acetonitrile and *Buffer* (7:18)
Diluent: Acetonitrile and water (1:1)
System suitability solution: 0.5 mg/mL of USP Zaleplon RS and 0.5 µg/mL each of USP Zaleplon Related Compound A RS and USP Zaleplon Related Compound B RS in *Diluent*
Standard solution: 50 µg/mL of USP Zaleplon RS in *Diluent*
Sample solution: 50 µg/mL of Zaleplon in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm
Column: 4-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of zaleplon
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for zaleplon and zaleplon related compound B are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between zaleplon and zaleplon related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of zaleplon ($C_{17}H_{15}N_5O$) in the portion of Zaleplon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zaleplon from the *Sample solution*
r_S = peak response of zaleplon from the *Standard solution*
C_S = concentration of USP Zaleplon RS in the *Standard solution* (µg/mL)
C_U = concentration of Zaleplon in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A: 1.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
15	60	40
25	45	55
26	80	20
35	80	20

Diluent: Acetonitrile and water (250:750)
System suitability solution: 0.5 mg/mL of USP Zaleplon RS and 0.5 µg/mL each of USP Zaleplon Related Compound A RS, USP Zaleplon Related Compound B RS, and USP Zaleplon Related Compound C RS in *Diluent*
Standard solution: 0.5 µg/mL of USP Zaleplon RS in *Diluent*
Sample solution: 500 µg/mL of Zaleplon in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between zaleplon related compound A and zaleplon related compound C; NLT 2.0 between zaleplon and zaleplon related compound B, *System suitability solution*

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Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Zaleplon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of zaleplon from the *Standard solution*

C_S = concentration of USP Zaleplon RS in the *Standard solution* (µg/mL)

C_U = concentration of Zaleplon in the *Sample solution* (µg/mL)

F = relative response factor for the corresponding impurity peak (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyanopyrazolamine[a]	0.15	0.50	0.15
Zaleplon related compound A	0.54	0.36	0.15
Zaleplon related compound C[b]	0.57	0.80	0.1
Desethylzaleplon[b, c]	0.70	1.1	0.1
Zaleplon	1.0	—	—
Zaleplon related compound B	1.11	0.65	0.15
Zaleplon oxopropenyl analog[b, d]	1.59	0.51	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 3-Aminopyrazole-4-carbonitrile.

[b] Process impurity; may not be found in all manufacturing processes.

[c] *N*-[3-(3-Cyanopyrazolo[1,5-*a*]pyrimidin-7-yl)phenyl]acetamide.

[d] (*E*)-*N*-[3-(3-Cyano-6-{3-[3-(*N*-ethylacetamido)phenyl]-3-oxoprop-1-en-1-yl}pyrazolo[1,5-*a*]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide.

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Zaleplon RS

USP Zaleplon Related Compound A RS
(*E*)-*N*-{3-[3-(Dimethylamino)acryloyl]phenyl}-*N*-ethylacetamide.
$C_{15}H_{20}N_2O_2$ 260.33

USP Zaleplon Related Compound B RS
N-[3-(3-Cyanopyrazolo[1,5-*a*]pyrimidin-5-yl)phenyl]-*N*-ethylacetamide.
$C_{17}H_{15}N_5O$ 305.33

USP Zaleplon Related Compound C RS
7-[3-(*N*-Ethylacetamido)phenyl]pyrazolo[1,5-*a*]pyrimidine-3-carboxamide.
$C_{17}H_{17}N_5O_2$ 323.35

Zaleplon Capsules

DEFINITION

Zaleplon Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of zaleplon ($C_{17}H_{15}N_5O$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.3 g/L of ammonium formate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
30	50	50
31	95	5

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.1 mg/mL of USP Zaleplon RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of zaleplon from NLT 5 Capsules in *Diluent*. Pass through a filter of 0.45-µm pore size. [NOTE—Use the entire Capsule with its contents. Shake for 10 min.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.4 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zaleplon ($C_{17}H_{15}N_5O$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Zaleplon RS in the *Standard solution* (mg/mL)

USP Monographs

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C_U = nominal concentration of zaleplon in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION**

Test 1

Medium: Water; 900 mL

Apparatus 2: 75 rpm, with sinkers if necessary

Time: 20 min

Determine the percentage of zaleplon dissolved using one of the following procedures.

Spectrophotometric procedure

Standard stock solution: 0.044 mg/mL of USP Zaleplon RS prepared as follows. Transfer a suitable quantity of USP Zaleplon RS to an appropriate volumetric flask. Add 10.0% of the total flask volume of methanol to dissolve. Dilute with *Medium* to volume. [NOTE—This solution is stable for 7 days.]

Standard solution: (L/1000) mg/mL of USP Zaleplon RS from *Standard stock solution* in *Medium*, where L is the label claim in mg/Capsule. [NOTE—This solution is stable for 7 days.]

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: About 333 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zaleplon ($C_{17}H_{15}N_5O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Chromatographic procedure

Buffer: 0.77 g/L of ammonium acetate in water

Mobile phase: Methanol and *Buffer* (33:67)

Standard stock solution: 0.22 mg/mL of USP Zaleplon RS prepared as follows. Dissolve first in methanol using 5% of total flask volume and dilute with *Medium* to volume.

Standard solution: (L/900) mg/mL of USP Zaleplon RS from *Standard stock solution* in *Medium*, where L is the label claim in mg/Capsule

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 5-cm; 3-µm packing L1

Column temperature: 35°

Flow rate: 2.0 mL/min

Injection volume: 50 µL

Run time: 2 times the retention time of zaleplon

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zaleplon ($C_{17}H_{15}N_5O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zaleplon RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of zaleplon ($C_{17}H_{15}N_5O$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 75 rpm, with sinkers

Time: 20 min

Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (30:70)

Standard stock solution: 1.1 mg/mL of USP Zaleplon RS in *Mobile phase*

Standard solution: (L/900) mg/mL of USP Zaleplon RS from *Standard stock solution* in *Medium*, where L is the label claim in mg/Capsule

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 28°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: 2 times the retention time of zaleplon

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zaleplon ($C_{17}H_{15}N_5O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zaleplon RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of zaleplon ($C_{17}H_{15}N_5O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

USP Monographs

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IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.5 mg/mL of USP Zaleplon RS in *Diluent*

System suitability solution: 0.5 µg/mL of USP Zaleplon Related Compound B RS and 0.5 mg/mL of USP Zaleplon RS in *Diluent*

Sensitivity solution: 0.05 µg/mL of USP Zaleplon RS in *Diluent*, from the *Standard stock solution*

Standard solution: 0.1 mg/mL of USP Zaleplon RS in *Diluent*, from the *Standard stock solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between zaleplon and zaleplon related compound B, *System suitability solution*

Tailing factor: NMT 1.5 for the zaleplon peak, *System suitability solution*

Relative standard deviation: NMT 2.0% for the zaleplon peak, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of any individual degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*

r_S = peak response of USP Zaleplon RS from the *Standard solution*

C_S = concentration of USP Zaleplon RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zaleplon in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyanopyrazolamine[a, b]	0.16	—
Zaleplon related compound A[b, c]	0.76	—
Zaleplon	1.0	—
Zaleplon related compound B[b]	1.05	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.0

[a] 3-Aminopyrazole-4-carbonitrile.

[b] This is a process impurity that is included in the table for identification purposes only. It is controlled in the drug substance and is not to be reported or included in the total degradation products for the drug product.

[c] (*E*)-*N*-{3-[3-(Dimethylamino)acryloyl]phenyl}-*N*-ethylacetamide.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

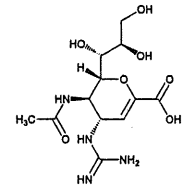
USP Zaleplon RS

USP Zaleplon Related Compound B RS

N-[3-(3-Cyanopyrazolo[1,5-*a*]pyrimidin-5-yl)phenyl]-*N*-ethylacetamide.

$C_{17}H_{15}N_5O$ 305.33

Zanamivir



$C_{12}H_{20}N_4O_7$ 332.31

D-*glycero*-D-*galacto*-Non-2-enonic acid, 5-(acetylamino)-4-[(aminoiminomethyl)amino]-2,6-anhydro-3,4,5-trideoxy-; 5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*galacto*-non-2-enonic acid [139110-80-8].

DEFINITION

Zanamivir contains NLT 98.0% and NMT 102.0% of zanamivir ($C_{12}H_{20}N_4O_7$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K or 197M▲ (CN 1-May-2020)

Wavenumber range: 4000 cm^{-1} to 400 cm^{-1}

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 7.5 mM sulfuric acid (60:40). Adjust with ammonia TS to a pH of 6.2.

Resolution solution: Prepare 2.5 µg/mL of *talo*-zanamivir and 0.05 mg/mL of zanamivir from USP Zanamivir Resolution Mixture RS in *Mobile phase*.

Standard solution: 0.045 mg/mL of USP Zanamivir RS in *Mobile phase*

Sample solution: 0.045 mg/mL of Zanamivir in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 234 nm

Column: 4.6-mm × 25-cm; 5-µm packing L82

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Resolution solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between *talo*-zanamivir and zanamivir, *Resolution solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zanamivir ($C_{12}H_{20}N_4O_7$) in the portion of Zanamivir taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zanamivir RS in the *Standard solution* (mg/mL)
C_U = concentration of Zanamivir in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: Prepare 0.45 mg/mL of zanamivir and 9 µg/mL each of imidazole, imidazole carboximidamide, zanamivir urea analog, 4-amino zanamivir, 4-biguanide zanamivir, and *talo*-zanamivir from USP Zanamivir Related Compounds Mixture RS in *Mobile phase*.

Sample solution: 0.45 mg/mL of Zanamivir prepared as follows. Dissolve the sample using 40% of the final volume with water, and dilute with acetonitrile to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm and 234 nm
Column: 4.6-mm × 25-cm; 5-µm packing L82
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between the peaks of *talo*-zanamivir and zanamivir at 234 nm

Analysis
Sample: *Sample solution*
Calculate the percentage of imidazole in the portion of Zanamivir taken:

$$\text{Result} = [(r_i/F)/(r_i/F + r_z)] \times 100$$

r_i = peak response of imidazole at 210 nm
F = relative response factor for imidazole (see *Table 1*)
r_z = peak response of zanamivir at 210 nm

Calculate the percentage of imidazole carboximidamide and 4-biguanide zanamivir in the portion of Zanamivir taken:

$$\text{Result} = [(r_i/F)/(r_i/F + r_z)] \times 100$$

r_i = peak response of imidazole carboximidamide or 4-biguanide zanamivir at 234 nm
F = relative response factor for imidazole carboximidamide or 4-biguanide zanamivir (see *Table 1*)
r_z = sum of the responses of all the peaks including the zanamivir peak at 234 nm

Calculate the percentage of *O*-triazinyl zanamivir, zanamivir urea analog, 4-amino zanamivir, *talo*-zanamivir, zanamivir dimer, and any other unspecified impurity in the portion of Zanamivir taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of *O*-triazinyl zanamivir, zanamivir urea analog, 4-amino zanamivir, *talo*-zanamivir, zanamivir dimer, or any other unspecified impurity at 234 nm
r_T = sum of the responses of all the peaks including the zanamivir peak at 234 nm

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazole[a]	0.26	2.5	—
Imidazole carboximidamide[b]	0.30	3.3	0.01
O-Triazinyl zanamivir[c]	0.60	—	0.3
Zanamivir urea analog[d]	0.70	—	0.2
4-Amino zanamivir[e]	0.77	—	0.2
4-Biguanide zanamivir[f]	0.83	1.6	0.2
Zanamivir	1.00	—	—
talo-Zanamivir[g]	1.14	—	—
Zanamivir dimer[h]	2.75	—	0.5
Any other unspecified impurity	—	—	0.1
Total impurities	—	—	1.2

[a] 1*H*-Imidazole (No individual limit. Included in the determination of total impurities.)
[b] 1*H*-Imidazole-1-carboximidamide.
[c] 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*galacto*-non-2-enonic acid.
[d] 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-ureido-D-*glycero*-D-*galacto*-non-2-enonic acid.
[e] 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-amino-D-*glycero*-D-*galacto*-non-2-enonic acid.
[f] 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-(1-biguanidyl)-D-*glycero*-D-*galacto*-non-2-enonic acid.
[g] 5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*talo*-non-2-enonic acid (No individual limit. Included in the determination of total impurities.)
[h] 4,4′-(2-Amino-4-oxo-1,3,5-triazapent-2-ene-1,5-diyl) bis(5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid.)

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in water
Acceptance criteria: +36.0° to +38.0°, measured at 20°, determined on the anhydrous and solvent-free basis

• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** 4.0%–9.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in a well-closed container at controlled room temperature.

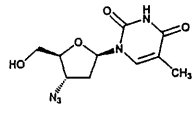
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Zanamivir RS
USP Zanamivir Related Compounds Mixture RS
This Reference Standard is a mixture of zanamivir, imidazole, imidazole carboximidamide, zanamivir urea analog, 4-amino zanamivir, 4-biguanide zanamivir, *talo*-zanamivir, and zanamivir dimer. The chemical names are given below:
Imidazole: 1*H*-Imidazole.
Imidazole carboximidamide: 1*H*-Imidazole-1-carboximidamide.

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Zanamivir urea analog: 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-ureido-D-*glycero*-D-*galacto*-non-2-enonic acid.
4-Amino zanamivir: 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-amino-D-*glycero*-D-*galacto*-non-2-enonic acid.
4-Biguanide zanamivir: 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-(1-biguanidyl)-D-*glycero*-D-*galacto*-non-2-enonic acid.
talo-Zanamivir: 5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*talo*-non-2-enonic acid.
Zanamivir dimer: 4,4′-(2-Amino-4-oxo-1,3,5-triazapent-2-ene-1,5-diyl) bis(5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid.)

USP Zanamivir Resolution Mixture RS
This Reference Standard is a mixture of zanamivir and *talo*-zanamivir.

Zidovudine



$C_{10}H_{13}N_5O_4$ 267.24
Thymidine, 3′-azido-3′-deoxy-;
3′-Azido-3′-deoxythymidine [30516-87-1].

DEFINITION
Zidovudine contains NLT 97.0% and NMT 102.0% of zidovudine ($C_{10}H_{13}N_5O_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Methanol and water (20:80)
Standard stock solution: 1.0 mg/mL of USP Zidovudine RS in methanol
Zidovudine related compound B standard stock solution: 0.1 mg/mL of USP Zidovudine Related Compound B RS in methanol
Zidovudine related compound C standard stock solution: 0.2 mg/mL of USP Zidovudine Related Compound C RS in methanol prepared as follows. Transfer 20 mg of USP Zidovudine Related Compound C RS to a 100-mL volumetric flask, add 75 mL of methanol, sonicate for 15 min, and dilute with methanol to volume.
Standard solution: 0.1 mg/mL USP Zidovudine RS, 1 µg/mL of USP Zidovudine Related Compound B RS, and 2 µg/mL of USP Zidovudine Related Compound C RS in methanol from an appropriate volume of *Standard stock solution*, *Zidovudine related compound B standard stock solution*, and *Zidovudine related compound C standard stock solution*, respectively.
Sample solution: 0.1 mg/mL of Zidovudine in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Columns
Guard: With recommended dimensions of 3.2-mm × 1.5-cm; packing L1
Analytical: 4.0-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for zidovudine related compound C, zidovudine, and zidovudine related compound B are 0.25, 1.0, and 1.17, respectively.]
Suitability requirements
Resolution: NLT 1.4 between the zidovudine and zidovudine related compound B peaks
Tailing factor: NMT 1.5 for the zidovudine peak
Relative standard deviation: NMT 2.0% for the zidovudine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of zidovudine ($C_{10}H_{13}N_5O_4$) in the portion of Zidovudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zidovudine from the *Sample solution*
r_S = peak response of zidovudine from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.25%
- **LIMIT OF TRIPHENYLMETHANOL AND OTHER IMPURITIES**

Standard solution: 0.1 mg/mL each of USP Zidovudine RS and triphenylmethanol in methanol
Sample solution: 20 mg/mL of Zidovudine in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator having an optimal intensity at 254 nm
Application volume: 10 µL
Developing solvent system: Chloroform and methanol (9:1)
Spray reagent: 5 mg/mL of carbazole in alcohol and sulfuric acid (95:5)
Analysis: Develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spot in the chromatogram of the *Standard solution*.
Acceptance criteria 1: No secondary spot from the chromatogram of the *Sample solution* is larger or more intense than the principal spot from the *Standard solution*, and the sum of the intensities of the secondary spots obtained from the *Sample solution* corresponds to NMT 3.0%.

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Spray the plate with *Spray reagent*, heat for 10 min at 120°, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spots in the chromatogram of the *Standard solution*.

Acceptance criteria 2: No spot corresponding to triphenylmethanol (R_F about 2.3 relative to the R_F of zidovudine) is more intense than the corresponding spot from the *Standard solution*. No secondary spot from the chromatogram of the *Sample solution* is larger or more intense than the principal spot obtained from the *Standard solution*. The sum of the intensities of the secondary spots from the *Sample solution* corresponds to NMT 3.0%.

• **LIMIT OF ZIDOVUDINE RELATED COMPOUNDS B AND C**

Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of zidovudine related compound B and zidovudine related compound C in the portion of Zidovudine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for zidovudine related compound B or zidovudine related compound C

r_T = sum of the responses of all of the peaks

Acceptance criteria

Individual impurities: NMT 1.0% for zidovudine related compound B and NMT 2.0% for zidovudine related compound C

Total impurities: NMT 3.0% for all impurities from the tests for *Limit of Triphenylmethanol and Other Impurities* and *Limit of Zidovudine Related Compounds B and C*.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL, in alcohol

Acceptance criteria: +60.5° to +63°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Zidovudine RS

USP Zidovudine Related Compound B RS

3′-Chloro-3′-deoxythymidine.

$C_{10}H_{13}ClN_2O_4$ 260.68

USP Zidovudine Related Compound C RS

Thymine.

$C_5H_6N_2O_2$ 126.12

Zidovudine Capsules

DEFINITION

Zidovudine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Solution A: Methanol and water (75:25)

Sample solution: 15 µg/mL prepared as follows. Mix Capsule contents, equivalent to 300 mg of zidovudine, with 50 mL of *Solution A* in a 200-mL volumetric flask. Sonicate for 5 min, and dilute with methanol to volume. Allow insoluble solids to settle, and dilute the supernatant 100-fold with *Solution A*.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and water (20:80)

Diluent: Methanol and water (75:25)

Standard stock solution: 1.0 mg/mL of USP Zidovudine RS in methanol

Zidovudine related compound C standard stock solution: 0.2 mg/mL of USP Zidovudine Related Compound C RS in methanol prepared as follows. Transfer 20 mg of USP Zidovudine Related Compound C RS to a 100-mL volumetric flask, add 75 mL of methanol, sonicate for 15 min, dilute with methanol to volume.

Standard solution: 0.1 mg/mL of USP Zidovudine RS and 2 µg/mL of USP Zidovudine Related Compound C RS prepared as follows. Transfer 10 mL of *Standard stock solution* and 1.0 mL of *Zidovudine related compound C standard stock solution* to a 100-mL volumetric flask, add 25 mL of water, and dilute with methanol to volume.

Sample stock solution: Nominally 1 mg/mL of zidovudine in *Diluent* prepared as follows. Weigh the contents of Capsules (NLT 20), mix, and transfer a portion of the powder, equivalent to 100 mg of zidovudine, to a 100-mL volumetric flask. Dissolve in *Diluent*, sonicate for 20 min, and dilute with *Diluent* to volume. Allow the solids to settle, and use the supernatant layer to prepare the *Sample solution*.

Sample solution: Nominally 0.1 mg/mL of zidovudine from a suitable volume of supernatant of *Sample stock solution* in *Diluent*. Filter, discarding the first 4 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Columns

Guard: Recommended dimensions are 3.2-mm × 1.5-cm; packing L1.

Analytical: 4.0-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for zidovudine related compound C and zidovudine are about 0.2 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the zidovudine and zidovudine related compound C peaks

Tailing factor: NMT 2.0 for the zidovudine peak

Relative standard deviation: NMT 2.0% for the zidovudine peak

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zidovudine from the *Sample solution*
r_S = peak response of zidovudine from the *Standard solution*
C_S = concentration of USP Zidovudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: Prepare a solution having a known concentration of USP Zidovudine RS in *Medium*.
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of zidovudine ($C_{10}H_{13}N_5O_4$) dissolved by using the procedure set forth in the *Assay*, making any necessary modifications.
Tolerances: NLT 75% (Q) of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES
• **Organic Impurities**
Mobile phase, Diluent, Zidovudine related compound C standard stock solution, Standard stock solution, Standard solution, Sample stock solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of zidovudine related compound C in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zidovudine related compound C from the *Sample solution*
r_S = peak response of zidovudine related compound C from the *Standard solution*
C_S = concentration of USP Zidovudine Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Zidovudine RS
USP Zidovudine Related Compound C RS
Thymine.
$C_5H_6N_2O_2$ 126.12

Zidovudine Injection

DEFINITION
Zidovudine Injection is a sterile solution of Zidovudine in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Solution A: Methanol and water (75:25)
Sample solution: Nominally 15 µg/mL of zidovudine from Injection in *Solution A*
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Methanol and water (20:80)
Standard stock solution: 1.0 mg/mL of USP Zidovudine RS in methanol
Zidovudine related compound C standard stock solution: 0.2 mg/mL of USP Zidovudine Related Compound C RS in methanol prepared as follows. Transfer 20 mg of USP Zidovudine Related Compound C RS to a 100-mL volumetric flask, add 75 mL of methanol, sonicate for 15 min, and dilute with methanol to volume.
Standard solution: 0.1 mg/mL of USP Zidovudine RS and 4 µg/mL of USP Zidovudine Related Compound C RS prepared as follows. Transfer a suitable volume of *Standard stock solution* and *Zidovudine related compound C standard stock solution* to a suitable volumetric flask. Add 25% of the flask volume of water, and dilute with methanol to volume.
Sample solution: Nominally 0.1 mg/mL of zidovudine from Injection in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Columns
Guard: Recommended dimensions are 3.2-mm × 1.5-cm; packing L1.
Analytical: 4.0-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for zidovudine related compound C and zidovudine are about 0.2 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between zidovudine and zidovudine related compound C
Tailing factor: NMT 1.5 for the zidovudine peak
Relative standard deviation: NMT 2.0% for the zidovudine peak
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zidovudine from the *Sample solution*
r_S = peak response of zidovudine from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Standard stock solution, Zidovudine related compound C standard stock solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution: 0.1 mg/mL of USP Zidovudine RS and 2 µg/mL of USP Zidovudine Related Compound C RS prepared as follows. Transfer a suitable volume of *Standard stock solution* and *Zidovudine related compound C standard stock solution* to a suitable volumetric flask. Add 25% of the flask volume of water, and dilute with methanol to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zidovudine related compound C in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zidovudine related compound C from the *Sample solution*
r_S = peak response of zidovudine related compound C from the *Standard solution*
C_S = concentration of USP Zidovudine Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirement when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*

• **pH** ⟨791⟩

Sample solution: A mixture containing a volume of Injection equivalent to 150 mg of zidovudine and 5 mL of 0.12 M potassium chloride

Acceptance criteria: 3.5–7.0

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 1.0 USP Endotoxin Unit/mg of zidovudine

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Zidovudine RS
USP Zidovudine Related Compound C RS
Thymine.
$C_5H_6N_2O_2$ 126.12

Zidovudine Oral Solution

DEFINITION

Zidovudine Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION

• The retention time of the zidovudine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, acetonitrile, glacial acetic acid, and 0.040 M sodium acetate (90:10:2:900)

Standard stock solution: 1 mg/mL of USP Zidovudine RS in *Mobile phase*

Zidovudine related compound C stock solution: 0.1 mg/mL of USP Zidovudine Related Compound C RS in *Mobile phase*. [NOTE—Sonicate for 10 min before making final volume.]

Standard solution: Transfer 10.0 mL of the *Standard stock solution* and 2.0 mL of the *Zidovudine related compound C stock solution* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

Sample solution: Equivalent to 0.1 mg/mL of zidovudine in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: UV 240 nm
Column: 4.0-mm × 12.5-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for zidovudine related compound C and zidovudine are about 0.12 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between zidovudine and zidovudine related compound C
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{10}H_{13}N_5O_4$ in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*.]

• **Procedure 1**

Mobile phase: Methanol, acetonitrile, glacial acetic acid, and 0.040 M sodium acetate (90:10:2:900)

Standard stock solution: 1 mg/mL of USP Zidovudine RS in *Mobile phase*

Zidovudine related compound C stock solution: 0.1 mg/mL of USP Zidovudine Related Compound C RS in

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Mobile phase. [NOTE—Sonicate for 10 min before making final volume.]

Standard solution: Transfer 10.0 mL of the *Standard stock solution* and 2.0 mL of the *Zidovudine related compound C stock solution* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Sample solution: Equivalent to 0.1 mg/mL of zidovudine in *Mobile phase*

Chromatographic system and **System suitability:** Proceed as directed in the *Assay.*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zidovudine related compound C (thymine) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Zidovudine Related Compound C RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

• Procedure 2

Buffer: 5.44 mg/mL of sodium acetate trihydrate in water. Pass the solution through a suitable filter of 0.45-µm pore size.

Mobile phase: Methanol, acetonitrile, glacial acetic acid, and *Buffer* (50:10:2:940)

Standard stock solution: 0.25 mg/mL of USP Zidovudine RS in *Mobile phase.* [NOTE—Sonicate to dissolve if necessary.]

Standard solution: 0.002 mg/mL of USP Zidovudine RS in *Mobile phase* from the *Standard stock solution*

Impurity stock solution: 0.625 mg/mL of USP Zidovudine Related Compound C RS, 0.375 mg/mL of thymidine, 0.375 mg/mL of USP Stavudine RS, and 0.25 mg/mL of USP Zidovudine Related Compound B RS dissolved initially at 25% of final volume with methanol. Dilute with *Mobile phase* to final volume. [NOTE—Sonicate with intermittent shaking to dissolve, if necessary, and cool to room temperature before diluting to final volume.]

Impurity solution: 0.05 mg/mL of USP Zidovudine Related Compound C RS, 0.03 mg/mL of thymidine, 0.03 mg/mL of USP Stavudine RS, and 0.02 mg/mL of USP Zidovudine Related Compound B RS in *Mobile phase* from the *Impurity stock solution*

System suitability solution: 1 mg/mL of USP Zidovudine RS, 0.005 mg/mL of USP Zidovudine Related Compound C RS, 0.003 mg/mL of thymidine, 0.003 mg/mL of USP Stavudine RS, 0.002 mg/mL of USP Zidovudine Related Compound B RS in *Mobile phase* from USP Zidovudine RS and the *Impurity solution*

Sample solution: Equivalent to 1 mg/mL of zidovudine in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Column temperature: 25°

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times are listed in *Impurity Table 1.*]

Suitability requirements

Resolution: NLT 1.4 between zidovudine and zidovudine related compound B, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

r_U = peak response of each impurity in the *Sample solution*

r_S = peak response of zidovudine in the *Standard solution*

C_S = concentration of USP Zidovudine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

F = relative response factor for each impurity (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1.*

Total impurities: NMT 2.0%. [NOTE—All impurities listed in *Impurity Table 1* excluding zidovudine related compound C]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zidovudine related compound C[a]	0.08	1.9	3.0
Zidovudine (thymidine)[b]	0.14	1.0	0.30
Stavudine[c]	0.27	1.0	0.30
Zidovudine	1.00	—	—
Zidovudine related compound B[d]	1.22	1.0	0.20
Individual unspecified impurity	—	1.0	0.20

[a] 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.
[b] [1-(2-Deoxy-β-D-ribofuranosyl)]thymine.
[c] Thymidine, 2′,3′-didehydro-3′-deoxy-.
[d] 3′-Chloro-3′-deoxythymidine.

SPECIFIC TESTS

• MICROBIAL ENUMERATION TESTS ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** Meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*

• pH ⟨791⟩**:** 3.0–4.0, in a mixture containing a volume of Oral Solution equivalent to 150 mg of zidovudine and 5 mL of 0.12 M potassium chloride (3:1)

PERFORMANCE TESTS

• DELIVERABLE VOLUME ⟨698⟩**:** Meets the requirements for oral solution packaged in multiple-unit containers

• UNIFORMITY OF DOSAGE UNITS ⟨905⟩**:** Meets the requirements for oral solution packaged in single-unit containers

USP Monographs

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ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Stavudine RS
  USP Zidovudine RS
  USP Zidovudine Related Compound B RS
  3′-Chloro-3′-deoxythymidine.
  $C_{10}H_{13}ClN_2O_4$ 260.68
  USP Zidovudine Related Compound C RS
  Thymine.
  $C_5H_6N_2O_2$ 126.12

Zidovudine Tablets

DEFINITION

Zidovudine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Grind 1 Tablet in a mortar so that no large pieces remain, and remove the coating film so that about 5 mg of ground Tablet remain.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Dissolve 3.0 g of sodium acetate and 1.3 g of sodium 1-octane sulfonate in 900 mL of water. Add 90 mL of methanol and 40 mL of acetonitrile. Adjust with glacial acetic acid to a pH of 5.3.
  Zidovudine related compound B standard stock solution: 0.1 mg/mL of USP Zidovudine Related Compound B RS in methanol
  Zidovudine related compound C standard stock solution: 0.2 mg/mL of USP Zidovudine Related Compound C RS in methanol prepared as follows. Dissolve by sonicating for 15 min before bringing the solution to volume.
  Standard solution: 0.12 mg/mL of USP Zidovudine RS, 1 µg/mL of USP Zidovudine Related Compound B RS, and 4 µg/mL of USP Zidovudine Related Compound C RS prepared as follows. Transfer 30 mg of USP Zidovudine RS to a 250-mL volumetric flask, and dissolve in 3.0 mL of methanol. Add 2.5 mL of *Zidovudine related compound B standard stock solution* and 5.0 mL of *Zidovudine related compound C standard stock solution*, and dilute with water to volume.
  Sample stock solution: Nominally 3 mg/mL of zidovudine prepared as follows. Transfer a number of Tablets, equivalent to 1500 mg of zidovudine, to a 500-mL volumetric flask. Add 50 mL of water, and shake by mechanical means for 30 min to disperse the Tablets. Add 150 mL of methanol, and sonicate for 10 min. Dilute with water to volume.
  Sample solution: Nominally 0.12 mg/mL of zidovudine in water from *Sample stock solution*. Pass the solution through a nylon filter, discarding the first 2 mL of the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 265 nm
  Column: 4.6-mm × 15-cm; packing L1
  Flow rate: 1.3 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for zidovudine related compound C, zidovudine, and zidovudine related compound B are about 0.17, 1.0, and 1.2, respectively.]
  Suitability requirements
  Resolution: NLT 2.5 between the zidovudine and zidovudine related compound B peaks
  Tailing factor: NMT 2.0 for the zidovudine peak
  Relative standard deviation: NMT 2.0% for the zidovudine peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of zidovudine ($C_{10}H_{13}N_5O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zidovudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Standard solution: Prepare a solution having a known concentration of USP Zidovudine RS in *Medium*
  Sample solution: A filtered portion of the solution under test suitably diluted with *Medium*
  Analysis:
  Samples: *Standard solution* and *Sample solution*
  Determine the percentage of zidovudine ($C_{10}H_{13}N_5O_4$) dissolved by using the procedure set forth in the *Assay*.
  Tolerances: NLT 80% (*Q*) of the labeled amount of $C_{10}H_{13}N_5O_4$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  Procedure for content uniformity
  Mobile phase: Methanol and water (1:4)
  Standard solution: Proceed as directed in the *Assay*.
  Sample solution: Transfer 1 Tablet to a 100-mL volumetric flask, add 20 mL of water, and shake by mechanical means to disperse the Tablet. Add 30 mL of methanol, and sonicate for 10 min. Dilute with water to volume. Pipet 4.0 mL of the resulting solution into a 100-mL volumetric flask, and dilute with water to volume. Pass a portion of the solution through a suitable nylon filter, discarding the first 2 mL of the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 265 nm
  Column: 4.6-mm × 15-cm; base-deactivated packing L1

Supplement 3

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Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the zidovudine peak
Relative standard deviation: NMT 2.0% for the zidovudine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of zidovudine ($C_{10}H_{13}N_5O_4$), based on the label claim, in the portion of Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zidovudine from the *Sample solution*
r_S = peak response of zidovudine from the *Standard solution*
C_S = concentration of USP Zidovudine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of zidovudine from the *Standard solution*
C_S = concentration of USP Zidovudine RS in the Standard solution (mg/mL)
C_U = nominal concentration of Zidovudine in the Sample solution (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

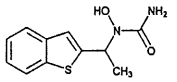
Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zidovudine related compound C	0.17	1.7	1.5
Zidovudine	1.00	—	—
Zidovudine related compound B[a]	1.2	—	—
Any other individual unidentified impurity	—	1.0	0.2
Total impurities	—	—	2.0

[a] Process impurity. Included for identification purpose only. Not to be included in the *Total Impurities*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Zidovudine RS
USP Zidovudine Related Compound B RS
3′-Chloro-3′-deoxythymidine.
$C_{10}H_{13}ClN_2O_4$ 260.68
USP Zidovudine Related Compound C RS
Thymine.
$C_5H_6N_2O_2$ 126.12

Zileuton



$C_{11}H_{12}N_2O_2S$ 236.29
Urea, *N*-(1-benzo[*b*]thien-2-ylethyl)-*N*-hydroxy-, (±)-.
(±)-1-(1-Benzo[*b*]thien-2-ylethyl)-1-hydroxyurea [111406-87-2].

» Zileuton contains not less than 98.5 percent and not more than 101.5 percent of $C_{11}H_{12}N_2O_2S$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at room temperature.
USP Reference standards ⟨11⟩—
USP Zileuton RS
USP Zileuton Related Compound A RS
N-(1-Benzo-[*b*]thien-2-ylethyl)urea.
$C_{11}H_{12}N_2OS$ 220.30
USP Zileuton Related Compound B RS
2-(Benzo[*b*]thien-2-oyl)benzo[*b*]thiopene.
$C_{17}H_{10}OS_2$ 294.40
USP Zileuton Related Compound C RS
1-Benzo-[*b*]thien-2-ylethanone.
$C_{10}H_8OS$ 176.24

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.
Specific rotation ⟨781S⟩**:** between –0.5° and +0.5°.
Test solution: 10 mg per mL, in methanol.
Water Determination, *Method I* ⟨921⟩**:** not more than 1.5%.
Residue on ignition ⟨281⟩**:** not more than 0.2%.
Specific surface area, *Method I* ⟨846⟩—Outgas a portion of the test sample, about 100 mg, at 90° for 1 hour at ambient pressure using 0.001 mole fraction of krypton in helium as the adsorbate gas: between 0.9 and 3.1. m^2 per g.
Arsenic, *Method II* ⟨211⟩**:** 2 µg per g.

Limit of boron—
Sulfuric acid solution—Carefully add 50 mL of sulfuric acid to 450 mL of water, and mix.
Standard solution—Prepare a solution in *Sulfuric acid solution* having a concentration of about 2.0 µg of boron per

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mL. Use of a commercially prepared boron ICP standard solution is recommended.

Test solution—Accurately weigh approximately 1.0 g of Zileuton into a 125-mL conical flask. Add 1 to 1.5 mL of sulfuric acid, and digest in a fume hood on a hot plate until charring begins. Add 2 mL of nitric acid to the cooled sample to aid digestion, and heat until brown fumes are not evolved. Cautiously add 30 percent hydrogen peroxide, dropwise, allowing the reaction to subside, and heating between drops. Add the first few drops very slowly with sufficient mixing in order to prevent a rapid reaction. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously, rotating the flask occasionally to prevent the sample from caking on glass exposed to the heating unit. Maintain oxidizing conditions at all times during the digestion by adding small quantities of the 30 percent hydrogen peroxide, whenever the mixture turns brown or darkens. Approximately 1 to 2 mL of nitric acid can be added, if necessary, which will create a refluxing effect to wash down any particles adhering to the neck of the flask. Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Transfer the solution to a 25-mL volumetric flask using about 7 mL of water. Repeat the washing twice more, and combine the washings in the volumetric flask. Dilute with water to volume, and mix.

Procedure—The inductively coupled plasma-atomic emission spectrometer is set up with wavelength of 249.7 nm, RF power of 1.25 KW, argon torch flow of about 13 L per minute, argon nebulizer flow of about 1 L per minute, and argon auxillary flow of about 0.5 L per minute. Analyze the *Standard solution* and the *Test solution,* using *Sulfuric acid solution* as the blank. Calculate the quantity, in µg of boron per g, in the portion of Zileuton taken by the formula:

$$25C/W,$$

where C is the concentration, in µg per mL, of boron in the *Test solution* determined from the instrument; and W is the weight, in g, of the Zileuton: not more than 10 µg per g is found.

Limit of pyridine—

Standard solution—Dissolve an accurately weighed quantity of pyridine, approximately 250 mg, in dimethyl sulfoxide, and dilute with dimethyl sulfoxide to 50 mL. Transfer 5 µL to a 100-mL sealed headspace vial.

Test solution—Transfer about 100 mg of Zileuton, accurately weighed, to a vial, add 5.0 mL of dimethyl sulfoxide and 1 g of anhydrous sodium sulfate, and seal with a septum and crimp cap. Heat the sealed vial at 80° for 60 minutes.

Chromatographic system (see *Chromatography* ⟨621⟩)—[NOTE—The use of a headspace apparatus is allowed.] The gas chromatograph is equipped with a flame-ionization detector, a 0.53-mm × 30-m fused silica analytical column coated with a 3.0-µm G43 stationary phase. The carrier gas is helium with a linear velocity of about 35 cm per second. The injection port and detector temperatures are maintained at 140° and 260°, respectively. The column temperature is programmed according to the following steps. It is maintained at 40° for 20 minutes, then increased rapidly to 240°, and maintained at 240° for 20 minutes. Inject the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 15%.

Procedure—Using a heated gas-tight syringe separately inject equal volumes (about 1 mL) of the headspace of the *Standard solution* and the *Test solution* into the gas chromatograph, and record the peak responses. Calculate the quantity, in ppm, of pyridine in the portion of Zileuton taken by the formula:

$$100(r_U/r_S)(W_S/W_U)$$

in which r_U and r_S are the peak responses for pyridine in the *Test solution* and the *Standard solution,* respectively; W_S is the weight, in mg, of pyridine used to prepare the *Standard solution;* and W_U is the weight, in mg, of Zileuton: not more than 100 ppm is found.

Chromatographic purity— [NOTE—For *Test 1* and *Test 2,* the *System suitability solution,* the *Standard solution,* and the *Test solution* are to be refrigerated at or below 5° immediately after preparation and during analysis using a refrigerated autosampler. The solutions are stable at or below 5° for about 36 hours.]

TEST 1—

Buffer solution—Prepare as directed in the *Assay.*

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (82:18). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve accurately weighed quantities of USP Zileuton RS and USP Zileuton Related Compound A RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 5 µg of each USP Reference Standard per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Zileuton RS in acetonitrile to obtain a solution having a known concentration of about 10 µg per mL.

Test solution—Transfer about 125 mg of Zileuton, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system—Prepare as directed in the *Assay,* except to use a flow rate of 2.2 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between zileuton and zileuton related compound A is not less than 1.5; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Zileuton taken by the formula:

$$100F(C_S/C_U)(r_i/r_S)$$

in which F is the relative response factor for each impurity, which is 1.0 for any peak with a relative retention time of 0.5, 0.7, 1.2, 1.6, 3.2, or 3.4, and is 1.2, 1.4, and 1.7 for peaks with relative retention times of 0.8, 2.1, and 2.8, respectively; C_S is the concentration, in mg per mL, of USP Zileuton RS in the *Standard solution;* C_U is the concentration, in mg per mL, of zileuton in the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for zileuton obtained from the *Standard solution:* not more than 0.1% of any individual impurity with a relative retention time of 0.8, 1.6, or 2.1 is found; not more than 0.10% of any individual impurity with a relative retention time of 0.7, 3.2, or 3.4 is found; not more than 0.20% of any individual impurity with a relative retention time of 0.5 or 1.2 is found; and not more than 0.07% of any individual impurity with a relative retention time of 2.8 is found.

TEST 2—

Perchloric acid solution—Dissolve 5.0 mL of perchloric acid in 1000 mL of water.

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Mobile phase—Prepare a filtered and degassed mixture of *Perchloric acid solution* and acetonitrile (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard stock solution—Dissolve an accurately weighed quantity of USP Zileuton Related Compound B RS in acetonitrile to obtain a solution having a known concentration of about 0.25 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.
System suitability solution—Dissolve an accurately weighed quantity of USP Zileuton Related Compound C RS in acetonitrile to obtain a solution having a known concentration of about 10 µg per mL. Transfer 5.0 mL of this solution and 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.
Standard solution—Transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.
Test solution—Proceed as directed for *Test solution* under *Test 1*.
Chromatographic system—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between zileuton related compound B and zileuton related compound C is not less than 20. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Zileuton taken by the formula:

$$100(C_S/C_U)(r_i/r_S)$$

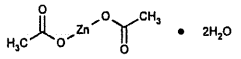
in which C_S is the concentration, in mg per mL, of USP Zileuton Related Compound B RS in the *Standard solution;* C_U is the concentration, in mg per mL, of zileuton in the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for zileuton related compound B obtained from the *Standard solution:* not more than 0.1% of any individual impurity is found; and not more than 0.7% of total impurities is found, the results for *Test 1* and *Test 2* being added.

Assay—
[NOTE—The *Standard preparation* and the *Assay preparation* are to be refrigerated at or below 5° immediately after preparation and during analysis using a refrigerated autosampler. The solutions are stable at or below 5° for about 36 hours.]
Buffer solution—Dissolve 7.7 g of ammonium acetate and 0.25 g of acetohydroxamic acid in about 900 mL of water in a 1000-mL volumetric flask, adjust with perchloric acid to a pH of 2.0, dilute with water to volume, and mix.
Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (72:28). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard preparation—Transfer about 30 mg of methylparaben, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.
Standard stock preparation—Dissolve an accurately weighed quantity of USP Zileuton RS in acetonitrile to obtain a solution having a known concentration of about 1 mg per mL.
Standard preparation—Transfer 5.0 mL of the *Standard stock preparation* and 4.0 mL of the *Internal standard preparation* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.
Assay preparation—Transfer about 100 mg of Zileuton, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Transfer 5.0 mL of this solution and 4.0 mL of the *Internal standard preparation* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 1.5 mL per minute.
Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between zileuton and methylparaben is not less than 5.0; the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 0.6%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the quantity, in mg, of $C_{11}H_{12}N_2O_2S$ in the portion of Zileuton taken by the formula:

$$1000C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Zileuton RS in the *Standard preparation;* and R_U and R_S are the peak area ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Zinc Acetate



$C_4H_6O_4Zn \cdot 2H_2O$ 219.50
$C_4H_6O_4Zn$ 183.48
Acetic acid, zinc salt, dihydrate;
Zinc acetate dihydrate [5970-45-6].
Zinc acetate anhydrous [557-34-6].

DEFINITION
Zinc Acetate contains NLT 98.0% and NMT 102.0% of $C_4H_6O_4Zn \cdot 2H_2O$.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Zinc* ⟨191⟩: A 50 mg/mL solution meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL,** *Acetate* ⟨191⟩: A 50 mg/mL solution meets the requirements.

ASSAY
- **PROCEDURE**

Sample solution: To 400 mg of Zinc Acetate in 100 mL of water add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS.
Analysis: Titrate the *Sample solution* with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 10.98 mg of zinc acetate dihydrate ($C_4H_6O_4Zn \cdot 2H_2O$).
Acceptance criteria: 98.0%–102.0%

USP Monographs

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IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩: A 1.5-g portion shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.005%).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩: A 1.0-g portion shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.010%).
- **ARSENIC,** *Method I* ⟨211⟩: NMT 3 ppm
- **LEAD** ⟨251⟩

Standard solution: To 20 mL of dilute nitric acid (1 in 100) add 0.01 mg of lead, 4 mL of *Ammonia–cyanide solution*, and 2 drops of *Hydroxylamine hydrochloride solution*, and shake for 30 s with 10.0 mL of *Standard dithizone solution*. Pass through acid-washed filter paper into a color-comparison tube.

Sample solution: Dissolve 0.5 g of zinc acetate in 1 mL of a mixture of nitric acid and water (1:1) in a separator. Add 3 mL of *Ammonium citrate solution* and 0.5 mL of *Hydroxylamine hydrochloride solution*, and render alkaline with ammonium hydroxide to phenol red TS. Add 10 mL of *Potassium cyanide solution*, and immediately extract the solution with successive 5-mL portions of *Dithizone extraction solution*, draining off each extract into another separator, until the last portion of dithizone solution retains its green color. Shake the combined extracts for 30 s with 20 mL of dilute nitric acid (1 in 100), and discard the chloroform layer. Add to the acid solution 4.0 mL of *Ammonia–cyanide solution* and 2 drops of *Hydroxylamine hydrochloride solution*. Add 10.0 mL of *Standard dithizone solution*, and shake the mixture for 30 s. Filter the chloroform layer through acid-washed filter paper into a color-comparison tube.

Analysis: Compare the color of the *Sample solution* with that of a *Standard solution*.

Acceptance criteria: The color of the *Sample solution* does not exceed the color of the *Standard solution* (0.002%).

- **ALKALIES AND ALKALINE EARTHS**

Sample solution: Dissolve 2.0 g in 150 mL of water in a 200-mL volumetric flask. Add sufficient ammonium sulfide TS to precipitate the zinc completely, and dilute with water to volume. Pass through a dry filter, rejecting the first portion of the filtrate.

Analysis: To 100 mL of the filtrate add 5 drops of sulfuric acid. Evaporate to dryness, and ignite.

Acceptance criteria: The weight of the residue does not exceed 2 mg (0.2%).

SPECIFIC TESTS

- **INSOLUBLE MATTER:** A 20-g portion, dissolved in 150 mL of water containing 1 mL of glacial acetic acid, shows NMT 1.0 mg of insoluble matter (0.005%).
- **pH** ⟨791⟩: 6.0–8.0, in a solution (1 in 20)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Zinc Acetate Oral Solution

DEFINITION

Zinc Acetate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of zinc (Zn) as zinc acetate dihydrate ($C_4H_6O_4Zn \cdot 2H_2O$). It may contain one or more suitable flavors and sweeteners, which are recognized as safe, in amounts that are unobjectionable.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Zinc* ⟨191⟩and *Acetate* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Sample: An accurately measured volume of Oral Solution, equivalent to 40 mg of zinc

Blank: 100 mL of water, 5 mL of ammonia–ammonium chloride buffer TS, and 0.1 mL of eriochrome black TS

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL flask. Add 100 mL of water, 5 mL of ammonia–ammonium chloride buffer TS, and 0.1 mL of eriochrome black TS. Titrate with *Titrant* to a green endpoint.

Calculate the percentage of the labeled amount of zinc (Zn) in the portion of Oral Solution taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mmol/mL)
F = equivalency factor, 65.4 mg/mmol
W = nominal amount of zinc in the *Sample* (mg)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 5.7–6.3
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/mL. The total molds and yeasts count does not exceed 10^1 cfu/mL. It meets the requirement of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store in a cool, dry place protected from light.
- **LABELING:** Label the Oral Solution in terms of zinc acetate dihydrate ($C_4H_6O_4Zn \cdot 2H_2O$) and in terms of elemental zinc.

Zinc Carbonate

$3Zn(OH)_2 \cdot 2ZnCO_3$ 548.96

Basic zinc carbonate;
Zinc subcarbonate [5263-02-5].

DEFINITION

Zinc Carbonate contains the equivalent of NLT 70.0% of zinc oxide (ZnO).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Zinc* ⟨191⟩

Sample solution: Dissolve in a slight excess of hydrochloric acid.

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Analysis: Transfer 2.0 g to a 125-mL conical flask, add 50.0 mL of 1 N sulfuric acid VS, and swirl to dissolve. Add 3 drops of methyl orange TS, and titrate with 1 N sodium hydroxide VS to a yellow endpoint. Each mL of 1 N sulfuric acid is equivalent to 40.69 mg of zinc oxide (ZnO).

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Acceptance criteria: NLT 70.0%

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: Dissolve 1.0 g in a mixture of 20 mL of water and 3 mL of nitric acid.
Acceptance criteria: The *Sample solution* shows no more chloride than corresponds to 0.03 mL of 0.02 N hydrochloric acid (0.002%).

• **SULFATE**
Sample solution: Dissolve 10.0 g in a mixture of 75 mL of water and 10 mL of hydrochloric acid, and filter. Neutralize the filtrate with ammonium hydroxide, and dilute with water to 100 mL.
Analysis: To 10.0 mL of the *Sample solution* add 1 mL of 0.6 N hydrochloric acid and 1 mL of barium chloride TS, and allow to stand for 10 min.
Acceptance criteria: The *Sample solution* shows no more turbidity than that produced in a solution containing 0.10 mL of 0.02 N sulfuric acid and the same quantities of reagents used to prepare the *Sample solution* (0.01%).

• **IRON** ⟨241⟩
Test preparation: Dissolve 0.5 g in 20 mL of water and 3 mL of hydrochloric acid.
Acceptance criteria: NMT 0.002%

• **LEAD**
Standard lead solution: Use standard lead solution TS.
Sample solution: Transfer 10.0 g to a 100-mL volumetric flask, add 20 mL of nitric acid and 10 mL of water, swirl to dissolve, and dilute with water to volume.
Blank: Nitric acid in water (1 in 25)
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 217.0 nm (lead emission line)
Lamp: Lead hollow-cathode
Flame: Air–acetylene
Analysis: Add 10.0 mL of the *Sample solution* to each of three 25-mL volumetric flasks. To the respective volumetric flasks add 0, 5.0, and 10.0 mL of *Standard lead solution*, and dilute with water to volume. These solutions contain 0, 0.002, and 0.004 mg/mL of added lead, respectively. Determine the absorbances of these three solutions. Plot the absorbances of the three solutions versus their contents of added lead, in mg/mL, as furnished by the *Standard lead solution*. Draw a straight line (calibration curve) best fitting the three points, and extrapolate the line until it intersects the concentration axis. From the intercept determine the concentration, in mg/mL, of lead in the *Sample solution*. Calculate the quantity, in ppm, of lead in the specimen:

$$\text{Result} = (V_1/W) \times (V_2/V_3) \times (C/F)$$

V_1 = total volume of the *Sample solution*, 100 mL
W = weight of specimen (g)
V_2 = final volume of the solution used for analysis, 25 mL
V_3 = volume of the *Sample solution* used for analysis, 10 mL
C = concentration of lead in the *Sample solution*, as determined from the calibration curve (mg/mL)
F = conversion factor, 0.001 µg/mg

Acceptance criteria: NMT 5 ppm

• **INSOLUBLE MATTER**
Sample solution: Dissolve 10 g in a mixture of 100 mL of water and 7 mL of sulfuric acid.
Analysis: Heat the *Sample solution* on a steam bath for 1 h. Filter the solution through a tared sintered-glass crucible, wash with hot water, and dry the crucible at 105°. Cool, and weigh.
Acceptance criteria: The residue weighs NMT 2 mg (0.02%).

• **SUBSTANCES NOT PRECIPITATED BY AMMONIUM SULFIDE**
Sample solution: Dissolve 1.0 g in 10 mL of water and 2 mL of sulfuric acid. Dilute with water to 80 mL, add 10 mL of ammonium hydroxide, and pass hydrogen sulfide through the solution for 30 min. Dilute with water to 100 mL, and allow the precipitate to settle. Decant the supernatant through a filter. Use the clear filtrate.
Analysis: Transfer 50 mL of the *Sample solution* to a tared dish, and evaporate to dryness. Ignite, gently at first, and finally at 800 ± 25°. Cool, and weigh.
Acceptance criteria: The weight of the residue is NMT 2 mg (0.4%).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Zinc Chloride

$ZnCl_2$ 136.29
Zinc chloride [7646-85-7].

DEFINITION
Zinc Chloride contains NLT 97.0% and NMT 100.5% of zinc chloride ($ZnCl_2$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Zinc* ⟨191⟩**:** Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY

• **PROCEDURE**
Sample solution: Dissolve 12 g of Zinc Chloride in 500 mL of water in a 1-L volumetric flask. Add 12 g of ammonium chloride, and dilute with water to volume.
Analysis: Pipet 25 mL of the *Sample solution* into a 400-mL beaker. Add 100 mL of water, 10 mL of ammonia–ammonium chloride buffer TS, and 1 mL of 0.5 mg/mL of eriochrome black T solution. Titrate with 0.05 M edetate disodium VS to a deep blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 6.815 mg of zinc chloride ($ZnCl_2$).
Acceptance criteria: 97.0%–100.5%

IMPURITIES

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample solution: 1.0 g in 30 mL of water
Acceptance criteria: 20 mL of the *Sample solution* shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.03%).

• **LEAD** ⟨251⟩
Standard solution: Add 5 mL of water, 2.50 mL of standard lead solution TS, and 15 mL of *Potassium cyanide solution* (100 mg/mL) to a similar color-comparison tube.
Sample solution: Dissolve 0.50 g in 5 mL of water, and transfer the solution to a color-comparison tube. Add 15 mL of *Potassium cyanide solution* (100 mg/mL), and allow the mixture to become clear.
Analysis: To each tube add 0.1 mL of sodium sulfide TS. Mix the contents of each tube, and allow to stand for 5 min.
Acceptance criteria: Viewed downward over a white surface, the *Sample solution* is not darker than the *Standard solution* (NMT 0.005%).

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• **LIMIT OF AMMONIUM SALTS**
Sample solution: 100 mg/mL
Analysis: To 5 mL of *Sample solution* add 1 N sodium hydroxide until the precipitate first formed is redissolved, then warm the solution.
Acceptance criteria: No odor of ammonia is perceptible.
• **LIMIT OF OXYCHLORIDE**
Sample solution: Dissolve 1.0 g in 20 mL of water, and add 20 mL of alcohol.
Analysis: To 10 mL of the *Sample solution* add 0.30 mL of 1.0 N hydrochloric acid.
Acceptance criteria: The solution becomes perfectly clear.
• **ALKALIES AND ALKALINE EARTHS**
Sample solution: Dissolve 2.0 g in 150 mL of water contained in a 200-mL volumetric flask. Add sufficient ammonium sulfide TS to precipitate the zinc completely, and dilute with water to volume. Pass through a dry filter, and reject the first portion of the filtrate.
Analysis: To 100 mL of the *Sample solution* add 5 drops of sulfuric acid. Evaporate to dryness, and ignite.
Acceptance criteria: The weight of the residue is NMT 10 mg (1.0%).

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

Zinc Chloride Injection

DEFINITION
Zinc Chloride Injection is a sterile solution of Zinc Chloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of zinc (Zn).

IDENTIFICATION
• **A.** The *Sample solution*, prepared as directed in the *Assay*, exhibits an absorption maximum at 213.8 nm, when determined as directed in the *Assay*.

ASSAY
• **PROCEDURE**
[NOTE—The *Standard solutions* and the *Sample solution* may be diluted quantitatively with water, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]
Sodium chloride solution: 0.9 mg/mL of sodium chloride in water
Standard stock solution A: 10 mg/mL of zinc in water prepared as follows. Transfer 3.11 g of zinc oxide to a 250-mL volumetric flask. Add 80 mL of 1 N sulfuric acid, warm to dissolve, and cool. Dilute with water to volume.
Standard stock solution B: 125 µg/mL of zinc in water from *Standard stock solution A*
Standard solution A: Transfer 2.0 mL of *Standard stock solution B* and 5 mL of *Sodium chloride solution* into a 500-mL volumetric flask, and dilute with water (0.50 µg/mL of zinc).
Standard solution B: Transfer 3.0 mL of *Standard stock solution B* and 5 mL of *Sodium chloride solution* into a 500-mL volumetric flask, and dilute with water (0.75 µg/mL of zinc).
Standard solution C: Transfer 4.0 mL of *Standard stock solution B* and 5 mL of *Sodium chloride solution* into a 500-mL volumetric flask, and dilute with water (1.0 µg/mL of zinc).
Sample solution: Transfer a volume of Injection, equivalent to 5 mg of zinc, to a 500-mL volumetric flask. Dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the 10.0-mL portion, and add a sufficient amount of *Sodium chloride solution* to bring the total sodium chloride content of the 100-mL volumetric flask to 0.9 mg. Dilute with water to volume.
Blank: Water
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Zinc emission line at 213.8 nm
Lamp: Zinc hollow-cathode
Flame: Air–acetylene
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of zinc, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in µg/mL, of zinc in the *Sample solution*.
Calculate the percentage of the labeled amount of zinc (Zn) in the portion of Injection taken:

$$\text{Result} = (C_O/C_U) \times 100$$

C_O = concentration of zinc in the *Sample solution* as determined from the standard curve (observed concentration, µg/mL)
C_U = nominal concentration of the *Sample solution*, based on the label claim of the Injection and the dilution factor (calculated concentration, µg/mL)

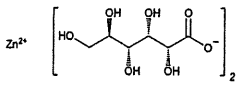
Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 25.0 USP Endotoxin Units/mg of zinc.
• **pH** ⟨791⟩: 1.5–2.5
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.
• **LABELING:** Label the Injection to indicate that it is to be diluted with Water for Injection or other suitable fluid to appropriate strength before administration.

Zinc Gluconate



$C_{12}H_{22}O_{14}Zn$ 455.67
Bis(D-gluconato-O^1,O^2) zinc;
Zinc D-gluconate (1:2) [4468-02-4].

DEFINITION
Zinc Gluconate contains NLT 97.0% and NMT 102.0% of zinc gluconate ($C_{12}H_{22}O_{14}Zn$), calculated on the anhydrous basis.

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IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Zinc* ⟨191⟩**:** A 100-mg/mL solution meets the requirements.

• **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Zinc Gluconate, heating in a water bath at 60°, if necessary, to dissolve

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Sample: 700 mg of Zinc Gluconate

Blank: 100 mL of water

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 100 mL of water. Add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with *Titrant* until the solution is deep blue in color. Perform a blank determination.

Calculate the percentage of zinc gluconate ($C_{12}H_{22}O_{14}Zn$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = *Titrant* molarity (mmol/mL)
F = equivalency factor, 455.7 mg/mmol
W = *Sample* weight (mg)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Standard solution: 0.7 mL of 0.020 N hydrochloric acid

Sample: 1.0 g

Acceptance criteria: NMT 0.05%

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Standard solution: 1.0 mL of 0.020 N sulfuric acid

Sample: 2.0 g

Acceptance criteria: NMT 0.05%

• **ARSENIC,** *Method I* ⟨211⟩

Test preparation: 1 g in 35 mL of water

Acceptance criteria: NMT 3 ppm

• **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Ascorbic acid–sodium iodide solution: 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

Trioctylphosphine oxide solution: 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone.

[**CAUTION**—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

Standard solution: Transfer 5.0 mL of lead nitrate stock solution TS, to a 100-mL volumetric flask, and dilute with water to volume. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask, and add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution*, and it contains 2 µg/mL of lead.

Sample solution: To a 50-mL volumetric flask add 1.0 g of Zinc Gluconate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

Blank: To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 283.3 nm

Lamp: Lead hollow-cathode

Flame: Air–acetylene

System suitability

Samples: *Standard solution* and *Blank*

Suitability requirements: The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and the *Sample solution*. Use the *Blank* to set the instrument to zero.

Acceptance criteria: NMT 10 ppm: the absorbance of the *Sample solution* does not exceed that of the *Standard solution*.

• **LIMIT OF CADMIUM**

Standard stock solution: 0.1372 mg/mL of cadmium nitrate

Standard solution: Pipet 25 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 1 mL of hydrochloric acid, dilute with water to volume, and mix. It contains 12.5 µg/mL of cadmium (Cd).

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Sample stock solution: Transfer 10.0 g of Zinc Gluconate into a 50-mL volumetric flask, and dissolve in and dilute with water to volume.
Sample solution A: Transfer 5.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume. This solution contains 0 µg/mL of added cadmium from the *Standard solution*.
Sample solution B: Transfer 5.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 2.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 1.0 µg/mL of added cadmium from the *Standard solution*.
Sample solution C: Transfer 5.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 4.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 2.0 µg/mL of added cadmium from the *Standard solution*.
Blank: Water
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 228.8 nm
Lamp: Cadmium hollow-cathode
Flame: Air–acetylene
Analysis
Samples: *Sample solution A*, *Sample solution B*, and *Sample solution C*
Determine the absorbances. Correct the absorbance values of *Sample solution A*, *Sample solution B*, and *Sample solution C* from that of the *Blank*. Plot the corrected absorbances of *Sample solution A*, *Sample solution B*, and *Sample solution C* versus their added cadmium concentrations, in µg/mL. Draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the amount, in µg/mL, of cadmium in *Sample solution A*.
Calculate the content of cadmium in the portion of Zinc Gluconate taken:

$$\text{Result} = (C \times V)/W$$

C = concentration of cadmium in *Sample solution A* (µg/mL), determined from the intercept of the linear regression line
V = volume of solvent taken to prepare *Sample solution A* (mL)
W = weight of Zinc Gluconate taken to prepare *Sample solution A* (g)

Acceptance criteria: NMT 5 ppm

• **REDUCING SUBSTANCES**
Sample: 1.0 g of Zinc Gluconate
Blank: 10 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant 0.1 N iodine VS
Back titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the blank determination.
Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = *Back titrant* volume consumed by the *Blank* (mL)
V_S = *Back titrant* volume consumed by the *Sample* (mL)
N = *Back titrant* normality (mEq/mL)
F = equivalency factor, 27 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 5.5–7.5
• **WATER DETERMINATION,** *Method Ib* ⟨921⟩**:** NMT 11.6%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Potassium Gluconate RS

Zinc Gluconate Tablets

DEFINITION
Zinc Gluconate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of Zn, in the form of zinc gluconate ($C_{12}H_{22}O_{14}Zn$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHY**
Standard solution: USP Potassium Gluconate RS in water; 10 mg/mL
Sample solution: Transfer a weighed quantity of powdered Tablets to a suitable flask, and add the necessary volume of water to obtain a solution containing about 10 mg/mL of zinc gluconate. Shake and sonicate, heating in a water bath at 60°, if necessary, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Alcohol, water, ammonium hydroxide, and ethyl acetate (50:30:10:10)
Spray reagent: Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.
Analysis
Samples: *Standard solution* and *Sample solution*
When the solvent front has moved about three-fourths the length of the plate, remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, spray with *Spray reagent*, and heat the plate at 110° for about 10 min.
Acceptance criteria: The principal spot from the *Sample solution* corresponds in color, size, and R_F value to that from the *Standard solution*.
• **B. PROCEDURE**
Sodium hydroxide solution: 42 mg/mL of sodium hydroxide
Ammonium chloride solution: 107 mg/mL of ammonium chloride
Glycerin solution: A mixture of glycerin and water (85:15)

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Sodium sulfide solution: Dissolve 12 g of sodium sulfide with heating in a 45-mL mixture of *Glycerin solution* and water (29:10), allow to cool, and dilute with the same mixture of solvents to 100 mL. The solution should be colorless.

Sample solution: Shake and sonicate the amount of powdered Tablets with the necessary volume of water to obtain a solution containing 100 mg/mL of zinc gluconate. Heat in a water bath at 60°, if necessary, and filter.

Analysis: To 5 mL of the *Sample solution* add 0.2 mL of *Sodium hydroxide solution*, add an additional 2 mL of *Sodium hydroxide solution*, and add 10 mL of *Ammonium chloride solution*. Add 0.1 mL of *Sodium sulfide solution*.

Acceptance criteria: A white precipitate is formed after the first addition of *Sodium hydroxide solution*. The precipitate dissolves after the second addition of *Sodium hydroxide solution*. The solution remains clear after addition of *Ammonium chloride solution*, and a white precipitate forms after addition of *Sodium sulfide solution*.

STRENGTH

• **PROCEDURE**

Sample solution: Weigh and finely powder NLT 20 Tablets. Weigh a portion of the powder, equivalent to about 80 mg of zinc, transfer to a suitable crucible, and ignite, gently at first, until free from carbon. Cool the crucible, add 25 mL of water and 5 mL of hydrochloric acid, and stir. Heat on a steam bath for 5 min, and filter, rinsing the filter with several portions of water. Dilute the combined filtrate and washes with water to about 100 mL.

Analysis: Add ammonia–ammonium chloride buffer TS until the solution is neutral to litmus. Add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 3.27 mg of Zn or 22.78 mg of zinc gluconate ($C_{12}H_{22}O_{14}Zn$).

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISINTEGRATION ⟨701⟩:** For Tablets intended to be mixed with water prior to intake as oral liquids

Time: NMT 60 s

• **DISINTEGRATION AND DISSOLUTION OF DIETARY SUPPLEMENTS ⟨2040⟩:** For Tablets not to be mixed with water prior to ingestion

Medium: Hydrochloric acid 0.01 N; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Analysis: Determine the amount of $C_{12}H_{22}O_{14}Zn$ dissolved, employing atomic absorption spectrophotometry at the resonance emission line for zinc, at 213.8 nm, on filtered portions of the solution under test, suitably diluted with water, in comparison with a standard solution having a known concentration of zinc in the same *Medium*.

Tolerances: NLT 75% of the labeled amount of $C_{12}H_{22}O_{14}Zn$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.

• **LABELING:** Label the Tablets in terms of elemental zinc, and also in terms of zinc gluconate ($C_{12}H_{22}O_{14}Zn$). The labeling indicates whether the Tablets are intended to be mixed with water before intake.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Potassium Gluconate RS

Zinc Oxide

ZnO 81.38

Zinc oxide [1314-13-2].

DEFINITION

Zinc Oxide, freshly ignited, contains NLT 98.0% and NMT 102.0% of zinc oxide (ZnO).

IDENTIFICATION

• **A.** When strongly heated, it assumes a yellow color that disappears on cooling.

• **B.** The retention time of the zinc peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

Proceed as directed in ▲*Zinc Determination ⟨591⟩, Ion Chromatographic Method*▲ (CN 1-May-2020).

Diluent: 0.2% (w/v) hydrochloric acid

Standard solution: 20 µg/mL of USP Zinc Oxide RS in *Diluent*, prepared as directed in the chapter

Sample solution: 20 µg/mL of Zinc Oxide from the freshly ignited Zinc Oxide in *Diluent*, prepared as directed in the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zinc oxide (ZnO) in the portion of Zinc Oxide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zinc from the *Sample solution*

r_S = peak response of zinc from the *Standard solution*

C_S = concentration of USP Zinc Oxide RS in the *Standard solution* (µg/mL)

C_U = concentration of Zinc Oxide in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the ignited basis

IMPURITIES

• **ARSENIC ⟨211⟩, *Procedures, Method I*:** NMT 6 ppm

• **LEAD**

Sample solution: Add 2 g to 20 mL of water, stir well, and add 5 mL of glacial acetic acid. Warm on a steam bath until dissolved.

Analysis: Add 5 drops of potassium chromate TS.

Acceptance criteria: No turbidity or precipitate is produced.

• **IRON AND OTHER HEAVY METALS**

Sample solution: Use the solution from *Carbonate and Color of Solution*.

Analysis: Cool 2 separate 5-mL aliquots of the *Sample solution*. Add potassium ferrocyanide TS to the first aliquot, and add sodium sulfide TS to the second aliquot.

Acceptance criteria: White precipitates are formed in both aliquots.

• **LOSS ON IGNITION ⟨733⟩**

Sample: 2 g

Analysis: Ignite the *Sample* at 500° to constant weight.

Acceptance criteria: NMT 1.0%

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SPECIFIC TESTS

- **ALKALINITY**

Analysis: Mix 1.0 g with 10 mL of hot water, add 2 drops of phenolphthalein TS, and pass through a suitable filter of appropriate pore size.

Acceptance criteria: If a red color is produced, NMT 0.30 mL of 0.10 N hydrochloric acid is required to discharge it.

- **CARBONATE AND COLOR OF SOLUTION**

Analysis: Mix 2.0 g with 10 mL of water, add 30 mL of 2 N sulfuric acid, and heat on a steam bath with constant stirring.

Acceptance criteria: No effervescence occurs, and the resulting solution is clear and colorless. [NOTE—Use this solution in the test for *Iron and Other Heavy Metals*.]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Zinc Oxide Neutral

ZnO 81.39

DEFINITION

Zinc Oxide Neutral, freshly ignited, contains NLT 95.0% and NMT 98.0% of zinc oxide (ZnO).

IDENTIFICATION

- **A.** When strongly heated, it assumes a yellow color that disappears on cooling.
- **B.** The retention time of the zinc peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Proceed as directed in ▲*Zinc Determination* ⟨591⟩, *Ion Chromatographic Method*▲ (CN 1-May-2020).

Diluent: 0.2% (w/v) hydrochloric acid

Standard solution: 20 µg/mL of USP Zinc Oxide RS in *Diluent*, prepared as directed in the chapter

Sample solution: 20 µg/mL of Zinc Oxide Neutral from the freshly ignited Zinc Oxide Neutral in *Diluent*, prepared as directed in the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zinc oxide (ZnO) in the portion of Zinc Oxide Neutral taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zinc from the *Sample solution*

r_S = peak response of zinc from the *Standard solution*

C_S = concentration of USP Zinc Oxide RS in the *Standard solution* (µg/mL)

C_U = concentration of Zinc Oxide Neutral in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–98.0%

IMPURITIES

- **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*

Standard solution: 0.020 N sulfuric acid

Sample: 0.1 g

Acceptance criteria: The *Sample* shows no more sulfate than corresponds to 2.3 mL of the *Standard solution* (2.2%).

- **ARSENIC** ⟨211⟩, *Procedures, Method I*: NMT 2 ppm
- **LEAD**

Sample solution: Add 2 g to 20 mL of water, and stir well. Add 5 mL of glacial acetic acid, and warm on a steam bath until the solution is dissolved.

Analysis: Add 5 drops of potassium chromate TS.

Acceptance criteria: No turbidity or precipitate is produced.

- **MERCURY**

Diluent A: Carefully add 50 mL of nitric acid to 450 mL of water, and mix.

Diluent B: Carefully add 10 mL of nitric acid to 490 mL of water, and mix.

Aqua regia: [NOTE—Prepare immediately before use.] Hydrochloric acid and nitric acid (3:1)

Stannous sulfate solution: [NOTE—The mixture is a suspension and should be stirred continuously during use.] Add 25 g of stannous sulfate to 250 mL of 0.5 N sulfuric acid.

Sodium chloride–hydroxylamine sulfate solution: 120 mg/mL each of sodium chloride and hydroxylamine sulfate in water

Potassium permanganate solution: 50 mg/mL of potassium permanganate in water

Standard stock mercury solution: [NOTE—Use of a commercially prepared mercury standard is recommended.] 1.0 mg/mL of mercury from mercuric chloride in *Diluent A*

Standard working mercury solution: 0.5 µg/mL of mercury in *Diluent B*, from *Standard stock mercury solution*

Standard solutions: Transfer 1-, 2-, 3-, and 4-mL aliquots of *Standard working mercury solution* to four separate 300-mL biological oxygen-demand (BOD) bottles. To each bottle add 5 mL of water and 5 mL of *Aqua regia*. Heat the bottle for 2 min in a water bath at 95°. Cool, and add 50 mL of water and 15 mL of *Potassium permanganate solution*. Mix thoroughly, and place in a water bath for 30 min at 95°. Cool, add 5 mL of *Sodium chloride–hydroxylamine sulfate solution*, and dilute with water to 200 mL. These solutions contain the equivalent of 2.5, 5, 7.5, and 10 ng/mL of mercury, respectively.

Sample solution: Transfer 2.0 g of Zinc Oxide Neutral into a 300-mL BOD bottle. To the bottle add 5 mL of water and 5 mL of *Aqua regia*. Heat the sample in a water bath for 2 min at 95°. Cool, and add 50 mL of water and 15 mL of *Potassium permanganate solution*. Mix thoroughly, and place in a water bath for 30 min at 95°. Cool, add 5 mL of *Sodium chloride–hydroxylamine sulfate solution*, and dilute with water to 200 mL.

Blank solution: To a 300-mL BOD bottle add 5 mL of water and 5 mL of *Aqua regia*. Heat the solution for 2 min in a water bath at 95°. Cool, and add 50 mL of water and 15 mL of *Potassium permanganate solution*. Mix thoroughly, and place in a water bath for 30 min at 95°. Cool, add 5 mL of *Sodium chloride–hydroxylamine sulfate solution*, and dilute with water to 200 mL.

Mercury detection instrument and **Aeration apparatus:** Proceed as directed in *Mercury* ⟨261⟩, *Method IIa and Method IIb*.

Analysis: Add 5 mL of *Stannous sulfate solution* to a *Standard solution*, and immediately insert the bottle into the *Aeration apparatus*. Measure the absorbances of the *Standard solutions*. Repeat with the remaining *Standard solutions* and *Sample solution*. Perform a blank determination using the *Blank solution*, and make any necessary corrections. Plot the absorbances of the *Standard solutions* versus concentrations, in µg/mL, and draw the straight line best fitting the plotted points. From the graph

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so obtained, determine the concentration, in ppm of mercury, in the *Sample solution*.
Acceptance criteria: NMT 1 ppm

• **IRON AND OTHER HEAVY METALS**
Sample solution: Use the solution from the test for *Carbonate and Color of Solution*.
Analysis: Cool two separate 5-mL aliquots of the *Sample solution*. Add potassium ferrocyanide TS to the first aliquot, and add sodium sulfide TS to the second aliquot.
Acceptance criteria: White precipitates are formed in both aliquots.

• **MAGNESIUM OXIDE**
Blank: Carefully add 10 mL of concentrated nitric acid to 490 mL of water, and mix.
Standard solution: [NOTE—Use of a commercially prepared magnesium–inductively coupled plasma standard solution is recommended.] 25 µg/mL of magnesium in *Blank*
Sample solution: 4 mg/mL of Zinc Oxide Neutral in *Blank*
Instrumental conditions
(See *Plasma Spectrochemistry* ⟨730⟩.)
Mode: Inductively coupled plasma–atomic emission spectrometry
Analytical wavelength: 279.1 nm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of magnesium oxide in the portion of Zinc Oxide Neutral taken:

$$\text{Result} = (C_U/C_S) \times (M_{r1}/M_{r2}) \times 100$$

C_U = concentration of magnesium in the *Sample solution*, determined from the instrument (µg/mL)
C_S = concentration of Zinc Oxide Neutral in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of magnesium oxide, 40.30
M_{r2} = molecular weight of magnesium, 24.31

Acceptance criteria: NMT 0.7%

• **LOSS ON IGNITION** ⟨733⟩
Sample: 1 g
Analysis: Ignite the *Sample* at 750° for 15 min.
Acceptance criteria: NMT 5.0%

SPECIFIC TESTS

• **ALKALINITY**
Analysis: Mix 1.0 g with 10 mL of hot water, and add two drops of phenolphthalein TS.
Acceptance criteria: No color change occurs.

• **CARBONATE AND COLOR OF SOLUTION**
Analysis: Mix 2.0 g with 10 mL of water, add 30 mL of 2 N sulfuric acid, and heat on a steam bath, with constant stirring.
Acceptance criteria: No effervescence occurs and the resulting solution is clear and colorless. [NOTE—Use this solution in the test for *Iron and Other Heavy Metals*.]

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

• **LABELING:** Label it to indicate that it is for use in sunscreen preparations only.

Change to read:

Zinc Oxide ▲Compounded▲ (USP 1-May-2019) Ointment

DEFINITION

Change to read:

Zinc Oxide ▲Compounded▲ (USP 1-May-2019) Ointment contains NLT 18.5% and NMT 21.5% of zinc oxide (ZnO).
Prepare Zinc Oxide ▲Compounded▲ (USP 1-May-2019) Ointment as follows.

Zinc Oxide	200 g
Mineral Oil	150 g
White Ointment	650 g
To make	1000 g

Levigate the *Zinc Oxide* with the *Mineral Oil* to a smooth paste, and incorporate the *White Ointment* [see *General Notices, 5.20.20 Added Substances (Excipients and Ingredients) in Official Products*].

IDENTIFICATION

• **A.** The residue obtained in the *Assay* is yellow when hot and white when cool.

ASSAY

Change to read:

• **PROCEDURE**
Sample solution: Weigh 700 mg of Ointment in a porcelain crucible, heat gently until melted, and continue heating, gradually raising the temperature until the mass is thoroughly charred. Ignite the mass until the residue is uniformly yellow, and cool. Dissolve the residue in 10 mL of 2 N sulfuric acid, warming if necessary to dissolve.
Titrimetric system
▲(See *Titrimetry* ⟨541⟩.)▲ (USP 1-May-2019)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Transfer the *Sample solution* to a beaker, and rinse the crucible with small portions of water until the combined solution and rinsings measure 50 mL. Add 15 mL of ammonia–ammonium chloride buffer TS and 1 mL of eriochrome black TS, and titrate with *Titrant* until the solution is blue in color. Each milliliter of *Titrant* is equivalent to 4.069 mg of zinc oxide (ZnO).
Acceptance criteria: 18.5%–21.5%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS

• **CALCIUM, MAGNESIUM, AND OTHER FOREIGN SUBSTANCES**
Sample: Heat 2 g gently until melted, and continue heating, gradually raising the temperature until the mass is thoroughly charred. Ignite the mass until the residue is uniformly yellow.
Analysis 1: To the *Sample* add 6 mL of 3 N hydrochloric acid.
Acceptance criteria 1: No effervescence occurs.
Analysis 2: Heat the mixture obtained in *Analysis 1* on a steam bath for 10–15 min.
Acceptance criteria 2: NMT a trace of insoluble residue remains.

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Analysis 3: Filter the solution from *Analysis 2*, dilute with water to 10 mL, add 6 N ammonium hydroxide until the precipitate first formed redissolves, and add 2 mL each of ammonium oxalate TS and dibasic sodium phosphate TS.
Acceptance criteria 3: NMT a slight turbidity is produced in 5 min.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in well-closed containers, and avoid prolonged exposure to temperatures exceeding 30°.

Change to read:

Zinc Oxide ▲Compounded▲ (USP 1-May-2019) Paste

DEFINITION

Change to read:

Zinc Oxide ▲Compounded▲ (USP 1-May-2019) Paste contains NLT 24.0% and NMT 26.0% of zinc oxide (ZnO). Prepare Zinc Oxide ▲Compounded▲ (USP 1-May-2019) Paste as follows.

Zinc Oxide	250 g
Starch	250 g
White Petrolatum	500 g
To make	1000 g

Mix the ingredients.

IDENTIFICATION
- **A.** The residue obtained in the *Assay* is yellow when hot and white when cool.

ASSAY

Change to read:

- **PROCEDURE**

Sample solution: Transfer 600 mg of Paste to a porcelain crucible. Heat gently until melted, and continue heating, gradually raising the temperature until the mass is thoroughly charred. Ignite the mass until the residue is uniformly yellow, and cool. Dissolve the residue in 10 mL of 2 N sulfuric acid, warming if necessary to dissolve.
Titrimetric system
▲(See *Titrimetry* ⟨541⟩.)▲ (USP 1-May-2019)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Transfer the *Sample solution* to a beaker, and rinse the crucible with small portions of water until the combined solution and rinsings measure 50 mL. Add 15 mL of ammonia–ammonium chloride buffer TS and 1 mL of eriochrome black TS, and titrate with *Titrant* until the solution is blue in color. Each milliliter of *Titrant* is equivalent to 4.069 mg of zinc oxide (ZnO).
Acceptance criteria: 24.0%–26.0%

PERFORMANCE TESTS
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in well-closed containers, and avoid prolonged exposure to temperatures exceeding 30°.

Zinc Oxide Powder

DEFINITION
Zinc Oxide Powder contains NLT 90.0% and NMT 110.0% of the labeled amount of zinc oxide (ZnO).

IDENTIFICATION
- **A.** The retention time of the zinc peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Proceed as directed in ▲*Zinc Determination* ⟨591⟩, *Ion Chromatographic Method*▲ (CN 1-May-2020).
Diluent: 0.2% (w/v) hydrochloric acid
Standard solution: 20 µg/mL of USP Zinc Oxide RS in *Diluent*, prepared as directed in the chapter
Sample stock solution: Nominally 500 µg/mL of zinc oxide prepared as follows. Transfer an appropriate amount of Powder to a suitable volumetric flask. Add hydrochloric acid to about 15% of the final volume of the flask. [NOTE—Sonication or shaking may be necessary.] Dilute with water to volume. Centrifuge or pass a portion of this solution through a suitable filter.
Sample solution: Nominally 20 µg/mL of zinc oxide in water from the *Sample stock solution*
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zinc oxide (ZnO) in the portion of Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zinc from the *Sample solution*
r_S = peak response of zinc from the *Standard solution*
C_S = concentration of USP Zinc Oxide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of zinc oxide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

Zinc Oxide and Salicylic Acid Paste

» Zinc Oxide and Salicylic Acid Paste contains not less than 23.5 percent and not more than 25.5

USP Monographs

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percent of zinc oxide (ZnO), and not less than 1.9 percent and not more than 2.1 percent of salicylic acid ($C_7H_6O_3$).

It may be prepared as follows:

Salicylic Acid, in fine powder	20 g
Zinc Oxide Paste, a sufficient quantity to make	1000 g

Thoroughly triturate the Salicylic Acid with a portion of the paste, then add the remaining paste, and triturate until a smooth mixture is obtained.

Packaging and storage—Preserve in well-closed containers.

Identification—

A: The residue obtained in the *Assay for zinc oxide* is yellow when hot and white when cool.

B: Shake 1 g of it with 10 mL of water, and filter. To the filtrate add 1 mL of ferric chloride TS: an intense reddish violet color is produced. To this solution add 1 mL of acetic acid: the color is not dispersed. To this solution add 2 mL of 2 N hydrochloric acid: the color is dispersed and a white crystalline precipitate is formed.

Minimum fill ⟨755⟩**:** meets the requirements.

Assay for zinc oxide—Weigh accurately in a tared porcelain crucible about 500 mg of Paste, heat gently until melted, and continue the heating, gradually raising the temperature until the mass is thoroughly charred. Ignite the mass strongly until all of the carbonaceous material has been dissipated, the residue is uniformly yellow, and the weight of the cooled residue is constant. The weight of the residue represents the quantity of ZnO in the weight of the Paste taken for the assay.

Assay for salicylic acid—Transfer to a suitable beaker about 5 g of Paste, accurately weighed. Add 40 mL of alcohol, previously neutralized with 0.1 N sodium hydroxide to a phenol red endpoint, and heat on a water bath for 5 minutes, with frequent swirling. While still hot, add phenol red TS, and titrate with 0.1 N sodium hydroxide VS to a red endpoint. Each mL of 0.1 N sodium hydroxide is equivalent to 13.81 mg of salicylic acid ($C_7H_6O_3$).

Zinc Stearate

Octadecanoic acid, zinc salt;
Zinc stearate [557-05-1].

DEFINITION

Zinc Stearate is a compound of zinc with a mixture of solid organic acids obtained from fats, and consists chiefly of variable proportions of zinc stearate and zinc palmitate. It contains the equivalent of NLT 12.5% and NMT 14.0% of ZnO.

IDENTIFICATION

• **A. Identification Tests—General,** *Zinc* ⟨191⟩

Sample solution: Mix 25 g with 200 mL of hot water, add 60 mL of 2 N sulfuric acid, and boil until the fatty acids separate as a transparent layer. Cool the mixture, and remove the solidified layer of fatty acids.

Acceptance criteria: A portion of the water layer meets the requirements.

• **B. Congealing Temperature** ⟨651⟩

Analysis: Place the separated fatty acids obtained in *Identification* test *A* in a filter wetted with water, and wash with boiling water until free from sulfate. Collect the fatty acids in a small beaker, allow to cool, pour off the separated water, then melt the acids. Filter into a dry beaker while hot, and dry at 105° for 20 min.

Acceptance criteria: The fatty acids congeal at a temperature not below 54°.

ASSAY

• **Procedure**

Sample solution: Boil 1 g of Zinc Stearate with 50 mL of 0.1 N sulfuric acid for at least 10 min, or until the fatty acids layer is clear, adding more water as necessary to maintain the original volume. Cool, and filter. Wash the filter and the flask thoroughly with water until the last washing is not acid to litmus paper.

Analysis: Add to the combined filtrate and washings 15 mL of ammonia–ammonium chloride buffer TS and 0.2 mL of eriochrome black TS. Heat the solution to about 40°, and titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 4.069 mg of ZnO.

Acceptance criteria: 12.5%–14.0% of ZnO

IMPURITIES

• **Arsenic,** *Method I* ⟨211⟩

Test preparation: Mix 5.0 g with 50 mL of water. Cautiously add 5 mL of sulfuric acid, and boil gently until the fatty acids layer is clear and the volume is reduced to about 25 mL. Filter while hot, cool the filtrate, and dilute with water to 50 mL. Transfer a 20-mL aliquot to the arsine generator flask, and dilute with water to 35 mL.

Acceptance criteria: NMT 1.5 ppm

• **Lead** ⟨251⟩

Standard solution: To 20 mL of 0.2 N nitric acid add 5 µg of lead, 4 mL of *Ammonia–Cyanide Solution*, and 2 drops of *Hydroxylamine Hydrochloride Solution*, and shake with 10.0 mL of *Standard Dithizone Solution* for 30 s. Pass through an acid-washed filter paper into a color-comparison tube.

Test preparation: Ignite 0.50 g in a platinum crucible for 15–20 min in a muffle furnace at 475°–500°. Cool, add 3 drops of nitric acid, evaporate over a low flame to dryness, and ignite again at 475°–500° for 30 min. Dissolve the residue in 1 mL of 8 N nitric acid, add 3 mL of *Ammonium Citrate Solution* and 0.5 mL of *Hydroxylamine Hydrochloride Solution*, and render alkaline to phenol red TS with ammonium hydroxide. Add 10 mL of *Potassium Cyanide Solution*. Immediately extract the solution with successive 5-mL portions of *Dithizone Extraction Solution*, draining off each extract into another separator, until the last portion of dithizone solution retains its green color. Shake the combined extracts for 30 s with 20 mL of 0.2 N nitric acid, and discard the chloroform layer. Add to the acid solution 4.0 mL of *Ammonia–Cyanide Solution* and 2 drops of *Hydroxylamine Hydrochloride Solution*. Add 10.0 mL of *Standard Dithizone Solution*, and shake the mixture for 30 s. Pass the chloroform layer through an acid-washed filter paper into a color-comparison tube.

Acceptance criteria: The color of the *Test preparation* does not exceed that of the *Standard solution* (NMT 10 ppm).

• **Alkalies and Alkaline Earths**

Sample solution: Mix 2.0 g with 50 mL of water, and add 10 mL of hydrochloric acid. Boil until the solution is clear, filter while hot, and wash the separated fatty acids with about 50 mL of hot water. Render the combined filtrate and washings alkaline with 6 N ammonium hydroxide. Add

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ammonium sulfide TS to precipitate the zinc completely, dilute with water to 200 mL, mix, and filter.
Analysis: To 100 mL of the clear filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite to constant weight.
Acceptance criteria: The weight of the residue does not exceed 10 mg (1.0%).

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Zinc Sulfate

$ZnSO_4 \cdot xH_2O$
Sulfuric acid, zinc salt (1:1), hydrate;
Zinc sulfate (1:1) monohydrate 179.46
Zinc sulfate (1:1) heptahydrate 287.56
[7446-20-0].
Anhydrous 161.44
[7733-02-0].

DEFINITION
Zinc Sulfate contains one or seven molecules of water of hydration. The monohydrate contains NLT 89.0% and NMT 90.4% of zinc sulfate ($ZnSO_4$), corresponding to NLT 99.0% and NMT 100.5% of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$), and the heptahydrate contains NLT 55.6% and NMT 61.0% of zinc sulfate ($ZnSO_4$), corresponding to NLT 99.0% and NMT 108.7% of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$).

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Zinc* ⟨191⟩**:** Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**

Sample: An amount of Zinc Sulfate equivalent to 170 mg of zinc sulfate ($ZnSO_4$)
Analysis: Dissolve the *Sample* in 100 mL of water. Add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 8.072 mg of zinc sulfate ($ZnSO_4$).
Acceptance criteria
Monohydrate: 89.0%–90.4% of zinc sulfate ($ZnSO_4$), corresponding to 99.0%–100.5% of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$)
Heptahydrate: 55.6%–61.0% of zinc sulfate ($ZnSO_4$), corresponding to 99.0%–108.7% of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)

IMPURITIES
- **ARSENIC,** *Method I* ⟨211⟩

Test preparation: Dissolve a portion equivalent to 215 mg of zinc sulfate ($ZnSO_4$) in 35 mL of water.
Acceptance criteria: NMT 14 ppm
- **LEAD** ⟨251⟩

Standard solution: Combine 5 mL of water, 0.50 mL of standard lead solution TS, and 10 mL of *Potassium Cyanide Solution* (1 in 10) in a color-comparison tube.
Test preparation: Dissolve an amount equivalent to 0.25 g of zinc sulfate ($ZnSO_4$) in 5 mL of water, and transfer the solution to a similar, matched color-comparison tube. Add 10 mL of *Potassium Cyanide Solution* (1 in 10), and allow the mixture to become clear.
Analysis: To the *Standard solution* and the *Test preparation* add 0.1 mL of sodium sulfide TS. Mix the contents of each tube, and allow to stand for 5 min.
Acceptance criteria: Viewed downward over a white surface, the *Test preparation* is not darker than the *Standard solution* (NMT 20 ppm of lead).
- **ALKALIES AND ALKALINE EARTHS**

Sample solution: Dissolve the equivalent of 1.12 g of zinc sulfate ($ZnSO_4$) in 150 mL of water contained in a 200-mL volumetric flask. Add sufficient ammonium sulfide TS to precipitate the zinc completely, and dilute with water to volume. Pass through a dry filter, and reject the first portion of the filtrate.
Analysis: To 100 mL of the *Sample solution* filtrate add a few drops of sulfuric acid, evaporate to dryness in a tared dish, and ignite to constant weight.
Acceptance criteria: The weight of the residue is NMT 5 mg (0.9%).

SPECIFIC TESTS
- **ACIDITY**

Sample solution: 28 mg/mL of zinc sulfate ($ZnSO_4$)
Acceptance criteria: The *Sample solution* is not colored pink by methyl orange TS.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label indicates whether it is the monohydrate or the heptahydrate. Label any oral or parenteral preparations containing Zinc Sulfate to state the content of elemental zinc.

Zinc Sulfate Injection

DEFINITION
Zinc Sulfate Injection is a sterile solution of Zinc Sulfate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of zinc (Zn).

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Zinc* ⟨191⟩ and *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**

[NOTE—The *Standard solutions* and the *Sample solution* may be diluted quantitatively with water, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]
Sodium chloride solution: 0.9 mg/mL of sodium chloride in water
Standard stock solution A: 10 mg/mL of zinc in water prepared as follows. Transfer 3.11 g of zinc oxide to a 250-mL volumetric flask. Add 80 mL of 1 N sulfuric acid, warm to dissolve, and cool. Dilute with water to volume.
Standard stock solution B: 125 µg/mL of zinc in water, from *Standard stock solution A*
Standard solution A: Transfer 2.0 mL of the *Standard stock solution B* and 5 mL of *Sodium chloride solution* into a 500-mL volumetric flask, and dilute with water (0.50 µg/mL of zinc).
Standard solution B: Transfer 3.0 mL of the *Standard stock solution B* and 5 mL of *Sodium chloride solution* into a

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500-mL volumetric flask, and dilute with water (0.75 µg/mL of zinc).
Standard solution C: Transfer 4.0 mL of the *Standard stock solution B* and 5 mL of *Sodium chloride solution* into a 500-mL volumetric flask, and dilute with water (1.0 µg/mL of zinc).
Sample solution: Transfer a volume of Injection, equivalent to 5 mg of zinc, to a 500-mL volumetric flask. Dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the 10.0-mL portion and add sufficient *Sodium chloride solution* to bring the total sodium chloride content of the 100-mL volumetric flask to 0.9 mg. Dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Zinc emission line at 213.8 nm
Lamp: Zinc hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of zinc, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in µg/mL, of zinc in the *Sample solution*.
Calculate the percentage of zinc (Zn) in the Injection:

$$\text{Result} = (C_O/C_U) \times 100$$

C_O = concentration of zinc in the *Sample solution* as determined from the standard curve (observed concentration, µg/mL)
C_U = nominal concentration of the *Sample solution*, based on the label claim of the Injection and the dilution factor (calculated concentration, µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections

SPECIFIC TESTS
- **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 25.0 USP Endotoxin Units/mg of zinc.
- **pH** ⟨791⟩: 2.0–4.0
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers.
- **Labeling:** Label the Injection in terms of its content of anhydrous zinc sulfate ($ZnSO_4$) and in terms of its content of elemental zinc. Label it to state that it is not intended for direct injection but is to be added to other intravenous solutions.

Zinc Sulfate Ophthalmic Solution

DEFINITION
Zinc Sulfate Ophthalmic Solution is a sterile solution of Zinc Sulfate in Water rendered isotonic by the addition of suitable salts. It contains NLT 95.0% and NMT 105.0% of the labeled amount of zinc sulfate ($ZnSO_4$).

IDENTIFICATION
- **A. Identification Tests—General** ⟨191⟩, *Chemical Identification Tests, Sulfate*: Meets the requirements
- **B.** The retention time of the zinc peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **Procedure**

Proceed as directed in ▲*Zinc Determination* ⟨591⟩, *Ion Chromatographic Method*▲ (CN 1-May-2020).
Diluent: 0.2% (w/v) hydrochloric acid
Standard stock solution: Prepare as directed in the chapter.
Standard solution: 20 µg/mL of USP Zinc Oxide RS in *Diluent* from the *Standard stock solution*
Sample solution: Nominally 40 µg/mL of zinc sulfate from a suitable volume of Ophthalmic Solution in *Diluent*
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zinc sulfate ($ZnSO_4$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of zinc from the *Sample solution*
r_S = peak response of zinc from the *Standard solution*
C_S = concentration of USP Zinc Oxide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of zinc sulfate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of zinc sulfate, 161.44
M_{r2} = molecular weight of zinc oxide, 81.38

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 5.8–6.2, or if it contains sodium citrate, 7.2–7.8
- **Other Requirements:** It meets the requirements in *Ophthalmic Products—Quality Tests* ⟨771⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.

Zinc Sulfate Oral Solution

DEFINITION
Zinc Sulfate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$). It may contain one or more suitable flavors and sweeteners.

IDENTIFICATION
- **Identification Tests—General,** *Zinc* ⟨191⟩ and *Sulfate* ⟨191⟩: Meets the requirements

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ASSAY

• **PROCEDURE**

Sample: An accurately measured volume of Oral Solution, equivalent to 99 mg of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$)

Blank: 60 mL of water and 10 mL of ammonia–ammonium chloride buffer TS. Add 0.1 mL of eriochrome black TS.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL flask. Add 50 mL of water and 10 mL of ammonia–ammonium chloride buffer TS and 0.3 mL of eriochrome black TS. Titrate with *Titrant* to a green endpoint.

Calculate the percentage of the labeled amount of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) in the portion of Oral Solution taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 179.46 mg/mM
W = nominal amount of zinc sulfate monohydrate in the *Sample* (mg)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩**:** 1.18–1.24

• **PH** ⟨791⟩**:** 2.5–4.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store in a cool, dry place protected from light.

• **LABELING:** Label the Oral Solution in terms of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) and in terms of elemental zinc.

Zinc Sulfate Tablets

DEFINITION

Zinc Sulfate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$). It may contain one or more suitable flavors and sweeteners.

IDENTIFICATION

• **A. SULFATE**

Sample solution: Dissolve a portion of powdered Tablets, nominally equivalent to 5 g of zinc sulfate, in 100 mL of water, filter in a Buchner funnel under vacuum, and use the filtrate.

Hydrochloric acid solution: Dilute 20 g of hydrochloric acid with water to 100 mL.

Barium chloride solution: 61 mg/mL of barium chloride

Analysis: To 5 mL of the *Sample solution* add 1 mL of *Hydrochloric acid solution* and 1 mL of *Barium chloride solution*.

Acceptance criteria: A white precipitate is formed.

• **B. ZINC**

Sample solution: Proceed as directed in *Identification* test *A*.

Sodium hydroxide solution: 420 mg/mL of sodium hydroxide

Ammonium chloride solution: 107 mg/mL of ammonium chloride

Glycerin solution: A mixture of glycerin and water (85:15)

Sodium sulfide solution: Dissolve 12 g of sodium sulfide with heating in a 45-mL mixture of *Glycerin solution* and water (29:10). Allow to cool, and dilute with the same mixture of solvents to 100 mL. The solution should be colorless.

Analysis: To 5 mL of the *Sample solution* add 0.2 mL of *Sodium hydroxide solution*.

Acceptance criteria: A white precipitate is formed. When an additional 2 mL of *Sodium hydroxide solution* is added, the precipitate dissolves. When 10 mL of *Ammonium chloride solution* is added, the solution remains clear. When 0.1 mL of *Sodium sulfide solution* is added, a white precipitate is formed.

ASSAY

• **PROCEDURE**

Sample: Portion of the powder, equivalent to 90 mg of zinc (about 247 mg of zinc sulfate monohydrate), from NLT 20 finely powdered Tablets

Blank: Proceed as directed in the *Analysis*, but without the *Sample*.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 M edetate disodium VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a suitable flask or beaker. Dissolve in 15 mL of dilute acetic acid, and sonicate for 15 min. Add 200 mL of water and 50 mg of xylenol orange triturate, and mix. [NOTE—For preparation of xylenol orange triturate, triturate 1 part of xylenol orange with 99 parts of potassium nitrate. Xylenol orange triturate, 1% is also commercially available.[1]] Neutralize the solution with 2 g of methenamine until the solution is a violet-pink color. Titrate with *Titrant* until the solution is yellow. [NOTE—Add the titrant slowly near the endpoint with expedited stirring.] Perform a *Blank* determination.

Calculate the percentage of the labeled amount of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 179.46 mg/mM
W = nominal amount of zinc sulfate monohydrate in the *Sample* (mg)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Time: 60 s

Acceptance criteria: Meet the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **LABELING:** Label the Tablets in terms of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) and in terms of elemental zinc.

[1] Ricca Chemical Company, part number 9395-100.

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Zinc Sulfide Topical Suspension

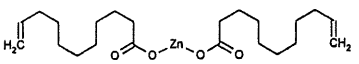
DEFINITION
Prepare Zinc Sulfide Topical Suspension as follows.

Zinc Sulfate	40 g
Sulfurated Potash	40 g
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Zinc Sulfate* and the *Sulfurated Potash* separately, each in 450 mL of *Purified Water*, and filter each solution. Add the sulfurated potash solution slowly to the zinc sulfate solution with constant stirring. Then add sufficient *Purified Water* to bring to final volume, and mix.
[NOTE—Prepare the Topical Suspension fresh, and shake it thoroughly before dispensing.]

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Dispense in tight containers.

Zinc Undecylenate



$C_{22}H_{38}O_4Zn$ 431.91
10-Undecenoic acid, zinc(2+) salt.
Zinc 10-undecenoate [557-08-4].

» Zinc Undecylenate contains not less than 98.0 percent and not more than 102.0 percent of $C_{22}H_{38}O_4Zn$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

Identification—
A: Acidify about 5 g with 25 mL of 2 N sulfuric acid, add 20 mL of water, and extract in a separator with two 25-mL portions of ether. Evaporate the ether solution until the odor of ether no longer is perceptible. Add potassium permanganate TS dropwise to a 1-mL portion of this residue: the permanganate color is discharged.
B: A 3-mL portion of the residue of undecylenic acid obtained in *Identification* test *A* responds to *Identification* test *B* under *Undecylenic Acid*.
C: Dissolve about 100 mg in a mixture of 10 mL of water and 1 mL of ammonium hydroxide, and add a few drops of sodium sulfide TS: a white, flocculent precipitate of zinc sulfide is formed.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 1.25% of its weight.
Alkalies and alkaline earths—Boil 1.50 g with a mixture of 50 mL of water and 10 mL of hydrochloric acid, filter while hot, and wash the separated acid with about 50 mL of hot water. Render the combined filtrate and washings alkaline with 6 N ammonium hydroxide, add ammonium sulfide TS to precipitate the zinc completely, dilute with water to 200 mL, mix, and filter. To 100 mL of the clear filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite over a low flame to constant weight: the weight of the residue does not exceed 7.5 mg (1.0%).

Assay—Boil 50.0 mL of 0.1 N sulfuric acid VS with about 1 g of Zinc Undecylenate, accurately weighed, for 10 minutes, or until the undecylenic acid layer is clear, adding water, as necessary, to maintain the original volume. Cool, and transfer the mixture, with the aid of water, to a 500-mL separator. Dilute with water to about 250 mL, and extract with two 100-mL portions of solvent hexane. Wash the combined extracts with water until the last washing is neutral to litmus, add the washings to the original water layer, and evaporate on a steam bath to about 100 mL. Cool, add 3 drops of methyl orange TS, and titrate the excess sulfuric acid with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.1 N sulfuric acid is equivalent to 21.60 mg of $C_{22}H_{38}O_4Zn$.

Ziprasidone Capsules

DEFINITION
Ziprasidone Capsules contain an amount of ziprasidone hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 0.3% (v/v) of triethylamine in water
Mobile phase: Acetonitrile and *Buffer* (35:65). Adjust with glacial acetic acid to a pH of 6.0.
Diluent: Acetonitrile, water, and glacial acetic acid (70:30:5)
Standard stock solution: 1.0 mg/mL of USP Ziprasidone Hydrochloride RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Ziprasidone Hydrochloride RS from the *Standard stock solution* in *Mobile phase*
Sample stock solution: Nominally 1 mg/mL of ziprasidone prepared as follows. Empty the contents of NLT 20 Capsules into a container. Blend the contents. Transfer an amount of the contents, equivalent to NLT 50 mg of ziprasidone, to a suitable volumetric flask. Dissolve the contents in 60% of the flask volume of *Diluent*. Sonicate for NLT 5 min. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size and use the filtrate to prepare the *Sample solution*.
Sample solution: Nominally 0.2 mg/mL of ziprasidone prepared from the filtered *Sample stock solution* and *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, a diode array detector may be used in the wavelength range of 200–300 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 20 µL
Run time: 1.5 times the retention time of ziprasidone
System suitability
Sample: *Standard solution*

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Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ziprasidone from the *Sample solution*
r_S = peak response of ziprasidone from the *Standard solution*
C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ziprasidone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ziprasidone free base, 412.94
M_{r2} = molecular weight of ziprasidone hydrochloride; 467.41 for the monohydrate, 449.40 for the anhydrous form

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Tier 1
Phosphate buffer, pH 7.5: Dissolve 7.8 g of monobasic sodium phosphate dihydrate and 20 g of sodium dodecyl sulfate in 1 L water. Sonicate to dissolve and adjust with phosphoric acid or sodium hydroxide to a pH of 7.5.
Medium: *Phosphate buffer, pH 7.5*; 900 mL
Apparatus 2: 75 rpm. Use a suitable sinker, if necessary.
Time: 45 min
Buffer: 0.3% (v/v) of triethylamine in water. Adjust with glacial acetic acid to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Diluent: Acetonitrile, water, and glacial acetic acid (70:30:5)
Standard stock solution: 0.24 mg/mL of USP Ziprasidone Hydrochloride RS prepared as follows. Dissolve a suitable amount of USP Ziprasidone Hydrochloride RS in a suitable volumetric flask first in 60% of the flask volume of *Diluent*, and then dilute with *Diluent* to volume.
Standard solution: 0.024 mg/mL of USP Ziprasidone Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 1.5 times the retention time of ziprasidone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ziprasidone from the *Sample solution*
r_S = peak response of ziprasidone from the *Standard solution*
C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of ziprasidone free base, 412.94
M_{r2} = molecular weight of ziprasidone hydrochloride; 467.41 for the monohydrate, 449.40 for the anhydrous form

Tolerances: NLT 75% (*Q*) of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) is dissolved.
If the above tolerance cannot be met, proceed to *Tier 2*.
Tier 2
Solution A: Dissolve 7.8 g of monobasic sodium phosphate dihydrate in 1 L of water. Sonicate to dissolve and adjust with phosphoric acid or sodium hydroxide to a pH of 7.5. Dissolve 10 g of pancreatin in the resulting solution.
Solution B: Dissolve 7.8 g of monobasic sodium phosphate dihydrate in 1 L of water. Adjust with phosphoric acid or sodium hydroxide to a pH of 7.5. Dissolve 90 g of sodium dodecyl sulfate in the resulting solution. Sonicate to dissolve.
Medium: Transfer 700 mL of *Solution A* to the dissolution vessel and equilibrate at 37° for 15 min. Add 200 mL of *Solution B*; 900 mL.
Apparatus 2: 75 rpm. Use a suitable sinker, if necessary.
Time: 45 min
Analyze the *Sample solution* using the liquid chromatographic procedure described in *Tier 1*.
Tolerances: NLT 75% (*Q*) of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Tier 1
Medium: 2% sodium lauryl sulfate in pH 7.5 phosphate buffer (dissolve 6.9 g of monobasic sodium phosphate monohydrate and 1.6 g of sodium hydroxide in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 7.5 and dilute with water to 1000 mL); 900 mL
Apparatus 2: 75 rpm. Use a suitable sinker, if necessary.
Time: 60 min
Tier 2
Medium A: pH 7.5 phosphate buffer (dissolve 6.9 g of monobasic sodium phosphate monohydrate and 1.6 g of sodium hydroxide in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 7.5 and dilute with water to 1000 mL) with 1% pancreatin; 700 mL
Medium B: pH 7.5 phosphate buffer with 9% of sodium lauryl sulfate; 200 mL
Apparatus 2: 75 rpm. Use a suitable sinker, if necessary.
Time: 15 min for *Medium A*; 45 min for *Medium A* with the addition of *Medium B*

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Solution A: Dissolve 2.7 g of monobasic sodium phosphate monohydrate in 1 L of water. Adjust with 1 N sodium hydroxide to a pH of 6.0.
Mobile phase: Acetonitrile and *Solution A* (50:50)
Diluent: Acetonitrile and water (50:50)
Standard stock solution: 0.48 mg/mL of USP Ziprasidone Hydrochloride RS in *Diluent*
Standard solution: (*L*/900) mg/mL of USP Ziprasidone Hydrochloride RS in *Medium* from *Standard stock solution*, where *L* is the label claim of ziprasidone in mg/Capsules
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Procedure: Perform the test using the conditions in *Tier 1*. In the presence of cross-linking repeat the test with new Capsules using the conditions in *Tier 2* as follows. After 15 min with 700 mL of *Medium A*, stop the dissolution bath and timer and add 200 mL of *Medium B* pre-equilibrated at 37 ± 0.5°. Restart the bath and timer, and continue the dissolution for an additional 45 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 1.8 times the retention time of ziprasidone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ziprasidone from the *Sample solution*
r_S = peak response of ziprasidone from the *Standard solution*
C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of ziprasidone, 412.94
M_{r2} = molecular weight of ziprasidone hydrochloride; 467.41 for the monohydrate form, 449.40 for the anhydrous form

Tolerances: NLT 75% (*Q*) of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) is dissolved.

▲**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Tier 1
Medium: 2% sodium lauryl sulfate in pH 7.5 phosphate buffer (6.9 g/L of monobasic sodium phosphate pH adjusted with 5 N sodium hydroxide); 900 mL
Apparatus 2: 75 rpm. Use a suitable sinker.
Time: 60 min

Tier 2
Medium A: pH 7.5 phosphate buffer (6.9 g/L of monobasic sodium phosphate pH adjusted with 5 N sodium hydroxide) with 1% pancreatin; 700 mL
Medium B: pH 7.5 phosphate buffer (6.9 g/L of monobasic sodium phosphate pH adjusted with 5 N sodium hydroxide) with 9% sodium lauryl sulfate; 200 mL
Apparatus 2: 75 rpm. Use a suitable sinker.
Time: 15 min for *Medium A*; 45 min for *Medium A* with the addition of *Medium B*
Buffer: 6.8 g/L g of monobasic sodium phosphate. To each liter of this solution, add 1 mL of triethylamine and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (30:70)
Diluent
Diluent 1: Acetonitrile and methanol (35:65)
Diluent 2
Tier 1: *Medium*
Tier 2: *Medium A* and *Medium B* (70:20)
Standard stock solution 1: 0.5 mg/mL of USP Ziprasidone Hydrochloride RS in *Diluent 1*
Standard stock solution 2: Prepare solutions of USP Ziprasidone Hydrochloride RS in *Diluent 2* at concentrations given in *Table 1* as follows. Transfer a suitable volume of *Standard stock solution* into a suitable volumetric flask and dilute with *Diluent 2* to volume.

Table 1

Strength of Ziprasidone Capsules (mg)	Concentration of Ziprasidone (mg/mL)
20	0.025
40	0.050
60	0.080
80	0.100

Standard solution: Transfer 5 mL of *Standard stock solution 2* to a 25-mL volumetric flask and dilute with *Mobile phase* to volume.
Sample solution: Centrifuge a portion of the solution under test. Dilute the supernatant with *Mobile phase* to volume to obtain nominal concentration of ziprasidone similar to that of the *Standard solution*. Pass through a suitable filter of 0.45-µm pore size. [NOTE—A centrifuge speed of 4000 rpm for 10 min may be suitable.]
Procedure: Perform the test using the conditions in *Tier 1*. In the presence of cross-linking repeat the test with new Capsules using the conditions in *Tier 2* as follows. After 15 min with 700 mL of *Medium A*, stop the dissolution bath and timer and add 200 mL of *Medium B* pre-equilibrated at 37 ± 0.5°. Restart the bath and timer, and continue the dissolution for an additional 45 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.3 mL/min
Injection volume: 10 µL
Run time: 1.3 times the retention time of ziprasidone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0

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Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ziprasidone from the *Sample solution*

r_S = peak response of ziprasidone from the *Standard solution*

C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)

V = volume of *Medium* (*Tier 1* or *Tier 2*), 900 mL

D = dilution factor for the *Sample solution*, 5

L = label claim (mg/Capsule)

M_{r1} = molecular weight of ziprasidone, 412.94

M_{r2} = molecular weight of ziprasidone hydrochloride; 467.41 for the monohydrate form, 449.40 for the anhydrous form

Tolerances: NLT 70% (*Q*) of the labeled amount of ziprasidone ($C_{21}H_{21}ClN_4OS$) is dissolved. ▲(RB 1-May-2018)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**

Buffer: 0.05 M monobasic potassium phosphate

Solution A: Methanol and *Buffer* (33:67). Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile, methanol, and *Buffer* (55:5:40). Adjust with potassium hydroxide to a pH of 6.0.

Mobile phase: See ▲*Table 2*.

Table 2 ▲(RB 1-May-2018)

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	85	15
30	85	15
40	55	45
55	40	60
65	25	75
70	20	80
71	100	0
75	100	0

Diluent: Acetonitrile, methanol, and water (40:10:50). Adjust with phosphoric acid to a pH of 2.5.

System suitability solution: 0.5 mg/mL of USP Ziprasidone Hydrochloride RS and 0.05 mg/mL each of USP Ziprasidone Related Compound B RS and USP Ziprasidone Related Compound F RS in *Diluent*

Standard solution: 0.002 mg/mL each of USP Ziprasidone Hydrochloride RS and USP Ziprasidone Related Compound B RS in *Diluent*. Sonication may be used to aid in dissolution.

Sample solution: Nominally 1.0 mg/mL of ziprasidone in *Diluent* from a portion of contents of Capsules (NLT 20) prepared as follows. Transfer a suitable amount of Capsule contents to a suitable volumetric flask. Add 60% of the flask volume of *Diluent*. Sonicate for 10 min. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 229 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between ziprasidone related compound B and related compound F; NLT 2.0 between ziprasidone related compound F and ziprasidone, *System suitability solution*

Tailing factor: NMT 1.5 for ziprasidone, *Standard solution*

Relative standard deviation: NMT 5.0% for both ziprasidone and ziprasidone related compound B, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ziprasidone related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ziprasidone related compound B from the *Sample solution*

r_S = peak response of ziprasidone related compound B from the *Standard solution*

C_S = concentration of USP Ziprasidone Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ziprasidone in the *Sample solution* (mg/mL)

Calculate the percentage of any other unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of ziprasidone from the *Standard solution*

C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ziprasidone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ziprasidone free base, 412.94

M_{r2} = molecular weight of ziprasidone hydrochloride; 467.41 for the monohydrate, 449.40 for the anhydrous form

Acceptance criteria: See ▲*Table 3*. ▲(RB 1-May-2018) Disregard any peak with an area below 0.05% in the *Sample solution*.

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▲Table 3▲ (RB 1-May-2018)

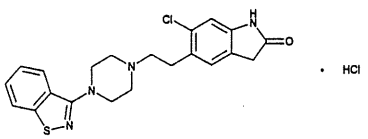
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ziprasidone related compound A[a, b]	0.22	—
Chloroindolinone[a, c]	0.59	—
Ziprasidone related compound B	0.70	0.20
Ziprasidone related compound F[a]	0.84	—
Ziprasidone	1.0	—
Ziprasidone related compound C[a, d]	1.84	—
Ziprasidone related compound D[a, e]	2.18	—
Any individual unspecified degradation product	—	0.20
Total degradation products	—	0.50

[a] Process impurity included in the table for identification only; controlled in the drug substance. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.
[b] 3-(Piperazin-1-yl)benzo[*d*]isothiazole.
[c] 6-Chloroindolin-2-one.
[d] 5,5′-Bis{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-6,6′-dichloro-3-hydroxy-3,3′-biindoline-2,2′-dione.
[e] 3-(Benzo[*d*]isothiazol-3-yl)-5-{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-6-chloroindolin-2-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ziprasidone Hydrochloride RS
  USP Ziprasidone Related Compound B RS
  5-{2-[4-(Benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-6-chloroindoline-2,3-dione.
  $C_{21}H_{19}ClN_4O_2S$ 426.92
  USP Ziprasidone Related Compound F RS
  2-(2-Amino-5-{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-4-chlorophenyl)acetic acid.
  $C_{21}H_{23}ClN_4O_2S$ 430.95

Ziprasidone Hydrochloride



$C_{21}H_{21}ClN_4OS \cdot HCl$ 449.40
$C_{21}H_{21}ClN_4OS \cdot HCl \cdot H_2O$ 467.41
2*H*-Indol-2-one, 5-{2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl}-6-chloro-1,3-dihydro-, monohydrochloride;
5-{2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]ethyl}-6-chloro-2-indolinone monohydrochloride.
Monohydrate [138982-67-9].
Anhydrous [122883-93-6].

DEFINITION

Ziprasidone Hydrochloride contains NLT 97.5% and NMT 102.0% of ziprasidone hydrochloride ($C_{21}H_{21}ClN_4OS \cdot HCl$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020) If a difference appears in the spectra of the analyte and the Reference Standard, dissolve equal portions of the analyte and the Reference Standard in equal volumes of methanol separately, evaporate to dryness, and record new spectra using the residues.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
  Sample solution: Suspend 30 mg of the analyte in 2 mL of water, acidify with 0.15 mL of 2 M nitric acid, and filter. Use the filtrate.
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 85% phosphoric acid to a pH of 3.0.
  Mobile phase: Methanol and *Buffer* (40:60)
  Diluent: Methanol and water (60:40)
  Standard solution: 0.23 mg/mL of USP Ziprasidone Hydrochloride RS in *Diluent*
  Sample solution: 0.23 mg/mL of Ziprasidone Hydrochloride in *Diluent*. [NOTE—Because of its hygroscopic nature, the anhydrous form may be dried at 70° under vacuum for 2 h prior to the preparation of the *Sample solution*.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 229 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L7
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of ziprasidone hydrochloride ($C_{21}H_{21}ClN_4OS \cdot HCl$) in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Ziprasidone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous and solvent-free basis

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IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **LIMIT OF TETRAHYDROFURAN**

[NOTE—Perform this test only if tetrahydrofuran is likely to be present as the result of the manufacturing process.]

Standard solution: 0.05 mg/mL of tetrahydrofuran in dimethyl sulfoxide. Transfer 4 mL of this solution to a 20-mL headspace vial, and seal.

Sample solution: Transfer 40 mg of Ziprasidone Hydrochloride and 4.0 mL of dimethyl sulfoxide to a 20-mL headspace vial, seal, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC with headspace injector

Detector: Flame ionization

Column: 30-m × 0.32-mm fused silica; coated with a 1.8-µm film of phase G43

Temperatures

Injection port: 180°

Detector: 260°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	2	90	0
90	30	225	2

Carrier gas: Helium

Flow rate: 1.6 mL/min

Injection volume: 2 mL

Injection type: Headspace; split ratio, 30:1

Vial treatment: Maintain at 105° for 60 min prior to injection

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tetrahydrofuran in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tetrahydrofuran from the *Sample solution*

r_S = peak response of tetrahydrofuran from the *Standard solution*

C_S = concentration of tetrahydrofuran in the *Standard solution* (mg/mL)

C_U = concentration of Ziprasidone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.4% of tetrahydrofuran

• **ORGANIC IMPURITIES**

Buffer: 0.05 M monobasic potassium phosphate in water

Solution A: Methanol and *Buffer* (33:67). Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile, methanol, and *Buffer* (55:5:40). Adjust with potassium hydroxide TS to a pH of 6.0.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	85	15
30	85	15
40	55	45
55	40	60
65	25	75
70	20	80
71	100	0
75	100	0

Diluent: Acetonitrile, methanol, and water (40:10:50). Adjust with phosphoric acid to a pH of 2.5.

System suitability solution: 0.5 mg/mL of USP Ziprasidone Hydrochloride RS and 0.05 mg/mL each of USP Ziprasidone Related Compound A RS, USP Ziprasidone Related Compound B RS, USP Ziprasidone Related Compound C RS, USP Ziprasidone Related Compound D RS, and USP Ziprasidone Related Compound F RS in *Diluent*. [NOTE—Ziprasidone related compound F is stable for 6 h at 10°.]

Standard solution: 0.001 mg/mL of USP Ziprasidone Hydrochloride RS and 0.002 mg/mL each of USP Ziprasidone Related Compound A RS, USP Ziprasidone Related Compound B RS, USP Ziprasidone Related Compound C RS, and USP Ziprasidone Related Compound D RS in *Diluent*

Sample solution: 1.0 mg/mL of Ziprasidone Hydrochloride in *Diluent*. [NOTE—Because ziprasidone related compound F is stable only for 6 h at 10°, it is recommended that the freshly prepared *Sample solution* be analyzed.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 229 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Temperatures

Autosampler: 10°

Column: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between ziprasidone related compound B and related compound F; NLT 2.0 between ziprasidone related compound F and ziprasidone, *System suitability solution*

Tailing factor: NMT 1.5 for ziprasidone, *Standard solution*

Relative standard deviation: NMT 5.0% for ziprasidone, ziprasidone related compound B, ziprasidone related compound C, and ziprasidone related compound D, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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r_U = peak response of each specified impurity from the *Sample solution*
r_S = peak response of the corresponding specified impurity from the *Standard solution*
C_S = concentration of the corresponding specified impurity Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Ziprasidone Hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ziprasidone free base, 412.94
M_{r2} = molecular weight of ziprasidone hydrochloride, 467.41 for the monohydrate, 449.40 for the anhydrous form

Calculate the percentage of chloroindolinone, ziprasidone related compound F, and any individual unspecified impurity in the portion of Ziprasidone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of chloroindolinone, ziprasidone related compound F, or any unspecified impurity from the *Sample solution*
r_S = peak response of ziprasidone from the *Standard solution*
C_S = concentration of USP Ziprasidone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Ziprasidone Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for chloroindolinone or ziprasidone related compound F or any individual unspecified impurity from *Table 3*
M_{r1} = molecular weight of ziprasidone free base, 412.94
M_{r2} = molecular weight of ziprasidone hydrochloride, 467.41 for the monohydrate, 449.40 for the anhydrous form

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05% for any peak area in the *Sample solution*.

Table 3

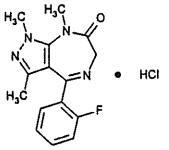
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ziprasidone related compound A	0.22	—	0.1
Chloroindolinone[a]	0.59	0.47	0.15
Ziprasidone related compound B	0.70	—	0.2
Ziprasidone related compound F	0.84	0.83	0.2
Ziprasidone	1.0	—	—
Ziprasidone related compound C	1.84	—	0.2
Ziprasidone related compound D	2.18	—	0.2
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] 6-Chloroindolin-2-one. A process impurity specific to a manufacturing process; may not be present in all manufacturing processes.

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I*
  Monohydrate: 3.7%–5.0%
  Anhydrous: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
- **LABELING:** If this is an anhydrous form, it is so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ziprasidone Hydrochloride RS
  USP Ziprasidone Related Compound A RS
  3-(Piperazin-1-yl)benzo[*d*]isothiazole monohydrochloride.
  $C_{11}H_{13}N_3S \cdot HCl$ 255.77
  USP Ziprasidone Related Compound B RS
  5-{2-[4-(Benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-6-chloroindoline-2,3-dione.
  $C_{21}H_{19}ClN_4O_2S$ 426.92
  USP Ziprasidone Related Compound C RS
  5,5′-Bis{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl] ethyl}-6,6′-dichloro-3-hydroxy-3,3′-biindoline-2,2′-dione.
  $C_{42}H_{40}Cl_2N_8O_3S_2$ 839.85
  USP Ziprasidone Related Compound D RS
  3-(Benzo[*d*]isothiazol-3-yl)-5-{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-6-chloroindolin-2-one.
  $C_{28}H_{24}ClN_5OS_2$ 546.11
  USP Ziprasidone Related Compound F RS
  2-(2-Amino-5-{2-[4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl]ethyl}-4-chlorophenyl)acetic acid.
  $C_{21}H_{23}ClN_4O_2S$ 430.95

Zolazepam Hydrochloride



$C_{15}H_{15}FN_4O \cdot HCl$ 322.77
Pyrazolo[3,4-*e*][1,4]diazepin-7(1*H*)-one, 4-(2-fluorophenyl)-6,8-dihydro-1,3,8-trimethyl-, monohydrochloride.
4-(*o*-Fluorophenyl)-6,8-dihydro-1,3,8-trimethylpyrazolo [3,4-*e*][1,4]diazepin-7(1*H*)-one monohydrochloride [33754-49-3].

» Zolazepam Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{15}H_{15}FN_4O \cdot HCl$.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is for veterinary use only. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Zolazepam Hydrochloride RS
Clarity of solution—Dissolve 2.0 g of it in 10 mL of water: the solution is clear.

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Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 0.015 mg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 223 nm do not differ by more than 3.0%.

C: It responds to the tests for *Chloride* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩ —Where the label states that Zolazepam Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.07 USP Endotoxin Unit per mg of zolazepam.

Sterility Tests ⟨71⟩ —Where the label states that Zolazepam Hydrochloride is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩**:** between 1.5 and 3.5, in a solution (1 in 10).

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.5%.

Chromatographic purity—

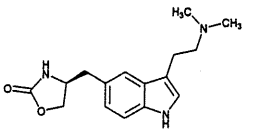
Modified Dragendorff's reagent—Dissolve 1.7 g of bismuth subnitrate in 80 mL of water and 20 mL of glacial acetic acid, warming, if necessary. Cool, add 100 mL of potassium iodide solution (1 in 2), and mix. Refrigerate this stock solution for prolonged storage. For use, dilute 10 mL of this stock solution with water to 100 mL, add 10 mL of glacial acetic acid, and mix. Then add 120 mg of iodine crystals, and shake until the iodine has completely dissolved. Store refrigerated, and discard after 2 weeks.

Procedure—Prepare a test solution of Zolazepam Hydrochloride in methanol containing 100 mg per mL. Prepare a Standard solution in methanol containing 2.0 mg of USP Zolazepam Hydrochloride RS per mL. Prepare a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Separately apply 5 µL of the test solution and the Standard solution to the plate, and allow the spots to dry. Place the plate in a saturated chamber containing a solvent system consisting of a mixture of toluene, acetone, and ammonium hydroxide (75:18:7), and lined with filter paper. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, allow the plate to air-dry. Spray the plate with *Modified Dragendorff's reagent*, and examine the plate: no individual secondary spot observed in the chromatogram obtained from the test solution is greater in size or intensity than the principal spot observed in the chromatogram obtained from the Standard solution, corresponding to 2%, and the total of any such spots observed does not exceed 3%.

Chloride content—Transfer about 400 mg of it, accurately weighed, to a conical flask, add 5 mL of water, 5 mL of glacial acetic acid, and 50 mL of methanol, and swirl to dissolve. Add 1 drop of eosin Y TS, and titrate with 0.1 N silver nitrate VS to the endpoint when the granular precipitate first turns to a permanent pink color. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride: between 10.5% and 11.5% is found.

Assay—Transfer about 480 mg of Zolazepam Hydrochloride, accurately weighed, to a conical flask, add 70 mL of glacial acetic acid, 10 mL of mercuric acetate TS, and swirl to dissolve. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 32.28 mg of $C_{15}H_{15}FN_4O \cdot HCl$.

Zolmitriptan



$C_{16}H_{21}N_3O_2$ 287.36

2-Oxazolidinone, 4-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-, (*S*)-;
(*S*)-4-[[3-[2-(Dimethylamino)ethyl]indol-5-yl]methyl]-2-oxazolidinone [139264-17-8].

DEFINITION

Zolmitriptan contains NLT 97.0% and NMT 102.0% of zolmitriptan ($C_{16}H_{21}N_3O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The migration time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Limit of Zolmitriptan R-Isomer and Other Impurities*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Acetonitrile and water ▲(135:865). For every liter of the mixture add 1 mL of trifluoroacetic acid and 0.25 mL of triethylamine.▲ (USP 1-Aug-2019)
System suitability solution: 0.12 µg/mL of USP Zolmitriptan Related Compound E RS and 25 µg/mL of USP Zolmitriptan RS in *Mobile phase*
Standard solution: 0.025 mg/mL of USP Zolmitriptan RS in *Mobile phase*
Sample solution: 0.025 mg/mL of Zolmitriptan in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.0-mm × 12.5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: About 3 times the retention time of zolmitriptan
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for zolmitriptan and zolmitriptan related compound E are 1.0 and 1.6, respectively.]
Suitability requirements
Resolution: NLT 5 between zolmitriptan and zolmitriptan related compound E

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Tailing factor: NMT 2.0 for zolmitriptan

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zolmitriptan ($C_{16}H_{21}N_3O_2$) in the portion of Zolmitriptan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)
C_U = concentration of Zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF ZOLMITRIPTAN RELATED COMPOUND H**

Perform this test only if zolmitriptan related compound H is a known process impurity. If this test is performed, then this is to be included in total impurities.

Standard solution: 0.1 mg/mL of USP Zolmitriptan Related Compound H RS in methanol

Sample solution: 200 mg/mL of Zolmitriptan in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 15-m; 1-µm coating of dimethylpolysiloxane phase G1

Temperatures

Injection port: 200°

Column: 130°

Detector: 250°

Carrier gas: Helium at 6 mL/min

Injection volume: 3 µL

Injection type: Split ratio, 10:1

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zolmitriptan related compound H in the portion of Zolmitriptan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zolmitriptan related compound H from the *Sample solution*
r_S = peak response of zolmitriptan related compound H from the *Standard solution*
C_S = concentration of USP Zolmitriptan Related Compound H RS in the *Standard solution* (mg/mL)
C_U = concentration of Zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1% of zolmitriptan related compound H

Change to read:

- **LIMIT OF ZOLMITRIPTAN *R*-ISOMER AND OTHER IMPURITIES**

Buffer: 19.1 g/L of sodium borate decahydrate in water. Adjust with phosphoric acid to a pH of 2.2.

Run buffer: 50 mg/mL of ▲hydroxypropyl-β-cyclodextrin▲ (USP 1-Aug-2019) in *Buffer*

Diluent: 0.02 M hydrochloric acid

Internal standard solution: ▲0.05 mg/mL▲ (USP 1-Aug-2019) of tryptamine hydrochloride in *Diluent*

System suitability solution: 0.01 mg/mL of tryptamine hydrochloride from the *Internal standard solution*; 1 mg/mL of USP Zolmitriptan RS; and 0.01 mg/mL each of USP Zolmitriptan Related Compound F RS, USP Zolmitriptan Related Compound G RS, and USP Zolmitriptan *R*-Isomer RS in *Diluent*

Standard solution: 0.01 mg/mL of tryptamine hydrochloride from *Internal standard solution* and 0.001 mg/mL of USP Zolmitriptan RS in *Diluent*

Sample solution: 0.01 mg/mL of tryptamine hydrochloride from *Internal standard solution* and 1 mg/mL of Zolmitriptan in *Diluent*. Filter the solution and protect from light.

▲Capillary rinsing procedure: Use separate *Run buffer* vials for the capillary rinse and sample analysis. Condition the capillary by rinsing with 0.1 N sodium hydroxide followed by *Run buffer* before each injection. [NOTE—It may be suitable to rinse with 0.1 N sodium hydroxide using a pressure of 20 psi for NLT 1 min and then to rinse with *Run buffer* using a pressure of 20 psi for NLT 3 min.]▲ (USP 1-Aug-2019)

Electrophoretic system

Mode: CE

Detector: UV 200 nm

Column: 75-µm ▲(ID)▲ (USP 1-Aug-2019) × 50-cm ▲effective length, 60-cm total length capillary▲ (USP 1-Aug-2019) uncoated fused-silica

▲Capillary temperature: 25°

Injection pressure: 0.5 psi for 5 s▲ (USP 1-Aug-2019)

Applied voltage: 15 kV

Run time: ▲NLT▲ (USP 1-Aug-2019) 1.5 times the migration time of zolmitriptan

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative migration times.]

Suitability requirements

Resolution: NLT 1.5 between the zolmitriptan and zolmitriptan *R*-isomer peaks, *System suitability solution*

Relative standard deviation: NMT 5% for the peak response ratio of the zolmitriptan and tryptamine peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the corrected peak responses:

$$\text{Result} = (r/m)$$

r = peak response
m = migration time of the peak (min)

Calculate the percentage of each impurity in the portion of Zolmitriptan taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (1/F) \times 100$$

R_U = corrected peak response ratio of the impurity to the internal standard from the *Sample solution*
R_S = corrected peak response ratio of zolmitriptan to the internal standard from the *Standard solution*
C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)
C_U = concentration of Zolmitriptan in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

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Acceptance criteria: See *Table 1*. Disregard the peak due to zolmitriptan related compound E. ▲The reporting threshold is 0.10%.▲ (USP 1-Aug-2019)

Table 1

Name	Relative Migration Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolmitriptan related compound ▲G	0.68	0.52▲ (USP 1-Aug-2019)	0.1
Zolmitriptan related compound ▲F	0.71	0.41▲ (USP 1-Aug-2019)	1.2
Tryptamine	0.78	1.0	—
Zolmitriptan	1.0	—	—
Zolmitriptan *R*-isomer	1.07	1.0	0.2
Any individual unspecified impurity	—	1.0	0.1

Change to read:

- **ORGANIC IMPURITIES**

Mobile phase: Acetonitrile and water ▲(135:865). For every liter of the mixture add 1 mL of trifluoroacetic acid and 0.25 mL of triethylamine.▲ (USP 1-Aug-2019)

System suitability solution: 0.5 µg/mL of USP Zolmitriptan Related Compound E RS and 0.1 mg/mL of USP Zolmitriptan RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Zolmitriptan in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.0-mm × 12.5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: ▲NLT▲ (USP 1-Aug-2019) 6 times the retention time of zolmitriptan

System suitability
Sample: *System suitability solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 5 between the zolmitriptan and zolmitriptan related compound E peaks
Tailing factor: NMT 3.0 for zolmitriptan

Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Zolmitriptan taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. ▲The reporting threshold is 0.05%.▲ (USP 1-Aug-2019)

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zolmitriptan related compound B[a]	0.36	0.2
Zolmitriptan	1.0	—
Zolmitriptan related compound E	1.6	0.2
Zolmitriptan related compound F[b]	2.3	—
Any individual unspecified impurity	—	0.1
Total impurities[c]	—	0.5

[a] (*S*)-2-Amino-3-{3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl}propan-1-ol.
[b] Included for identification purposes only. Not reported here as it is monitored under the *Limit of Zolmitriptan R-Isomer and Other Impurities* test. Not to be included in total impurities.
[c] Includes all impurities except zolmitriptan related compound F. Also includes zolmitriptan related compound H from the test for *Limit of Zolmitriptan Related Compound H* if it is a known process impurity, as well as impurities from the test for *Limit of Zolmitriptan R-Isomer and Other Impurities* with the exception of zolmitriptan related compound F.

SPECIFIC TESTS
- **WATER DETERMINATION ⟨921⟩, *Method I, Method Ia*:** NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Zolmitriptan RS
USP Zolmitriptan *R*-Isomer RS
(*R*)-4-[[3-[2-(Dimethylamino)ethyl]indol-5-yl]methyl]-2-oxazolidinone.
$C_{16}H_{21}N_3O_2$ 287.36
USP Zolmitriptan Related Compound E RS
(*S*)-*N*,*N*-Dimethyl-2-{5-[(2-oxooxazolidin-4-yl)methyl]-1*H*-indol-3-yl}ethanamine oxide.
$C_{16}H_{21}N_3O_3$ 303.36
USP Zolmitriptan Related Compound F RS
2,2′-[4-(Dimethylamino)butane-1,1-diyl]bis{5-[(*S*)-(2-oxooxazolidin-4-yl)methyl]-3-(2-dimethylaminoethyl) indole}.
$C_{38}H_{53}N_7O_4$ 671.87
USP Zolmitriptan Related Compound G RS
(*S*)-4-(4-Aminobenzyl)oxazolidin-2-one.
$C_{10}H_{12}N_2O_2$ 192.21
USP Zolmitriptan Related Compound H RS
4,4-Diethoxy-*N*,*N*-dimethylbutan-1-amine.
$C_{10}H_{23}NO_2$ 189.30

Zolmitriptan Nasal Spray

DEFINITION
Zolmitriptan Nasal Spray is an aqueous solution of zolmitriptan, supplied in a form suitable for nasal administration. It contains NLT 90.0% and NMT 110.0% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Standard: Add 300 µL of a solution of 2.5 mg/mL of USP Zolmitriptan RS in methylene chloride to about 200 mg of

USP Monographs

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dried potassium bromide. Allow the mixture to dry. Grind the residue.

Sample: Add the contents of NLT 5 units of Nasal Spray to a container with 3 mL of 0.1 M sodium hydroxide. Extract into a sufficient volume of methylene chloride to obtain a final concentration of about 1.0–2.5 mg/mL of zolmitriptan in the extract. Dry the extract over anhydrous sodium sulfate. Add a suitable volume of the extract containing about 0.8 mg of zolmitriptan to about 200 mg of dried potassium bromide. Allow the mixture to dry. Grind the residue.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: 0.02 M hydrochloric acid

Mobile phase: Acetonitrile and water (135:865). Add 1 mL of trifluoroacetic acid and 0.25 mL of triethylamine to each liter of the mixture.

Standard stock solution: 0.5 mg/mL of USP Zolmitriptan RS in *Diluent*

Standard solution: 0.025 mg/mL of USP Zolmitriptan RS from *Standard stock solution* and *Mobile phase*

System suitability stock solution: 0.005 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS in *Mobile phase*

System suitability solution: Dilute 1 mL of *System suitability stock solution* with 9 mL of *Standard solution*.

Sample stock solution: Nominally 0.5 mg/mL of zolmitriptan prepared as follows. Discharge the contents of NLT 10 units of Nasal Spray to a suitable container. Transfer an amount of the composite solution containing about 5 mg of zolmitriptan to a 10-mL volumetric flask. Dilute with *Diluent* to volume.

Sample solution: Nominally 0.025 mg/mL of zolmitriptan from *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Column temperature: 40°

Flow rate: 1.6 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of zolmitriptan

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between zolmitriptan and zolmitriptan related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for zolmitriptan, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DELIVERED DOSE UNIFORMITY**

Diluent, Mobile phase, Standard solution, and **System suitability solution:** Prepare as directed in the *Assay*.

Sample solution: Nominally 0.025 mg/mL of zolmitriptan prepared as follows. Discharge the contents of a single unit into a suitable volumetric flask. Dilute with *Mobile phase* to volume.

Repeat this procedure with 9 additional units.

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan in each dose of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

V = volume of the *Sample solution*

L = label claim of zolmitriptan (mg/dose)

Acceptance criteria

Tier 1

1. The mean of 10 units is within 85.0%–115.0% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$).
2. NMT 1 dosage unit outside of 80%–120% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$)
3. None outside of 75%–125% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) for 10 units

If criterion 2 in *Tier 1* cannot be met, proceed to *Tier 2*.

Tier 2: Test an additional 20 units. All 30 results (including the results from *Tier 1*) meet the following acceptance criteria.

1. NMT 3 of the 30 dosage units outside of 80%–120% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$)
2. None of the 30 dosage units are outside of 75%–125% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$).

IMPURITIES

- **ORGANIC IMPURITIES**

Diluent, Mobile phase, Standard solution, System suitability solution, and **Sample stock solution:** Prepare as directed in the *Assay*.

Sensitivity solution: 0.1 µg/mL of USP Zolmitriptan RS in *Mobile phase* from *Standard solution*

Sample solution: Nominally 0.1 mg/mL of zolmitriptan from *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Column temperature: 40°

Flow rate: 1.6 mL/min

Injection volume: 20 µL

Run time: NLT 8 times the retention time of zolmitriptan

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System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between zolmitriptan and zolmitriptan related compound E, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each degradation product in the portion of Nasal Spray taken:

$$\text{Result} = (r_I / r_U) \times 100$$

r_I = peak response of each degradation product from the *Sample solution*
r_U = peak response of zolmitriptan from the *Sample solution*

Acceptance criteria: See *Table 1*. The reporting threshold is 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zolmitriptan related compound G[a]	0.27	—
Zolmitriptan hydroxy ketone analog[b]	0.34	0.6
Zolmitriptan pyrrolo analog quaternary salt[c]	0.42	1.3
Zolmitriptan hydroxymethyl quaternary salt[d]	0.89	0.4
Zolmitriptan	1.0	—
Zolmitriptan related compound E[a]	1.3	—
Zolmitriptan related compound F[a, e]	1.8	—
Zolmitriptan methylene dimer[f]	6.0	0.3
Any other individual degradation product	—	0.2
Total degradation products	—	2.5

[a] Process impurity; included for peak identification and/or resolution check only; controlled in the drug substance. Not to be included in total degradation products.
[b] (4*S*)-4-({3-[2-(Dimethylamino)ethyl]-3-hydroxy-2-oxoindolin-5-yl}methyl) oxazolidin-2-one.
[c] 3a-Hydroxy-1,1-dimethyl-5-{[(*S*)-2-oxooxazolidin-4-yl]methyl}-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-1-ium; may also be known as (4*S*)-4-[(8*b*-Hydroxy-3,3-dimethyl-1,2,3a,4-tetrahydropyrrolo[2,3-*b*]indol-3-ium-7-yl) methyl]oxazolidin-2-one.
[d] (*S*)-*N*-(Hydroxymethyl)-*N*,*N*-dimethyl-2-{5-[(2-oxooxazolidin-4-yl)methyl]-1*H*-indol-3-yl}ethan-1-aminium; may also be known as (4*S*)-4-[[3-(2-Dimethylaminoethyl)-1-(hydroxymethyl)indol-5-yl]methyl]oxazolidin-2-one.
[e] 2,2′-[4-(Dimethylamino)butane-1,1-diyl]bis{5-[(*S*)-(2-oxooxazolidin-4-yl) methyl]-3-(2-dimethylaminoethyl)indole}.
[f] (*S*)-4-({3-[2-(Dimethylamino)ethyl]-1-[(3-[2-(dimethylamino)ethyl]-5-{[(*S*)-2-oxooxazolidin-4-yl]methyl}-1*H*-indol-2-yl)methyl]-1*H*-indol-5-yl}methyl) oxazolidin-2-one.

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.7–5.3
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩
  Total aerobic viable count: $\leq 10^2$ cfu/mL
  Total combined yeasts and molds count: $\leq 10^1$ cfu/mL
  Bile-tolerant Gram-negative bacteria per mL: NMT 10
  Tests for the absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in a tight, light-resistant container. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Zolmitriptan RS
  USP Zolmitriptan Related Compound E RS
  (*S*)-*N*,*N*-Dimethyl-2-{5-[(2-oxooxazolidin-4-yl)methyl]-1*H*-indol-3-yl}ethanamine oxide.
  $C_{16}H_{21}N_3O_3$ 303.36
  USP Zolmitriptan Related Compound G RS
  (*S*)-4-(4-Aminobenzyl)oxazolidin-2-one.
  $C_{10}H_{12}N_2O_2$ 192.21

Zolmitriptan Tablets

DEFINITION
Zolmitriptan Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$).

IDENTIFICATION
- **A.** The UV spectrum of the zolmitriptan peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: Dissolve 2 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.
  Mobile phase: Acetonitrile and *Buffer* (15:85)
  Standard stock solution: 0.25 mg/mL of USP Zolmitriptan RS in methanol. Sonicate if necessary to aid dissolution.
  Standard solution: 0.025 mg/mL of USP Zolmitriptan RS in *Mobile phase* from *Standard stock solution*
  Sample stock solution: Nominally 0.25 mg/mL of zolmitriptan from Tablets in methanol prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add 70% of the flask volume of methanol. Sonicate for 30 min. Cool to room temperature, and dilute with methanol to volume.
  Sample solution: Nominally 0.025 mg/mL of zolmitriptan in *Mobile phase* from *Sample stock solution* passed through a suitable membrane filter of 0.45-µm pore size
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 225 nm. For *Identification A*, a diode array detector can be used in the wavelength range of 200–300 nm.
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 30°
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  Run time: NLT 2.5 times the retention time of zolmitriptan
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zolmitriptan from the *Sample solution*
r_S = peak response of zolmitriptan from the *Standard solution*
C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 15 min
[Note—Analyze the sample under test using either the *Chromatographic procedure* or the *Instrumental procedure*.]
Chromatographic procedure
Buffer, Mobile phase, Standard stock solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: (*L*/500) mg/mL of USP Zolmitriptan RS in *Medium* from *Standard stock solution*, where *L* is the Tablet label claim in mg
Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of zolmitriptan from the *Sample solution*
r_S = peak response of zolmitriptan from the *Standard solution*
C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim of zolmitriptan (mg/Tablet)

Instrumental procedure
Standard solution: 0.01 mg/mL of USP Zolmitriptan RS from *Standard stock solution* in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.2-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: About 283 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim of zolmitriptan (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 5.8 g/L of ammonium dihydrogen phosphate in water. Adjust with triethylamine to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (15:85)
Standard solution: (*L*/500) mg/mL of USP Zolmitriptan RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a suitable portion of the solution under test through a suitable filter. Discard the first few mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.8 times the retention time of zolmitriptan
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim of zolmitriptan (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Solution A: 2.7 g/L of monobasic potassium phosphate in water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
10	95	5
45	86	14
55	55	45
60	55	45
62	95	5
75	95	5

Diluent: Methanol and water (25:75)

System suitability stock solution: 0.2 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS in methanol

System suitability solution: 0.25 mg/mL of USP Zolmitriptan RS and 0.002 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS from *System suitability stock solution* in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Zolmitriptan RS in a suitable volumetric flask containing 50% of the flask volume of *Diluent*. Sonicate to dissolve. Transfer a suitable volume of *System suitability stock solution* to the flask. Dilute with *Diluent* to volume.

Standard stock solution: 0.25 mg/mL of USP Zolmitriptan RS in methanol. Sonicate if necessary to aid dissolution.

Standard solution: 0.001 mg/mL of USP Zolmitriptan RS in *Diluent* from a suitable volume of *Standard stock solution*

Sample solution: Nominally 0.25 mg/mL of zolmitriptan from NLT 5 Tablets prepared as follows. Transfer the required number of Tablets to a suitable volumetric flask. Add 25% of the flask volume of methanol. Sonicate for 30 min with intermittent shaking. Cool to room temperature. Dilute with water to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector

For zolmitriptan and zolmitriptan related compound E and any other unspecified degradation product: UV 223 nm

For zolmitriptan and zolmitriptan related compound G: UV 235 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Use 223 nm for *System suitability* evaluation.

Resolution: NLT 5.0 between zolmitriptan and zolmitriptan related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for the zolmitriptan peak, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

▲**Samples:** *Standard solution* and *Sample solution*▲ (ERR 1-Nov-2018)

Calculate the percentage of zolmitriptan related compound G in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of zolmitriptan related compound G at 235 nm from the *Sample solution*

r_S = peak response of zolmitriptan at 235 nm from the *Standard solution*

C_S = concentration of the USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor for zolmitriptan related compound G (see *Table 2*)

Calculate the percentage of zolmitriptan related compound E and any other unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of zolmitriptan related compound E or any other unspecified degradation product at 223 nm from the *Sample solution*

r_S = peak response of zolmitriptan at 223 nm from the *Standard solution*

C_S = concentration of the USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolmitriptan related compound G	0.50	1.2	0.2
Zolmitriptan	1.0	—	—
Zolmitriptan related compound E	1.28	1.0	0.6
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Zolmitriptan RS

USP Zolmitriptan Related Compound E RS

(*S*)-*N*,*N*-Dimethyl-2-{5-[(2-oxooxazolidin-4-yl)methyl]-1*H*-indol-3-yl}ethanamine oxide.

$C_{16}H_{21}N_3O_3$ 303.36

USP Zolmitriptan Related Compound G RS

(*S*)-4-(4-Aminobenzyl)oxazolidin-2-one.

$C_{10}H_{12}N_2O_2$ 192.21

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Zolmitriptan Orally Disintegrating Tablets

DEFINITION

Zolmitriptan Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$).

IDENTIFICATION

- **A.** The UV spectrum of the zolmitriptan peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: Dissolve 2 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (15:85)

Standard stock solution: 0.25 mg/mL of USP Zolmitriptan RS in methanol

Standard solution: 0.025 mg/mL of USP Zolmitriptan RS in *Mobile phase* from a suitable volume of *Standard stock solution*

Sample stock solution: 0.25 mg/mL of zolmitriptan in methanol, prepared as follows. Transfer NLT 20 Tablets to a suitable volumetric flask. Add 75% of the flask volume of methanol. Sonicate for 30 min. Allow to cool to room temperature and dilute with methanol to volume.

Sample solution: Nominally 0.025 mg/mL of zolmitriptan in *Mobile phase* from a suitable volume of *Sample stock solution.* Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 225 nm. For *Identification* test *A,* use a diode-array detector in the wavelength range of 200–300 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2.5 times the retention time of zolmitriptan

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zolmitriptan from the *Sample solution*

r_S = peak response of zolmitriptan from the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩**:** NMT 30 s
- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

Analyze the sample under test using either the *Chromatographic procedure* or the *Instrumental procedure.*

Chromatographic procedure

Buffer, Mobile phase, Standard stock solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*

Standard solution: 0.005 mg/mL of USP Zolmitriptan RS from *Standard stock solution* in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of zolmitriptan from the *Sample solution*

r_S = peak response of zolmitriptan from the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

V = volume of *Medium,* 500 mL

L = label claim of zolmitriptan (mg/Tablet)

Instrumental procedure

Standard solution: 0.01 mg/mL of USP Zolmitriptan RS from *Standard stock solution* in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.2-µm pore size.

Instrumental conditions

Mode: UV

Analytical wavelength: About 283 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

V = volume of *Medium,* 500 mL

L = label claim of zolmitriptan (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of zolmitriptan ($C_{16}H_{21}N_3O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Diluent: Methanol and water (25:75)

Solution A: 2.7 g/L of monobasic potassium phosphate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1.*

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
15	92	8
45	86	14
55	55	45
60	55	45
62	95	5
75	95	5

Impurities stock solution: 0.2 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS in methanol

System suitability solution: 0.25 mg/mL of USP Zolmitriptan RS and 0.002 mg/mL each of USP Zolmitriptan Related Compound E RS and USP Zolmitriptan Related Compound G RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Zolmitriptan RS in a suitable volumetric flask containing 50% of the flask volume of *Diluent*. Sonicate to dissolve. Transfer a suitable volume of *Impurities stock solution* to the flask. Dilute with *Diluent* to volume.

Standard stock solution: 0.25 mg/mL of USP Zolmitriptan RS in methanol

Standard solution: 0.001 mg/mL of USP Zolmitriptan RS from *Standard stock solution* in *Diluent*

Sample solution: Nominally 0.25 mg/mL of zolmitriptan from NLT 5 Tablets prepared as follows. Transfer the required number of Tablets to a suitable volumetric flask. Add 25% of the flask volume of methanol. Sonicate for 30 min with intermittent shaking. Cool to room temperature. Dilute with water to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector

For zolmitriptan and zolmitriptan related compound E and any other unspecified degradation products: UV 223 nm

For zolmitriptan and zolmitriptan related compound G: UV 235 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Use 223 nm for system suitability evaluation.

Resolution: NLT 5.0 between zolmitriptan and zolmitriptan related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for zolmitriptan, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zolmitriptan related compound G in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of zolmitriptan related compound G at 235 nm from the *Sample solution*

r_S = peak response of zolmitriptan at 235 nm from the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor for zolmitriptan related compound G (see *Table 2*)

Calculate the percentage of zolmitriptan related compound E and any other unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of zolmitriptan related compound E or any other unspecified degradation product at 223 nm from the *Sample solution*

r_S = peak response of zolmitriptan at 223 nm from the *Standard solution*

C_S = concentration of USP Zolmitriptan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zolmitriptan in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolmitriptan related compound G	0.66	1.2	0.2
Zolmitriptan	1.0	—	—
Zolmitriptan related compound E	1.30	1.0	0.6
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Zolmitriptan RS

USP Zolmitriptan Related Compound E RS

(*S*)-*N*,*N*-Dimethyl-2-{5-[(2-oxooxazolidin-4-yl)methyl]-1*H*-indol-3-yl}ethanamine oxide.

$C_{16}H_{21}N_3O_3$ 303.36

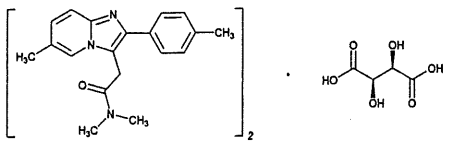
USP Zolmitriptan Related Compound G RS

(*S*)-4-(4-Aminobenzyl)oxazolidin-2-one.

$C_{10}H_{12}N_2O_2$ 192.21

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Zolpidem Tartrate



$(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6$ 764.87

Imidazo[1,2-*a*]pyridine-3-acetamide, *N*,*N*,6-trimethyl-2-(4-methylphenyl)-, [*R*-(*R**,*R**)]-2,3-dihydroxy butanedioate; *N*,*N*,6-Trimethyl-2-*p*-tolylimidazo[1,2-*a*]pyridine-3-acetamide L-(+)-tartrate [99294-93-6].

DEFINITION

Zolpidem Tartrate contains NLT 98.0% and NMT 102.0% of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K▲ (CN 1-May-2020)

  Sample: Dissolve 0.10 g of Zolpidem Tartrate in 10 mL of 0.1 M hydrochloric acid. Add 10 mL of water. Add dropwise with stirring 1 mL of diluted ammonia solution. Filter and collect the resulting precipitate. Wash the precipitate with water, and then dry at 100°–105° for 2 h. Use 2 mg of the dried residue.

  Analysis: The IR spectrum of the free base thus obtained corresponds to the IR spectrum of similarly prepared USP Zolpidem Tartrate RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- **C. Reaction of Tartrates**

  Sample: 0.1 g of Zolpidem Tartrate

  Analysis: Dissolve the *Sample* in 1 mL of methanol with gentle heating. To 0.1 mL of the solution, add 0.1 mL of a 100-g/L solution of potassium bromide, 0.1 mL of a 20-g/L solution of resorcinol, and 3 mL of sulfuric acid. Heat on a water bath for 5–10 min. A dark blue color develops. Allow to cool, then pour the solution into water.

  Acceptance criteria: The dark blue color changes to red.

ASSAY

- **Procedure**

  Buffer: 5.6 g/L of phosphoric acid. Adjust with triethylamine to a pH of 5.5.

  Mobile phase: Methanol, acetonitrile, and *Buffer* (23:18:59)

  System suitability solution: 0.05 mg/mL each of USP Zolpidem Tartrate RS and USP Zolpidem Related Compound A RS in *Mobile phase*

  Standard solution: 0.1 mg/mL of USP Zolpidem Tartrate RS in *Mobile phase*

  Sample solution: 0.1 mg/mL of Zolpidem Tartrate in *Mobile phase*

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *System Suitability*.)

  Mode: LC

  Detector: UV 254 nm

  Column: 3.9-mm × 15-cm; 4-µm packing L1

  Flow rate: 1.5 mL/min

  Injection volume: 20 µL

  Run time: NLT 3 times the retention time of zolpidem

  System suitability

  Samples: *System suitability solution* and *Standard solution*

  [Note—See *Table 1* for the relative retention times.]

  Suitability requirements

  Resolution: NLT 2.0 between zolpidem related compound A and zolpidem, *System suitability solution*

  Tailing factor: NMT 2.0, *Standard solution*

  Relative standard deviation: NMT 1.0%, *Standard solution*

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ in the portion of Zolpidem Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

  r_U = peak response of zolpidem from the *Sample solution*

  r_S = peak response of zolpidem from the *Standard solution*

  C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)

  C_U = concentration of Zolpidem Tartrate in the *Sample solution* (mg/mL)

  Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%

- **Organic Impurities**

  Buffer, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

  Standard solution: 0.001 mg/mL of USP Zolpidem Tartrate RS in *Mobile phase*

  Sample solution: 0.5 mg/mL of Zolpidem Tartrate in *Mobile phase*

  System suitability

  Samples: *System suitability solution* and *Standard solution*

  [Note—See *Table 1* for the relative retention times.]

  Suitability requirements

  Resolution: NLT 2.0 between zolpidem related compound A and zolpidem, *System suitability solution*

  Relative standard deviation: NMT 10.0%, *Standard solution*

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of each impurity in the portion of Zolpidem Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

  r_U = peak response of each impurity from the *Sample solution*

  r_S = peak response of zolpidem from the *Standard solution*

  C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)

  C_U = concentration of Zolpidem Tartrate in the *Sample solution* (mg/mL)

  Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

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Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tartaric acid[a]	0.16	—
Zolpidem related compound A	0.83	0.10
Zolpidem	1.0	—
Any individual impurity	—	0.10
Total impurities	—	0.2

[a] Counter ion peak; not to be reported and not to be included in total impurities.

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I*, *Method Ia*: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Zolpidem Tartrate RS
  USP Zolpidem Related Compound A RS
  N,*N*-Dimethyl-2-(7-methyl-2-*p*-tolylimidazo[1,2-*a*]pyridin-3-yl)acetamide.
  $C_{19}H_{21}N_3O$ 307.39

Zolpidem Tartrate Tablets

DEFINITION

Zolpidem Tartrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$.

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 3.4 g/L of monobasic potassium phosphate in water; adjusted with ammonium hydroxide to a pH of 5.5
Mobile phase: Acetonitrile, methanol, and *Buffer* (30:20:50)
Standard stock solution: 0.8 mg/mL of USP Zolpidem Tartrate RS in 0.01 N hydrochloric acid
Standard solution: 0.16 mg/mL of USP Zolpidem Tartrate RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: Nominally 0.4 mg/mL of zolpidem tartrate from NLT 20 Tablets prepared as follows. Transfer the Tablets to a suitable volumetric flask and add 40% of the flask volume of 0.125 N hydrochloric acid. Mix well until the Tablets disintegrate, and add 50% of the flask volume of *Mobile phase*. Dilute with water to volume, and stir for 30 min using a magnetic stirrer. Allow solid particles to settle, and pass the supernatant through a suitable filter. [NOTE—Whatman No. 40 filter or equivalent may be suitable.]
Sample solution: Nominally 0.16 mg/mL of zolpidem tartrate in *Mobile phase*, from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: NLT 2.5 times the retention time of zolpidem tartrate
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 3.0 for zolpidem
Relative standard deviation: NMT 2.0% for zolpidem
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zolpidem tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: 0.01 N hydrochloric acid; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 15 min
Standard solution: (*L*/1000) mg/mL of USP Zolpidem Tartrate RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: 295 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 20 min

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Standard solution: (*L*/900) mg/mL of USP Zolpidem Tartrate RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Analytical wavelength: 295 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Standard solution: (*L*/900) mg/mL of USP Zolpidem Tartrate RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: 295 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (*Q*) of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A: 3.3 mL/L of phosphoric acid in water. Adjust with triethylamine to a pH of 6.0.
Solution B: Acetonitrile
Solution C: Methanol
Diluent: *Solution B*, *Solution C*, and *Solution A* (15:10:75)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	76	14	10
35.0	48	16	36
36.5	48	16	36
37.5	76	14	10
42.0	76	14	10

System suitability solution: 0.5 mg/mL of USP Zolpidem Tartrate RS and 0.8 µg/mL of USP Zolpidem Related Compound A RS in *Diluent*
Standard solution: 5 µg/mL of USP Zolpidem Tartrate RS in *Diluent*
Sample solution: Nominally equivalent to 0.5 mg/mL of zolpidem tartrate in *Diluent* from NLT 10 Tablets, prepared as follows. Transfer the Tablets to a suitable volumetric flask, add 70% of the flask volume of *Diluent*, and stir for 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the zolpidem and zolpidem related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of zolpidem from the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zolpidem tartrate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tolyloyl propionic acid[a, b]	0.15	—	—
Zolpidem acid[c]	0.18	1.0	0.3
Tolyloyl propionamide[b, d]	0.54	—	—
Tolyloyl acrylamide[b, e]	0.65	—	—
Zolpidem related compound B[f]	0.83	1.1	0.3

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Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolpidem related compound A	0.91	1.1	0.20
Zolpidem	1.0	—	—
Tolyloyl bromopropionamide[b, g]	1.14	—	—
Zolpidem related compound C[h]	1.20	1.1	0.3
Zolpidem 3- carbaldehyde[i]	1.65	1.4	0.3
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	0.5

[a] 4-Oxo-4-(*p*-tolyl)butanoic acid.
[b] Process impurities related to specific manufacturing process of the drug substance; included for peak identification purposes only and are not to be reported or included in the total degradation products.
[c] 2-(6-Methyl-2-*p*-tolylimidazo[1,2-α]pyridin-3-yl)acetic acid.
[d] *N,N*-Dimethyl-4-oxo-4-(*p*-tolyl)butanamide.
[e] *N,N*-Dimethyl-4-oxo-4-(*p*-tolyl)but-2-enamide.
[f] *N,N*-Dimethyl-2-(6-methyl-2-*p*-tolylimidazo[1,2-α]pyridin-3-yl)-2-oxoacetamide.
[g] 3-Bromo-*N,N*-dimethyl-4-oxo-4-(*p*-tolyl)butanamide.
[h] 4-Methyl-*N*-(5-methylpyridin-2-yl)benzamide.
[i] 6-Methyl-2-*p*-tolylimidazo[1,2-α]pyridine-3-carbaldehyde.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Zolpidem Tartrate RS
  USP Zolpidem Related Compound A RS
  N,N-Dimethyl-2-(7-methyl-2-*p*-tolylimidazo[1,2-α]pyridin-3-yl)acetamide.
  $C_{19}H_{21}N_3O$ 307.39

Zolpidem Tartrate Extended-Release Tablets

DEFINITION

Zolpidem Tartrate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample: 25 µg/mL of zolpidem tartrate in 0.01 M hydrochloric acid from a suitable quantity of powder obtained by grinding 1 Tablet
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 3.3 mL of phosphoric acid in 1 L of water. Adjust with triethylamine to a pH of 5.5.
  Mobile phase: Acetonitrile, methanol, and *Buffer* (4:5:11)
  Standard stock solution: 0.5 mg/mL of USP Zolpidem Tartrate RS in a mixture of alcohol and 0.01 M hydrochloric acid (4:1)
  Standard solution: 0.1 mg/mL of USP Zolpidem Tartrate RS from the *Standard stock solution* in *Mobile phase*
  Sample stock solution: Finely powder NLT 8 Tablets. Transfer the powder quantitatively to a suitable volumetric flask to obtain nominally 0.5 mg/mL of zolpidem tartrate. Add 70% of the flask volume of a mixture of alcohol and 0.01 M hydrochloric acid (5:2), and stir on a magnetic stirrer for 1 h. Dilute with alcohol to volume. Allow solid particles to settle, and pass the supernatant through a suitable filter (Whatman 40 or equivalent).
  Alternatively, the *Sample stock solution* can be prepared as follows. Transfer NLT 20 Tablets to a suitable volumetric flask to obtain a nominal concentration of 0.5 mg/mL of zolpidem tartrate. Add 10% of the flask volume of alcohol and stir for 30 min or until the Tablets have completely disintegrated. Add another 10% of the flask volume of alcohol and stir for another 90 min. Add 10% of the flask volume of 0.01 M hydrochloric acid followed by 40% of the flask volume of a mixture of alcohol and 0.01 M hydrochloric acid (5:2). Continue to stir for another 30 min. Remove the stirrer bar and rinse with alcohol. Dilute with alcohol to volume.
  Sample solution: Nominally 0.1 mg/mL of zolpidem tartrate from filtered *Sample stock solution* in *Mobile phase*. If the *Sample stock solution* is prepared using the alternative procedure, dilute the required volume of the *Sample stock solution* with *Mobile phase* and centrifuge instead of filtering before use.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1 mL/min
  Injection volume: 15 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 3.0 for zolpidem
  Relative standard deviation: NMT 2.0% for zolpidem
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: 0.01 N hydrochloric acid; 500 mL

USP Monographs

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Apparatus 1: 100 rpm
Times: 30, 90, and 240 min
Standard solution: Solution of USP Zolpidem Tartrate RS in *Medium* containing (*L*/500) mg/mL, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Detector: UV 295 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: See *Table 1*.

Table 1

Time (min)	Amount Dissolved
30	50%–70%
90	70%–85%
240	NLT 90%

The percentages of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Apparatus, and **Times:** Proceed as directed in *Test 1*.
Standard solution: Solution of USP Zolpidem Tartrate RS in *Medium* containing (*L*/500) mg/mL, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Analytical wavelength: 295 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: See *Table 2*.

Table 2

Time (min)	Amount Dissolved
30	55%–75%

Table 2 *(continued)*

Time (min)	Amount Dissolved
90	70%–90%
240	NLT 85%

The percentages of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 1: 100 rpm
Times: 30, 90, and 120 min
Standard solution: Solution of USP Zolpidem Tartrate RS in *Medium* containing (*L*/500) mg/mL, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
Mode: UV
Analytical wavelength: 237 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: See *Table 3*.

Table 3

Time (min)	Amount Dissolved
30	25%–45%
90	65%–85%
120	NLT 80%

The percentages of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 1: 100 rpm
Times: 30, 60, and 120 min
Standard solution: Solution of USP Zolpidem Tartrate RS in *Medium* containing (*L*/500) mg/mL, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter. Replace the volume withdrawn with an equal volume of *Medium*.
Instrumental conditions
Mode: UV
Analytical wavelength: 294 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at each time point (Q_i):

$$Q_{30} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

$$Q_{60} = [(A_U/A_S) \times C_S \times V \times (1/L) \times 100] + [Q_{30} \times (V_S/V)]$$

$$Q_{120} = [(A_U/A_S) \times C_S \times V \times (1/L) \times 100] + [(Q_{30} + Q_{60}) \times (V_S/V)]$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Zolpidem Tartrate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the sample withdrawn (mL)

Tolerances: See *Table 4*.

Table 4

Time (min)	Amount Dissolved
30	30%–55%
60	55%–80%
120	NLT 85%

The percentages of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: pH 6.8 phosphate buffer (Dissolve 6.8 g of monobasic potassium phosphate and 0.8 g of sodium hydroxide in 1 L of water. Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 6.8.); 900 mL
Apparatus 1: 50 rpm
Times: 60, 180, and 360 min
Buffer: Dissolve 5.6 g of phosphoric acid in 1 L of water. Adjust with triethylamine to a pH of 5.5.
Mobile phase: Methanol, acetonitrile, and *Buffer* (28:22:50)
Standard stock solution: 0.5 mg/mL of USP Zolpidem Tartrate RS in methanol
Standard solution: (L/900) mg/mL of USP Zolpidem Tartrate RS from *Standard stock solution* in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 25 µL
Run time: NLT 2.5 times the retention time of zolpidem
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: See *Table 5*.

Table 5

Time (min)	Amount Dissolved
60	55%–75%
180	NLT 75%
360	NLT 85%

The percentages of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 1: 100 rpm
Times: 15, 60, and 120 min
Diluent: Methanol and water (50:50)
Standard stock solution: 0.25 mg/mL of USP Zolpidem Tartrate RS in *Diluent*. Sonication may be used to aid complete dissolution.
Standard solution: (L/500) mg/mL of USP Zolpidem Tartrate RS from *Standard stock solution* in *Medium*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the volume withdrawn with an equal volume of *Medium*.
Instrumental conditions
Mode: UV
Analytical wavelength: 295 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) in the sample withdrawn at each time point (i):

$$C_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount (Q_i) of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of zolpidem tartrate in the portion of sample withdrawn at time point i (mg/mL)
V = volume of *Medium*, 500 mL

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L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (*i*)	Time (min)	Amount Dissolved
1	15	45%–65%
2	60	63%–83%
3	120	NLT 80%

The percentages of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 1: 100 rpm
Times: 30, 90, and 240 min
Standard stock solution: 0.5 mg/mL of USP Zolpidem Tartrate RS in methanol. Sonication may be used to aid complete dissolution.
Standard solution: 0.0125 mg/mL of USP Zolpidem Tartrate RS from *Standard stock solution* in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discarding the first 2 mL of filtrate.
Instrumental conditions
Mode: UV
Analytical wavelength: 237 nm
Cell: 5.0 mm
Blank: *Medium*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Correct the instrument by using the *Blank*.
Calculate the concentration (C_i) of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at each time point (i):

$$C_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of zolpidem tartrate in the portion of sample withdrawn at the specified time point i (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 7*.

Table 7

Time Point (*i*)	Time (min)	Amount Dissolved
1	30	70%–90%
2	90	NLT 75%
3	240	NLT 80%

The percentages of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer, Mobile phase, Standard stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: Dissolve a suitable amount of USP Zolpidem Related Compound A RS in the *Standard stock solution* to obtain a solution containing 1 µg/mL of zolpidem related compound A. Dilute 1 mL of the resulting solution with *Mobile phase* to 5 mL.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between zolpidem related compound A and zolpidem
Tailing factor: NMT 3.0 for the zolpidem peak
Relative standard deviation: NMT 2.0% for the zolpidem peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
F = relative response factor of the corresponding impurity (see *Table 8*)

Acceptance criteria: See *Table 8*.

Table 8

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zolpidem acid[a]	0.3	1.2	0.20
Zolpidem related compound A[b]	0.9	1.0	0.20
Zolpidem	1.0	—	—
Any unspecified degradation product	—	1.0	0.20
Total impurities	—	—	0.5

[a] 2-(6-Methyl-2-*p*-tolylimidazo[1,2-*a*]pyridin-3-yl)acetic acid.
[b] *N*,*N*-Dimethyl-2-(7-methyl-2-*p*-tolylimidazo[1,2-*a*]pyridin-3-yl)acetamide.

ADDITIONAL REQUIREMENTS

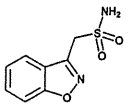
• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.

USP Monographs

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- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Zolpidem Related Compound A RS
  N,N-Dimethyl-2-(7-methyl-2-*p*-tolylimidazo[1,2-*a*] pyridin-3-yl)acetamide.
  $C_{19}H_{21}N_3O$ 307.39
  USP Zolpidem Tartrate RS

Zonisamide



$C_8H_8N_2O_3S$ 212.23
1,2-Benzisoxazole-3-methanesulfonamide [68291-97-4].

DEFINITION
Zonisamide contains NLT 98.0% and NMT 102.0% of $C_8H_8N_2O_3S$, calculated on the anhydrous basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨197K⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
  Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust the pH to 3.0 ± 0.1 with 10% phosphoric acid.
  Mobile phase: Acetonitrile, methanol, and *Buffer* (1:1:8)
  Standard solution: 0.1 mg/mL of USP Zonisamide RS in *Mobile phase*
  Sample solution: 0.1 mg/mL of Zonisamide in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 5000 theoretical plates
  Tailing: NMT 1.8
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_8H_8N_2O_3S$ in the portion of Zonisamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zonisamide from the *Sample solution*
r_S = peak response of zonisamide from the *Standard solution*
C_S = concentration of USP Zonisamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Zonisamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
INORGANIC IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

ORGANIC IMPURITIES
- **Procedure**
  Mobile phase: Prepare as directed in the *Assay.*
  Standard solution: 1 µg/mL of USP Zonisamide RS and 1.5 µg/mL of USP Zonisamide Related Compound A RS in *Mobile phase*
  Sample solution: 1.0 mg/mL of Zonisamide in *Mobile phase*
  Chromatographic system: Prepare as directed in the *Assay.*
  Run time: 2 times the retention time of the zonisamide peak
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 10.0 between zonisamide related compound A and zonisamide
  Relative standard deviation: NMT 10.0% for both zonisamide and zonisamide related compound A peaks
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of zonisamide related compound A in the portion of Zonisamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of zonisamide related compound A from the *Sample solution*
r_S = peak response of zonisamide related compound A from the *Standard solution*
C_S = concentration of USP Zonisamide Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of zonisamide ▲▲ (ERR 1-Jul-2019) in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of zonisamide related compound A (free acid), 213.23
M_{r2} = molecular weight of USP Zonisamide Related Compound A RS (sodium salt), 235.23

Calculate the percentage of any unspecified impurity in the portion of Zonisamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of zonisamide from the *Standard solution*
C_S = concentration of USP Zonisamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Zonisamide in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1.*
Total impurities: NMT 0.3%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zonisamide related compound A[a]	0.51	0.15

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Impurity Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zonisamide	1.0	—
Any unspecified individual impurity	—	0.10

[a] 1,2-Benzisoxazole-3-methane sulfonic acid.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.8%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Zonisamide RS
USP Zonisamide Related Compound A RS
1,2-Benzisoxazole-3-methanesulfonic acid sodium salt.
$C_8H_6NNaO_4S$ 235.19
[CAS-73101-64-1].

Zonisamide Capsules

DEFINITION
Zonisamide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of zonisamide ($C_8H_8N_2O_3S$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample solution: Shake the contents of a sufficient quantity of Capsules, equivalent to about 100 mg of zonisamide, with 5 mL of methanol. Sonicate for 2 min and pass through a filter of 0.45-µm pore size or equivalent. Discard the initial 1-mL portion and evaporate the solution at 85°. Cool the residue. Use 1–2 mg of the residue.
Analysis: The IR spectrum of the *Sample solution* corresponds to the IR spectrum of similarly prepared USP Zonisamide RS.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, methanol, and 0.1% trifluoroacetic acid TS (20:16:64)
Standard stock solution: 1 mg/mL of USP Zonisamide RS in methanol
Standard solution: 0.1 mg/mL of zonisamide in *Mobile phase*, from the *Standard stock solution*
Sample stock solution: Nominally 1.0 mg/mL of zonisamide prepared as follows. Transfer a suitable amount of Capsule contents (NLT 10) to a suitable volumetric flask and add a suitable volume of methanol. Sonicate and shake for 15 min. Dilute with methanol to volume.
Alternatively, the *Sample stock solution* can be prepared as follows. Transfer a suitable amount of Capsules (NLT 10) in a suitable volumetric flask, add 20% of the flask volume with water. Stir, and warm slightly for 30 min. Dilute with *Mobile phase* to volume.
Sample solution: 0.1 mg/mL of zonisamide in *Mobile phase*, from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Run time: 1.5 times the retention time of the zonisamide
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zonisamide ($C_8H_8N_2O_3S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zonisamide from the *Sample solution*
r_S = peak response of zonisamide from the *Standard solution*
C_S = concentration of USP Zonisamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zonisamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: ▲75 rpm. Use suitable sinkers, if necessary.▲ (ERR 1-May-2018)
Time: 45 min
Determine the percentage of zonisamide dissolved using one of the following procedures.
Spectrophotometric procedure
Standard stock solution: 0.6 mg/mL of USP Zonisamide RS in methanol
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain solutions with final concentrations as given in *Table 1*.

Table 1

Capsule Strength (mg)	Final Concentration (µg/mL)
25	13
50	13
100	22

Sample solution: 10 mL of the solution under test. Dilute the filtrate with *Medium* as given in *Table 2*.

Table 2

Capsule Strength (mg)	Volume of Filtrate (mL)	Final Volume (mL)
25	5.0	10.0
50	5.0	25.0

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Table 2 *(continued)*

Capsule Strength (mg)	Volume of Filtrate (mL)	Final Volume (mL)
100	5.0	25.0

Instrumental conditions
Mode: UV
Analytical wavelength: 241 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zonisamide ($C_8H_8N_2O_3S$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of USP Zonisamide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Chromatographic procedure
Solution A: Dilute 52 mL of 40% tetrabutylammonium hydroxide solution with 948 mL of water. Adjust with phosphoric acid to a pH of 7.5.
Mobile phase: Acetonitrile, methanol, and *Solution A* (25:5:70)
Standard stock solution: 0.4 mg/mL of USP Zonisamide RS prepared as follows. Transfer a suitable amount of USP Zonisamide RS to a suitable volumetric flask, and dissolve in 20% of the flask volume of acetonitrile. Dilute with *Medium* to volume.
Standard solution: (L/1000) mg/mL of USP Zonisamide RS in *Medium*, where L is the label claim in mg/Capsule from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 3.0%
Calculate the percentage of the labeled amount of zonisamide ($C_8H_8N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Zonisamide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (Q) of the labeled amount of zonisamide ($C_8H_8N_2O_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: Dilute 52 mL of 40% tetrabutylammonium hydroxide solution with 948 mL of water. Adjust with phosphoric acid to a pH of 7.5.
Mobile phase: Acetonitrile, methanol, and *Solution A* (25:5:70)
Standard stock solution: 0.32 mg/mL of USP Zonisamide RS. Transfer a suitable amount of USP Zonisamide RS in a suitable volumetric flask, dissolve in 20% of flask volume of acetonitrile and dilute with *Mobile phase* to volume.
Standard solution: 0.32 µg/mL of zonisamide in *Mobile phase*, from the *Standard stock solution*
System suitability stock solution: 0.4 mg/mL of zonisamide related compound C. Transfer a suitable amount of USP Zonisamide Related Compound C RS to a suitable volumetric flask, and dissolve in 20% flask volume of methanol. Dilute with *Mobile phase* to volume.
System suitability solution: Equal volumes of *Standard stock solution* and *System suitability stock solution*
Sample stock solution: Transfer a suitable number of Capsules (NLT 10) to a suitable volumetric flask, dissolve in 20% flask volume of water and dilute with *Mobile phase* to volume to obtain a solution of concentration as given in *Table 3*. [NOTE—Stir and warm slightly for 30 min to dissolve the Capsules.]

Table 3

Capsule Strength (mg)	Final Concentration (mg/mL)
25	2
50	2
100	4

Sample solution: 0.32 mg/mL of zonisamide in *Mobile phase*, from the *Sample stock solution*
Chromatographic system
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: 6 times the retention time of the zonisamide peak
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between zonisamide and zonisamide related compound C, *System suitability solution*
Tailing factor: NMT 1.5 for the zonisamide peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

USP Monographs

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r_S = peak response of zonisamide from the *Standard solution*
C_S = concentration of USP Zonisamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of zonisamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*. Disregard any peak below 0.1%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zonisamide	1.0	—
Zonisamide related compound C[a]	1.3	—
Methyl zonisamide[b]	1.6	0.2
Zonisamide related compound A[c]	2.5	0.2
Any unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] Process impurity included in the table for identification and system suitability only. Process impurities are controlled in the drug substance and are not to be reported or included in the total degradation products for the drug product.
[b] 1-Benzisoxazol-3-yl-*N*-methylmethanesulfonamide.
[c] 1,2-Benzisoxazole-3-methane sulfonic acid.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers and protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Zonisamide RS
  USP Zonisamide Related Compound C RS
  N'-(Benzisoxazol-3-ylmethylsulfonyl)-*N,N*-dimethylformimidamide.
  $C_{11}H_{13}N_3O_3S$ 267.30

Zonisamide Compounded Oral Suspension

DEFINITION
Zonisamide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of zonisamide ($C_8H_8N_2O_3S$).

Prepare Zonisamide Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Zonisamide capsules[a] equivalent to	1 g
Vehicle: a 1:1 mixture of Ora-Plus[b] and Ora-Sweet (regular or sugar-free),[b] a sufficient quantity to make	100 mL

[a] Zonisamide 100-mg capsules, Mylan, Morgantown, WV.
[b] Paddock Laboratories, Minneapolis, MN.

Empty the capsules, and pour the contents into a mortar or other suitable container. Crush the capsule contents into a fine powder using a pestle or other mechanical means. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add the *Vehicle* to make the mortar contents pourable. Transfer the contents, stepwise and quantitatively, to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume, and mix well. [**CAUTION**—Avoid skin contact or inhalation of zonisamide by using protective gloves and a fume hood or surgical mask.]

ASSAY
- **PROCEDURE**

Solution A: 50 mM sodium phosphate adjusted with phosphoric acid to a pH of 2.8
Mobile phase: Acetonitrile and *Solution A* (25:75)
Standard stock solution: 1 mg/mL of USP Zonisamide RS in *Mobile phase*. Sonicate for 10 min.
Standard solution: Transfer 1 mL of the *Standard stock solution* to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well. Centrifuge an aliquot of the solution for 10 min at 13,000 rpm.
Sample solution: Shake each bottle of Oral Suspension thoroughly. Transfer 1 mL of Oral Suspension to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well. Vortex for 30 s, and sonicate for 10 min. Transfer 1 mL of the resultant solution to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume. Centrifuge an aliquot of the solution for 10 min at 13,000 rpm.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 264 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 32°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for zonisamide is about 4.6 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of zonisamide ($C_8H_8N_2O_3S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zonisamide from the *Sample solution*
r_S = peak response of zonisamide from the *Standard solution*
C_S = concentration of zonisamide in the *Standard solution* (mg/mL)
C_U = nominal concentration of zonisamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.9–4.9

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Zonisamide RS

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Combined Index to USP 43 and NF 38, Volumes 1–5

Page citations refer to the pages of Volumes 1, 2, 3, 4 and 5 of USP 43–NF 38. This index is repeated in its entirety in each volume.

1–2482	Volume 1
2483–4722	Volume 2
4723–6108	Volume 3
6109–7208	Volume 4
7209–8760	Volume 5

Numbers in angle brackets such as ⟨421⟩ refer to chapter numbers in the General Chapters section.

A

Abacavir
  oral solution, 19
  sulfate, 23
  tablets, 20
  and lamivudine tablets, 21
Abiraterone
  acetate, 25
  acetate tablets, 26
Absolute
  alcohol, 6133
  ether, 6131
Absorbable
  dusting powder, 1569
  gelatin film, 2092
  gelatin sponge, 2093
  surgical suture, 4189
Absorbent
  cotton, 6132
  gauze, 2091
Acacia, 5597
  syrup, 5597
Acamprosate calcium, 28
Acarbose, 30
  tablets, 31
Acebutolol hydrochloride, 33
  capsules, 35
Acepromazine maleate, 36
  injection, 37
  tablets, 38
Acesulfame potassium, 5598
Acetal, 6132
Acetaldehyde, 6132
  TS, 6225
Acetaldehyde ammonia trimer trihydrate, 6132
Acetaminophen, 38
  aspirin and caffeine tablets, 49
  and aspirin tablets, 48
  butalbital and caffeine capsules, 645
  butalbital and caffeine tablets, 646
  and caffeine tablets, 51
  capsules, 40
  and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, capsules containing at least three of the following, 52
  and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, oral powder containing at least three of the following, 54
  and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, oral solution containing at least three of the following, 56
  and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, tablets containing at least three of the following, 58
  chlorpheniramine maleate, and dextromethorphan hydrobromide tablets, 60
  and codeine phosphate capsules, 52
  and codeine phosphate oral solution, 54
  and codeine phosphate oral suspension, 56
  and codeine phosphate tablets, 66
  dextromethorphan hydrobromide, doxylamine succinate, and pseudoephedrine hydrochloride oral solution, 67
  and diphenhydramine citrate tablets, 69
  diphenhydramine hydrochloride, and pseudoephedrine hydrochloride tablets, 70
  and hydrocodone bitartrate tablets, 2224
  isometheptene mucate, and dichloralphenazone capsules, 2423
  and oxycodone capsules, 3341
  and oxycodone tablets, 3343
  and pentazocine tablets, 3460
  and pseudoephedrine hydrochloride tablets, 72
  oral solution, 41
  for effervescent oral solution, 42
  suppositories, 44
  oral suspension, 42
  tablets, 45
  extended-release tablets, 47
  and tramadol hydrochloride oral suspension, 4452
Acetanilide, 6132
Acetate
  methyl, 6178
Acetate buffer, 6145
  TS, 6225
Acetazolamide, 73
  for injection, 74
  oral suspension, 76
  tablets, 76
Acetic acid, 5599, 6132
  ammonium acetate buffer TS, 6225
  diluted, 5599, 6132, 6159
  double-normal (2 N), 6238
  glacial, 77, 6132, 6168
  glacial, TS, 6229
  and hydrocortisone otic solution, 2235
  irrigation, 78
  metaphosphoric, TS, 6231
  otic solution, 78
  strong, TS, 6225
  1 M TS, 6225
  2 M TS, 6225
Acetic acid in peptides, 6741
0.008 M Acetic acid TS, 6225
0.3 N Acetic acid TS, 6225
Acetic anhydride, 6132
Acetohydroxamic acid, 78
  tablets, 79
Acetone, 5600, 6132
  anhydrous, 6132
  neutralized, 6132, 6227
Acetonitrile, 6132
  spectrophotometric, 6132
Acetophenone, 6132
p-Acetotoluidide, 6132
Acetylacetone, 6132
Acetyl chloride, 6132
Acetylcholine chloride, 80, 6132
  for ophthalmic solution, 81
Acetylcysteine, 82
Acetylcysteine
  compounded solution, 83
  and isoproterenol hydrochloride inhalation solution, 84

www.webofpharma.com

Acetylcysteine *(continued)*
solution, 82
N-Acetylglucosamine, 4745
2-Acetylresorcinol, 6133
3-Acetylthio-2-methylpropanoic acid, 6133
Acetyltributyl citrate, 5601
Acetyltriethyl citrate, 5601
N-Acetyltyrosine, 4746
N-Acetyl-L-tyrosine ethyl ester, 6133
Acid
acrylic, 6133
alpha lipoic, 5124
dehydroacetic, 5741
ferric chloride TS, 6225
ferrous sulfate TS, 6225
Folic, compounded oral soultion, 2027
iminodiacetic, 6172
stannous chloride TS, 6225
stannous chloride TS, stronger, 6225
Acid-neutralizing capacity ⟨301⟩, 6661
Acidulated phosphate and sodium fluoride topical solution, 4074
Acitretin, 85
capsules, 86
Acoustic emission ⟨1005⟩, 7230, 7644, 8117
Acrylic acid, 6133
Activated
alumina, 6133
charcoal, 923, 6133
magnesium silicate, 6133
Acyclovir, 88
capsules, 89
for injection, 90
ointment, 91
oral suspension, 92
tablets, 92
Adamantane, 6133
Adapalene, 93
gel, 96
Ademetionine disulfate tosylate, 4747
Adenine, 97
sulfate, 6133
Adenosine, 98
injection, 99
Adipic acid, 5602, 6133
Admissions
to *NF 38*,
to *USP 43*, xxxi

Aerosol

Bacitracin and polymyxin B sulfate topical, 468
Benzocaine, butamben, and tetracaine hydrochloride topical, 513
Benzocaine and menthol topical, 518
Benzocaine topical, 503
Fluticasone propionate and salmeterol inhalation, 2005
Fluticasone propionate inhalation, 1988
Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests ⟨601⟩, 6819
Isoproterenol sulfate inhalation, 2437
Lidocaine topical, 2622
Polymyxin B sulfate and bacitracin zinc topical, 3591
Povidone–iodine topical, 3635
Thimerosal topical, 4346
Tolnaftate topical, 4425
Triamcinolone acetonide topical, 4483

Agar, 5603, 6133
Agarose, 6133
Air, medical, 100
Air-helium certified standard, 6133
Alanine, 101
L-Alanyl-L-glutamine, 4748
Albendazole, 102
oral suspension, 102
tablets, 103
Albumen TS, 6225
Albumin
bovine serum, 6133
human, 104
rAlbumin human, 5604
Albuterol, 104
sulfate, 109
tablets, 105
extended-release tablets, 106
Alclometasone dipropionate, 110
cream, 111
ointment, 112
Alcohol, 113, 6133
70 percent, 80 percent, and 90 percent, 6133
absolute, 6133
aldehyde-free, 6134
alpha-(2-(methylamino)ethyl)benzyl, 6134
amyl, 6134
tert-amyl, 6134, 6137, 6173
butyl, 5648
dehydrated, 115, 6134, 6155
dehydrated isopropyl, 6134
denaturated, 6134
denaturated, TS, 6228
determination ⟨611⟩, 6848
in dextrose injection, 117
diluted, 5607, 6134
injection, dehydrated, 117
isobutyl, 6134
isopropyl, 6134
methyl, 6134
neutralized, 6134, 6182
phenol TS, 6225
n-propyl, 6134
rubbing, 118
secondary butyl, 6134
tertiary butyl, 6134
Alcoholic
ammonia TS, 6225
mercuric bromide TS, 6226
potassium hydroxide TS, 6226
potassium hydroxide TS 2, 6234
TS, 6225
Alcoholic potassium hydroxide
0.2 N VS, 6238
Alcoholometric table, 6346
Aldehyde dehydrogenase, 6134
Alendronate sodium, 119
tablets, 120
Alfadex, 5607
Alfentanil
hydrochloride, 122
injection, 123
Alfuzosin hydrochloride, 124
extended-release tablets, 125
Alginates assay ⟨311⟩, 6662
Alginic acid, 5608
Alizarin complexone, 6134
Alkaline
cupric citrate TS, 6226
cupric citrate TS 2, 6226
cupric iodide TS, 6226
mercuric-potassium iodide TS, 6226
phosphatase enzyme, 6134
picrate TS, 6226
pyrogallol TS, 6234
sodium hydrosulfite TS, 6226
Alkaline borate buffer, 6145
Alkaline cupric tartrate TS, 6226
Alkyl (C12-15) benzoate, 5609
Alkylphenoxypolyethoxyethanol, 6134
Allantoin, 130
Allopurinol, 131
oral suspension, 133
tablets, 134
Allyl isothiocyanate, 135
Almond oil, 5609
Almotriptan
tablets, 138
Almotriptan malate, 135
Aloe, 140
Alosetron
tablets, 142
Alosetron hydrochloride, 141
Alpha
lipoic acid, 5124
Alpha-chymotrypsin, 6134
Alpha cyclodextrin hydrate, 6134
Alpha-(2-(methylamino)ethyl)benzyl alcohol, 6134
Alphanaphthol, 6134
Alphazurine 2G, 6220
Alprazolam, 144
oral suspension, 145
tablets, 146
extended-release tablets, 148
orally disintegrating tablets, 152
Alprenolol hydrochloride, 6134
Alprostadil, 154
injection, 156
Alteplase, 157
for injection, 160
Alternative microbiological sampling methods for nonsterile inhaled and nasal products ⟨610⟩, 6846
Altretamine, 163
capsules, 164
Alum, 6134
ammonium, 165, 6134
potassium, 166, 6192
Alumina, 6134
activated, 6133, 6134, 6138
anhydrous, 6134
aspirin, codeine phosphate, and magnesia tablets, 404
aspirin, and magnesia tablets, 397
aspirin, and magnesium oxide tablets, 399
magnesia, and calcium carbonate chewable tablets, 169
magnesia, calcium carbonate, and simethicone chewable tablets, 170
magnesia, and calcium carbonate oral suspension, 168
magnesia, and simethicone oral suspension, 173
magnesia, and simethicone chewable tablets, 174
and magnesia oral suspension, 166
and magnesia tablets, 167
magnesium carbonate, and magnesium oxide tablets, 178
and magnesium carbonate oral suspension, 176
and magnesium carbonate tablets, 177
and magnesium trisilicate oral suspension, 179
and magnesium trisilicate tablets, 180
Aluminon, 6134
Aluminum, 6135
acetate topical solution, 181

www.webofpharma.com

Aluminum *(continued)*
chloride, 181
chlorohydrate, 182
chlorohydrate solution, 183
chlorohydrex polyethylene glycol, 184
chlorohydrex propylene glycol, 185
dichlorohydrate, 185
dichlorohydrate solution, 186
dichlorohydrex polyethylene glycol, 187
dichlorohydrex propylene glycol, 188
hydroxide gel, 188
hydroxide gel, dried, 189
hydroxide gel capsules, dried, 190
hydroxide gel tablets, dried, 190
monostearate, 5612
oxide, 5613
oxide, acid-washed, 6134, 6135
phosphate gel, 190
potassium sulfate, 6135
sesquichlorohydrate, 191
sesquichlorohydrate solution, 191
sesquichlorohydrex polyethylene glycol, 192
sesquichlorohydrex propylene glycol, 192
subacetate topical solution, 193
sulfate, 193
sulfate and calcium acetate tablets for topical solution, 195
zirconium octachlorohydrate, 195
zirconium octachlorohydrate solution, 196
zirconium octachlorohydrex gly, 197
zirconium octachlorohydrex gly solution, 198
zirconium pentachlorohydrate, 199
zirconium pentachlorohydrate solution, 199
zirconium pentachlorohydrex gly, 200
zirconium pentachlorohydrex gly solution, 201
zirconium tetrachlorohydrate, 202
zirconium tetrachlorohydrate solution, 203
zirconium tetrachlorohydrex gly, 203
zirconium tetrachlorohydrex gly solution, 204
zirconium trichlorohydrate, 205
zirconium trichlorohydrate solution, 206
zirconium trichlorohydrex gly, 206
zirconium trichlorohydrex gly solution, 207
Aluminum oxide strips, 6135
Aluminum tartrate, 6137
Aluminum ⟨206⟩, 6605
Aluminum chloride, 6135
Aluminum chloride hexahydrate, 6135
Aluminum sulfate
and calcium acetate for topical solution, 194
Amantadine hydrochloride, 208
capsules, 209
oral solution, 212
Amaranth, 6135
TS, 6226
Amcinonide, 214
cream, 214
ointment, 215
American ginseng, 4750
capsules, 4754
extract, powdered, 4753
powdered, 4752
tablets, 4757
Amifostine, 216
for injection, 217
Amikacin, 219
sulfate, 220
sulfate injection, 221
Amiloride hydrochloride, 222
and hydrochlorothiazide tablets, 224
tablets, 223
Amiloxate, 227
Aminoacetic acid, 6135
4-Aminoantipyrine, 6135
Aminobenzoate
potassium, 228
potassium capsules, 230
sodium, 231
Aminobenzoic acid, 233
gel, 234
p-Aminobenzoic acid, 6135
2-Aminobenzonitrile, 6135
Aminocaproic acid, 235
injection, 236
oral solution, 236
tablets, 237
4-Amino-6-chloro-1,3-benzenedisulfonamide, 6135
4-Amino-2-chlorobenzoic acid, 6135, 6151
2-Amino-5-chlorobenzophenone, 6151, 6151
7-Aminodesacetoxycephalosporanic acid, 6135
2-Aminoethyl diphenylborinate, 6135
1-(2-Aminoethyl)piperazine, 6135
Aminoguanidine bicarbonate, 6136
2-Aminoheptane, 6136
N-Aminohexamethyleneimine, 6136
Aminohippurate sodium injection, 237
Aminohippuric acid, 238
4-Amino-3-hydroxy-1-naphthalenesulfonic acid, 6136
Aminolevulinic acid
hydrochloride, 238
Amino methacrylate copolymer, 5614
1,2,4-Aminonaphtholsulfonic acid, 6136
Aminonaphtholsulfonic acid TS, 6226
Aminopentamide sulfate, 239
injection, 240
tablets, 240
2-Aminophenol, 6136
4-Aminophenol in acetaminophen-containing drug products ⟨227⟩, 6637
m-Aminophenol, 6136
p-Aminophenol, 6136
Aminophylline, 241
injection, 243
oral solution, 245
rectal solution, 246
suppositories, 247
tablets, 248
delayed-release tablets, 250
3-Amino-1-propanol, 6136
3-Aminopropionic acid, 6136
Aminosalicylic acid, 251
3-Aminosalicylic acid, 6136
Amiodarone
hydrochloride injection, 254
Amiodarone hydrochloride, 253
oral suspension, 257
Amiodarone hydrochloride
tablets, 257
Amitraz, 259
concentrate for dip, 260
Amitriptyline hydrochloride, 261
and chlordiazepoxide tablets, 938
and perphenazine tablets, 3487
tablets, 263
Amlodipine
oral suspension, 264
and olmesartan medoxomil tablets, 272
and benazepril hydrochloride capsules, 269
and valsartan tablets, 275
valsartan and hydrochlorothiazide tablets, 279
Amlodipine and atorvastatin
tablets, 265
Amlodipine besylate, 285
tablets, 286
Ammonia
alcoholic TS, 6225
detector tube, 6136
N 13 injection, 3183
nitrate TS, silver, 6234
solution, diluted, 6136
solution, strong, 5616
spirit, aromatic, 287
TS, 6136, 6226
TS 2, 6226
TS alcoholic, 6226
TS stronger, 6226
water, stronger, 6207, 6226
water, 25 percent, 6136
Ammonia-ammonium chloride buffer TS, 6226
Ammoniacal potassium ferricyanide TS, 6226
Ammonia-cyanide TS, 6226
Ammoniated cupric oxide TS, 6226
Ammonio methacrylate copolymer, 5616
dispersion, 5618
Ammonium
acetate, 6136
acetate TS, 6226
alum, 165
bicarbonate, 6136
bisulfate, 6136
bromide, 6136
carbonate, 5619, 6137
carbonate TS, 6226
carbonate TS 2, 6226
chloride, 288, 6137
chloride–ammonium hydroxide TS, 6226
chloride injection, 288
chloride, potassium gluconate, and potassium citrate oral solution, 3622
chloride delayed-release tablets, 288
chloride TS, 6226
citrate, dibasic, 6137, 6156
citrate, ferric, 289
citrate for oral solution, ferric, 290
dihydrogen phosphate, 6137
fluoride, 6137
formate, 6137
glycyrrhizate, 5619
hydroxide, 6137
hydroxide 6 N, 6137
molybdate, 290, 6137
molybdate injection, 291
molybdate TS, 6226
nitrate, 6137
nitrate, ceric TS, 6227
nitrate TS, silver, 6234
oxalate, 6137
oxalate TS, 6226
persulfate, 6137
phosphate, 5620
phosphate, dibasic, 6137, 6156
phosphate, dibasic, TS, 6226
phosphate, monobasic, 6137
polysulfide TS, 6226
pyrrolidinedithiocarbamate, 6137
pyrrolidinedithiocarbamate, saturated, TS, 6226
reineckate, 6137
reineckate TS, 6226
sulfamate, 6137
sulfate, 5621, 6137
sulfate, cupric TS, 6227

www.webofpharma.com

Ammonium *(continued)*
sulfate, ferric TS, 6229
sulfide TS, 6226
thiocyanate, 6137
thiocyanate, tenth-normal (0.1 N), 6238
thiocyanate TS, 6226
vanadate, 6137
vanadate TS, 6226
Ammonium hydrogen difluoride , 6137
Ammonium hydroxide
1 M TS, 6226
2 M TS, 6226
Amobarbital sodium, 291
for injection, 292
Amodiaquine, 292
hydrochloride, 293
hydrochloride tablets, 294
Amoxapine, 295
tablets, 297
Amoxicillin, 297
boluses, 299
capsules, 300
and clavulanate potassium for oral suspension, 308
and clavulanate potassium tablets, 308
and clavulanic acid extended-release tablets, 310
for injectable suspension, 302
intramammary infusion, 302
oral suspension, 303
for oral suspension, 303
tablets, 304
tablets for oral suspension, 306
Amphetamine
sulfate, 312
sulfate tablets, 313
Amphotericin B, 314
cream, 315
for injection, 315
lotion, 316
ointment, 316
Ampicillin, 316
boluses, 321
capsules, 321
for injectable suspension, 324
for injection, 323
and probenecid for oral suspension, 326
sodium, 327
soluble powder, 323
and sulbactam for injection, 328
for oral suspension, 324
tablets, 325
Amprolium, 329
soluble powder, 330
oral solution, 331
Amyl
acetate, 6134, 6137, 6173
alcohol, 6137
nitrite, 331
nitrite inhalant, 332
α-Amylase, 6137
Amylene hydrate, 5622
tert-Amyl alcohol, 6137
Anagrelide
capsules, 334
hydrochloride, 332
Analysis of biological assays ⟨1034⟩, 7351
Analytical data—interpretation and treatment ⟨1010⟩, 7233
Analytical instrument qualification ⟨1058⟩, 7522
Analytical methodologies based on scattering phenomena—electrophoretic light scattering (determination of zeta potential) ⟨1430.4⟩, 8389
Analytical methodologies based on scattering phenomena—general ⟨1430⟩, 8376
Analytical methodologies based on scattering phenomena—light diffraction measurements of particle size ⟨1430.2⟩, 8386
Analytical methodologies based on scattering phenomena—small angle x-ray scattering and small angle neutron scattering ⟨1430.5⟩, 8392
Analytical methodologies based on scattering phenomena—static light scattering ⟨1430.1⟩, 8379
Analytical procedures for recombinant therapeutic monoclonal antibodies ⟨129⟩, 6565
Anastrozole, 336
tablets, 337
Ancillary materials for cell, gene, and tissue-engineered products ⟨1043⟩, 7381
Andrographis, 4758
extract, powdered, 4762
powdered, 4760
Anethole, 5622
(*E*)-Anethole, 6138
Angustifolia
extract, powdered echinacea, 4941
powdered echinacea, 4938

Anhydrous

acetone, 6132
alumina, 6138
barium chloride, 6138
calcium chloride, 6138
calcium phosphate, dibasic, 710
citric acid, 1040
cupric sulfate, 6138
dibasic sodium phosphate, 6138
magnesium perchlorate, 6138
magnesium sulfate, 6138
methanol, 6138
potassium carbonate, 6138
sodium acetate, 6138
sodium carbonate, 6138
sodium phosphate, monobasic, 6205
sodium sulfate, 6138
sodium sulfite, 6138

Anileridine, 339
hydrochloride, 340
hydrochloride tablets, 341
injection, 339
Aniline, 6138
blue, 6138
sulfate, 6138
Animal drugs for use in animal feeds ⟨1152⟩, 7952
Anion-exchange resin
strong, lightly cross-linked, in the chloride form, 6138
50- to 100-mesh, styrene-divinylbenzene, 6138, 6208
styrene-divinylbenzene, 6138
p-Anisaldehyde, 6139
Anise oil, 5624
p-Anisidine, 6139
Anisole, 6139
Annotations
to *NF 38*,
to *USP 43*, xxxiii
Antazoline phosphate, 342
Anthracene, 6139
Anthralin, 343
cream, 344
ointment, 344
Anthrone, 6139
TS, 6226
Antibiotics—microbial assays ⟨81⟩, 6488
Anticoagulant
citrate dextrose solution, 345
citrate phosphate dextrose solution, 347
citrate phosphate dextrose adenine solution, 348
heparin solution, 2194
sodium citrate solution, 350
Anti-D reagent, 6139
Anti-D (Rh_o) reagent, 6139
Anti-factor Xa and anti-factor IIa assays for unfractionated and low molecular weight heparins ⟨208⟩, 6611
Antifoam reagent, 6139
Antihuman globulin reagent, 6139
Antimicrobial
agents—content ⟨341⟩, 6664
effectiveness testing ⟨51⟩, 6449
Antimony
pentachloride, 6139
potassium tartrate, 350
sodium tartrate, 351
trichloride, 6140
trichloride TS, 6226
Antipyrine, 351
and benzocaine otic solution, 352
benzocaine, and phenylephrine hydrochloride otic solution, 354
Antithrombin III, 6140
human, 355
Apomorphine hydrochloride, 357
tablets, 358
Apparent intrinsic dissolution—dissolution testing procedures for rotating disk and stationary disk ⟨1087⟩, 7681
Applications of mass spectrometry ⟨1736⟩, 8508
Applications of nuclear magnetic resonance spectroscopy ⟨1761⟩, 8529
Application of water activity determination to nonsterile pharmaceutical products ⟨1112⟩, 7820
Apraclonidine
hydrochloride, 359
ophthalmic solution, 359
Aprepitant, 360
capsules, 362
Aprobarbital, 6140
Aprotinin, 364
injection, 367
Aqua regia, 6140
Argatroban, 368
Arginine, 371
capsules, 4763
hydrochloride, 371
hydrochloride injection, 373
tablets, 4764
Aripiprazole, 373
orally disintegrating tablets, 377
tablets, 375
Aromatic
castor oil, 807
elixir, 5624
Arsanilic acid, 379
Arsenazo III acid, 6140
Arsenic
in reagents, 6128
trioxide, 6140

www.webofpharma.com

Arsenic ⟨211⟩, 6621
Articaine
  hydrochloride, 380
  hydrochloride and epinephrine injection, 381
Articles
  appearing in *USP 43* that were not included in *USP 40* including supplements, xxix
  of Incorporation, xxvii
Articles of botanical origin ⟨561⟩, 6774
Ascorbic acid, 383, 6140
  compounded oral solution, 385
  injection, 383
  oral solution, 384
  tablets, 385
  10 TS, 6227
Ascorbic acid chewable gels, 4765
Ascorbyl palmitate, 5624
Ashwagandha root, 4766
  extract, powdered, 4768
  powdered, 4771
Asian ginseng, 4774
  extract, powdered, 4776
  powdered, 4775
  tablets, 4778
Asparagine, 5625
L-Asparagine, 6140
Aspart
  insulin, 2339
Aspartame, 5626
  acesulfame, 5627
Aspartic acid, 386
L-Aspartic acid, 6140
Aspirin, 388
  acetaminophen and caffeine tablets, 49
  and acetaminophen tablets, 48
  alumina and magnesia tablets, 397
  alumina and magnesium oxide tablets, 399
  boluses, 388
  butalbital, and caffeine capsules, 648
  butalbital, caffeine, and codeine phosphate capsules, 651
  butalbital, and caffeine tablets, 649
  and butalbital tablets, 646
  caffeine, and dihydrocodeine bitartrate capsules, 402
  capsules, 389
  delayed-release capsules, 390
  carisoprodol, and codeine phosphate tablets, 780
  and carisoprodol tablets, 778
  codeine phosphate, alumina, and magnesia tablets, 404
  and codeine phosphate tablets, 403
  effervescent tablets for oral solution, 395
  orphenadrine citrate and caffeine tablets, 3288
  and oxycodone tablets, 3344
  and pentazocine tablets, 3461
  suppositories, 391
  tablets, 392
  tablets, buffered, 393
  delayed-release tablets, 394
  extended-release tablets, 396
Assay
  alginates ⟨311⟩, 6662
  antibiotics, iodometric ⟨425⟩, 6696
  for citric acid/citrate and phosphate ⟨345⟩, 6668
  dexpanthenol ⟨115⟩, 6547
  epinephrine ⟨391⟩, 6676
  folic acid ⟨411⟩, 6689
  niacin or niacinamide ⟨441⟩, 6704
  riboflavin ⟨481⟩, 6735
  single-steroid ⟨511⟩, 6750
  for steroids ⟨351⟩, 6669
  thiamine ⟨531⟩, 6756
  vitamin A ⟨571⟩, 6801
  vitamin B_{12} activity ⟨171⟩, 6584
  vitamin D ⟨581⟩, 6808
  vitamin E ⟨551⟩, 6767
Assays
  antibiotics—microbial ⟨81⟩, 6488
  design and analysis of biological ⟨111⟩, 6543
  insulin ⟨121⟩, 6547
Assessment of drug product performance—bioavailability, bioequivalence, and dissolution ⟨1090⟩, 7695
Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems ⟨1664⟩, 8455
Assessment of extractables associated with pharmaceutical packaging/delivery systems ⟨1663⟩, 8442
Astaxanthin esters, 4779
Astragalus root, 4782
  dry extract, 4787
  powder, 4785
Atenolol, 405
  and chlorthalidone tablets, 409
  injection, 406
  oral solution, 407
  tablets, 407
Atenolol compounded
  oral suspension, 408
Atenolol compounded, veterinary
  oral suspension, 409
Atomic absorption spectroscopy ⟨852⟩, 7142
Atomic absorption spectroscopy—theory and practice ⟨1852⟩, 8632
Atomic masses, 6345
Atomic weights, 6344
Atomoxetine
  capsules, 412
Atomoxetine hydrochloride, 411
Atorvastatin calcium, 414
Atorvastatin calcium
  tablets, 418
Atovaquone, 422
  oral suspension, 423
Atracurium besylate, 424
  injection, 426
Atropine, 428
  sulfate, 428
  sulfate and diphenoxylate hydrochloride oral solution, 1438
  sulfate and diphenoxylate hydrochloride tablets, 1439
  sulfate injection, 430
  sulfate ophthalmic ointment, 431
  sulfate ophthalmic solution, 432
Attapulgite, activated, 433
  colloidal, 433
Aurothioglucose, 434
  injectable suspension, 434
Auxiliary packaging components ⟨670⟩, 6916
Avobenzone, 435
Azaperone, 435
  injection, 436
Azatadine maleate, 437
  tablets, 438
Azathioprine, 438
  oral suspension, 440
  sodium for injection, 442
  tablets, 441
Azelastine hydrochloride, 443
Azelastine hydrochloride ophthalmic solution, 445
Azithromycin, 446
  capsules, 450
  for injection, 451
  for oral suspension, 454
  tablets, 457
Azo violet, 6220
Aztec marigold zeaxanthin extract, 4789
Aztreonam, 460
  injection, 462
  for injection, 463
Azure A, 6140

B

Bacillus coagulans, 4791
Bacillus coagulans capsules, 4794
Bacillus subtilis subsp. *subtilis* menaquinone-7 extract, 5152
Bacitracin, 464
  for injection, 466
  methylene disalicylate, soluble, 467
  methylene disalicylate soluble powder, 467
  neomycin and polymyxin B sulfates and hydrocortisone acetate ointment, 3116
  neomycin and polymyxin B sulfates and hydrocortisone acetate ophthalmic ointment, 3116
  neomycin and polymyxin B sulfates and lidocaine ointment, 3117
  and neomycin and polymyxin B sulfates ointment, 3115
  and neomycin and polymyxin B sulfates ophthalmic ointment, 3115
  and neomycin sulfate ointment, 3104
  ointment, 466
  ophthalmic ointment, 467
  and polymyxin B sulfate topical aerosol, 468
  zinc, 468
  zinc, neomycin and polymyxin B sulfates, and hydrocortisone ointment, 3118
  zinc, neomycin and polymyxin B sulfates, and hydrocortisone ophthalmic ointment, 3119
  zinc, neomycin and polymyxin B sulfates, and hydrocortisone acetate ophthalmic ointment, 3120
  zinc, neomycin and polymyxin B sulfates, and lidocaine ointment, 3120
  zinc and neomycin and polymyxin B sulfates ointment, 3118
  zinc and neomycin and polymyxin B sulfates ophthalmic ointment, 3118
  zinc and neomycin sulfate ointment, 3104
  zinc ointment, 470
  zinc and polymyxin B sulfate topical aerosol, 3591
  zinc and polymyxin B sulfate ointment, 471
  zinc and polymyxin B sulfate ophthalmic ointment, 471
  zinc and polymyxin B sulfate topical powder, 3591
  zinc soluble powder, 470
Baclofen, 471
  oral suspension, 473
  tablets, 473

www.webofpharma.com

Bacopa, 4797
extract, powdered, 4800
powdered, 4798
Bacterial
alkaline protease preparation, 6140
endotoxins test ⟨85⟩, 6508
Bacteriostatic
sodium chloride injection, 4067
water for injection, 4652
Balances ⟨41⟩, 6449
Balsalazide disodium, 475
capsules, 477
Banaba leaf, 4801
extract, dry, 4805
powder, 4803
Bandage
adhesive, 478
gauze, 478
Barbital sodium, 6140
Barbituric acid, 6140
Barium
acetate, 6140
chloride, 6140
chloride, anhydrous, 6138, 6140
chloride dihydrate, 6140
chloride TS, 6227
hydroxide, 6140
hydroxide lime, 479
hydroxide TS, 6227
nitrate, 6141
nitrate TS, 6227
sulfate, 479
sulfate for suspension, 482
sulfate paste, 480
sulfate suspension, 481
sulfate tablets, 483
0.05 M Barium perchlorate VS, 6238
Basic fuchsin, 6141
Beclomethasone, 6141
Beclomethasone dipropionate, 483
Beclomethasone dipropionate compounded oral solution, 484
Beef extract, 6141
Behenoyl polyoxylglycerides, 5628
Belladonna
leaf, 485
extract, 486
extract tablets, 487
tincture, 488
Benazepril hydrochloride, 488
and amlodipine hydrochloride capsules, 269
tablets, 491
Benazepril hydrochloride compounded, veterinary
oral suspension, 490
Bendamustine hydrochloride, 493
Bendamustine hydrochloride for injection, 495
Bendroflumethiazide, 496
and nadolol tablets, 3062
tablets, 497
Benoxinate hydrochloride, 498
and fluorescein sodium ophthalmic solution, 1944
ophthalmic solution, 499
Bentonite, 5629
magma, 5632
purified, 5630
Benzaldehyde, 5632, 6141
elixir, compound, 5634
Benzalkonium chloride, 5634, 6141
solution, 5636
Benzamidine hydrochloride hydrate, 6141
Benzanilide, 6141
Benzene, 6141
Benzenesulfonamide, 6141
Benzenesulfonyl chloride, 6141
Benzethonium chloride, 499
concentrate, 500
topical solution, 501
tincture, 501
Benzhydrol, 6141
Benzil, 6141
Benzocaine, 502
topical aerosol, 503
and antipyrine otic solution, 352
antipyrine, and phenylephrine hydrochloride otic solution, 354
butamben, and tetracaine hydrochloride topical aerosol, 513
butamben, and tetracaine hydrochloride gel, 514
butamben, and tetracaine hydrochloride ointment, 516
butamben, and tetracaine hydrochloride topical solution, 517
cream, 505
gel, 506
lozenges, 507
and menthol topical aerosol, 518
ointment, 509
otic solution, 510
topical solution, 511
Benzoic
acid, 520, 6141
and salicylic acids ointment, 521
Benzoin, 522
tincture, compound, 522
Benzonatate, 523
capsules, 523
Benzophenone, 6141
p-Benzoquinone, 6197
Benzoyl
chloride, 6141
peroxide and erythromycin topical gel, 1692
peroxide gel, 526
peroxide, hydrous, 525
peroxide lotion, 527
N-Benzoyl-L-arginine ethyl ester hydrochloride, 6141
3-Benzoylbenzoic acid, 6141
Benzoylformic acid, 6141
Benzphetamine hydrochloride, 6141
Benzphetamine hydrochloride, 528
tablets, 530
Benztropine mesylate, 532
injection, 532
tablets, 533
Benzyl
alcohol, 5639
benzoate, 534
benzoate lotion, 535
N-Benzylacetamide, 6142
2-Benzylaminopyridine, 6142
1-Benzylimidazole, 6142
Benzylpenicilloyl polylysine
concentrate, 535
injection, 536
Benzyltrimethylammonium chloride, 6142
Beta carotene, 536
capsules, 538
preparation, 4806
Betadex, 5641
sulfobutyl ether sodium, 5643
Beta glucan, 4808
Betahistine hydrochloride, 540
Betaine hydrochloride, 540
Beta-lactoglobulin, 6174
Beta-lactoglobulin A, 6174
Betamethasone, 541
acetate, 544
acetate and betamethasone sodium phosphate injectable suspension, 551
acetate and gentamicin sulfate ophthalmic solution, 2102
benzoate, 544
benzoate gel, 545
cream, 541
dipropionate, 546
dipropionate and clotrimazole cream, 1124
dipropionate cream, 547
dipropionate lotion, 548
dipropionate ointment, 548
sodium phosphate, 549
sodium phosphate and betamethasone acetate injectable suspension, 551
sodium phosphate injection, 551
oral solution, 542
valerate, 552
valerate cream, 553
valerate and gentamicin sulfate ointment, 2103
valerate and gentamicin sulfate otic solution, 2104
valerate and gentamicin sulfate topical solution, 2105
valerate lotion, 554
valerate ointment, 556
Betanaphthol, 6142
TS, 6227
Betaxolol
hydrochloride, 557
ophthalmic solution, 558
tablets, 559
Bethanechol chloride, 561
injection, 562
oral solution, 563
oral suspension, 564
tablets, 564
Beta-lactamase, 6142, 6186
Bibenzyl, 6142, 6156
Bicalutamide, 566
tablets, 567
Bifidobacterium animalis subsp. *lactis*, 4811
Bilberry
extract, powdered, 4813
Bile salts, 6142, 6203
(S)-Binol, 6142
Bioburden control of nonsterile drug substances and products ⟨1115⟩, 7827
Biocompatibility of materials used in drug containers, medical devices, and implants, the ⟨1031⟩, 7313
Biological
assay chapters—overview and glossary ⟨1030⟩, 7300
assay validation ⟨1033⟩, 7337
indicators—resistance performance tests ⟨55⟩, 6452
indicators for sterilization ⟨1229.5⟩, 8216
reactivity tests, in vitro ⟨87⟩, 6514
reactivity tests, in vivo ⟨88⟩, 6516
Biologics ⟨1041⟩, 7379
Biotechnology products: stability testing of biotechnological/biological products, quality of ⟨1049⟩, 7454
Biotechnology-derived articles
amino acid analysis ⟨1052⟩, 7481
isoelectric focusing ⟨1054⟩, 7499
peptide mapping ⟨1055⟩, 7501
polyacrylamide gel electrophoresis ⟨1056⟩, 7508
total protein assay ⟨1057⟩, 7515

www.webofpharma.com

Biotechnology products derived from cell lines of human or animal origin, viral safety evaluation of ⟨1050⟩, 7459
Biotin, 569
capsules, 570
tablets, 571
Biotin compounded oral suspension, 571
Biphenyl, 6142
2,2′-Bipyridine, 6162
Bis(4-sulfobutyl) ether disodium, 6143
Bisacodyl, 572
rectal suspension, 574
suppositories, 573
delayed-release tablets, 574
4,4′-Bis(4-amino-naphthylazo)-2,2′-stilbenedisulfonic acid, 6143
Bis(2-ethylhexyl)
maleate, 6143
(phosphoric acid), 6143
phthalate, 6143
sebacate, 6143
Bismuth, 6143
citrate, 576
iodide TS, potassium, 6233
milk of, 575
nitrate pentahydrate, 6143
nitrate, 0.01 mol/L, 6239
subcarbonate, 576
subgallate, 577
subnitrate, 578, 6143
subsalicylate, 579
subsalicylate magma, 580
subsalicylate oral suspension, 582
subsalicylate tablets, 583
sulfite, 6220
sulfite agar, 6143
Bisoctrizole, 583
Bisoprolol fumarate, 585
and hydrochlorothiazide tablets, 587
tablets, 586
Bis(trimethylsilyl)
acetamide, 6143
trifluoroacetamide, 6143
trifluoroacetamide with trimethylchlorosilane, 6143
Biuret reagent TS, 6227
Black cohosh, 4815
fluidextract, 4821
powdered, 4817
powdered extract, 4820
tablets, 4823
Black pepper, 4825
powdered, extract, 4829
powdered, 4827
Bleomycin
for injection, 588
sulfate, 589

Blood

Blood, 6143
Grouping reagent, anti-A, grouping reagent, anti-B, and grouping reagent, anti-AB, 6143
Technetium Tc 99m red blood cells injection, 4235

Blue
G TS, brilliant , 6227
tetrazolium, 6144
tetrazolium TS, 6227
Board of trustees
USP Convention (2015–2020), ix
Boiling or distilling range for reagents, 6128
Boldine, 6144
Boluses
amoxicillin, 299
ampicillin, 321
aspirin, 388
dihydrostreptomycin sulfate, 1391
neomycin, 3102
phenylbutazone, 3511
tetracycline, 4302
Borage seed oil, 4830
capsules, 4831
Boric acid, 5646, 6144
(–)-Bornyl acetate, 6144
Boron trifluoride, 6144
14% Boron trifluoride–methanol, 6144
Boswellia serrata, 4832
extract, 4834
Botanical
extracts ⟨565⟩, 6798
origin, identification of articles of ⟨563⟩, 6788
Bovine collagen, 6144
Bovine serum ⟨1024⟩, 7260
7 percent bovine serum albumin certified standard, 6144
Branched polymeric sucrose, 6144
Bretylium tosylate, 590
in dextrose injection, 591
injection, 590
Brilliant
blue G TS, 6227
green, 6220
yellow, 6220
Brimonidine tartrate, 591
Brinzolamide, 592
ophthalmic suspension, 594
Bromelain, 6144
Bromine, 6145
sodium acetate TS, 6227
tenth-normal (0.1 N), 6239
TS, 6227
α-Bromo-2′-acetonaphthone, 6145
p-Bromoaniline, 6145
TS, 6227
Bromocresol
blue, 6220
blue TS, 6220
green, 6220
green-methyl red TS, 6227
green sodium salt, 6220
green TS, 6220, 6227
purple, 6220
purple sodium salt, 6220
purple TS, 6227
Bromocriptine mesylate, 595
capsules, 596
tablets, 597
Bromodiphenhydramine hydrochloride, 599
and codeine phosphate oral solution, 600
oral solution, 599
Bromofluoromethane, 6145
Bromophenol blue, 6220
sodium, 6220
TS, 6227
N-Bromosuccinimide, 6145
Bromothymol blue, 6220
TS, 6227
Brompheniramine maleate, 601
injection, 602
and pseudoephedrine sulfate oral solution, 603
oral solution, 602
tablets, 603
Brucine sulfate, 6145
Budesonide, 604

Buffer

Acetate TS, 6225
Acetic acid–ammonium acetate TS, 6225
Acetone buffered, TS, 6227
Acid phthalate, 6145
Alkaline borate, 6145
Hydrochloric acid, 6145
Neutralized phthalate, 6145

Buffered acetone TS, 6227
Buffers, 6145
Buffer solutions, 6145, 6223
acetate buffer, 6145
alkaline borate buffer, 6145
hydrochloric acid buffer, 6145
neutralized phthalate buffer, 6145
phosphate buffer, 6145
Bulk density and tapped density of powders ⟨616⟩, 6850
Bulk pharmaceutical excipients—certificate of analysis ⟨1080⟩, 7647
Bulk powder sampling procedures ⟨1097⟩, 7732
Bumetanide, 606
injection, 607
tablets, 608
Bupivacaine hydrochloride, 610
in dextrose injection, 612
and epinephrine injection, 612
injection, 611
Bupivacaine Hydrochloride
fentanyl citrate compounded injection, 1850
Buprenorphine
hydrochloride, 614
Buprenorphine and naloxone sublingual tablets, 616
Buprenorphine compounded, veterinary buccal solution, 615
Bupropion hydrochloride, 618
tablets, 620
extended-release tablets, 621
Buspirone hydrochloride, 634
tablets, 637
Busulfan, 639
tablets, 640
Butabarbital, 640
sodium, 641
sodium oral solution, 642
sodium tablets, 643
Butalbital, 644
acetaminophen, and caffeine capsules, 645
acetaminophen, and caffeine tablets, 646
aspirin, and caffeine capsules, 648
aspirin, caffeine, and codeine phosphate capsules, 651
aspirin, and caffeine tablets, 649
and aspirin tablets, 646
Butamben, 653
benzocaine, and tetracaine hydrochloride topical aerosol, 513
benzocaine, and tetracaine hydrochloride gel, 514
benzocaine, and tetracaine hydrochloride ointment, 516

www.webofpharma.com

Butamben *(continued)*
benzocaine, and tetracaine hydrochloride topical solution, 517
Butane, 5647
Butane-1,2-diol, 6145
Butane-1,4-diol, 6145
Butane-2,3-diol, 6145
1,3-Butanediol, 6145
2,3-Butanedione, 6145, 6156
1-Butanesulfonic acid sodium salt, 6145
1,4-Butane sultone, 6145
Butanol, 6145
Butoconazole nitrate, 654
vaginal cream, 655
Butorphanol tartrate, 655
injection, 656
nasal spray, 657
Butyl
acetate, normal, 6145
alcohol, 5648, 6145, 6146, 6184
alcohol, normal, 6145
alcohol, secondary, 6134, 6146, 6198
alcohol, tertiary, 6134, 6209
benzoate, 6146
ether, 6146
methacrylate, 6146
palmitostearate, 5649
stearate, 5650
n-Butyl chloride, 6146, 6151
tert-Butyl methyl ether, 6146
n-Butylamine, 6146, 6184
tert-Butylamine, 6146, 6184
4-(Butylamino)benzoic acid, 6146
Butylated
hydroxyanisole, 5651
n-Butylboronic acid, 6146
tert-Butyldimethylchlorosilane in *N*-methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide, (1 in 100), 6146
Butylene glycol, 5653
Butylparaben, 5655, 6146
4-*tert*-Butylphenol, 6146
t-Butylthiol, 6146
Butyraldehyde, 6146
Butyric acid, 6146
Butyrolactone, 6146
Butyrophenone, 6147

C

C 13
for oral solution, urea, 767
urea, 766
C 14
capsules, urea, 768
Cabergoline, 658
tablets, 659
Cadmium
acetate, 6147
nitrate, 6147
Caffeine, 661
acetaminophen and aspirin tablets, 49
and acetaminophen tablets, 51
aspirin and dihydrocodeine bitartrate capsules, 402
butalbital, and acetaminophen capsules, 645
butalbital, and acetaminophen tablets, 646
butalbital, and aspirin capsules, 648
butalbital, aspirin, and codeine phosphate capsules, 651
butalbital, and aspirin tablets, 649
citrate injection, 662
citrate oral solution, 663
and ergotamine tartrate suppositories, 1680
and ergotamine tartrate tablets, 1681
orphenadrine citrate and aspirin tablets, 3288
and sodium benzoate injection, 664
Calamine, 664
topical suspension, phenolated, 665
topical suspension, 665
Calcifediol, 666
capsules, 666
Calcipotriene, 667
ointment, 668
Calcitonin salmon, 670
injection, 674
nasal solution, 675
Calcitriol, 675
injection, 677
Calcium
acetate, 677, 6147
acetate and aluminum sulfate tablets for topical solution, 195
acetate tablets, 682
ascorbate, 682
carbonate, 683, 5656, 6147
carbonate, alumina, and magnesia chewable tablets, 169
carbonate, alumina, magnesia, and simethicone chewable tablets, 170
carbonate, alumina, and magnesia oral suspension, 168
carbonate, chelometric standard, 6147
carbonate lozenges, 685
carbonate, magnesia, and simethicone chewable tablets, 689
carbonate and magnesia chewable tablets, 688
carbonate oral suspension, 686
carbonate tablets, 687
caseinate, 6147
chloride, 692, 6147
chloride, anhydrous, 6138, 6147
chloride injection, 693
chloride TS, 6227
citrate, 693, 6147
citrate tablets, 4835
glubionate syrup, 695
gluceptate, 696
gluceptate injection, 697
gluconate, 697
gluconate injection, 700
gluconate tablets, 701
glycerophosphate, 4836
hydroxide, 701, 6147
hydroxide topical solution, 702
hydroxide TS, 6227
lactate, 702, 6147
lactate tablets, 703
lactobionate, 704
Leucovorin comounded oral solution, 2570
levulinate, 705
levulinate injection, 705
and magnesium carbonates oral suspension, 691
and magnesium carbonates tablets, 691
nitrate, 6148
pantothenate, 706
pantothenate assay ⟨91⟩, 6536
pantothenate, dextro, 6148
pantothenate, racemic, 708
pantothenate tablets, 707
phosphate, anhydrous dibasic, 710
phosphate tablets, dibasic, 712
phosphate, tribasic, 5657
phosphate dihydrate, dibasic, 708
polycarbophil, 713
propionate, 5658
saccharate, 713
silicate, 5659
stearate, 5661
succinate, 714
sulfate, 5662, 6148
sulfate TS, 6227
undecylenate, 714
and vitamin D with minerals tablets, 4845
with vitamin D tablets, 4843
Calcium acetate
and aluminum sulfate for topical solution, 194
Calcium acetate
capsules, 680
Calcium citrate malate, 694
Calcium glubionate
syrup, 695
Calcium L-5-methyltetrahydrofolate, 4838
capsules, 4841
tablets, 4842
Calconcarboxylic acid, 6148
triturate, 6148
Calf thymus DNA, 6148
dl-Camphene, 6148
Camphor, 715
spirit, 715
d-10-Camphorsulfonic acid, 6148
dl-10-Camphorsulfonic acid, 6148
Canada balsam, 6148
Candelilla wax, 5663
Candesartan cilexetil, 716
and hydrochlorothiazide tablets, 719
tablets, 717
Canola oil, 5664
Capecitabine, 722
tablets, 723
Capillary electrophoresis ⟨1053⟩, 7492
Capreomycin
for injection, 726
sulfate, 725
Capric acid, 6148
Caprylic acid, 5664
Caprylocaproyl polyoxylglycerides, 5665
Capsaicin, 727
Capsicum, 728
oleoresin, 730
tincture, 732

Capsules

Acebutolol hydrochloride, 35
Acetaminophen, 40
Containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 52
Acetaminophen and codeine phosphate, 52
Acitretin, 86
Acyclovir, 89
Altretamine, 164
Aluminum hydroxide gel, dried, 190
Amantadine hydrochloride, 209
Aminobenzoate potassium, 230
Amlodipine and benazepril hydrochloride, 269
Amoxicillin, 300
Ampicillin, 321

www.webofpharma.com

Capsules *(continued)*
Anagrelide, 334
Aprepitant, 362
Arginine, 4763
Aspirin, 389
Aspirin, caffeine, and dihydrocodeine bitartrate, 402
Aspirin delayed-release, 390
Atomoxetine, 412
Azithromycin, 450
Bacillus coagulans, 4794
Balsalazide disodium, 477
Benzonatate, 523
Beta carotene, 538
Biotin, 570
Borage seed oil, 4831
Bromocriptine mesylate, 596
Butalbital, acetaminophen, and caffeine, 645
Butalbital, aspirin, and caffeine, 648
Butalbital, aspirin, caffeine, and codeine phosphate, 651
Calcifediol, 666
Calcium acetate, 680
Calcium L-5-methyltetrahydrofolate, 4841
C 14, urea, 768
Castor oil, 805
Cat's claw, 4857
Cefaclor, 809
Cefadroxil, 815
Cefdinir, 827
Cephalexin, 897
Cephradine, 907
Chloral hydrate, 924
Chloramphenicol, 928
Chlordiazepoxide hydrochloride, 941
Chlordiazepoxide hydrochloride and clidinium bromide, 943
Chlorpheniramine maleate extended-release, 970
Chlorpheniramine maleate and pseudoephedrine hydrochloride extended-release, 972
Cholecalciferol, 986
Clindamycin hydrochloride, 1065
Clofazimine, 1085
Clofibrate, 1088
Clomipramine hydrochloride, 1093
Cloxacillin sodium, 1129
Cod liver oil, 4910
Crypthecodinium cohnii oil, 4925
Curcuminoids, 4929
Cyclobenzaprine hydrochloride extended-release capsules, 1197
Cycloserine, 1211
Cyclosporine, 1213
Danazol, 1234
Dantrolene sodium, 1236
Diazoxide, 1333
Dicloxacillin sodium, 1358
Dicyclomine hydrochloride, 1360
Didanosine delayed-release, 1364
Digitalis, 1380
Dihydrotachysterol, 1392
Diltiazem hydrochloride extended-release, 1399
Diphenhydramine hydrochloride, 1425
Diphenhydramine hydrochloride and ibuprofen, 1431
Diphenhydramine and pseudoephedrine, 1435
Disopyramide phosphate, 1450
Disopyramide phosphate extended-release, 1450
Divalproex sodium delayed-release, 1454
Docusate calcium, 1476
Docusate potassium, 1478
Docusate sodium, 1479
Doxepin hydrochloride, 1509
Doxycycline, 1519
Doxycycline extended-release, 1521
Doxycycline hyclate, 1534
Doxycycline hyclate delayed-release, 1536
Dronabinol, 1549
Duloxetine delayed-release, 1559
Echinacea species, 4966
Echinacea species dry extract, 4960
Efavirenz, 1588
Eleuthero root and rhizome dry extract, 4972
Eleuthero root and rhizome powder, 4976
Ephedrine sulfate, 1642
Ergocalciferol, 1666
Ergoloid mesylates, 1670
Erythromycin delayed-release, 1686
Erythromycin estolate, 1693
Esomeprazole magnesium delayed-release, 1716
Ethchlorvynol, 1766
Ethosuximide, 1774
Etodolac, 1786
Etoposide, 1794
Evening primrose oil, 4980
Fenofibrate, 1834
Fenoprofen calcium, 1845
Ferrous gluconate, 1859
Fexofenadine hydrochloride, 1870
Fish oil containing omega-3 acids, 4994
Fish oil containing omega-3 acids, delayed-release, 4997
Flax seed oil, 4999
Flucytosine, 1916
Fluoxetine, 1958
Fluoxetine delayed-release, 1959
Flurazepam hydrochloride, 1978
Flutamide, 1984
Fluvastatin, 2020
Gabapentin, 2058
Galantamine extended-release, 2074
Gemfibrozil, 2097
Ginger, 5032
Ginkgo, 5037
Ginseng, American, 4754
Griseofulvin, 2165
Guaifenesin, 2170
Guaifenesin and pseudoephedrine hydrochloride, 2174
Guaifenesin, pseudoephedrine hydrochloride, and dextromethorphan hydrobromide, 2175
Hydrochlorothiazide, 2219
Hydroxyurea, 2263
Hydroxyzine pamoate, 2271
Imipramine pamoate, 2304
Indomethacin, 2324
Indomethacin extended-release, 2325
Sodium iodide I 123, 2365
Sodium iodide I 131, 2368
Isometheptene mucate, dichloralphenazone, and acetaminophen, 2423
Isosorbide dinitrate extended-release, 2441
Isotretinoin, 2458
Isradipine, 2465
itraconazole, 2468
Kanamycin sulfate, 2503
Ketoprofen, 2510
Ketoprofen extended-release, 2511
Krill oil, 5104
Krill oil delayed-release, 5107
Lansoprazole delayed-release, 2553
Lincomycin hydrochloride, 2634
Alpha lipoic acid, 5125
Lithium carbonate, 2650
Lomustine, 2656
Loperamide hydrochloride, 2660
Loxapine, 2697
Lutein, 5128
Magnesium oxide, 2721
Meclofenamate sodium, 2754
Mefenamic acid, 2758
Menaquinone-7, 5147
Mesalamine extended-release, 2802
Methoxsalen, 2863
Methsuximide, 2870
Methyltestosterone, 2906
Metronidazole, 2934
Metyrosine, 2942
Mexiletine hydrochloride, 2944
Milk thistle, 5162
Minerals, 5164
Minocycline hydrochloride, 2965
Morphine sulfate extended-release, 3024
Mycophenolate mofetil, 3044
Nifedipine, 3161
Nitrofurantoin, 3174
Nizatidine, 3192
Nortriptyline hydrochloride, 3216
Oil- and water-soluble vitamins with minerals, 5435
Olanzapine and fluoxetine, 3237
Oleovitamin A and D, 3242
Omega-3 ethyl esters, 3253
Omeprazole delayed-release, 3258
Orlistat, 3280
Oseltamivir phosphate, 3293
Oxazepam, 3314
Oxycodone and acetaminophen, 3341
Oxytetracycline hydrochloride, 3362
Pancrelipase, 3384
Pancrelipase delayed-release, 3384
Paricalcitol, 3404
Paromomycin sulfate, 3408
Penicillamine, 3427
Phendimetrazine tartrate, 3493
Phenoxybenzamine hydrochloride, 3503
Phentermine hydrochloride, 3506
Phenytoin sodium, extended, 3531
Piroxicam, 3578
Potassium chloride extended-release, 3602
Prazosin hydrochloride, 3653
Procainamide hydrochloride, 3689
Procarbazine hydrochloride, 3696
Propranolol hydrochloride extended-release, 3747
Pseudoephedrine hydrochloride extended-release, 3762
Pygeum, 5200
Quinidine sulfate, 3813
Quinine sulfate, 3819
Ramipril, 3839
Rhodiola rosea, 5224
Ribavirin, 3863
Rifabutin, 3873
Rifampin, 3876
Rifampin and isoniazid, 3878
Ritonavir, 3914
Rivastigmine tartrate, 3925
Propafenone hydrochloride extended-release, 3729
St. John's wort flowering top dry extract, 5242
Salsalate, 3981
Saquinavir, 3984
Saw palmetto, 5259

www.webofpharma.com

Capsules *(continued)*
Schizochytrium oil, 5269
Secobarbital sodium, 4015
Selegiline hydrochloride, 4018
Simethicone, 4043
Soy isoflavones, 5276
Stavudine, 4120
Sulfinpyrazone, 4172
Tacrolimus, 4196
Tamsulosin hydrochloride, 4213
Temazepam, 4251
Temozolomide, 4253
Terazosin, 4261
Tetracycline hydrochloride, 4305
Tetracycline hydrochloride and nystatin, 4313
Thalidomide, 4318
Theophylline, 4321
Theophylline extended-release, 4322
Theophylline and guaifenesin, 4331
Thiothixene, 4359
Tienchi ginseng root and rhizome dry extract, 5309
Tienchi ginseng root and rhizome powder, 5304
Tolmetin sodium, 4423
Topiramate, 4432
Triamterene, 4495
Triamterene and hydrochlorothiazide, 4496
Trientine hydrochloride, 4510
Trihexyphenidyl hydrochloride extended-release, 4518
Trimethobenzamide hydrochloride, 4524
Ubidecarenone, 5320
Ursodiol, 4558
Valerian root dry extract , 5332
Valerian root powder, 5333
Valproic acid, 4573
Vancomycin hydrochloride, 4588
Venlafaxine hydrochloride extended-release, 4598
Verapamil hydrochloride extended-release, 4605
Vinpocetine, 5336
Vitamin A, 4633
Vitamin E, 4639
Vitamins with minerals, oil-soluble, 5361
Vitamins with minerals, oil- and water-soluble, 5435
Vitamins with minerals, water-soluble, 5523
Vitamins, oil-soluble, 5346
Vitamins, oil- and water-soluble, 5389
Vitamins, water-soluble, 5499
Zaleplon, 4669
Zidovudine, 4674
Ziprasidone, 4695
Zonisamide, 4719

Capsules—dissolution testing and related quality attributes ⟨1094⟩, 7725
Captopril, 733
and hydrochlorothiazide tablets, 737
oral solution, 734
oral suspension, 735
tablets, 735
Caramel, 5666
Caraway, 5666
oil, 5667
Carbachol, 738
intraocular solution, 739
ophthalmic solution, 739
Carbamazepine, 740
oral suspension, 742
tablets, 743
extended-release tablets, 746
Carbamide peroxide, 747
topical solution, 747
Carbazole sulfate, 6148
Carbenicillin
disodium, 748
indanyl sodium, 748
indanyl sodium tablets, 749
for injection, 748
Carbidopa, 750
and levodopa extended-release tablets, 753
and levodopa orally disintegrating tablets, 759
and levodopa tablets, 751
Carbinoxamine maleate, 761
pseudoephedrine hydrochloride, and dextromethorphan hydrobromide oral solution, 3767
tablets, 763
Carbol-fuchsin topical solution, 765
Carbomer
934, 5667
934P, 5668
940, 5670
941, 5671
1342, 5672
copolymer, 5673
homopolymer, 5676
interpolymer, 5679
Carbon
C 13 for oral solution, urea, 767
C 13, urea, 766
C 14 capsules, urea, 768
dioxide, 765
dioxide detector tube, 6148
disulfide, chromatographic, 6148
disulfide, CS, 6148
monoxide detector tube, 6148
tetrachloride, 6148
Carbonates
calcium and magnesium, oral suspension, 691
calcium and magnesium, tablets, 691
Carboplatin, 768
for injection, 769
Carboprost
tromethamine, 771
tromethamine injection, 772
Carboxylate (sodium form) cation-exchange resin (50- to 100-mesh), 6148
Carboxymethoxylamine hemihydrochloride, 6149
Carboxymethylcellulose
calcium, 5682
sodium, 773
sodium 12, 5684
sodium, low-substituted, 5682
sodium and microcrystalline cellulose, 5698
sodium paste, 774
sodium tablets, 774
Carboxymethylcellulose sodium
enzymatically-hydrolyzed, 5685
Cardamom
oil, 5688
seed, 5688
tincture, compound, 5689
Carisoprodol, 776
aspirin and codeine phosphate tablets, 780
and aspirin tablets, 778
tablets, 777
Carmellose, 5689
Carmine, 6149
Carmustine, 782
for injection, 784
Carotenes, 4850
Carprofen, 785
tablets, 786
Carrageenan, 5690
Carteolol hydrochloride, 788
ophthalmic solution, 789
tablets, 790
Carvedilol, 791
tablets, 793
(*R*)-(–)-Carvone, 6149
Casanthranol, 795
Cascara
fluidextract, aromatic, 801
sagrada, 797
sagrada extract, 798
sagrada fluidextract, 801
tablets, 800
Casein, 6149
hammersten, 6149
Castor oil, 802
aromatic, 807
capsules, 805
emulsion, 806
hydrogenated, 5691
polyoxyl 35, 5956
Catechol, 6149
Cation-exchange resin, 6149
carboxylate (sodium form) (50- to 100-mesh), 6148, 6149
polystyrene, 6149, 6192
styrene-divinylbenzene, 6149
styrene-divinylbenzene, strongly acidic, 6149
sulfonic acid, 6149, 6209
Cat's claw, 4852
capsules, 4857
extract, powdered, 4855
powdered, 4854
tablets, 4858
Cedar oil, 6149
Cefaclor, 807
capsules, 809
for oral suspension, 810
extended-release tablets, 811
Cefadroxil, 813
capsules, 815
for oral suspension, 816
tablets, 817
Cefamandole nafate, 818
for injection, 819
Cefazolin, 820
injection, 822
for injection, 823
sodium, 824
Cefdinir, 826
capsules, 827
for oral suspension, 830
Cefepime
hydrochloride, 834
for injection, 832
Cefixime, 836
for oral suspension, 837
tablets, 838
Cefmenoxime
hydrochloride, 839
for injection, 839
Cefmetazole, 840
injection, 841
for injection, 841
sodium, 842

www.webofpharma.com

Cefonicid
for injection, 842
sodium, 843
Cefoperazone
injection, 844
for injection, 844
sodium, 845
Ceforanide, 846
for injection, 846
Cefotaxime
injection, 847
for injection, 848
sodium, 850
Cefotetan, 853
disodium, 855
injection, 853
for injection, 854
Cefotiam
hydrochloride, 856
for injection, 857
Cefoxitin
injection, 859
for injection, 860
sodium, 858
Cefpiramide, 860
for injection, 862
Cefpodoxime proxetil, 863
for oral suspension, 864
tablets, 864
Cefprozil, 865
for oral suspension, 869
tablets, 870
Ceftazidime, 872
injection, 872
for injection, 873
Ceftiofur
hydrochloride, 875
sodium, 877
Ceftizoxime
injection, 880
for injection, 881
sodium, 880
Ceftriaxone
injection, 881
for injection, 882
sodium, 884
Cefuroxime
axetil, 887
axetil for oral suspension, 888
axetil tablets, 889
injection, 886
for injection, 886
sodium, 891
Celecoxib, 892
Cellaburate, 5694
Cellacefate, 5696
Cellular and tissue-based products ⟨1046⟩, 7400
Cellulose
acetate, 5701
chromatographic, 6149
microcrystalline, 5696, 6150
microcrystalline and carboxymethylcellulose sodium, 5698
mixture, chromatographic, 6150
oxidized, 893
oxidized regenerated, 894
powdered, 5700
silicified microcrystalline, 5699
sodium phosphate, 895
sodium phosphate for oral suspension, 896
Cellulose diethylaminoethyl, 6150
Centella asiatica, 4859
extract, powdered, 4863
powdered, 4861
triterpenes, 4864
Cephalexin, 896
capsules, 897
hydrochloride, 900
for oral suspension, 898
tablets, 899
tablets for oral suspension, 899
Cephalothin
injection, 901
for injection, 901
sodium, 902
Cephapirin
benzathine, 904
benzathine intramammary infusion, 905
for injection, 903
sodium, 905
sodium intramammary infusion, 906
Cephradine, 906
capsules, 907
for injection, 908
for oral suspension, 909
tablets, 909
Ceric
ammonium nitrate, 6150
ammonium nitrate TS, 6227
ammonium nitrate, twentieth-normal (0.05 N), 6239
ammonium sulfate, 6150
sulfate, 6150
sulfate, tenth-normal (0.1 N), 6239
Cesium chloride, 6150
Cetirizine hydrochloride, 910
and pseudoephedrine hydrochloride extended-release tablets, 917
oral solution, 912
tablets, 913
orally disintegrating tablets, 915
Cetostearyl alcohol, 5702
Cetrimide, 6150
Cetrimonium bromide, 5704
Cetyl
alcohol, 5705
esters wax, 5706
palmitate, 5708
Cetylpyridinium chloride, 921
lozenges, 922
topical solution, 923
Cetyltrimethylammonium bromide, 6150, 6169
Cetyltrimethylammonium chloride, 25 percent in water, 6150
Chamomile, 4866
Characterization of crystalline and partially crystalline solids by X-ray powder diffraction (XRPD) ⟨941⟩, 7201
Characterization of crystalline solids by microcalorimetry and solution calorimetry ⟨696⟩, 6931
Charcoal
activated, 923, 6133, 6150
Chaste tree, 4868
powdered, 4870
powdered, extract, 4872
Chelating ion exchange resin, 6150
Chemometrics ⟨1039⟩, 7363
Chenodeoxycholic acid, 6150
Cherry
juice, 5709
syrup, 5709
Chia seed
oil, 4877
Chinese salvia, 4878
powdered, 4880
Chinese skullcap root, 4883
powder, 4885
Chinese skullcap root dry extract, 4887
Chitosan, 5710
Chloral hydrate, 924
capsules, 924
oral solution, 924
TS, 6227
Chlorambucil, 925
tablets, 926
Chloramine T, 6150
Chloramphenicol, 927
capsules, 928
cream, 928
and hydrocortisone acetate for ophthalmic suspension, 932
injection, 928
ophthalmic ointment, 929
ophthalmic solution, 929
for ophthalmic solution, 930
otic solution, 931
palmitate, 933
palmitate oral suspension, 934
and polymyxin B sulfate ophthalmic ointment, 933
sodium succinate, 935
sodium succinate for injection, 936
oral solution, 930
tablets, 932
Chlordiazepoxide, 936
and amitriptyline hydrochloride tablets, 938
hydrochloride, 940
hydrochloride capsules, 941
hydrochloride and clidinium bromide capsules, 943
tablets, 937
Chlorhexidine
acetate, 945
acetate topical solution, 947
gluconate oral rinse, 949
gluconate solution, 948
gluconate topical solution, 951
hydrochloride, 952
Chlorhexidine gluconate
topical gel, 4741
Chloride
cobaltous, TS, 6227
ferric, TS, 6229
ferrous tetrahydrate, 6166
gold, 6169
gold, TS, 6229
platinic, 6191
platinic, TS, 6233
in reagents, 6129
stannous, 6041
and sulfate ⟨221⟩, 6636
Chlorine, 6150
detector tube, 6150
TS, 6227
m-Chloroacetanilide, 6150
p-Chloroacetanilide, 6150
1-Chloroadamantane, 6151
2-Chloro-4-aminobenzoic acid, 6151
5-Chloro-2-aminobenzophenone, 6151
3-Chloroaniline, 6151
p-Chloroaniline, 6151
Chlorobenzene, 6151
4-Chlorobenzoic acid, 6151
m-Chlorobenzoic acid, 6151
4-Chlorobenzophenone, 6151
2-Chlorobenzophenone, 6151
1-Chlorobutane, 6151
Chlorobutanol, 5714
Chlorocresol, 5715
2-Chloroethanol, 6151

www.webofpharma.com

2-Chloroethylamine monohydrochloride, 6151
Chloroform, 6151
alcohol-free, 6151
methyl, 6151
Chlorogenic acid, 6151
Chloromethylated polystyrene-divinylbenzene anion-exchange resin, 6151
1-Chloronaphthalene, 6151
4-Chloro-1-naphthol, 6151
2-Chloronicotinic acid, 6151
2-Chloro-4-nitroaniline, 99%, 6152
Chlorophyllin copper complex sodium, 954
Chloroplatinic acid, 6152
Chloroprocaine hydrochloride, 956
injection, 957
Chloroquine, 957
hydrochloride injection, 958
phosphate, 959
phosphate oral suspension, 960
phosphate tablets, 961
5-Chlorosalicylic acid, 6152
Chlorothiazide, 962
and reserpine tablets, 3859
sodium for injection, 966
oral suspension, 963
tablets, 964
Chlorothiazide compounded oral suspension, 963
1-Chloro-2,2,2-trifluoroethylchlorodifluoromethyl ether, 6152
Chlorotrimethylsilane, 6152, 6215
Chloroxylenol, 966
Chlorpheniramine
dextromethorphan, pseudoephedrine, (salts of), and acetaminophen, capsules containing at least three of the following, 52
dextromethorphan, pseudoephedrine (salts of), and acetaminophen, oral powder containing at least three of the following, 54
dextromethorphan, pseudoephedrine (salts of), and acetaminophen, oral solution containing at least three of the following, 56
dextromethorphan, pseudoephedrine (salts of), and acetaminophen, tablets containing at least three of the following, 58
maleate, 968
maleate extended-release capsules, 970
maleate injection, 971
maleate, penicillin G procaine, dihydrostreptomycin sulfate, and dexamethasone injectable suspension, 3445
maleate and pseudoephedrine hydrochloride extended-release capsules, 972
maleate and pseudoephedrine hydrochloride oral solution, 973
maleate oral solution, 971
maleate tablets, 971
maleate, acetaminophen, and dextromethorphan hydrobromide tablets, 60
Chlorpromazine, 974
hydrochloride, 975
hydrochloride injection, 976
hydrochloride oral concentrate, 975
hydrochloride syrup, 976
hydrochloride tablets, 977
suppositories, 974
Chlorpropamide, 978
tablets, 978
Chlortetracycline
bisulfate, 979
hydrochloride, 980, 6152
hydrochloride ointment, 980
hydrochloride ophthalmic ointment, 987
hydrochloride soluble powder, 987
hydrochloride tablets, 987
and sulfamethazine bisulfates soluble powder, 980
Chlorthalidone, 982
and atenolol tablets, 409
and clonidine hydrochloride tablets, 1104
tablets, 983
Chlorzoxazone, 983
tablets, 984
Chocolate, 5715
syrup, 5716
Cholecalciferol, 985
capsules, 986
solution, 987
tablets, 988
Cholecalciferol chewable gels, 4889
Cholestane, 6152
Cholestanol, 6152
Cholesterol, 5716, 6152
Cholesteryl
benzoate, 6152
n-heptylate, 6152
Cholestyramine
resin, 989
for oral suspension, 990
Choline
bitartrate, 4891
chloride, 4892, 6152
Chondroitin sulfate sodium, 4894
and glucosamine tablets, 5043
glucosamine, and methylsulfonylmethane tablets, 5051
shark, 4898
tablets, 4897
Chromate, sodium, Cr 51 injection, 992
Chromatographic
columns, 6252
fuller's earth, 6152
n-heptane, 6152
magnesium oxide, 6152
reagents, 6152
silica gel, 6152
silica gel mixture, 6152
siliceous earth, 6152
siliceous earth, silanized, 6152
solvent hexane, 6152
Chromatography ⟨621⟩, 6853
Chromatography, ion ⟨1065⟩, 7588
Chromic chloride, 990
injection, 991
Chromium
Cr 51 injection, sodium chromate, 992
picolinate, 4901
picolinate tablets, 4902
potassium sulfate dodecahydrate, 6152
trioxide, 6152
Chromogenic
substrate for amidolytic test, 6152
Chromotrope 2R, 6152
Chromotropic acid, 6152, 6202
disodium salt, 6153
TS, 6227
Chymotrypsin, 993
for ophthalmic solution, 994
Ciclopirox, 995
olamine, 997
olamine cream, 998
olamine topical suspension, 998
topical solution, 996
Cidofovir, 999
injection, 1000
Cilastatin
and imipenem for injectable suspension, 2298
and imipenem for injection, 2297
sodium, 1001
Cilostazol, 1003
tablets, 1004
Cimetidine, 1005
hydrochloride, 1008
injection, 1006
in sodium chloride injection, 1007
tablets, 1007
Cinchonidine, 6153
Cinchonine, 6153
Cinnamomum cassia
twig, 4902
twig powder, 4904
Ciprofloxacin, 1009
and dexamethasone otic suspension, 1021
extended-release tablets, 1017
hydrochloride, 1011
injection, 1012
ophthalmic ointment, 1014
ophthalmic solution, 1015
for oral suspension, 1019
tablets, 1015
Cisapride, 1024
Cisapride compounded, veterinary
injection, 1024
oral suspension, 1025
Cisatracurium besylate, 1026
injection, 1028
Cisplatin, 1030
for injection, 1032
Citalopram
hydrobromide, 1037
oral solution, 1034
tablets, 1035
Citicoline sodium, 4906
Citrate
cupric TS, alkaline, 6226, 6228
cupric TS 2, alkaline, 6226, 6228
Citric acid, 6153
anhydrous, 1040, 6153
and magnesium carbonate for oral solution, 2710
magnesium carbonate, and potassium citrate for oral solution, 2711
magnesium oxide, and sodium carbonate irrigation, 1044
monohydrate, 1042
and potassium citrate oral solution, 3617
and potassium and sodium bicarbonates effervescent tablets for oral solution, 3598
and sodium citrate oral solution, 4069
TS, 6227
Citrulline, 4908
Cladribine, 1045
injection, 1046
Clarithromycin, 1047
for oral suspension, 1049
tablets, 1049
extended-release tablets, 1051
Clavulanate
potassium, 1055
potassium and amoxicillin for oral suspension, 308
potassium and amoxicillin tablets, 308

www.webofpharma.com

Clavulanic acid
and amoxicillin extended-release tablets, 310
Clavulanic acid
and ticarcillin injection, 4371
and ticarcillin for injection, 4372
Cleaning glass apparatus ⟨1051⟩, 7481
Clemastine fumarate, 1057
tablets, 1059
Clenbuterol hydrochloride, 1060
Clidinium bromide, 1061
and chlordiazepoxide hydrochloride capsules, 943
Clindamycin
hydrochloride, 1063
hydrochloride capsules, 1065
hydrochloride oral solution, 1066
injection, 1062
for injection, 1063
palmitate hydrochloride, 1068
palmitate hydrochloride for oral solution, 1068
phosphate, 1069
phosphate gel, 1071
phosphate topical solution, 1072
phosphate topical suspension, 1072
phosphate vaginal cream, 1071
phosphate vaginal inserts, 1073
Clindamycin hydrochloride compounded oral solution, 1066
Clioquinol, 1074
cream, 1075
and hydrocortisone cream, 1077
and hydrocortisone ointment, 1078
ointment, 1076
topical powder, compound, 1076
Clobetasol propionate, 1079
cream, 1080
ointment, 1081
topical solution, 1082
Clocortolone pivalate, 1083
cream, 1084
Clofazimine, 1084
capsules, 1085
Clofibrate, 1087
capsules, 1088
Clomiphene citrate, 1088
tablets, 1090
Clomipramine compounded
oral suspension, veterinary, 1091
Clomipramine hydrochloride, 1092
capsules, 1093
Clonazepam, 1096
oral suspension, 1097
tablets, 1098
orally disintegrating tablets, 1099
Clonidine, 1100
hydrochloride, 1101
hydrochloride and chlorthalidone tablets, 1104
hydrochloride tablets, 1103
transdermal system, 1105
Clonidine hydrochloride compounded
oral suspension, 1103
Clopidogrel
bisulfate, 1109
tablets, 1111
Clopidogrel compounded
oral suspension, 1111
Cloprostenol
injection, 1113
sodium, 1113
Clorazepate dipotassium, 1114
tablets, 1115
Clorsulon, 1117
and ivermectin injection, 2477
Clotrimazole, 1118
and betamethasone dipropionate cream, 1124
cream, 1119
lotion, 1119
lozenges, 1120
topical solution, 1122
vaginal inserts, 1123
Clove oil, 5718
Clover, red, 5203
extract, powdered, 5210
powdered, 5207
tablets, 5212
Cloxacillin
benzathine, 1125
benzathine intramammary infusion, 1126
sodium, 1127
sodium capsules, 1129
sodium intramammary infusion, 1129
sodium for oral solution, 1130
Clozapine, 1130
tablets, 1132
Cloxapine N-oxide, 6153
Coal tar, 1133
ointment, 1133
topical solution, 1133
Cobalt
chloride, 6153
nitrate, 6153
platinum, TS, 6233
uranyl acetate TS, 6227
Cobaltous
acetate, 6153
chloride, 6153
chloride CS, 6224
chloride TS, 6227
Cocaine, 1133
hydrochloride, 1134
hydrochloride tablets for topical solution, 1134
Cocoa butter, 5718
Coconut
oil, 5719
oil, hydrogenated, 5720
Codeine, 1137
phosphate, 1137
phosphate and acetaminophen capsules, 52
phosphate and acetaminophen oral solution, 54
phosphate and acetaminophen oral suspension, 56
phosphate and acetaminophen tablets, 66
phosphate, aspirin, alumina, and magnesia tablets, 404
phosphate and aspirin tablets, 403
phosphate and bromodiphenhydramine hydrochloride oral solution, 600
phosphate, butalbital, aspirin, and caffeine capsules, 651
phosphate, carisoprodol, and aspirin tablets, 780
phosphate and guaifenesin oral solution, 2172
phosphate injection, 1138
phosphate tablets, 1139
phosphate and promethazine and phenylephrine hydrochloride oral solution, 3724
phosphate oral solution, 1138
sulfate, 1140
sulfate oral solution, 1141
sulfate tablets, 1142
and terpin hydrate oral solution, 4284
Cod liver oil, 1135
capsules, 4910
Coenzyme Q9, 6153
Coffee fruit dry
extract, 4912
Cohosh
black fluidextract, 4821
Coix seed, 4915
powder, 4917
Colchicine, 1143
injection, 1144
and probenecid tablets, 3687
tablets, 1145
Colestipol hydrochloride, 1145
for oral suspension, 1147
tablets, 1147
Colistimethate
for injection, 1149
sodium, 1148
Colistin
and neomycin sulfates and hydrocortisone acetate otic suspension, 1150
sulfate, 1149
sulfate for oral suspension, 1150
Collagen, 6153
rat tail, 6153
Collagenase, 6153
Collagenase I ⟨89.1⟩, 6524
Collagenase II ⟨89.2⟩, 6529
Collodion, 1151
flexible, 1151
Colloidal oatmeal, 1152
Color
and achromicity ⟨631⟩, 6865
instrumental measurement ⟨1061⟩, 7553
Colorimetric solutions (CS), 6224
Compactin, 6153
Completeness of solution ⟨641⟩, 6866
Compound cardamom tincture, 5689
Compounded topical gel
ondansetron, 3272
Compounding for phase I investigational studies, 7985
Conductivity of solutions ⟨644⟩, 6868
Congealing temperature ⟨651⟩, 6874
Congo red, 6153, 6220
TS, 6227
Constitution and bylaws, xxviii
Construct human fibroblasts in bilayer synthetic scaffold, 1153
Construct human fibroblasts in polyglactin scaffold, 1157
Container content for injections ⟨697⟩, 6934
Containers
glass ⟨660⟩, 6881
performance testing ⟨671⟩, 6922
Container specifications for capsules and tablets, 6259
Coomassie
blue G-250, 6153
brilliant blue R-250, 6153
Copovidone, 5721
Copper, 6153
gluconate, 1168
Copper sulfate pentahydrate, 6153
Coriander oil, 5723
Corn
oil, 5723
starch, 6044
syrup, 5724
high fructose syrup, 5727
syrup solids, 5731
Corticotropin
injection, 1170

www.webofpharma.com

Corticotropin *(continued)*
for injection, 1172
injection, repository, 1174
Cortisone, 6153
acetate, 1175
acetate tablets, 1176
Cosyntropin, 1178
Cotton
absorbent, 6153
purified, 1180
Cotton ⟨691⟩, 6929
Cottonseed oil, 5733
hydrogenated, 5734
Council of experts
(2015–2020), ix
Cr 51
injection, sodium chromate, 992
Cranberry
liquid preparation, 4919

Cream

Alclometasone dipropionate, 111
Amcinonide, 214
Amphotericin B, 315
Anthralin, 344
Benzocaine, 505
Betamethasone, 541
Betamethasone dipropionate, 547
Betamethasone valerate, 553
Butoconazole nitrate, vaginal, 655
Chloramphenicol, 928
Ciclopirox olamine, 998
Clindamycin phosphate, vaginal, 1071
Clioquinol, 1075
Clioquinol and hydrocortisone, 1077
Clobetasol propionate, 1080
Clocortolone pivalate, 1084
Clotrimazole, 1119
Clotrimazole and betamethasone dipropionate, 1124
Crotamiton, 1187
Desoximetasone, 1274
Dibucaine, 1336
Diflorasone diacetate, 1374
Dioxybenzone and oxybenzone, 1418
Estradiol, vaginal, 1725
estriol compounded vaginal, 1743
Estropipate, vaginal, 1754
Flumethasone pivalate, 1928
Fluocinolone acetonide, 1935
Fluocinonide, 1937
Fluorometholone, 1952
Fluorouracil, 1956
Flurandrenolide, 1975
Fluticasone propionate, 1987
Gentamicin sulfate, 2101
Gentian violet, 2108
Halcinonide, 2184
Hydrocortisone, 2229
Hydrocortisone acetate, 2237
Hydrocortisone butyrate, 2239
Hydrocortisone valerate, 2245
Hydroquinone, 2255
Lidocaine and prilocaine, 2630
Lindane, 2635
Mafenide acetate, 2703
Miconazole nitrate, 2949
Mometasone furoate, 3002
Mupirocin, 3040
Naftifine hydrochloride, 3065
Neomycin and polymyxin B sulfates, 3114
Neomycin and polymyxin B sulfates and gramicidin, 3122
Neomycin and polymyxin B sulfates, gramicidin, and hydrocortisone acetate, 3123
Neomycin and polymyxin B sulfates and hydrocortisone acetate, 3125
Neomycin and polymyxin B sulfates and lidocaine, 3126
Neomycin and polymyxin B sulfates and pramoxine hydrochloride, 3126
Neomycin sulfate, 3103
Neomycin sulfate and dexamethasone sodium phosphate, 3104
Neomycin sulfate and fluocinolone acetonide, 3107
Neomycin sulfate and flurandrenolide, 3107
Neomycin sulfate and hydrocortisone, 3109
Neomycin sulfate and hydrocortisone acetate, 3110
Neomycin sulfate and methylprednisolone acetate, 3113
Neomycin sulfate and triamcinolone acetonide, 3129
Nystatin, 3219
Nystatin, neomycin sulfate, gramicidin, and triamcinolone acetonide, 3222
Nystatin, neomycin sulfate, thiostrepton, and triamcinolone acetonide, 3223
Nystatin and triamcinolone acetonide, 3224
Piroxicam, 3579
Pramoxine hydrochloride, 3641
Prednicarbate, 3656
Prednisolone, 3660
Sulfadiazine, silver, 4150
Sulfa, vaginal, triple, 4137
Tetracaine hydrochloride, 4296
Tolnaftate, 4426
Tretinoin, 4478
Triamcinolone acetonide, 4484

Creatine, 4920
Creatinine, 5734
Cresol, 5735
red, 6220
red–thymol blue TS, 6227
red TS, 6227
m-Cresol purple, 6154
TS, 6227
Cromolyn sodium, 1180
inhalation powder, 1182
inhalation solution, 1182
nasal solution, 1184
ophthalmic solution, 1185
Croscarmellose sodium, 5736
Crospovidone, 5737
Crotamiton, 1186
cream, 1187
Cryopreservation of cells ⟨1044⟩, 7389
Crypthecodinium cohnii oil, 4922
capsules, 4925
Crystallinity ⟨695⟩, 6931
Crystal violet, 6220
TS, 6232
Cupric
acetate, 6154
acetate TS, 6227
acetate TS, stronger, 6236
ammonium sulfate TS, 6227
chloride, 1187, 6154
chloride injection, 1188
citrate, 6154
citrate TS, 6228
citrate TS, alkaline, 6226, 6228
citrate TS 2, alkaline, 6226, 6228
iodide TS, alkaline, 6226, 6228
nitrate hydrate, 6154
nitrate, tenth-normal (0.1 N), 6239
oxide, ammoniated, TS, 6226, 6228, 6234, 6234
sulfate, 1189, 6154
sulfate, anhydrous, 6138, 6154
sulfate CS, 6225
sulfate injection, 1190
sulfate test paper, 6222
sulfate TS, 6222, 6228
tartrate, alkaline, solution (Fehling's solution), 6239
tartrate TS, alkaline, 6226, 6228, 6229
Cupriethylenediamine hydroxide solution, 1.0 M, 6154
Curcuminoids, 4927
capsules, 4929
tablets, 4930
Cyanoacetic acid, 6154
Cyanocobalamin, 1190
injection, 1191
tablets, 1192
Cyanocobalamin chewable gels, 4931
Cyanogen bromide, 6154
4-Cyanophenol, 6154
4-Cyanopyridine, 6154
Cyclam, 6154
Cyclandelate, 1193
Cyclizine hydrochloride, 1194
tablets, 1195
Cyclobenzaprine hydrochloride, 1196
extended-release capsules, 1197
tablets, 1199
1,1-Cyclobutanedicarboxylic acid, 6154
α-Cyclodextrin, 6154
β-Cyclodextrin, 6154
Cyclohexane, 6154
Cyclohexanol, 6154
(1,2-Cyclohexylenedinitrilo)tetraacetic acid, 6154
Cyclohexylmethanol, 6154
3-Cyclohexylpropionic acid, 6154
Cyclomethicone, 5740
Cyclopentolate hydrochloride, 1200
ophthalmic solution, 1201
Cyclophosphamide, 1202
for injection, 1204
tablets, 1209
Cyclophosphamide compounded oral suspension, 1208
Cyclopropane, 1210
Cycloserine, 1211
capsules, 1211
Cyclosporine, 1212
capsules, 1213
injection, 1214
oral solution, 1216
Cyclosporine compounded, veterinary ophthalmic solution, 1217
Cyproheptadine hydrochloride, 1218
oral solution, 1219
tablets, 1220
Cyromazine, 1221
Cysteine hydrochloride, 1221
injection, 1222

www.webofpharma.com

Cystine, 4933
L-Cystine, 6154
Cytarabine, 1223
for injection, 1224

D

Dacarbazine, 1225
for injection, 1226
Dactinomycin, 1227
for injection, 1228
Dalfampridine, 1228
Dalteparin
sodium, 1230
Danazol, 1233
capsules, 1234
Dantrolene sodium, 1234
capsules, 1236
for injection, 1238
Dapsone, 1239
oral suspension, 1240
tablets, 1241
Daunorubicin hydrochloride, 1242
for injection, 1242
DEAE-Agarose, 6154
Decanol, 6154
Decoquinate, 1243
premix, 1243
Decyl sodium sulfate, 6154
Deferoxamine mesylate, 1244
for injection, 1245
Dehydrated alcohol, 6155
Dehydroacetic acid, 5741
Dehydrocholic acid, 1246
Delafield's hematoxylin TS, 6228
Deliverable volume ⟨698⟩, 6935
Delta-8-tetrahydrocannabinol, 6211
Demecarium bromide, 1246
ophthalmic solution, 1247
Demeclocycline, 1247
hydrochloride, 1248
hydrochloride tablets, 1249
Denatonium benzoate, 5741
Denaturated alcohol TS, 6228
Denigès' reagent, 6228
Density of solids ⟨699⟩, 6938
Dental paste
triamcinolone acetonide, 4485
Deoxyadenosine triphosphate, 6155
Deoxycytidine triphosphate, 6155
Deoxyguanosine triphosphate, 6155
Deoxyribonucleic acid polymerase, 6155
Deoxythymidine triphosphate, 6155
Depyrogenation ⟨1228⟩, 8176
Depyrogenation by filtration ⟨1228.3⟩, 8184
Depyrogenation by Rinsing, 8187
Deschloroclotrimazole, 6155
Description and relative solubility of USP and NF articles, 6273
Desflurane, 1253
Design, evaluation and characterization of viral clearance procedures ⟨1050.1⟩, 7472
Design and analysis of biological assays ⟨111⟩, 6543
Design and development of biological assays ⟨1032⟩, 7320
Desipramine hydrochloride, 1255
tablets, 1256
Deslanoside, 1258
injection, 1259
Desloratadine, 1259
tablets, 1261
orally disintegrating tablets, 1263
Desmopressin acetate, 1265
injection, 1266
nasal spray, 1267
Desogestrel, 1268
and ethinyl estradiol tablets, 1270
Desonide, 1271
Desoximetasone, 1272
cream, 1274
gel, 1275
ointment, 1275
Desoxycholic acid, 1276
Desoxycorticosterone
pivalate, 1278
pivalate injectable suspension, 1279
Desoxycorticosterone acetate, 6155
Desvenlafaxine, 1280
Desvenlafaxine fumarate, 1281
Desvenlafaxine succinate, 1283
Detection of irradiated dietary supplements ⟨2250⟩, 8722
Determination
methoxy ⟨431⟩, 6702
nitrogen ⟨461⟩, 6709
Deuterated ethanol, 6155
Deuterated methanol, 6155
Deuterated water, 6155
Deuterium
chloride, 6155
oxide, 6155
Deuterium chloride
solution, 6155
Deuterochloroform, 6155
Devarda's alloy, 6155
Dexamethasone, 1284
acetate, 1290
acetate injectable suspension, 1291
and ciprofloxacin otic suspension, 1021
elixir, 1286
injection, 1286
and neomycin and polymyxin B sulfates ophthalmic ointment, 3121
and neomycin and polymyxin B sulfates ophthalmic suspension, 3122
ophthalmic suspension, 1287
penicillin G procaine, dihydrostreptomycin sulfate, and chlorpheniramine maleate injectable suspension, 3445
sodium phosphate, 1292
sodium phosphate injection, 1294
sodium phosphate and neomycin sulfate cream, 3104
sodium phosphate and neomycin sulfate ophthalmic ointment, 3105
sodium phosphate and neomycin sulfate ophthalmic solution, 3106
sodium phosphate ophthalmic solution, 1296
oral solution, 1288
tablets, 1289
and tobramycin ophthalmic ointment, 4409
and tobramycin ophthalmic suspension, 4411
Dexamethasone sodium phosphate compounded
injection, 1295
Dexbrompheniramine maleate, 1296
and pseudoephedrine sulfate oral solution, 1298
Dexchlorpheniramine maleate, 1299
oral solution, 1301
tablets, 1301
Dexmedetomidine
injection, 1304
Dexmedetomidine hydrochloride, 1302
Dexpanthenol, 1305
assay ⟨115⟩, 6547
preparation, 1306
Dextran
1, 1307
40, 1309
40 in dextrose injection, 1311
40 in sodium chloride injection, 1312
70, 1313
70 in dextrose injection, 1315
70 in sodium chloride injection, 1316
high molecular weight, 6155
Dextrates, 5742
Dextrin, 5743, 6155
Dextro calcium pantothenate, 6155
Dextroamphetamine sulfate, 1316
tablets, 1318
Dextromethorphan, 1319
chlorpheniramine, pseudoephedrine (salts of), and acetaminophen, capsules containing at least three of the following, 52
chlorpheniramine, pseudoephedrine (salts of), and acetaminophen, oral powder containing at least three of the following, 54
chlorpheniramine, pseudoephedrine (salts of), and acetaminophen, oral solution containing at least three of the following, 56
chlorpheniramine, pseudoephedrine (salts of), and acetaminophen, tablets containing at least three of the following, 58
hydrobromide, 1320
hydrobromide, acetaminophen, doxylamine succinate, and pseudoephedrine hydrochloride oral solution, 67
hydrobromide, guaifenesin, and pseudoephedrine hydrochloride capsules, 2175
hydrobromide, pseudoephedrine hydrochloride, and carbinoxamine maleate oral solution, 3767
hydrobromide oral solution, 1321
hydrobromide, acetaminophen, and chlorpheniramine maleate tablets, 60
Dextrose, 1321
adenine solution, anticoagulant citrate phosphate, 348
anhydrous, 6156
and dopamine hydrochloride injection, 1495
excipient, 5743
and half-strength lactated Ringer's injection, 3895
injection, 1323
injection, alcohol in, 117
injection, bretylium tosylate in, 591
injection, bupivacaine hydrochloride in, 612
injection, dobutamine in, 1470
injection, magnesium sulfate in, 2727
injection, potassium chloride in, 3607
injection and potassium chloride in lactated ringer's, 3610
injection and sodium chloride injection, potassium chloride in, 3608
injection, tetracaine hydrochloride in, 4300
injection, theophylline in, 4328

www.webofpharma.com

Dextrose *(continued)*
injection type 1 and multiple electrolytes, 1598
injection type 2 and multiple electrolytes, 1601
injection type 3 and multiple electrolytes, 1605
and lactated Ringer's injection, 3893
and lidocaine hydrochloride injection, 2628
and modified lactated Ringer's injection, 3898
and Ringer's injection, 3889
and sodium chloride injection, 1323
solution, anticoagulant citrate, 345
solution, anticoagulant citrate phosphate, 347
Diacetyl, 6156
Diacetylated monoglycerides, 5745
3,3′-Diaminobenzidine hydrochloride, 6156
2,3-Diaminonaphthalene, 6156
2,6-Diaminopyridine, 6156
Diatomaceous earth, 6156
flux-calcined, 6156
silanized, 6156
Diatomaceous silica
calcined, 6156, 6199
Diatrizoate
meglumine, 1324
meglumine and diatrizoate sodium injection, 1326
meglumine and diatrizoate sodium solution, 1327
meglumine injection, 1325
sodium, 1327
sodium and diatrizoate meglumine injection, 1326
sodium and diatrizoate meglumine solution, 1327
sodium injection, 1328
sodium solution, 1329
Diatrizoic acid, 1329
Diaveridine, 6156
Diazepam, 1330
injection, 1331
tablets, 1332
Diazobenzenesulfonic acid TS, 6228
Diazoxide, 1333
capsules, 1333
injection, 1334
oral suspension, 1335
Dibasic
ammonium citrate, 6156
ammonium phosphate, 6156
calcium phosphate, anhydrous, 710
calcium phosphate dihydrate, 708
calcium phosphate tablets, 712
potassium phosphate, 3628, 6156
sodium phosphate, 4087
Dibenzyl, 6156
2,6-Dibromoquinone-chlorimide, 6156
Dibucaine, 1336
cream, 1336
hydrochloride, 1337
hydrochloride injection, 1338
ointment, 1337
Dibutyl
phthalate, 5745, 6156
sebacate, 5746
Dibutylamine, 6156
Dibutylammonium phosphate, 6156
1,3-Dicaffeoylquinic acid, 6157
Dichloralphenazone, 1338
isometheptene mucate and acetaminophen capsules, 2423
Dichloroacetic acid, 6157
2,5-Dichloroaniline, 6157
2,6-Dichloroaniline, 6157
o-Dichlorobenzene, 6157
1,2-Dichloroethane, 6157
Dichlorofluorescein, 6157
TS, 6228
Dichlorofluoromethane, 6157
2,6-Dichloroindophenol sodium, 6157
Dichloromethane, 6157
2,4-Dichloro-1-naphthol, 6157
2,6-Dichlorophenol-indophenol sodium, 6157, 6157
Dichlorophenol-indophenol solution, standard, 6240
2,6-Dichlorophenylacetic acid, 6157
2,6-Dichloroquinone-chlorimide, 6157
Dichlorphenamide, 1339
tablets, 1340
Diclazuril, 1341
Diclofenac potassium, 1343
tablets, 1345
Diclofenac potassium for oral solution, 1344
Diclofenac sodium, 1347
and misoprostol delayed-release tablets, 1353
delayed-release tablets, 1348
extended-release tablets, 1350
Diclofenac sodium
topical solution, 1352
Dicloxacillin sodium, 1357
capsules, 1358
Dicyclohexyl, 6157
Dicyclohexylamine, 6158
Dicyclohexyl phthalate, 6157
Dicyclomine hydrochloride, 1359
capsules, 1360
injection, 1361
oral solution, 1361
tablets, 1362
Didanosine, 1363
delayed-release capsules, 1364
for oral solution, 1366
tablets for oral suspension, 1367

Dietary Supplements

Chinese skullcap root, 4883
Chinese skullcap root dry extract, 4887
Chinese skullcap root powder, 4885
L-alpha-glycerylphosphorylcholine, 5055
Omega-3 free fatty acids, 5188
N-acetylglucosamine, 4745
Ademetionine disulfate tosylate, 4747
L-Alanyl-L-glutamine, 4748
Andrographis, 4758
Andrographis, powdered, 4760
Andrographis extract, powdered, 4762
Arginine capsules, 4763
Arginine tablets, 4764
Ascorbic acid chewable gels, 4765
Ashwagandha root, 4766
Ashwagandha root extract, powdered, 4768
Ashwagandha root, powdered, 4771
Astaxanthin esters, 4779
Astragalus root, 4782
Astragalus root dry extract, 4787
Astragalus root powder, 4785
Aztec marigold zeaxanthin extract, 4789
Bacillus coagulans, 4791
Bacillus coagulans capsules, 4794
Bacillus subtilis subsp. *subtilis* menaquinone-7 extract, 5152
Bacopa, 4797
Bacopa, powdered, 4798
Bacopa extract, powdered, 4800
Banaba leaf, 4801
Banaba leaf dry extract, 4805
Banaba leaf powder, 4803
Beta carotene preparation, 4806
Beta glucan, 4808
Bifidobacterium animalis subsp. *lactis*, 4811
Bilberry extract, powdered, 4813
Black cohosh, 4815
Black cohosh, powdered, 4817
Black cohosh extract, powdered, 4820
Black cohosh tablets, 4823
Black pepper, 4825
Powdered black pepper extract, 4829
Powdered black pepper, 4827
Borage seed oil, 4830
Borage seed oil capsules, 4831
Boswellia serrata, 4832
Boswellia serrata extract, 4834
Calcium citrate tablets, 4835
Calcium L-5-methyltetrahydrofolate, 4838
Calcium L-5-methyltetrahydrofolate capsules, 4841
Calcium L-5-methyltetrahydrofolate tablets, 4842
Calcium and vitamin D with minerals tablets, 4845
Calcium with vitamin D tablets, 4843
Carotenes, 4850
Cat's claw, 4852
Cat's claw capsules, 4857
Cat's claw extract, powdered, 4855
Cat's claw, powdered, 4854
Cat's claw tablets, 4858
Centella asiatica, 4859
Centella asiatica, powdered, 4861
Centella asiatica extract, powdered, 4863
Centella asiatica triterpenes, 4864
Chamomile, 4866
Chaste tree, 4868
Chaste tree, powdered, 4870
Chaste tree extract, powdered, 4872
Chia seed oil, 4877
Chinese salvia, 4878
Chinese salvia, powdered, 4880
Cholecalciferol chewable gels, 4889
Choline bitartrate, 4891
Choline chloride, 4892
Chondroitin sulfate sodium, 4894
Chondroitin sulfate sodium, shark, 4898
Chondroitin sulfate sodium tablets, 4897
Chromium picolinate, 4901
Chromium picolinate tablets, 4902
Cinnamomum cassia twig, 4902
Cinnamomum cassia twig powder, 4904
Citrulline, 4908
Clover, red, 5203
Clover, powdered red, 5207
Clover extract, powdered red, 5210
Clover tablets, red, 5212
Cod liver oil capsules, 4910
Coffee fruit dry extract, 4912
Cohosh, black, fluidextract, 4821
Coix seed, 4915
Coixseedpowder, 4917
Cranberry liquid preparation, 4919
Crypthecodinium cohnii oil, 4922
Crypthecodinium cohnii oil capsules, 4925
Curcuminoids, 4927
Curcuminoids capsules, 4929
Curcuminoids tablets, 4930

www.webofpharma.com

Dietary Supplements *(continued)*
Cyanocobalamin chewable gels, 4931
Diosmin, 4934
Echinacea angustifolia, 4935
Echinacea angustifolia, powdered, 4938
Echinacea angustifolia extract, powdered, 4941
Echinacea pallida, 4944
Echinacea pallida, powdered, 4946
Echinacea pallida, powdered, extract, 4948
Echinacea purpurea aerial parts, 4950
Echinacea purpurea, powdered, 4955
Echinacea purpurea, powdered, extract, 4958
Echinacea purpurea root, 4953
Echinacea Species Dry Extract Capsules, 4960
Echinacea Species Dry Extract Tablets, 4963
Echinacea species powder capsules, 4966
Eleuthero, 4969
Eleuthero, powdered, 4975
Eleuthero extract, powdered, 4970
Eleuthero root and rhizome dry extract capsules, 4972
Eleuthero root and rhizome dry extract tablets, 4973
Eleuthero root and rhizome powder capsules, 4976
European elder berry dry extract, 4978
Evening primrose oil, 4980
Evening primrose oil capsules, 4980
Fenugreek seed, 4982
Fenugreek seed powder, 4984
Fenugreek seed powdered extract, 4987
Feverfew, 4989
Feverfew, powdered, 4990
Fish oil containing omega-3 acids, 4992
Fish oil containing omega-3 acids capsules, 4994
Fish oil containing omega-3 acids delayed-release capsules, 4997
Flax seed oil, 4998
Flax seed oil capsules, 4999
Forskohlii, 5000
Powdered forskohlii, 5002
Powdered forskohlii extract, 5003
Ganoderma lucidum fruiting body, 5004
Ganoderma lucidum fruiting body powder, 5008
Garcinia cambogia, 5011
Garcinia cambogia, powdered, 5012
Garcinia hydroxycitrate extract, powdered, 5014
Garcinia indica, 5015
Garcinia indica, powdered, 5016
Garlic, 5018
Garlic, powdered, 5020
Garlic extract, powdered, 5022
Garlic fluidextract, 5023
Garlic delayed-release tablets, 5024
Ginger, 5026
Ginger, powdered, 5028
Ginger capsules, 5032
Ginger tincture, 5030
Ginkgo, 5034
Ginkgo extract, powdered, 5038
Ginkgo capsules, 5037
Ginkgo tablets, 5041
Ginseng, American, 4750
Ginseng, American, capsules, 4754
Ginseng, American, powdered, 4752
Ginseng, American extract, powdered, 4753
Ginseng, American, tablets, 4757
Ginseng, Asian, 4774
Ginseng, Asian, powdered, 4775
Ginseng, Asian extract, powdered, 4776
Ginseng, Asian, tablets, 4778
Glucosamine and chondroitin sulfate sodium tablets, 5043
Glucosamine hydrochloride, 5045
Glucosamine tablets, 5046
Glucosamine sulfate potassium chloride, 5047
Glucosamine sulfate sodium chloride, 5048
Glucosamine and methylsulfonylmethane tablets, 5049
Glucosamine, chondroitin sulfate sodium, and methylsulfonylmethane tablets, 5051
Glutamic acid, 5053
Glutathione, 5054
Glycyl-L-glutamine, 5058
Glycyl-L-tyrosine, 5059
Goldenseal, 5061
Goldenseal, powdered extract, 5064
Goldenseal, powdered, 5063
Grape seeds oligomeric proanthocyanidins, 5065
Green tea extract, decaffeinated, powdered, 5067
Guggul, 5069
Guggul extract, native, 5070
Guggul extract, purified, 5071
Guggul tablets, 5072
Gymnema, 5074
Gymnema extract, native, 5077
Gymnema, powdered, 5075
Gymnema extract, purified, 5078
Hawthorn leaf with flower, 5080
Hawthorn leaf with flower, powdered, 5082
Hesperidin, 5084
Holy basil leaf, 5085
Holy basil leaf powdered, 5087
Holy basil leaf extract, powdered, 5089
Horse chestnut, 4873
Horse chestnut, powdered, 4875
Horse chestnut extract, powdered, 4876
5-Hydroxy-L-tryptophan, 5313
Japanese honeysuckle flower, 5091
Japanese honeysuckle flower dry extract, 5094
Japanese honeysuckle flower powder, 5097
Krill oil capsules, 5104
Krill oil delayed-release capsules, 5107
Lactobacillus acidophilus La-14, 5111
Lactobacillus acidophilus NCFM, 5113
Lactobacillus paracasei LPC-37, 5115
Lactobacillus rhamnosus HN001, 5117
Licorice, 5119
Licorice, powdered, 5120
Licorice extract, powdered, 5121
Ground limestone, 5121
Linoleic acids-free fatty acids, conjugated, 5123
Lipoic acid, alpha, 5124
Lipoic acid capsules, alpha, 5125
Lipoic acid tablets, alpha, 5126
Lutein, 5126
Lutein capsules, 5128
Lutein preparation, 5128
Lycopene, 5130
Lycopene preparation, 5131
Lysine hydrochloride tablets, 5135
Malabar-nut-tree, leaf, 5136
Malabar-nut-tree, leaf, powdered, 5137
Malabar-nut-tree, leaf extract, powdered, 5138
Maritime pine, 5139
Maritime pine extract, 5141
Melatonin, 5143
Melatonin tablets, 5144
Menaquinone-4, 5145
Menaquinone-7, 5146
Menaquinone-7 capsules, 5147
Menaquinone-7 preparation, 5149
Menaquinone-7 tablets, 5151
Methylcobalamin, 5153
Methylcobalamin tablets, 5154
Methylsulfonylmethane, 5155
Methylsulfonylmethane tablets, 5156
Milk thistle, 5157
Milk thistle, powdered, 5159
Milk thistle extract, powdered, 5160
Milk thistle capsules, 5162
Milk thistle tablets, 5163
Minerals capsules, 5164
Minerals tablets, 5172
Northern schisandra fruit, 5261
Northern schisandra fruit dry extract, 5263
Northern schisandra fruit powder, 5265
Oliveleaf, 5181
Olive leaf dry extract, 5182
Olive leaf powder, 5184
Omega-3 acids triglycerides, 5185
Phyllanthus amarus, 5191
Phyllanthus amarus, powdered, 5193
Plant stanol esters, 5194
Potassium citrate tablets, 5196
Powdered *Rhodiola rosea*, 5220
Powdered *Rhodiola rosea* extract, 5221
Powdered rosemary, 5232
Pygeum extract, 5198
Quercetin, 5202
Red clover aerial parts isoflavone aglycones dry extract, 5206
Rhodiola crenulata root and rhizome, 5213
Rhodiola crenulata root and rhizome dry extract, 5215
Rhodiola crenulata root and rhizome powder, 5216
Rhodiola rosea, 5218
Rhodiola rosea capsules, 5224
Rhodiola rosea tablets, 5227
Rhodiola rosea tincture, 5223
Ribose, 5229
Rosemary, 5230
Rosemary leaf dry aqueous extract, 5234
Rutin, 5235
St. John's wort flowering top, 5237
St. John's wort flowering top dry extract capsules, 5242
St. John's wort flowering top extract, dry, 5240
St. John's wort flowering top powder, 5238
St. John's wort flowering top dry extract tablets, 5244
Salix species bark, 5245
Salix species bark dry extract, 5247
Salix species bark powder, 5249
Saw palmetto, 5251
Saw palmetto, powdered, 5254
Saw palmetto capsules, 5259
Saw palmetto extract, 5256
Schizochytrium oil, 5266
Schizochytrium oil capsules, 5269
Selenomethionine, 5272
Sodium ferrous citrate, 5273
Soy isoflavones capsules, 5276
Soy isoflavones extract, powdered, 5274
Soy isoflavones tablets, 5278
Spirulina, 5279

www.webofpharma.com

Dietary Supplements *(continued)*
Spirulina tablets, 5283
Stinging nettle, 5286
Stinging nettle, powdered extract, 5290
Stinging nettle, powdered, 5288
Tangerine peel, 5292
Tangerine peel dry extract, 5294
Tangerine peel powder, 5296
Tienchi ginseng root and rhizome, 5299
Tienchi ginseng root and rhizome dry extract capsules, 5309
Tienchi ginseng root and rhizome dry extract, 5307
Tienchi ginseng root and rhizome powder, 5301
Tienchi ginseng root and rhizome powder capsules, 5304
Tienchi ginseng root and rhizome dry extract tablets, 5311
Tienchi ginseng root and rhizome powder tablets, 5306
Tomato extract containing lycopene, 5133
Turmeric, 5314
Turmeric, powdered, 5316
Turmeric extract, powdered, 5317
Ubidecarenone, 5319
Ubidecarenone capsules, 5320
Ubidecarenone tablets, 5321
Ubiquinol, 5322
Ubiquinol capsules, 5323
Valerian, 5324
Valerian, powdered, 5326
Valerian extract, powdered, 5327
Valerian root dry extract capsules, 5332
Valerian root powder capsules, 5333
Valerian tablets, 5329
Valerian tincture, 5330
Vinpocetine, 5335
Vinpocetine capsules, 5336
Vinpocetine tablets, 5337
Vitamin A oral liquid preparation, 4634
Vitamins capsules, oil- and water-soluble, 5389
Vitamins with minerals capsules, oil- and water-soluble, 5435
Vitamins with minerals capsules, water-soluble, 5523
Vitamins with minerals oral solution, water-soluble, 5543
Vitamins with minerals tablets, oil- and water-soluble, 5476
Vitamins with minerals tablets, water-soluble, 5552
Vitamins tablets, oil- and water-soluble, 5419
Vitamins capsules, oil-soluble, 5346
Vitamins capsules, water-soluble, 5499
Vitamins with minerals oral solution, oil- and water-soluble, 5462
Oil-soluble vitamins with minerals capsules, 5361
Oil-soluble vitamins with minerals oral solution, 5372
Oil-soluble vitamins with minerals tablets, 5378
Vitamins oral solution, oil- and water-soluble, 5409
Oil-soluble vitamins oral solution, 5353
Vitamins tablets, oil-soluble, 5356
Vitamins tablets, water-soluble, 5512
Vitamins with minerals oral powder, 5338
meso-Zeaxanthin, 5570
meso-Zeaxanthin preparation, 5572
Zinc citrate, 5574
Zinc citrate tablets, 5575
Zinc and vitamin C lozenges, 5576

Diethanolamine, 5747
Diethyl phthalate, 6158
Diethylamine, 6158
Diethylamine phosphate, 6158
N,N-Diethylaniline, 6158
Diethylcarbamazine citrate, 1368
tablets, 1369
Diethylene glycol, 6158
monoethyl ether, 5749
stearates, 5751
succinate polyester, 6158
Di(ethylene glycol) methyl ether, 6158
Diethylenetriamine, 6158
Di(2-ethylhexyl)phthalate, 6158
Diethyl phthalate, 5748
Diethylpropion hydrochloride, 1370
tablets, 1371
Diethylpyrocarbonate, 6158
Diethyl sebacate, 5748
Diethylstilbestrol, 1372
Diethyl sulfone, 6158
Diethyltoluamide, 1372
topical solution, 1373
Diflorasone diacetate, 1374
cream, 1374
ointment, 1375
Diflunisal, 1375
tablets, 1376
Digitalis, 1377
capsules, 1380
powdered, 1379
tablets, 1380
Digitonin, 6158
Digitoxin, 1381
injection, 1381
tablets, 1382
Digoxigenin, 6158
Digoxin, 1383
injection, 1384
oral solution, 1385
tablets, 1385
Dihydrocodeine bitartrate, 1386
aspirin and caffeine capsules, 402
Dihydroergotamine mesylate, 1388
injection, 1389
7,8-Dihydrofolic acid, 6158
24,25-Dihydrolanosterol, 6158
Dihydroquinidine hydrochloride, 6158
Dihydroquinine, 6158
Dihydrostreptomycin
injection, 1391
sulfate, 1390
sulfate boluses, 1391
sulfate, penicillin G procaine, chlorpheniramine maleate, and dexamethasone injectable suspension, 3445
sulfate and penicillin G procaine injectable suspension, 3444
sulfate and penicillin G procaine intramammary infusion, 3444
sulfate, penicillin G procaine, and prednisolone injectable suspension, 3447
Dihydrotachysterol, 1391
capsules, 1392
oral solution, 1392
tablets, 1393
Dihydroxyacetone, 1393
Dihydroxyaluminum
aminoacetate, 1394
aminoacetate magma, 1395
sodium carbonate, 1395
sodium carbonate chewable tablets, 1396
Dihydroxybenzaldehyde, 6159
2,5-Dihydroxybenzoic acid, 6159
2,7-Dihydroxynaphthalene, 6159
2,7-Dihydroxynaphthalene TS, 6228
Dihydroxyphenylacetone, 6159
4,5-Dihydroxy-3-(*p*-sulfophenylazo)-2,7-napthalenedisulfonic acid, trisodium salt, 6220
Diiodofluorescein, 6159
TS, 6228
Diisodecyl phthalate, 6159
Diisopropanolamine, 5752
Diisopropyl ether, 6159, 6163, 6174
Diisopropylamine, 6159
Diisopropylethylamine, 6159
n,n′-Diisopropylethylenediamine, 6159
1,2-Dilinoleoyl-3-oleoyl-rac-glycerol, 6159
1,2-Dilinoleoyl-3-palmitoyl-rac-glycerol, 6159
Diloxanide furoate, 1397
Diltiazem hydrochloride, 1398
extended-release capsules, 1399
oral solution, 1405
oral suspension, 1406
tablets, 1406
Diluted
acetic acid, 5599, 6159
alcohol, 6159
hydrochloric acid, 6159
lead subacetate TS, 6228
nitric acid, 6159
sulfuric acid, 6159
Dimenhydrinate, 1408
injection, 1409
oral solution, 1410
tablets, 1410
Dimercaprol, 1411
injection, 1412
Dimethicone, 5753
viscosity 500 centistokes, 6159
2,5-Dimethoxybenzaldehyde, 6159
1,2-Dimethoxyethane, 6160
Dimethoxymethane, 6160
(3,4-Dimethoxyphenyl)-acetonitrile, 6160
Dimethyl
phthalate, 6160
sulfone, 6160
sulfoxide, 1413, 6180
sulfoxide gel, 1413
sulfoxide irrigation, 1414
sulfoxide topical solution, 1414
sulfoxide spectrophotometric grade, 6160
N,N-Dimethylacetamide, 6160
p-Dimethylaminoazobenzene, 6160
p-Dimethylaminobenzaldehyde, 6160
TS, 6228
p-Dimethylaminocinnamaldehyde, 6160
2-Dimethylaminoethyl methacrylate, 6160
Dimethylaminophenol, 6160
Dimethylaniline ⟨223⟩, 6636
2,6-Dimethylaniline, 6160
N,N-Dimethylaniline, 6160
3,4-Dimethylbenzophenone, 6160
Dimethylcarbonate, 6161
5,5-Dimethyl-1,3-cyclohexanedione, 6161
N,N-Dimethyldecylamine, 6161
1,5-Dimethyl-1,5-diazaundecamethylene polymethobromide, 6161
N,N-Dimethyldodecylamine-*N*-oxide, 6161
Dimethylethyl(3-hydroxyphenyl)ammonium chloride, 6161
Dimethylformamide, 6161
N,N-Dimethylformamide diethyl acetal, 6161
1,3-Dimethyl-2-imidazolidinone, 6161

www.webofpharma.com

1,9-Dimethyl-methylene blue, 6161
N,N-Dimethyl-1-naphthylamine, 6161
N,N-Dimethyloctylamine, 6161
2,5-Dimethylphenol, 6161
2,6-Dimethylphenol, 6161
3,5-Dimethylphenol, 6161
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 6161
Dimethyltin dibromide, 6161
N,N-Dimethyl-*p*-phenylenediamine dihydrochloride, 6161
Dimidium
bromide, 6162
m-Dinitrobenzene, 6162
3,5-Dinitrobenzoyl chloride, 6162
2,4-Dinitrochlorobenzene, 6162
2,4-Dinitrofluorobenzene, 6162
2,4-Dinitrophenylhydrazine, 6162
Dinitrophenylhydrazine TS, 6228
Dinoprost tromethamine, 1415
injection, 1416
Dinoprostone, 1417
Dioctyl sodium sulfosuccinate, 6162
Diosmin, 4934
Dioxane, 6162
Dioxybenzone, 1418
and oxybenzone cream, 1418
Diphenhydramine
citrate, 1419
citrate and acetaminophen tablets, 69
citrate and ibuprofen tablets, 1421
hydrochloride, 1423
hydrochloride, acetaminophen, and pseudoephedrine hydrochloride tablets, 70
hydrochloride capsules, 1425
hydrochloride injection, 1426
hydrochloride oral solution, 1429
hydrochloride and ibuprofen capsules, 1431
and phenylephrine hydrochloride tablets, 1433
and pseudoephedrine capsules, 1435
Diphenoxylate hydrochloride, 1437
and atropine sulfate oral solution, 1438
and atropine sulfate tablets, 1439
Diphenyl ether, 6163, 6189
Diphenylamine, 6162
TS, 6228
Diphenylborinic acid, ethanolamine ester, 6135, 6162
Diphenylcarbazide, 6162
Diphenylcarbazone, 6162
TS, 6228
2,2-Diphenylglycine, 6162
Diphtheria antitoxin potency testing for human immune globulins ⟨162⟩, 6582
Dipicolinic acid, 6162
Dipicrylamine, 6162
Dipivefrin hydrochloride, 1440
ophthalmic solution, 1442
Dipropyl phthalate, 6162
Dipyridamole, 1443
injection, 1445
oral suspension, 1445
tablets, 1446
4,4′-Dipyridyl, 6162
α,α′-Dipyridyl, 6162
Direct red 80, 6197
Dirithromycin, 1447
delayed-release tablets, 1448
Disinfectants and antiseptics ⟨1072⟩, 7608
Disintegration
⟨701⟩, 6940
and dissolution of dietary supplements ⟨2040⟩, 8711
Disodium
chromotropate, 6162
ethylenediaminetetraacetate, 6162
Disopyramide phosphate, 1449
capsules, 1450
extended-release capsules, 1450
Dissolution ⟨711⟩, 6945
The dissolution procedure: development and validation ⟨1092⟩, 7707
Distilling range ⟨721⟩, 6955
Disulfiram, 1451
tablets, 1452
5,5′-Dithiobis (2-nitrobenzoic acid), 6162
Dithiothreitol, 6162
Dithizone, 6162
TS, 6228
Divalproex sodium, 1453
delayed-release capsules, 1454
delayed-release tablets, 1457
extended-release tablets, 1458
Dobutamine
in dextrose injection, 1470
hydrochloride, 1466
injection, 1468
for injection, 1469
Docetaxel, 1471
injection, 1474
Docusate
calcium, 1475
calcium capsules, 1476
potassium, 1477
potassium capsules, 1478
sodium, 1479
sodium capsules, 1479
sodium and ferrous fumarate extended-release tablets, 1856
sodium solution, 1480
sodium syrup, 1481
sodium tablets, 1481
1-Dodecanol, 6162
Dodecyl
alcohol, 6162
lithium sulfate, 6162
sodium sulfonate, 6163
3-(Dodecyldimethylammonio) propanesulfonate, 6162
Dodecyltriethylammonium phosphate, 0.5 M, 6163
Dodecyltrimethylammonium bromide, 6163
Dofetilide, 1482
Dolasetron mesylate, 1483
oral solution, 1485
oral suspension, 1485
Donepezil hydrochloride, 1486
tablets, 1488
orally disintegrating tablets, 1492
Dopamine hydrochloride, 1494
and dextrose injection, 1495
injection, 1495
Dorzolamide
Hydrochloride and timolol maleate ophthalmic solution, 1499
Dorzolamide hydrochloride
ophthalmic solution, 1497
Dorzolamide hydrochloride, 1496
Doxapram hydrochloride, 1502
injection, 1503
Doxazosin mesylate, 1504
tablets, 1506
Doxepin hydrochloride, 1507
capsules, 1509
oral solution, 1510
Doxercalciferol, 1510
Doxorubicin hydrochloride, 1512
injection, 1514
for injection, 1516
Doxycycline, 1518
calcium oral suspension, 1529
capsules, 1519
extended-release capsules, 1521
hyclate, 1532
hyclate capsules, 1534
hyclate delayed-release capsules, 1536
hyclate tablets, 1537
hyclate delayed-release tablets, 1539
for injection, 1523
for oral suspension, 1525
tablets, 1527
Doxycycline compounded, veterinary oral suspension, 1530
Doxylamine succinate, 1544
acetaminophen, dextromethorphan hydrobromide, and pseudoephedrine hydrochloride oral solution, 67
oral solution, 1546
tablets, 1546
Drabkin's reagent, 6163
Dragendorff's TS, 6228
Dried peptone, 6163
Dronabinol, 1547
capsules, 1549
Dronedarone
hydrochloride, 1549
tablets, 1551
Droperidol, 1552
injection, 1553
Drospirenone, 1554
and ethinyl estradiol tablets, 1556
Drug release ⟨724⟩, 6957
Dry heat depyrogenation ⟨1228.1⟩, 8181
Dry heat sterilization ⟨1229.8⟩, 8225
Duloxetine
delayed-release capsules, 1559
Duloxetine hydrochloride, 1565
Dusting powder, absorbable, 1569
Dutasteride, 1567
Dyclonine hydrochloride, 1570
gel, 1570
topical solution, 1571
Dydrogesterone, 1571
tablets, 1572
Dyphylline, 1572
and guaifenesin oral solution, 1575
and guaifenesin tablets, 1575
injection, 1573
oral solution, 1573
tablets, 1574

E

Earth, chromatographic, silanized, acid-base washed, 6163
Ecamsule
solution, 1576
Echinacea
angustifolia, 4935
angustifolia, powdered, 4938
angustifolia extract, powdered, 4941
pallida, 4944
pallida, powdered, 4946
pallida extract, powdered, 4948
purpurea aerial parts, 4950
purpurea, powdered, 4955
purpurea root, 4953

www.webofpharma.com

Echinacea *(continued)*
purpurea extract, powdered, 4958
Echinacea species
dry extract capsules, 4960
dry extract tablets, 4963
powder capsules, 4966
Echothiophate
iodide, 1578
iodide for ophthalmic solution, 1579
Econazole nitrate, 1580
Edetate
calcium disodium, 1582
calcium disodium injection, 1583
disodium, 1583, 6162, 6163
disodium injection, 1584
disodium TS, 6228
disodium, twentieth-molar (0.05 M), 6240
Edetate disodium
0.01 M TS, 6228
0.002 M VS, 6240
0.01 M VS, 6240
0.02 M VS, 6240
Edetic acid, 5754, 6163
Edrophonium
chloride, 1584
chloride injection, 1585
Efavirenz, 1585
capsules, 1588
Tablets, 1591
Egg phospholipids, 5755
n-Eicosane, 6163
Eicosanol, 6163
Elastomeric closures for injections ⟨381⟩, 6669
Electrolytes
and dextrose injection type 1, multiple, 1598
and dextrose injection type 2, multiple, 1601
and dextrose injection type 3, multiple, 1605
and polyethylene glycol 3350 for oral solution, 3587
injection type 1, multiple, 1593
injection type 2, multiple, 1595
Elemental contaminants in dietary supplements ⟨2232⟩, 8719
Elemental impurities—limits ⟨232⟩, 6641
Elemental impurities—procedures ⟨233⟩, 6645
Elements
injection, trace, 1608
Eleuthero, 4969
extract, powdered, 4970
powdered, 4975
Eleuthero
root and rhizome dry extract capsules, 4972
root and rhizome dry extract tablets, 4973
root and rhizome powder capsules, 4976

Elixir

Aromatic, 5624
Benzaldehyde, compound, 5634
Dexamethasone, 1286
Fluphenazine hydrochloride, 1971
Hyoscyamine sulfate, 2276

Elm, 1609
Emedastine
difumarate, 1610
ophthalmic solution, 1610
Enalapril maleate, 1611
and hydrochlorothiazide tablets, 1616
tablets, 1614
Enalaprilat, 1619
injection, 1619
Enalapril maleate
oral suspension, 1613
Enalapril maleate compounded, veterinary
oral suspension, 1613
Endotoxin indicator for depyrogenation, 1621
Endotoxin indicators for depyrogenation ⟨1228.5⟩, 8190
Enflurane, 1622
Enoxaparin sodium, 1623
injection, 1626
Enrofloxacin, 1628
Enrofloxacin compounded, veterinary
oral suspension, 1630
Ensulizole, 1631
Entacapone, 1631
tablets, 1632
Entecavir, 1634
oral solution, 1636
tablets, 1637
Enzacamene, 1639
Enzymatically-hydrolyzed carboxymethylcellulose sodium, 5685
Enzymes used as ancillary materials in pharmaceutical manufacturing ⟨89⟩, 6521
Eosin Y, 6163, 6220
TS, 6228
Ephedrine, 1640
hydrochloride, 1641
hydrochloride, theophylline, and phenobarbital tablets, 4330
sulfate, 1642
sulfate capsules, 1642
sulfate injection, 1643
sulfate nasal solution, 1644
sulfate oral solution, 1644
Epiandrosterone, 6163
4-Epianhydrotetracycline ⟨226⟩, 6636
15-Epicarboprost, 6163
Epinephrine, 1645
and articaine hydrochloride injection, 381
assay ⟨391⟩, 6676
bitartrate, 1650
bitartrate ophthalmic solution, 1651
bitartrate for ophthalmic solution, 1652
and bupivacaine hydrochloride injection, 612
inhalation solution, 1648
injection, 1648
and lidocaine hydrochloride injection, 2629
nasal solution, 1649
ophthalmic solution, 1649
and prilocaine injection, 3679
and procaine hydrochloride injection, 3694
Epinephryl borate ophthalmic solution, 1652
Epirubicin hydrochloride, 1653
injection, 1654
Epitetracycline hydrochloride, 1656
Epoetin, 1657
Eprinomectin, 1660
Eprosartan mesylate, 1662
Equilenin, 6163
Equilin, 1664
Ergocalciferol, 1665
capsules, 1666
oral solution, 1667
tablets, 1668
α-Ergocryptine, 6163
Ergoloid mesylates, 1669
capsules, 1670
oral solution, 1671
tablets, 1671
Ergonovine maleate, 1672
injection, 1673
tablets, 1674
Ergotamine tartrate, 1675
and caffeine suppositories, 1680
and caffeine tablets, 1681
injection, 1677
sublingual tablets, 1679
tablets, 1678
Eriochrome
black T, 6220
black TS, 6229
black T–sodium chloride indicator, 6222
black T trituration, 6221
cyanine R, 6163
cyanine TS, 6229
Erythorbic acid, 5756
Erythritol, 5757
Erythromycin, 1683
and benzoyl peroxide topical gel, 1692
delayed-release capsules, 1686
estolate, 1693
estolate capsules, 1693
estolate and sulfisoxazole acetyl oral suspension, 1695
estolate oral suspension, 1693
estolate for oral suspension, 1694
estolate tablets, 1694
ethylsuccinate, 1696
ethylsuccinate injection, 1698
ethylsuccinate, sterile, 1698
ethylsuccinate and sulfisoxazole acetyl for oral suspension, 1701
ethylsuccinate oral suspension, 1698
ethylsuccinate for oral suspension, 1699
ethylsuccinate tablets, 1699
topical gel, 1686
gluceptate, sterile, 1702
injection, 1687
intramammary infusion, 1687
lactobionate for injection, 1703
lactobionate, sterile, 1703
ointment, 1688
ophthalmic ointment, 1689
pledgets, 1690
topical solution, 1690
stearate, 1704
stearate tablets, 1706
tablets, 1691
delayed-release tablets, 1691
Erythropoietin bioassays ⟨124⟩, 6557
Escin, 6163
Escitalopram
oral solution, 1706
Escitalopram oxalate, 1711
Escitalopram
tablets, 1708
Esmolol hydrochloride, 1712
Esomeprazole magnesium, 1714
delayed-release capsules, 1716
Esomeprazole strontium, 1720
Estazolam, 1722
tablets, 1723
Estradiol, 1724
vaginal cream, 1725
vaginal inserts, 1726
transdermal system, 1728
tablets, 1732
benzoate, 1736

www.webofpharma.com

Estradiol *(continued)*
cypionate, 1737
cypionate injection, 1738
and norethindrone acetate tablets, 1733
valerate, 1739
valerate injection, 1741
Estriol, 1741
Estriol compounded vaginal cream, 1743
Estrogens
conjugated, 1744
esterified, 1749
tablets, conjugated, 1746
tablets, esterified, 1750
Estrone, 1751
injectable suspension, 1752
Estropipate, 1753
tablets, 1754
vaginal cream, 1754
Eszopiclone, 1755
tablets, 1757
Ethacrynate sodium for injection, 1759
Ethacrynic acid, 1760
tablets, 1761
Ethambutol hydrochloride, 1762
rifampin, isoniazid, and pyrazinamide tablets, 3881
Compounded oral suspension, 1763
tablets, 1764
Ethanesulfonic acid, 6163
Ethchlorvynol, 1765
capsules, 1766
Ether, 1767, 6163
absolute, 6131, 6163
diphenyl, 6163
isopropyl, 6163
nonyl phenyl polyethylene glycol, 6163
peroxide-free, 6164
Ethidium bromide, 6164
Ethinyl estradiol, 1768
and desogestrel tablets, 1270
and drospirenone tablets, 1556
and ethynodiol diacetate tablets, 1781
and levonorgestrel tablets, 2612
and norethindrone acetate tablets, 3208
and norethindrone tablets, 3201
and norgestimate tablets, 3211
and norgestrel tablets, 3214
tablets, 1768
Ethiodized oil injection, 1770
Ethionamide, 1771
tablets, 1771
Ethopabate, 1772
Ethosuximide, 1773
capsules, 1774
oral solution, 1776
Ethotoin, 1777
tablets, 1778
4′-Ethoxyacetophenone, 6164
2-Ethoxyethanol, 6164
Ethyl
acetate, 5759, 6164
acrylate, 6164
acrylate and methacrylic acid copolymer, 5878
acrylate and methacrylic acid copolymer, partially-neutralized, 5881
acrylate and methyl methacrylate copolymer dispersion, 5760
alcohol, 6164
arachidate, 6164
benzoate, 6164
chloride, 1779
cyanoacetate, 6164
ether, 6163, 6164
ether, anhydrous, 6131, 6163, 6164
maltol, 5761
oleate, 5762
salicylate, 6164
vanillin, 5763
Ethyl p-toluenesulfonate, 6164
2-Ethylaminopropiophenone hydrochloride, 6164
4-Ethylbenzaldehyde, 6164
Ethylbenzene, 6164
Ethylcellulose, 5763
aqueous dispersion, 5765
dispersion type b, 5766
Ethylene
dichloride, 6157, 6164
glycol, 6164
glycol, diethylene glycol, and triethylene glycol in ethoxylated substances ⟨469⟩, 6733
glycol monoethyl ether, 6164
glycol stearates, 5771
glycol and vinyl alcohol graft copolymer, 5769
oxide and dioxane ⟨228⟩, 6639
oxide in methylene chloride (50 mg/mL), 6165
Ethylenediamine, 1779, 6165
2-Ethylhexanoic acid, 6165
N-Ethylmaleimide, 6165
2-Ethyl-2-methylsuccinic acid, 6165
Ethylparaben, 5772, 6165
Ethylparaben sodium, 5773
1-Ethylquinaldinium iodide, 6165
Ethynodiol diacetate, 1780
and ethinyl estradiol tablets, 1781
and mestranol tablets, 1781
Etidronate disodium, 1782
tablets, 1784
Etodolac, 1785
capsules, 1786
tablets, 1787
extended-release tablets, 1788
Etomidate, 1789
injection, 1790
Etoposide, 1792
capsules, 1794
injection, 1795
Eucalyptol, 1797
Eucalyptus oil, 5774
Eugenol, 1797
European elder berry dry extract, 4978
Evaluation of plastic packaging systems and their materials of construction with respect to their user safety impact ⟨1661⟩, 8434
Evaluation of screening technologies for assessing medicine quality ⟨1850⟩, 8626
Evaluation of the inner surface durability of glass containers ⟨1660⟩, 8429
Evening primrose oil, 4980
capsules, 4980
Excipient biological safety evaluation guidelines ⟨1074⟩, 7613
Excipient performance ⟨1059⟩, 7528
Excipients
USP and NF, listed by category, 5584
Exemestane, 1798
Exenatide, 1800
injection, 1803
Expert committees (2015–2020), x
Food Chemicals Codex, xvi
National Formulary, xiv
United States Pharmacopeia, x
United States Pharmacopeia and the *Dietary Supplements Compendium*, xv
United States Pharmacopeia and *USP on Compounding*, xvi
Expert Panels for the Council of Experts Executive Committee, x
Extended release tablets
nevirapine, 3135

Extract

Andrographis, powdered, 4762
Ashwagandha root, powdered, 4768
Astragalus root, dry, 4787
Aztec Marigold Zeaxanthin Extract, 4789
Bacillus subtilis subsp. *subtilis* menaquinone-7, 5152
Bacopa, powdered, 4800
Banaba leaf, dry, 4805
Beef, 6141
Belladonna, 486
Belladonna tablets, 487
Bilberry, powdered, 4813
Black cohosh, powdered, 4820
Black pepper, powdered, 4829
Boswellia serrata, 4834
Cascara fluidextract, aromatic, 801
Cascara sagrada, 798
Cascara sagrada fluidextract, 801
Cat's claw, powdered, 4855
Centella asiatica, powdered, 4863
Chaste tree, powdered, 4872
Chinese skullcap root dry , 4887
Clover, red, powdered, 5210
coffee fruit dry, 4912
Echinacea angustifolia, powdered, 4941
Echinacea pallida, powdered, 4948
Echinacea purpurea, powdered, 4958
Eleuthero, powdered, 4970
European elder berry dry, 4978
Fenugreek seed, powdered, 4987
Garcinia hydroxycitrate, powdered, 5014
Garlic, powdered, 5022
Garlic fluidextract, 5023
Ginkgo, powdered, 5038
Ginseng, American, powdered, 4753
Ginseng, Asian, powdered, 4776
Goldenseal, powdered, 5064
Green tea, decaffeinated, powdered, 5067
Guggul, native, 5070
Guggul, purified, 5071
Gymnema, native, 5077
Gymnema, purified, 5078
Holy basil leaf powdered, 5089
Horse chestnut, powdered, 4876
Japanese honeysuckle flower, dry, 5094
Licorice, powdered, 5121
Licorice fluidextract, 5857
Malabar-nut-tree, leaf, powdered, 5138
Maritime pine, 5141
Milk thistle, powdered, 5160
Northern schisandra fruit, dry, 5263
Olive leaf dry, 5182
Powdered *Rhodiola rosea*, 5221
Pygeum, 5198
Pyrethrum, 3778
Red clover aerial parts isoflavone aglycones, dry, 5206
Rhodiola crenulata root and rhizome dry, 5215
Salix species bark dry, 5247
Saw palmetto, 5256
Senna fluidextract, 4025
Soy isoflavones, powdered, 5274
Stinging nettle, powdered, 5290

www.webofpharma.com

Extract *(continued)*
St. John's wort flowering top, dry, 5240
Tangerine peel, dry, 5294
Tienchi ginseng root and rhizome, dry, 5307
Tomato, containing lycopene, 5133
Turmeric, powdered, 5317
Valerian, powdered, 5327
Valerian root dry, capsules, 5332
Yeast, 6219

Ezetimibe, 1804
tablets, 1806

F

F 18
injection, fludeoxyglucose, 1946
injection, sodium fluoride, 1950
Factor IX complex, 1808
Factor X_a (activated factor X) for anti-factor X_a test, 6165
Famciclovir, 1808
tablets, 1812
Famciclovir compounded
oral suspension, 1812
Famotidine, 1815
injection, 1816
for oral suspension, 1818
tablets, 1819
Fast
blue B salt, 6165
blue BB salt, 6165
green FCF, 6165
Fat, hard, 5776
Fats and fixed oils ⟨401⟩, 6676
FD&C blue no. 1, 6165
Fehling's solution, 6229
Felbamate, 1821
oral suspension, 1822
tablets, 1824
Felodipine, 1826
extended-release tablets, 1827
Fenbendazole, 1831
Fennel oil, 5776
Fenofibrate, 1833
capsules, 1834
tablets, 1837
Fenoldopam mesylate, 1840
injection, 1842
Fenoprofen calcium, 1843
capsules, 1845
tablets, 1845
Fentanyl, 1846
Fentanyl citrate, 1848
injection, 1849
Fentanyl citrate
bupivacaine hydrochloride compounded injection, 1850
Fentanyl citrate compounded
injection, 1848
Fenugreek seed, 4982
powdered extract, 4987
powder, 4984
Ferric
ammonium citrate, 289, 6165
ammonium citrate for oral solution, 290
ammonium sulfate, 6166
ammonium sulfate, tenth-normal (0.1 N), 6241
ammonium sulfate TS, 6229
chloride, 6166
chloride CS, 6225
chloride TS, 6229
nitrate, 6166
oxide, 5776
subsulfate solution, 1852
sulfate, 1852, 6166
Ferrocyphen, 6166
Ferroin TS, 6229
Ferrosoferric oxide, 5777
Ferrous
ammonium sulfate, 6166
ammonium sulfate, tenth-normal (0.1 N), 6241
fumarate, 1853
fumarate and docusate sodium extended-release tablets, 1856
fumarate tablets, 1855
gluconate, 1857
gluconate capsules, 1859
gluconate oral solution, 1860
gluconate tablets, 1861
sulfate, 1862, 6166
sulfate, dried, 1864
sulfate oral solution, 1863
sulfate syrup, 1863
sulfate tablets, 1864
sulfate TS, 6229
sulfate, acid, TS, 6225, 6229
0.07 N Ferrous ammonium sulfate, 6241
Ferulic acid, 6166
Ferumoxides injection, 1866
Ferumoxsil oral suspension, 1868
Fetal bovine serum—quality attributes and functionality tests ⟨90⟩, 6533
Feverfew, 4989
powdered, 4990
Fexofenadine hydrochloride, 1869
capsules, 1870
and pseudoephedrine hydrochloride extended-release tablets, 1876
tablets, 1872
Fibroblast growth factor-2, 6166
Fibroblasts
bilayer synthetic scaffold, construct human, 1153
polyglactin scaffold, construct human, 1157
Filgrastim, 1885
Finasteride, 1889
tablets, 1890
Fingolimod
hydrochloride, 1891
Fish oil containing omega-3 acids, 4992
capsules, 4994
delayed-release capsules, 4997
Flame photometry for reagents, 6129
Flavoxate hydrochloride, 1892
tablets, 1894
Flax seed oil, 4998
capsules, 4999
Flecainide acetate, 1895
oral suspension, 1897
tablets, 1898
Flow cytometric enumeration of CD34+ cells ⟨127⟩, 6560
Flow cytometry ⟨1027⟩, 7281
Floxuridine, 1899
for injection, 1901
Fluconazole, 1901
in dextrose injection, 1907
for oral suspension, 1912
injection, 1903
in sodium chloride injection, 1909
tablets, 1914
Flucytosine, 1915
capsules, 1916
oral suspension, 1917
Fludarabine phosphate, 1917
injection, 1919
for injection, 1921
Fludeoxyglucose F18 injection, 1946
Fludrocortisone acetate, 1922
tablets, 1923
Flumazenil, 1925
injection, 1926
Flumethasone pivalate, 1927
cream, 1928
Flunisolide, 1929
nasal solution, 1929
Flunixin meglumine, 1930
granules, 1931
injection, 1932
paste, 1933
Fluocinolone acetonide, 1934
cream, 1935
and neomycin sulfate cream, 3107
ointment, 1935
topical solution, 1936
Fluocinonide, 1937
cream, 1937
gel, 1938
ointment, 1938
topical solution, 1939
Fluorene, 6166
(–)-1-(9-Fluorenyl) ethanol, 6166
(+)-1-(9-Fluorenyl) ethyl chloroformate solution, 6166
9-Fluorenylmethyl chloroformate, 6167
Fluorescamine, 6167
Fluorescein, 1940
injection, 1940
sodium, 1942
sodium and benoxinate hydrochloride ophthalmic solution, 1944
sodium ophthalmic strips, 1943
sodium and proparacaine hydrochloride ophthalmic solution, 1945
Fluorescence spectroscopy ⟨853⟩, 7146
Fluorescence spectroscopy—theory and practice ⟨1853⟩, 8641
Fluorine
F 18 injection, fludeoxyglucose, 1946
F 18 injection, sodium fluoride, 1950
4′-Fluoroacetophenone, 6167
Fluoroandrostadiene carboxylic acid, 6167
Fluorometholone, 1950
acetate, 1953
acetate and tobramycin ophthalmic suspension, 4412
cream, 1952
and neomycin sulfate ointment, 3107
ophthalmic suspension, 1952
Fluorouracil, 1954
cream, 1956
injection, 1957
topical solution, 1957
Fluoxetine
capsules, 1958
delayed-release capsules, 1959
hydrochloride, 1964
and olanzapine capsules, 3237
oral solution, 1961
tablets, 1962
Fluoxymesterone, 1965
tablets, 1966

www.webofpharma.com

Fluphenazine
decanoate, 1967
decanoate injection, 1968
enanthate, 1969
enanthate injection, 1970
hydrochloride, 1970
hydrochloride elixir, 1971
hydrochloride injection, 1972
hydrochloride oral solution, 1973
hydrochloride tablets, 1973
Flurandrenolide, 1974
cream, 1975
lotion, 1976
and neomycin sulfate cream, 3107
and neomycin sulfate lotion, 3108
and neomycin sulfate ointment, 3108
ointment, 1976
tape, 1976
Flurazepam hydrochloride, 1977
capsules, 1978
Flurbiprofen, 1979
sodium, 1981
sodium ophthalmic solution, 1982
tablets, 1980
Flutamide, 1983
capsules, 1984
Fluticasone
propionate and salmeterol inhalation aerosol, 2005
propionate and salmeterol inhalation powder, 2010
Fluticasone propionate, 1985
cream, 1987
inhalation aerosol, 1988
inhalation powder, 1993
lotion, 1998
nasal spray, 2000
ointment, 2003
Fluvastatin
capsules, 2020
sodium, 2018
Fluvoxamine maleate, 2022
tablets, 2023
Folic acid, 2026
assay ⟨411⟩, 6689
compounded oral solution, 2027
injection, 2027
tablets, 2028
Folin-ciocalteu phenol TS, 6229
Fondaparinux sodium, 2029
injection, 2033
Formaldehyde
solution, 2035, 6167, 6229
TS, 6229
Formamide, 6167
anhydrous, 6167
Formic acid, 6167
96 percent, 6167
anhydrous, 6168
Formoterol fumarate, 2036
Forskohlii, 5000
extract, powdered, 5003
powdered, 5002
Foscarnet sodium, 2037
Fosfomycin tromethamine, 2039
Fosinopril sodium, 2040
and hydrochlorothiazide tablets, 2043
tablets, 2042
Fosphenytoin sodium, 2045
injection, 2047
Fructose, 2048
injection, 2049
and sodium chloride injection, 2049
Fuchsin
basic, 2050, 6141, 6168
pyrogallol TS, 6229
sulfurous acid TS, 6229
Fuller's earth, chromatographic, 6152, 6168
Fulvestrant, 2050
Fumaric acid, 5780
Fuming
nitric acid, 6168
sulfuric acid, 6168
Furazolidone, 2052
oral suspension, 2052
tablets, 2053
Furfural, 6168
Furosemide, 2053
injection, 2054
oral solution, 2055
tablets, 2056

G

G designations, 6168
Ga 67 injection, gallium citrate, 2088
Gabapentin, 2057
capsules, 2058
tablets, 2060
Gadodiamide, 2061
injection, 2064
Gadolinium (Gd III) acetate hydrate, 6168
Gadolinium sulfate, 6168
Gadopentetate dimeglumine injection, 2065
Gadoteridol, 2067
injection, 2069
Gadoversetamide, 2070
injection, 2073
Galactose, 5781
Galageenan, 5782
Galantamine
extended-release capsules, 2074
hydrobromide, 2083
oral solution, 2079
tablets, 2081
Gallamine triethiodide, 2087
injection, 2087
Gallium citrate Ga 67 injection, 2088
Gamma cyclodextrin, 5739
Ganciclovir, 2088
for injection, 2089
oral suspension, 2090
Ganoderma lucidum fruiting body, 5004
Ganoderma lucidum fruiting body powder, 5008
Garcinia cambogia, 5011
powdered, 5012
Garcinia hydroxycitrate
extract, powdered, 5014
Garcinia indica, 5015
powdered, 5016
Garlic, 5018
delayed-release tablets, 5024
extract, powdered, 5022
fluidextract, 5023
powdered, 5020
Gaseous sterilization ⟨1229.7⟩, 8222
Gastric fluid, simulated, TS, 6234
Gauze
absorbent, 2091
petrolatum, 2092

Gel

Adapalene, 96
Aluminum hydroxide, 188
Aluminum hydroxide, dried, 189
Aluminum hydroxide capsules, dried, 190
Aluminum hydroxide tablets, dried, 190
Aluminum phosphate, 190
Aminobenzoic acid, 234
ascorbic acid chewable, 4765
Benzocaine, 506
Benzocaine, butamben, and tetracaine hydrochloride, 514
Benzoyl peroxide, 526
Betamethasone benzoate, 545
cholecalciferol chewable, 4889
Chromatographic silica, 6152
Chromatographic silica mixture, 6152
Clindamycin phosphate, 1071
Cyanocobalamin chewable, 4931
Desoximetasone, 1275
Dimethyl sulfoxide, 1413
Dyclonine hydrochloride, 1570
Erythromycin and benzoyl peroxide, topical, 1692
Erythromycin, topical, 1686
Fluocinonide, 1938
Gelatin, 5783
Gelatin film, absorbable, 2092
Gelatin sponge, absorbable, 2093
Gelatin TS, 6229
Hydrocortisone, 2230
Indomethacin, topical, 2330
Metronidazole, 2935
Naftifine hydrochloride, 3066
Phenol topical, camphorated, 3501
Phenytoin compounded topical, 3525
Salicylic acid, 3972
Selegiline compounded topical, 4021
Silica, 6199
Silica, binder-free, 6199
Silica, chromatographic, 6152, 6199
Silica, impregnated glass microfiber sheet, 6199
Silica mixture, chromatographic, 6152, 6199
Silica mixture, chromatographic, with chemically bound amino groups, 6199
Silica mixture, dimethylsilanized, chromatographic, 6199
Silica mixture, octadecylsilanized chromatographic, 6199
Silica mixture, octylsilanized, chromatographic, 6199
Silica, octadecylsilanized chromatographic, 6199
Silica, porous, 6199
Sodium fluoride and phosphoric acid, 4075
Sodium sulfide topical, 4096
Stannous fluoride, 4115
Tolnaftate, 4426
Tretinoin, 4479

Gelatin, 5783, 6168
film, absorbable, 2092
sponge, absorbable, 2093
TS, 6229
Gellan gum, 5785
Gemcitabine
for injection, 2094

www.webofpharma.com

Gemcitabine *(continued)*
hydrochloride, 2093
Gemfibrozil, 2096
capsules, 2097
tablets, 2098
Gene therapy products ⟨1047⟩, 7426

General chapters

⟨1⟩ Injections and implanted drug products (parenterals)—product quality tests, 6405
⟨2⟩ Oral drug products—product quality tests, 6410
⟨3⟩ Topical and transdermal drug products—product quality tests, 6415
⟨4⟩ Mucosal drug products—product quality tests, 6423
⟨5⟩ Inhalation and nasal drug products general information and product quality tests, 6427
⟨7⟩ Labeling, 6435
⟨11⟩ USP reference standards, 6442
⟨17⟩ Prescription container labeling, 6445
⟨31⟩ Volumetric apparatus, 6448
⟨41⟩ Balances, 6449
⟨51⟩ Antimicrobial effectiveness testing, 6449
⟨55⟩ Biological indicators—resistance performance tests, 6452
⟨60⟩ Microbiological examination of nonsterile products tests for Burkholderia Cepacia complex, 6454
⟨61⟩ Microbiological examination of nonsterile products: microbial enumeration tests, 6457
⟨62⟩ Microbiological examination of nonsterile products: tests for specified organisms, 6463
⟨63⟩ Mycoplasma tests, 6472
⟨71⟩ Sterility tests, 6481
⟨81⟩ Antibiotics—microbial assays, 6488
⟨85⟩ Bacterial endotoxins test, 6508
⟨87⟩ Biological reactivity tests, in vitro, 6514
⟨88⟩ Biological reactivity tests, in vivo, 6516
⟨89.1⟩ Collagenase I, 6524
⟨89.2⟩ Collagenase II, 6529
⟨89⟩ Enzymes used as ancillary materials in pharmaceutical manufacturing, 6521
⟨90⟩ Fetal bovine serum—quality attributes and functionality tests, 6533
⟨91⟩ Calcium pantothenate assay, 6536
⟨92⟩ Growth factors and cytokines used in cell therapy manufacturing, 6539
⟨111⟩ Design and analysis of biological assays, 6543
⟨115⟩ Dexpanthenol assay, 6547
⟨121⟩ Insulin assays, 6547
⟨121.1⟩ Physicochemical analytical procedures for insulins, 6550
⟨123⟩ Glucagon bioidentity tests, 6552
⟨124⟩ Erythropoietin bioassays, 6557
⟨126⟩ Somatropin bioidentity tests, 6558
⟨127⟩ Flow cytometric enumeration of CD34+ cells, 6560
⟨129⟩ Analytical procedures for recombinant therapeutic monoclonal antibodies, 6565
⟨130⟩ Protein A quality attributes, 6570
⟨151⟩ Pyrogen test, 6577
⟨161⟩ Medical devices—bacterial endotoxin and pyrogen tests, 6579
⟨162⟩ Diphtheria antitoxin potency testing for human immune globulins, 6582
⟨165⟩ Prekallikrein activator, 6583
⟨171⟩ Vitamin B_{12} activity assay, 6584
⟨181⟩ Identification—organic nitrogenous bases, 6587
⟨191⟩ Identification tests—general, 6587
⟨193⟩ Identification—tetracyclines, 6592
⟨197⟩ Spectrophotometric identification tests, 6593
⟨201⟩ Thin-layer chromatographic identification test, 6600
⟨202⟩ Identification of fixed oils by thin-layer chromatography, 6601
⟨203⟩ High-performance thin-layer chromatography procedure for identification of articles of botanical origin, 6602
⟨206⟩ Aluminum, 6605
⟨207⟩ Test for 1,6-anhydro derivative for enoxaparin sodium, 6605
⟨208⟩ Anti-factor Xa and anti-factor IIa assays for unfractionated and low molecular weight heparins, 6611
⟨209⟩ Low molecular weight heparin molecular weight determinations, 6614
⟨210⟩ Monosaccharide analysis, 6616
⟨211⟩ Arsenic, 6621
⟨212⟩ Oligosaccharide analysis, 6623
⟨221⟩ Chloride and sulfate, 6636
⟨223⟩ Dimethylaniline, 6636
⟨226⟩ 4-Epianhydrotetracycline, 6636
⟨227⟩ 4-Aminophenol in acetaminophen-containing drug products, 6637
⟨228⟩ Ethylene oxide and dioxane, 6639
⟨232⟩ Elemental impurities—limits, 6641
⟨233⟩ Elemental impurities—procedures, 6645
⟨241⟩ Iron, 6648
⟨251⟩ Lead, 6649
⟨261⟩ Mercury, 6650
⟨267⟩ Porosimetry by mercury intrusion, 6653
⟨268⟩ Porosity by nitrogen adsorption–desorption, 6655
⟨271⟩ Readily carbonizable substances test, 6660
⟨281⟩ Residue on ignition, 6660
⟨291⟩ Selenium, 6661
⟨301⟩ Acid-neutralizing capacity, 6661
⟨311⟩ Alginates assay, 6662
⟨341⟩ Antimicrobial agents—content, 6664
⟨345⟩ Assay for citric acid/citrate and phosphate, 6668
⟨351⟩ Assay for steroids, 6669
⟨381⟩ Elastomeric closures for injections, 6669
⟨391⟩ Epinephrine assay, 6676
⟨401⟩ Fats and fixed oils, 6676
⟨411⟩ Folic acid assay, 6689
⟨413⟩ Impurities testing in medical gases, 6692
⟨415⟩ Medical gases assay, 6693
⟨425⟩ Iodometric assay—antibiotics, 6696
⟨429⟩ Light diffraction measurement of particle size, 6697
⟨431⟩ Methoxy determination, 6702
⟨441⟩ Niacin or niacinamide assay, 6704
⟨451⟩ Nitrite titration, 6709
⟨461⟩ Nitrogen determination, 6709
⟨466⟩ Ordinary impurities, 6710
⟨467⟩ Residual solvents, 6712
⟨469⟩ Ethylene glycol, diethylene glycol, and triethylene glycol in ethoxylated substances, 6733
⟨471⟩ Oxygen flask combustion, 6735
⟨481⟩ Riboflavin assay, 6735
⟨501⟩ Salts of organic nitrogenous bases, 6741
⟨503⟩ Acetic acid in peptides, 6741
⟨503.1⟩ Trifluoroacetic acid (TFA) in peptides, 6743
⟨507⟩ Protein determination procedures, 6744
⟨509⟩ Residual DNA testing, 6748
⟨511⟩ Single-steroid assay, 6750
⟨525⟩ Sulfur dioxide, 6751
⟨531⟩ Thiamine assay, 6756
⟨541⟩ Titrimetry, 6764
⟨551⟩ Vitamin E assay, 6767
⟨561⟩ Articles of botanical origin, 6774
⟨563⟩ Identification of articles of botanical origin, 6788
⟨565⟩ Botanical extracts, 6798
⟨571⟩ Vitamin A assay, 6801
⟨580⟩ Vitamin C assay, 6805
⟨581⟩ Vitamin D assay, 6808
⟨591⟩ Zinc determination, 6817
⟨601⟩ Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests, 6819
⟨602⟩ Propellants, 6844
⟨603⟩ Topical aerosols, 6845
⟨604⟩ Leak rate, 6846
⟨610⟩ Alternative microbiological sampling methods for nonsterile inhaled and nasal products, 6846
⟨611⟩ Alcohol determination, 6848
⟨616⟩ Bulk density and tapped density of powders, 6850
⟨621⟩ Chromatography, 6853
⟨630⟩ Visiual Comparison , 6865
⟨631⟩ Color and achromicity, 6865
⟨641⟩ Completeness of solution, 6866
⟨643⟩ Total organic carbon, 6867
⟨644⟩ Conductivity of solutions, 6868
⟨645⟩ Water conductivity, 6871
⟨651⟩ Congealing temperature, 6874
⟨659⟩ Packaging and storage requirements, 6876
⟨660⟩ Containers—glass, 6881
⟨661⟩ Plastic packaging systems and their materials of construction, 6887
⟨661.1⟩ Plastic materials of construction, 6893
⟨661.2⟩ Plastic packaging systems for pharmaceutical use, 6912
⟨670⟩ Auxiliary packaging components, 6916
⟨671⟩ Containers—performance testing, 6922
⟨691⟩ Cotton, 6929
⟨695⟩ Crystallinity, 6931
⟨696⟩ Characterization of crystalline solids by microcalorimetry and solution calorimetry, 6931
⟨697⟩ Container content for injections, 6934
⟨698⟩ Deliverable volume, 6935
⟨699⟩ Density of solids, 6938
⟨701⟩ Disintegration, 6940
⟨705⟩ Quality attributes of tablets labeled as having a functional score, 6944
⟨711⟩ Dissolution, 6945
⟨721⟩ Distilling range, 6955
⟨724⟩ Drug release, 6957

www.webofpharma.com

General chapters *(continued)*
⟨729⟩ Globule size distribution in lipid injectable emulsions, 6963
⟨730⟩ Plasma spectrochemistry, 6967
⟨731⟩ Loss on drying, 6970
⟨733⟩ Loss on ignition, 6970
⟨735⟩ X-ray fluorescence spectrometry, 6971
⟨736⟩ Mass spectrometry, 6975
⟨741⟩ Melting range or temperature, 6980
⟨755⟩ Minimum fill, 6982
⟨761⟩ Nuclear magnetic resonance spectroscopy, 6984
⟨771⟩ Ophthalmic products—quality tests, 6993
⟨776⟩ Optical microscopy, 6998
⟨781⟩ Optical rotation, 7001
⟨782⟩ Vibrational circular dichroism spectroscopy, 7002
⟨785⟩ Osmolality and osmolarity, 7008
⟨786⟩ Particle size distribution estimation by analytical sieving, 7010
⟨787⟩ Subvisible particulate matter in therapeutic protein injections, 7014
⟨788⟩ Particulate matter in injections, 7017
⟨789⟩ Particulate matter in ophthalmic solutions, 7020
⟨790⟩ Visible particulates in injections, 7021
⟨791⟩ pH, 7022
⟨795⟩ Pharmaceutical compounding—nonsterile preparations, 7025
⟨797⟩ Pharmaceutical compounding—sterile preparations, 7037
⟨800⟩ Hazardous drugs—handling in healthcare settings, 7071
⟨801⟩ Polarography, 7089
⟨811⟩ Powder fineness, 7093
⟨821⟩ Radioactivity, 7094
⟨823⟩ Positron emission tomography drugs for compounding, investigational, and research uses, 7100
⟨825⟩ Radiopharmaceuticals—preparation, compounding, dispensing, and repackaging, 7109
⟨831⟩ Refractive index, 7137
⟨841⟩ Specific gravity, 7137
⟨846⟩ Specific surface area, 7139
⟨852⟩ Atomic absorption spectroscopy, 7142
⟨853⟩ Fluorescence spectroscopy, 7146
⟨854⟩ Mid-infrared spectroscopy, 7151
⟨855⟩ Nephelometry, turbidimetry, and visual comparison, 7155
⟨856⟩ Near-infrared spectroscopy, 7161
⟨857⟩ Ultraviolet-visible spectroscopy, 7166
⟨861⟩ Sutures—diameter, 7175
⟨871⟩ Sutures—needle attachment, 7178
⟨881⟩ Tensile strength, 7179
⟨891⟩ Thermal analysis, 7180
⟨905⟩ Uniformity of dosage units, 7183
⟨911⟩ Viscosity—capillary methods, 7187
⟨912⟩ Viscosity—rotational methods, 7189
⟨913⟩ Viscosity—rolling ball method, 7193
⟨914⟩ Viscosity—pressure driven methods, 7195
⟨921⟩ Water determination, 7196
⟨941⟩ Characterization of crystalline and partially crystalline solids by X-ray powder diffraction (XRPD), 7201
⟨1004⟩ Mucosal drug products—performance tests, 7227
⟨1005⟩ Acoustic emission, 7230
⟨1010⟩ Analytical data—interpretation and treatment, 7233
⟨1024⟩ Bovine serum, 7260
⟨1025⟩ Pancreatin, 7272
⟨1027⟩ Flow cytometry, 7281
⟨1030⟩ Biological assay chapters—overview and glossary, 7300
⟨1031⟩ The biocompatibility of materials used in drug containers, medical devices, and implants, 7313
⟨1032⟩ Design and development of biological assays, 7320
⟨1033⟩ Biological assay validation, 7337
⟨1034⟩ Analysis of biological assays, 7351
⟨1039⟩ Chemometrics, 7363
⟨1041⟩ Biologics, 7379
⟨1043⟩ Ancillary materials for cell, gene, and tissue-engineered products, 7381
⟨1044⟩ Cryopreservation of cells, 7389
⟨1046⟩ Cellular and tissue-based products, 7400
⟨1047⟩ Gene therapy products, 7426
⟨1048⟩ Quality of biotechnological products: analysis of the expression construct in cells used for production of r-DNA derived protein products, 7452
⟨1049⟩ Quality of biotechnological products: stability testing of biotechnological/biological products, 7454
⟨1050⟩ Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin, 7459
⟨1050.1⟩ Design, evaluation and characterization of viral clearance procedures, 7472
⟨1051⟩ Cleaning glass apparatus, 7481
⟨1052⟩ Biotechnology-derived articles—amino acid analysis, 7481
⟨1053⟩ Capillary electrophoresis, 7492
⟨1054⟩ Biotechnology-derived articles—isoelectric focusing, 7499
⟨1055⟩ Biotechnology-derived articles—peptide mapping, 7501
⟨1056⟩ Biotechnology-derived articles—polyacrylamide gel electrophoresis, 7508
⟨1057⟩ Biotechnology-derived articles—total protein assay, 7515
⟨1058⟩ Analytical instrument qualification, 7522
⟨1059⟩ Excipient performance, 7528
⟨1061⟩ Color—instrumental measurement, 7553
⟨1062⟩ Tablet compression characterization, 7556
⟨1063⟩ Shear cell methodology for powder flow testing, 7566
⟨1064⟩ Identification of articles of botanical origin by high-performance thin-layer chromatography procedure, 7578
⟨1065⟩ Ion chromatography, 7588
⟨1066⟩ Physical environments that promote safe medication use, 7590
⟨1071⟩ Rapid sterility testing of short life products a risk based approach, 7602
⟨1072⟩ Disinfectants and antiseptics, 7608
⟨1074⟩ Excipient biological safety evaluation guidelines, 7613
⟨1078⟩ Good manufacturing practices for bulk pharmaceutical excipients, 7617
⟨1079.1⟩ Storage and transportation of investigational drug products, 7644
⟨1079⟩ Good storage and distribution practices for drug products, 7635
⟨1080⟩ Bulk pharmaceutical excipients—certificate of analysis, 7647
⟨1084⟩ Glycoprotein and glycan analysis—general considerations, 7655
⟨1085⟩ Guidelines on endotoxins test, 7665
⟨1086⟩ Impurities in drug substances and drug products, 7678
⟨1087⟩ Apparent intrinsic dissolution—dissolution testing procedures for rotating disk and stationary disk, 7681
⟨1088⟩ In vitro and in vivo evaluation of dosage forms, 7684
⟨1090⟩ Assessment of drug product performance—bioavailability, bioequivalence, and dissolution, 7695
⟨1091⟩ Labeling of inactive ingredients, 7706
⟨1092⟩ The dissolution procedure: development and validation, 7707
⟨1094⟩ Capsules—dissolution testing and related quality attributes, 7725
⟨1097⟩ Bulk powder sampling procedures, 7732
⟨1099⟩ Limit on number of large deviations when assessing content uniformity in large samples, 7744
⟨1102⟩ Immunological test methods—general considerations, 7746
⟨1103⟩ Immunological test methods—enzyme-linked immunosorbent assay (ELISA), 7753
⟨1104⟩ Immunological test methods—immunoblot analysis, 7763
⟨1105⟩ Immunological test methods—surface plasmon resonance, 7773
⟨1106⟩ Immunogenicity assays—design and validation of immunoassays to detect anti-drug antibodies, 7788
⟨1106.1⟩ Immunogenicity assays—design and validation of assays to detect anti-drug neutralizing antibody, 7802
⟨1111⟩ Microbiological examination of nonsterile products: acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use, 7819
⟨1112⟩ Application of water activity determination to nonsterile pharmaceutical products, 7820
⟨1113⟩ Microbial characterization, identification, and strain typing, 7823
⟨1115⟩ Bioburden control of nonsterile drug substances and products, 7827
⟨1116⟩ Microbiological control and monitoring of aseptic processing environments, 7833
⟨1117⟩ Microbiological best laboratory practices, 7845
⟨1118⟩ Monitoring devices—time, temperature, and humidity, 7850
⟨1120⟩ Raman spectroscopy, 7856
⟨1121⟩ Nomenclature, 7862
⟨1125⟩ Nucleic acid-based techniques—general, 7865
⟨1126⟩ Nucleic acid-based techniques—extraction, detection, and sequencing, 7870
⟨1127⟩ Nucleic acid-based techniques—amplification, 7879
⟨1128⟩ Nucleic acid-based techniques—microarray, 7887
⟨1129⟩ Nucleic acid-based techniques—genotyping, 7893
⟨1130⟩ Nucleic acid-based techniques—approaches for detecting trace nucleic acids (residual DNA testing), 7897

www.webofpharma.com

General chapters *(continued)*
⟨1132⟩ Residual host cell protein measurement in biopharmaceuticals, 7900
⟨1136⟩ Packaging and repackaging—single unit containers, 7919
⟨1151⟩ Pharmaceutical dosage forms, 7929
⟨1152⟩ Animal drugs for use in animal feeds, 7952
⟨1160⟩ Pharmaceutical calculations in pharmacy practice, 7953
⟨1163⟩ Quality assurance in pharmaceutical compounding, 7979
⟨1168⟩ Compounding for phase I investigational studies, 7985
⟨1174⟩ Powder flow, 7993
⟨1176⟩ Prescription balances and volumetric apparatus, 7997
⟨1177⟩ Good packaging practices, 8003
⟨1178⟩ Good repackaging practices, 8006
⟨1180⟩ Human plasma, 8008
⟨1181⟩ Scanning electron microscopy, 8025
⟨1184⟩ Sensitization testing, 8035
⟨1191⟩ Stability considerations in dispensing practice, 8044
⟨1195⟩ Significant change guide for bulk pharmaceutical excipients, 8049
⟨1197⟩ Good distribution practices for bulk pharmaceutical excipients, 8060
⟨1207.1⟩ Package integrity and test method selection, 8086
⟨1207.2⟩ Package integrity leak test technologies, 8097
⟨1207.3⟩ Package seal quality test technologies, 8111
⟨1207⟩ Sterile product packaging—integrity evaluation, 8079
⟨1208⟩ Sterility testing—validation of isolator systems, 8113
⟨1210⟩ Statistical tools for procedure validation, 8117
⟨1211⟩ Sterility assurance, 8129
⟨1216⟩ Tablet friability, 8137
⟨1217⟩ Tablet breaking force, 8138
⟨1222⟩ Terminally sterilized pharmaceutical products—parametric release, 8142
⟨1223⟩ Validation of alternative microbiological methods, 8144
⟨1223.1⟩ Validation of alternative methods to antibiotic microbial assays, 8157
⟨1224⟩ Transfer of analytical procedures, 8164
⟨1225⟩ Validation of compendial procedures, 8166
⟨1226⟩ Verification of compendial procedures, 8171
⟨1227⟩ Validation of microbial recovery from pharmacopeial articles, 8172
⟨1228.4⟩ Depyrogenation by Rinsing, 8187
⟨1228⟩ Depyrogenation, 8176
⟨1228.1⟩ Dry heat depyrogenation, 8181
⟨1228.3⟩ Depyrogenation by filtration, 8184
⟨1228.5⟩ Endotoxin indicators for depyrogenation, 8190
⟨1229⟩ Sterilization of compendial articles, 8194
⟨1229.1⟩ Steam sterilization by direct contact, 8199
⟨1229.2⟩ Moist heat sterilization of aqueous liquids, 8202
⟨1229.3⟩ Monitoring of bioburden, 8206
⟨1229.4⟩ Sterilizing filtration of liquids, 8209
⟨1229.5⟩ Biological indicators for sterilization, 8216
⟨1229.6⟩ Liquid-phase sterilization, 8219
⟨1229.7⟩ Gaseous sterilization, 8222
⟨1229.8⟩ Dry heat sterilization, 8225
⟨1229.9⟩ Physicochemical integrators and indicators for sterilization, 8227
⟨1229.10⟩ Radiation sterilization, 8228
⟨1229.11⟩ Vapor phase sterilization, 8231
⟨1229.12⟩ New sterilization methods, 8233
⟨1229.13⟩ Sterilization-in-place, 8233
⟨1229.14⟩ Sterilization cycle development, 8235
⟨1229.15⟩ Sterilization filtration of gases, 8238
⟨1229.16⟩ Prion inactivation, 8239
⟨1230⟩ Water for hemodialysis applications, 8240
⟨1231⟩ Water for pharmaceutical purposes, 8242
⟨1234⟩ Vaccines for human use—polysaccharide and glycoconjugate vaccines, 8276
⟨1235⟩ Vaccines for human use—general considerations, 8291
⟨1237⟩ Virology test methods, 8319
⟨1238⟩ Vaccines for human use—bacterial vaccines, 8338
⟨1240⟩ Virus testing of human plasma for further manufacture, 8350
⟨1241⟩ Water–solid interactions in pharmaceutical systems, 8359
⟨1251⟩ Weighing on an analytical balance, 8363
⟨1265⟩ Written prescription drug information—guidelines, 8368
⟨1285⟩ Preparation of biological specimens for histologic and immunohistochemical analysis, 8370
⟨1285.1⟩ Hematoxylin and eosin staining of sectioned tissue for microscopic examination, 8374
⟨1430.1⟩ Analytical methodologies based on scattering phenomena—static light scattering, 8379
⟨1430.2⟩ Analytical methodologies based on scattering phenomena- light diffraction measurements of particle size, 8386
⟨1430.4⟩ Analytical methodologies based on scattering phenomena—electrophoretic light scattering (determination of zeta potential), 8389
⟨1430.5⟩ Analytical methodologies based on scattering phenomena—small angle x-ray scattering and small angle neutron scattering, 8392
⟨1430⟩ Analytical methodologies based on scattering phenomena—general, 8376
⟨1467⟩ Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures, 8404
⟨1601⟩ Products for nebulization—characterization tests, 8407
⟨1602⟩ Spacers and valved holding chambers used with inhalation aerosols—characterization tests, 8411
⟨1644⟩ Theory and practice of electrical conductivity measurements of solutions, 8423
⟨1660⟩ Evaluation of the inner surface durability of glass containers, 8429
⟨1661⟩ Evaluation of plastic packaging systems and their materials of construction with respect to their user safety impact, 8434
⟨1663⟩ Assessment of extractables associated with pharmaceutical packaging/delivery systems, 8442
⟨1664⟩ Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems, 8455
⟨1664.1⟩ Orally inhaled and nasal drug products, 8467
⟨1724⟩ Semisolid drug products—performance tests, 8473
⟨1730⟩ Plasma spectrochemistry—theory and practice, 8484
⟨1735⟩ X-ray fluorescence spectrometry—theory and practice, 8490
⟨1736⟩ Applications of mass spectrometry, 8508
⟨1761⟩ Applications of nuclear magnetic resonance spectroscopy, 8529
⟨1771⟩ Ophthalmic products—performance tests, 8549
⟨1782⟩ Vibrational circular dichroism spectroscopytheory and practice, 8549
⟨1787⟩ Measurement of subvisible particulate matter in therapeutic protein injections, 8562
⟨1788⟩ Methods for the determination of particulate matter in injections and ophthalmic solutions, 8575
⟨1790⟩ Visual inspection of injections, 8587
⟨1821⟩ Radioactivity—theory and practice, 8604
⟨1823⟩ Positron emission tomography drugs—information, 8617
⟨1850⟩ Evaluation of screening technologies for assessing medicine quality, 8626
⟨1852⟩ Atomic absorption spectroscopy—theory and practice, 8632
⟨1853⟩ Fluorescence spectroscopy—theory and practice, 8641
⟨1854⟩ Mid-infrared spectroscopy—theory and practice, 8650
⟨1857⟩ Ultraviolet-visible spectroscopy—theory and practice, 8670
⟨1911⟩ Rheometry, 8679
⟨198⟩ Nuclear magnetic resonance spectroscopy identity testing of bacterial polysaccharides used in vaccine manufacture, 6596
⟨2021⟩ Microbial enumeration tests—nutritional and dietary supplements, 8687
⟨2022⟩ Microbiological procedures for absence of specified microorganisms—nutritional and dietary supplements, 8692
⟨2023⟩ Microbiological attributes of nonsterile nutritional and dietary supplements, 8698
⟨2030⟩ Supplemental information for articles of botanical origin, 8702
⟨2040⟩ Disintegration and dissolution of dietary supplements, 8711
⟨2091⟩ Weight variation of dietary supplements, 8718
⟨2232⟩ Elemental contaminants in dietary supplements, 8719
⟨2250⟩ Detection of irradiated dietary supplements, 8722

www.webofpharma.com

General chapters *(continued)*
⟨2251⟩ Screening for undeclared drugs and drug analogues, 8725
⟨2750⟩ Manufacturing practices for dietary supplements, 8742

General chapters

Applications of mass spectrometry ⟨1736⟩, 8508
Radiopharmaceuticals—preparation, compounding, dispensing, and repackaging ⟨825⟩, 7109
Acetic acid in peptides ⟨503⟩, 6741
Acid-neutralizing capacity ⟨301⟩, 6661
Acoustic emission ⟨1005⟩, 7230
Alcohol determination ⟨611⟩, 6848
Alginates assay ⟨311⟩, 6662
Alternative microbiological sampling methods for nonsterile inhaled and nasal products ⟨610⟩, 6846
Aluminum ⟨206⟩, 6605
4-Aminophenol in acetaminophen-containing drug products ⟨227⟩, 6637
Analysis of biological assays ⟨1034⟩, 7351
Analytical data—interpretation and treatment ⟨1010⟩, 7233
Analytical instrument qualification ⟨1058⟩, 7522
Analytical methodologies based on scattering phenomena—electrophoretic light scattering (determination of zeta potential) ⟨1430.4⟩, 8389
Analytical methodologies based on scattering phenomena—general ⟨1430⟩, 8376
Analytical methodologies based on scattering phenomena—light diffraction measurements of particle size ⟨1430.2⟩, 8386
Analytical methodologies based on scattering phenomena—small angle x-ray scattering and small angle neutron scattering ⟨1430.5⟩, 8392
Analytical methodologies based on scattering phenomena—static light scattering ⟨1430.1⟩, 8379
Analytical procedures for recombinant therapeutic monoclonal antibodies ⟨129⟩, 6565
Ancillary materials for cell, gene, and tissue-engineered products ⟨1043⟩, 7381
Animal drugs for use in animal feeds ⟨1152⟩, 7952
Antibiotics—microbial assays ⟨81⟩, 6488
Anti-factor Xa and anti-factor IIa assays for unfractionated and low molecular weight heparins ⟨208⟩, 6611
Antimicrobial agents—content ⟨341⟩, 6664
Antimicrobial effectiveness testing ⟨51⟩, 6449
Apparent intrinsic dissolution—dissolution testing procedures for rotating disk and stationary disk ⟨1087⟩, 7681
Applications of nuclear magnetic resonance spectroscopy ⟨1761⟩, 8529
Application of water activity determination to nonsterile pharmaceutical products ⟨1112⟩, 7820
Arsenic ⟨211⟩, 6621
Articles of botanical origin ⟨561⟩, 6774
Assay for citric acid/citrate and phosphate ⟨345⟩, 6668
Assay for steroids ⟨351⟩, 6669
Assessment of drug product performance—bioavailability, bioequivalence, and dissolution ⟨1090⟩, 7695
Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems ⟨1664⟩, 8455
Assessment of extractables associated with pharmaceutical packaging/delivery systems ⟨1663⟩, 8442
Atomic absorption spectroscopy ⟨852⟩, 7142
Atomic absorption spectroscopy—theory and practice ⟨1852⟩, 8632
Auxiliary packaging components ⟨670⟩, 6916
Bacterial endotoxins test ⟨85⟩, 6508
Balances ⟨41⟩, 6449
Bioburden control of nonsterile drug substances and products ⟨1115⟩, 7827
The biocompatibility of materials used in drug containers, medical devices, and implants ⟨1031⟩, 7313
Biological assay chapters—overview and glossary ⟨1030⟩, 7300
Biological assay validation ⟨1033⟩, 7337
Biological indicators—resistance performance tests ⟨55⟩, 6452
Biological indicators for sterilization ⟨1229.5⟩, 8216
Biological reactivity tests, in vitro ⟨87⟩, 6514
Biological reactivity tests, in vivo ⟨88⟩, 6516
Biologics ⟨1041⟩, 7379
Biotechnology-derived articles—amino acid analysis ⟨1052⟩, 7481
Biotechnology-derived articles—isoelectric focusing ⟨1054⟩, 7499
Biotechnology-derived articles—peptide mapping ⟨1055⟩, 7501
Biotechnology-derived articles—polyacrylamide gel electrophoresis ⟨1056⟩, 7508
Biotechnology-derived articles—total protein assay ⟨1057⟩, 7515
Botanical extracts ⟨565⟩, 6798
Bovine serum ⟨1024⟩, 7260
Bulk density and tapped density of powders ⟨616⟩, 6850
Bulk pharmaceutical excipients—certificate of analysis ⟨1080⟩, 7647
Bulk powder sampling procedures ⟨1097⟩, 7732
Calcium pantothenate assay ⟨91⟩, 6536
Capillary electrophoresis ⟨1053⟩, 7492
Capsules—dissolution testing and related quality attributes ⟨1094⟩, 7725
Cellular and tissue-based products ⟨1046⟩, 7400
Characterization of crystalline and partially crystalline solids by X-ray powder diffraction (XRPD) ⟨941⟩, 7201
Characterization of crystalline solids by microcalorimetry and solution calorimetry ⟨696⟩, 6931
Chemometrics ⟨1039⟩, 7363
Chloride and sulfate ⟨221⟩, 6636
Chromatography ⟨621⟩, 6853
Cleaning glass apparatus ⟨1051⟩, 7481
Collagenase I ⟨89.1⟩, 6524
Collagenase II ⟨89.2⟩, 6529
Color and achromicity ⟨631⟩, 6865
Color—instrumental measurement ⟨1061⟩, 7553
Completeness of solution ⟨641⟩, 6866
Compounding for phase I investigational studies ⟨1168⟩, 7985
Conductivity of solutions ⟨644⟩, 6868
Congealing temperature ⟨651⟩, 6874
Container content for injections ⟨697⟩, 6934
Containers—glass ⟨660⟩, 6881
Containers—performance testing ⟨671⟩, 6922
Cotton ⟨691⟩, 6929
Cryopreservation of cells ⟨1044⟩, 7389
Crystallinity ⟨695⟩, 6931
Deliverable volume ⟨698⟩, 6935
Density of solids ⟨699⟩, 6938
Depyrogenation ⟨1228⟩, 8176
Depyrogenation by filtration ⟨1228.3⟩, 8184
Depyrogenation by Rinsing ⟨1228.4⟩, 8187
Design, evaluation and characterization of viral clearance procedures ⟨1050.1⟩, 7472
Design and analysis of biological assays ⟨111⟩, 6543
Design and development of biological assays ⟨1032⟩, 7320
Detection of irradiated dietary supplements ⟨2250⟩, 8722
Dexpanthenol assay ⟨115⟩, 6547
Dimethylaniline ⟨223⟩, 6636
Diphtheria antitoxin potency testing for human immune globulins ⟨162⟩, 6582
Disinfectants and antiseptics ⟨1072⟩, 7608
Disintegration ⟨701⟩, 6940
Disintegration and dissolution of dietary supplements ⟨2040⟩, 8711
Dissolution ⟨711⟩, 6945
The dissolution procedure: development and validation ⟨1092⟩, 7707
Distilling range ⟨721⟩, 6955
Drug release ⟨724⟩, 6957
Dry heat depyrogenation ⟨1228.1⟩, 8181
Dry heat sterilization ⟨1229.8⟩, 8225
Elastomeric closures for injections ⟨381⟩, 6669
Elemental contaminants in dietary supplements ⟨2232⟩, 8719
Elemental impurities—limits ⟨232⟩, 6641
Elemental impurities—procedures ⟨233⟩, 6645
Endotoxin indicators for depyrogenation ⟨1228.5⟩, 8190
Enzymes used as ancillary materials in pharmaceutical manufacturing ⟨89⟩, 6521
4-Epianhydrotetracycline ⟨226⟩, 6636
Epinephrine assay ⟨391⟩, 6676
Erythropoietin bioassays ⟨124⟩, 6557
Ethylene glycol, diethylene glycol, and triethylene glycol in ethoxylated substances ⟨469⟩, 6733
Ethylene oxide and dioxane ⟨228⟩, 6639
Evaluation of plastic packaging systems and their materials of construction with respect to their user safety impact ⟨1661⟩, 8434
Evaluation of screening technologies for assessing medicine quality ⟨1850⟩, 8626
Evaluation of the inner surface durability of glass containers ⟨1660⟩, 8429
Excipient biological safety evaluation guidelines ⟨1074⟩, 7613
Excipient performance ⟨1059⟩, 7528

www.webofpharma.com

General chapters *(continued)*
Fats and fixed oils ⟨401⟩, 6676
Fetal bovine serum—quality attributes and functionality tests ⟨90⟩, 6533
Flow cytometric enumeration of CD34+ cells ⟨127⟩, 6560
Flow cytometry ⟨1027⟩, 7281
Fluorescence spectroscopy ⟨853⟩, 7146
Fluorescence spectroscopy—theory and practice ⟨1853⟩, 8641
Folic acid assay ⟨411⟩, 6689
Gaseous sterilization ⟨1229.7⟩, 8222
Gene therapy products ⟨1047⟩, 7426
Globule size distribution in lipid injectable emulsions ⟨729⟩, 6963
Glucagon bioidentity tests ⟨123⟩, 6552
Glycoprotein and glycan analysis—general considerations ⟨1084⟩, 7655
Good distribution practices for bulk pharmaceutical excipients ⟨1197⟩, 8060
Good manufacturing practices for bulk pharmaceutical excipients ⟨1078⟩, 7617
Good packaging practices ⟨1177⟩, 8003
Good repackaging practices ⟨1178⟩, 8006
Good storage and distribution practices for drug products ⟨1079⟩, 7635
Growth factors and cytokines used in cell therapy manufacturing ⟨92⟩, 6539
Guidelines on endotoxins test ⟨1085⟩, 7665
Hazardous drugs—handling in healthcare settings ⟨800⟩, 7071
Hematoxylin and eosin staining of sectioned tissue for microscopic examination ⟨1285.1⟩, 8374
High-performance thin-layer chromatography procedure for identification of articles of botanical origin ⟨203⟩, 6602
Human plasma ⟨1180⟩, 8008
Identification of articles of botanical origin ⟨563⟩, 6788
Identification of articles of botanical origin by high-performance thin-layer chromatography procedure ⟨1064⟩, 7578
Identification of fixed oils by thin-layer chromatography ⟨202⟩, 6601
Identification—organic nitrogenous bases ⟨181⟩, 6587
Identification tests—general ⟨191⟩, 6587
Identification—tetracyclines ⟨193⟩, 6592
Immunogenicity assays—design and validation of assays to detect anti-drug neutralizing antibody ⟨1106.1⟩, 7802
Immunogenicity assays—design and validation of immunoassays to detect anti-drug antibodies ⟨1106⟩, 7788
Immunological test methods—surface plasmon resonance ⟨1105⟩, 7773
Immunological test methods—enzyme-linked immunosorbent assay (ELISA) ⟨1103⟩, 7753
Immunological test methods—general considerations ⟨1102⟩, 7746
Immunological test methods—immunoblot analysis ⟨1104⟩, 7763
Impurities in drug substances and drug products ⟨1086⟩, 7678
Impurities testing in medical gases ⟨413⟩, 6692
Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests ⟨601⟩, 6819
Inhalation and nasal drug products—general information and product quality tests ⟨5⟩, 6427
Injections and implanted drug products (parenterals)—product quality tests ⟨1⟩, 6405
Insulin assays ⟨121⟩, 6547
In vitro and in vivo evaluation of dosage forms ⟨1088⟩, 7684
Iodometric assay—antibiotics ⟨425⟩, 6696
Ion chromatography ⟨1065⟩, 7588
Iron ⟨241⟩, 6648
Labeling ⟨7⟩, 6435
Labeling of inactive ingredients ⟨1091⟩, 7706
Lead ⟨251⟩, 6649
Leak rate ⟨604⟩, 6846
Light diffraction measurement of particle size ⟨429⟩, 6697
Limit on number of large deviations when assessing content uniformity in large samples ⟨1099⟩, 7744
Liquid-phase sterilization ⟨1229.6⟩, 8219
Loss on drying ⟨731⟩, 6970
Loss on ignition ⟨733⟩, 6970
Low molecular weight heparin molecular weight determinations ⟨209⟩, 6614
Manufacturing practices for dietary supplements ⟨2750⟩, 8742
Mass spectrometry ⟨736⟩, 6975
Measurement of subvisible particulate matter in therapeutic protein injections ⟨1787⟩, 8562
Medical devices—bacterial endotoxin and pyrogen tests ⟨161⟩, 6579
Medical gases assay ⟨415⟩, 6693
Melting range or temperature ⟨741⟩, 6980
Mercury ⟨261⟩, 6650
Methods for the determination of particulate matter in injections and ophthalmic solutions ⟨1788⟩, 8575
Methoxy determination ⟨431⟩, 6702
Microbial characterization, identification, and strain typing ⟨1113⟩, 7823
Microbial enumeration tests—nutritional and dietary supplements ⟨2021⟩, 8687
Microbiological attributes of nonsterile nutritional and dietary supplements ⟨2023⟩, 8698
Microbiological best laboratory practices ⟨1117⟩, 7845
Microbiological control and monitoring of aseptic processing environments ⟨1116⟩, 7833
Microbiological examination of nonsterile products: acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use ⟨1111⟩, 7819
Microbiological examination of nonsterile products: microbial enumeration tests ⟨61⟩, 6457
Microbiological examination of nonsterile products: tests for specified organisms ⟨62⟩, 6463
Microbiological examination of nonsterile products tests for Burkholderia Cepacia complex ⟨60⟩, 6454
Microbiological procedures for absence of specified microorganisms—nutritional and dietary supplements ⟨2022⟩, 8692
Mid-infrared spectroscopy ⟨854⟩, 7151
Mid-infrared spectroscopy—theory and practice ⟨1854⟩, 8650
Minimum fill ⟨755⟩, 6982
Moist heat sterilization of aqueous liquids ⟨1229.2⟩, 8202
Monitoring devices—time, temperature, and humidity ⟨1118⟩, 7850
Monitoring of bioburden ⟨1229.3⟩, 8206
Monosaccharide analysis ⟨210⟩, 6616
Mucosal drug products—performance tests ⟨1004⟩, 7227
Mucosal drug products—product quality tests ⟨4⟩, 6423
Mycoplasma tests ⟨63⟩, 6472
Near-infrared spectroscopy ⟨856⟩, 7161
Nephelometry, turbidimetry, and visual comparison ⟨855⟩, 7155
New sterilization methods ⟨1229.12⟩, 8233
Niacin or niacinamide assay ⟨441⟩, 6704
Nitrite titration ⟨451⟩, 6709
Nitrogen determination ⟨461⟩, 6709
Nomenclature ⟨1121⟩, 7862
Nuclear magnetic resonance spectroscopy ⟨761⟩, 6984
Nuclear magnetic resonance spectroscopy identity testing of bacterial polysaccharides used in vaccine manufacture ⟨198⟩, 6596
Nucleic acid-based techniques—amplification ⟨1127⟩, 7879
Nucleic acid-based techniques—approaches for detecting trace nucleic acids (residual DNA testing) ⟨1130⟩, 7897
Nucleic acid-based techniques—extraction, detection, and sequencing ⟨1126⟩, 7870
Nucleic acid-based techniques—general ⟨1125⟩, 7865
Nucleic acid-based techniques—genotyping ⟨1129⟩, 7893
Nucleic acid-based techniques—microarray ⟨1128⟩, 7887
Oligosaccharide analysis ⟨212⟩, 6623
Ophthalmic products—performance tests ⟨1771⟩, 8549
Ophthalmic products—quality tests ⟨771⟩, 6993
Optical microscopy ⟨776⟩, 6998
Optical rotation ⟨781⟩, 7001
Oral drug products—product quality tests ⟨2⟩, 6410
Orally inhaled and nasal drug products ⟨1664.1⟩, 8467
Ordinary impurities ⟨466⟩, 6710
Osmolality and osmolarity ⟨785⟩, 7008
Oxygen flask combustion ⟨471⟩, 6735
Package integrity and test method selection ⟨1207.1⟩, 8086
Package integrity leak test technologies ⟨1207.2⟩, 8097
Package seal quality test technologies ⟨1207.3⟩, 8111
Packaging and repackaging—single unit containers ⟨1136⟩, 7919
Packaging and storage requirements ⟨659⟩, 6876
Pancreatin ⟨1025⟩, 7272
Particle size distribution estimation by analytical sieving ⟨786⟩, 7010
Particulate matter in injections ⟨788⟩, 7017
Particulate matter in ophthalmic solutions ⟨789⟩, 7020
pH ⟨791⟩, 7022
Pharmaceutical calculations in pharmacy practice ⟨1160⟩, 7953
Pharmaceutical compounding—nonsterile preparations ⟨795⟩, 7025

www.webofpharma.com

General chapters *(continued)*
Pharmaceutical compounding—sterile preparations ⟨797⟩, 7037
Pharmaceutical dosage forms ⟨1151⟩, 7929
Physical environments that promote safe medication use ⟨1066⟩, 7590
Physicochemical analytical procedures for insulins ⟨121.1⟩, 6550
Physicochemical integrators and indicators for sterilization ⟨1229.9⟩, 8227
Plasma spectrochemistry ⟨730⟩, 6967
Plasma spectrochemistry—theory and practice ⟨1730⟩, 8484
Plastic materials of construction ⟨661.1⟩, 6893
Plastic packaging systems and their materials of construction ⟨661⟩, 6887
Plastic packaging systems for pharmaceutical use ⟨661.2⟩, 6912
Polarography ⟨801⟩, 7089
Porosimetry by mercury intrusion ⟨267⟩, 6653
Porosity by nitrogen adsorption–desorption ⟨268⟩, 6655
Positron emission tomography drugs for compounding, investigational, and research uses ⟨823⟩, 7100
Positron emission tomography drugs—information ⟨1823⟩, 8617
Powder fineness ⟨811⟩, 7093
Powder flow ⟨1174⟩, 7993
Prekallikrein activator ⟨165⟩, 6583
Preparation of biological specimens for histologic and immunohistochemical analysis ⟨1285⟩, 8370
Prescription balances and volumetric apparatus ⟨1176⟩, 7997
Prescription container labeling ⟨17⟩, 6445
Prion inactivation ⟨1229.16⟩, 8239
Products for nebulization—characterization tests ⟨1601⟩, 8407
Propellants ⟨602⟩, 6844
Protein A quality attributes ⟨130⟩, 6570
Protein determination procedures ⟨507⟩, 6744
Pyrogen test ⟨151⟩, 6577
Quality assurance in pharmaceutical compounding ⟨1163⟩, 7979
Quality attributes of tablets labeled as having a functional score ⟨705⟩, 6944
Quality of biotechnological products: analysis of the expression construct in cells used for production of r-DNA derived protein products ⟨1048⟩, 7452
Quality of biotechnological products: stability testing of biotechnological/biological products ⟨1049⟩, 7454
Radiation sterilization ⟨1229.10⟩, 8228
Radioactivity ⟨821⟩, 7094
Radioactivity—theory and practice ⟨1821⟩, 8604
Raman spectroscopy ⟨1120⟩, 7856
Rapid sterility testing of short life products a risk based approach ⟨1071⟩, 7602
Readily carbonizable substances test ⟨271⟩, 6660
Refractive index ⟨831⟩, 7137
Residual DNA testing ⟨509⟩, 6748
Residual host cell protein measurement in biopharmaceuticals ⟨1132⟩, 7900
Residual solvents ⟨467⟩, 6712
Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures ⟨1467⟩, 8404
Residue on ignition ⟨281⟩, 6660
Rheometry ⟨1911⟩, 8679
Riboflavin assay ⟨481⟩, 6735
Salts of organic nitrogenous bases ⟨501⟩, 6741
Scanning electron microscopy ⟨1181⟩, 8025
Screening for undeclared drugs and drug analogues ⟨2251⟩, 8725
Selenium ⟨291⟩, 6661
Semisolid drug products—performance tests ⟨1724⟩, 8473
Sensitization testing ⟨1184⟩, 8035
Shear cell methodology for powder flow testing ⟨1063⟩, 7566
Significant change guide for bulk pharmaceutical excipients ⟨1195⟩, 8049
Single-steroid assay ⟨511⟩, 6750
Somatropin bioidentity tests ⟨126⟩, 6558
Spacers and valved holding chambers used with inhalation aerosols—characterization tests ⟨1602⟩, 8411
Specific gravity ⟨841⟩, 7137
Specific surface area ⟨846⟩, 7139
Spectrophotometric identification tests ⟨197⟩, 6593
Stability considerations in dispensing practice ⟨1191⟩, 8044
Statistical tools for procedure validation ⟨1210⟩, 8117
Steam sterilization by direct contact ⟨1229.1⟩, 8199
Sterile product packaging—integrity evaluation ⟨1207⟩, 8079
Sterility Assurance ⟨1211⟩, 8129
Sterility testing—validation of isolator systems ⟨1208⟩, 8113
Sterility tests ⟨71⟩, 6481
Sterilization cycle development ⟨1229.14⟩, 8235
Sterilization filtration of gases ⟨1229.15⟩, 8238
Sterilization-in-place ⟨1229.13⟩, 8233
Sterilization of compendial articles ⟨1229⟩, 8194
Sterilizing filtration of liquids ⟨1229.4⟩, 8209
Storage and transportation of investigational drug products ⟨1079.1⟩, 7644
Subvisible particulate matter in therapeutic protein injections ⟨787⟩, 7014
Sulfur dioxide ⟨525⟩, 6751
Supplemental information for articles of botanical origin ⟨2030⟩, 8702
Sutures—diameter ⟨861⟩, 7175
Sutures—needle attachment ⟨871⟩, 7178
Tablet breaking force ⟨1217⟩, 8138
Tablet compression characterization ⟨1062⟩, 7556
Tablet friability ⟨1216⟩, 8137
Tensile strength ⟨881⟩, 7179
Terminally sterilized pharmaceutical products—parametric release ⟨1222⟩, 8142
Test for 1,6-anhydro derivative for enoxaparin sodium ⟨207⟩, 6605
Theory and practice of electrical conductivity measurements of solutions ⟨1644⟩, 8423
Thermal analysis ⟨891⟩, 7180
Thiamine assay ⟨531⟩, 6756
Thin-layer chromatographic identification test ⟨201⟩, 6600
Titrimetry ⟨541⟩, 6764
Topical aerosols ⟨603⟩, 6845
Topical and transdermal drug products—product quality tests ⟨3⟩, 6415
Total organic carbon ⟨643⟩, 6867
Transfer of analytical procedures ⟨1224⟩, 8164
Trifluoroacetic acid (TFA) in peptides ⟨503.1⟩, 6743
Ultraviolet-visible spectroscopy ⟨857⟩, 7166
Ultraviolet-visible spectroscopy—theory and practice ⟨1857⟩, 8670
Uniformity of dosage units ⟨905⟩, 7183
USP reference standards ⟨11⟩, 6442
Vaccines for human use—bacterial vaccines ⟨1238⟩, 8338
Vaccines for human use—general considerations ⟨1235⟩, 8291
Vaccines for human use—polysaccharide and glycoconjugate vaccines ⟨1234⟩, 8276
Validation of alternative microbiological methods ⟨1223⟩, 8144
Validation of compendial procedures ⟨1225⟩, 8166
Validation of microbial recovery from pharmacopeial articles ⟨1227⟩, 8172
Validation of alternative methods to antibiotic microbial assays ⟨1223.1⟩, 8157
Vapor phase sterilization ⟨1229.11⟩, 8231
Verification of compendial procedures ⟨1226⟩, 8171
Vibrational circular dichroism spectroscopytheory and practice ⟨1782⟩, 8549
Vibrational circular dichroism spectroscopy ⟨782⟩, 7002
Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin ⟨1050⟩, 7459
Virology test methods ⟨1237⟩, 8319
Virus testing of human plasma for further manufacture ⟨1240⟩, 8350
Viscosity—capillary methods ⟨911⟩, 7187
Viscosity—pressure driven methods ⟨914⟩, 7195
Viscosity—rolling ball method ⟨913⟩, 7193
Viscosity—rotational methods ⟨912⟩, 7189
Visible particulates in injections ⟨790⟩, 7021
Visual Comparison ⟨630⟩, 6865
Visual inspection of injections ⟨1790⟩, 8587
Vitamin A assay ⟨571⟩, 6801
Vitamin B_{12} activity assay ⟨171⟩, 6584
Vitamin C assay ⟨580⟩, 6805
Vitamin D assay ⟨581⟩, 6808
Vitamin E assay ⟨551⟩, 6767
Volumetric apparatus ⟨31⟩, 6448
Water conductivity ⟨645⟩, 6871
Water determination ⟨921⟩, 7196
Water for hemodialysis applications ⟨1230⟩, 8240
Water for pharmaceutical purposes ⟨1231⟩, 8242
Water–solid interactions in pharmaceutical systems ⟨1241⟩, 8359
Weighing on an analytical balance ⟨1251⟩, 8363
Weight variation of dietary supplements ⟨2091⟩, 8718
Written prescription drug information—guidelines ⟨1265⟩, 8368
X-ray fluorescence spectrometry ⟨735⟩, 6971
X-ray fluorescence spectrometry—theory and practice ⟨1735⟩, 8490

www.webofpharma.com

General chapters *(continued)*
Zinc determination ⟨591⟩, 6817

General notices and requirements, 1
Conformance to standards, 4
Monograph components, 6
Monographs and general chapters, 5
Official status and legal recognition, 3
Prescribing and dispensing, 12
Preservation, packaging, storage, and labeling, 12
Terms and definitions, 9
Test results, 9
Testing practices and procedures, 8
Title and revision, 3
General tests for reagents, 6128
Geneticin, 6168
Gentamicin
injection, 2099
and prednisolone acetate ophthalmic ointment, 2106
and prednisolone acetate ophthalmic suspension, 2107
sulfate, 2100
sulfate and betamethasone acetate ophthalmic solution, 2102
sulfate and betamethasone valerate ointment, 2103
sulfate and betamethasone valerate otic solution, 2104
sulfate and betamethasone valerate topical solution, 2105
sulfate cream, 2101
sulfate ointment, 2101
sulfate ophthalmic ointment, 2102
sulfate ophthalmic solution, 2102
uterine infusion, 2099
Gentian violet, 2107
cream, 2108
topical solution, 2109
Ginger, 5026
capsules, 5032
powdered, 5028
tincture, 5030
Ginkgo, 5034
capsules, 5037
extract, powdered, 5038
tablets, 5041
Ginseng
American, 4750
Asian, 4774
capsules, American, 4754
extract, powdered American, 4753
extract, powdered Asian, 4776
powdered, American, 4752
powdered, Asian, 4775
tablets, American, 4757
tablets, Asian, 4778
Tienchi, root and rhizome, 5299
Tienchi, root and rhizome dry extract, 5307
Tienchi, root and rhizome powder, 5301
Girard reagent T, 6168
Gitoxin, 6168
Glacial acetic acid, 77, 6168
TS, 6229
Glass wool, 6168
Glaze, pharmaceutical, 5786
Glimepiride, 2109
and pioglitazone tablets, 3554
tablets, 2111
Glipizide, 2113
and metformin hydrochloride tablets, 2116
tablets, 2115
Globule size distribution in lipid injectable emulsions ⟨729⟩, 6963
Globulin
immune, 2119
reagent, anti-human, 6139
RH_o (D) immune, 2120
Glucagon, 2120
for injection, 2122
Glucagon bioidentity tests ⟨123⟩, 6552
Glucoamylase, 6168
D-Gluconic acid, 50 percent in water, 6168
Gluconolactone, 2123
Glucosamine
and chondroitin sulfate sodium tablets, 5043
chondroitin sulfate sodium, and methylsulfonylmethane tablets, 5051
hydrochloride, 5045
and methylsulfonylmethane tablets, 5049
sulfate potassium chloride, 5047
sulfate sodium chloride, 5048
tablets, 5046
Glucose, 6168
enzymatic test strip, 2123
liquid, 5788
oxidase-chromogen TS, 6229
Glucose oxidase, 6168
D-Glucuronolactone, 6168
Glutamic acid, 5053, 6168
L-Glutamic acid, 6168
hydrochloride, 5789
Glutamine, 2123
L-Glutamine, 6168
Glutaral
concentrate, 2124
disinfectant solution, 5789
Glutathione, 5054
Glyburide, 2125
and metformin hydrochloride tablets, 2128
tablets, 2126
Glycerin, 2132, 6168
base TS, 6229
ophthalmic solution, 2133
oral solution, 2134
suppositories, 2134
Glyceryl
behenate, 5790
dibehenate, 5791
distearate, 5792
monocaprylate, 5799
monocaprylocaprate, 5802
monolinoleate, 5804
monooleate, 5805
monostearate, 5807
tristearate, 5810
Glyceryl mono and dicaprylate, 5794
Glyceryl mono and dicaprylocaprate, 5796
Glyceryl monostearate, 6168
Glyceryl tricaprylate, 5808
Glycine, 2134
irrigation, 2136
Glycolic acid, 6168
Glycoprotein and glycan analysis—general considerations ⟨1084⟩, 7655
Glycopyrrolate, 2136
injection, 2139
tablets, 2140
Glycyl-L-glutamine, 5058
Glycyl-L-tyrosine, 5059
Glyoxal solution, 6169
Gold
chloride, 6169
chloride TS, 6229
sodium thiomalate, 2142
sodium thiomalate injection, 2143
Goldenseal, 5061
extract, powdered, 5064
powdered, 5063
Gonadorelin
acetate, 2144
hydrochloride, 2146
for injection, 2143
Gonadotropin
chorionic, 2148
chorionic, for injection, 2149
Good distribution practices for bulk pharmaceutical excipients ⟨1197⟩, 8060
Good manufacturing practices for bulk pharmaceutical excipients ⟨1078⟩, 7617
Good packaging practices ⟨1177⟩, 8003
Good repackaging practices ⟨1178⟩, 8006
Good storage and distribution practices for drug products ⟨1079⟩, 7635
Goserelin acetate, 2150
Goserelin Implants, 2152
Government liaisons to expert committees and expert panels, xvi
Graftskin, 1162
Gramicidin, 2156
and neomycin and polymyxin B sulfates cream, 3122
and neomycin and polymyxin B sulfates and hydrocortisone acetate cream, 3123
and neomycin and polymyxin B sulfates ophthalmic solution, 3123
and neomycin sulfate ointment, 3108
nystatin, neomycin sulfate, and triamcinolone acetonide cream, 3222
nystatin, neomycin sulfate, and triamcinolone acetonide ointment, 3222
Granisetron, 2156
Granisetron hydrochloride, 2158
injection, 2159
oral suspension, 2161
tablets, 2161
Granules
Flunixin meglumine, 1931
Montelukast sodium, oral, 3012
Grape seeds oligomeric proanthocyanidins, 5065
Gravity, specific ⟨841⟩, 7137
Green
brilliant, 6144, 6176, 6220
FCF, fast, 6165
soap, 2163
soap tincture, 2164
Green tea
extract, decaffeinated, powdered, 5067
Griseofulvin, 2164
capsules, 2165
oral suspension, 2166
tablets, 2166
tablets, ultramicrosize, 2168
Growth factors and cytokines used in cell therapy manufacturing ⟨92⟩, 6539
Guaiacol, 6169
Guaifenesin, 2169, 6169
capsules, 2170
and codeine phosphate oral solution, 2172
compounded injection, veterinary, 2170
and dyphylline oral solution, 1575
and dyphylline tablets, 1575
and pseudoephedrine hydrochloride capsules, 2174
pseudoephedrine hydrochloride, and dextromethorphan hydrobromide capsules, 2175
and theophylline capsules, 4331
and theophylline oral solution, 4332

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Guaifenesin *(continued)*
for injection, 2171
oral solution, 2171
tablets, 2172
Guanabenz acetate, 2176
tablets, 2177
Guanethidine monosulfate, 2178
tablets, 2179
Guanfacine
hydrochloride, 2180
tablets, 2180
Guanidine hydrochloride, 6169
Guanidine isothiocyanate, 6169
Guanine hydrochloride, 6169
Guar gum, 5813
Guggul, 5069
extract, native, 5070
extract, purified, 5071
tablets, 5072
Guide to general chapters
charts, 6353
table of contents, 13
Guidelines on endotoxins test ⟨1085⟩, 7665
Gutta percha, 2182
Gymnema, 5074
extract, native, 5077
extract, purified, 5078
powdered, 5075

H

Halazone, 2182
tablets for solution, 2182
Halcinonide, 2183
cream, 2184
ointment, 2185
topical solution, 2186
Halobetasol propionate, 2187
Haloperidol, 2188
decanoate, 2191
injection, 2189
oral solution, 2189
tablets, 2190
Halothane, 2192
Hawthorn leaf
with flower, 5080
with flower, powdered, 5082
Hazardous drugs—handling in healthcare settings ⟨800⟩, 7071
Heavy metals in reagents, 6130
Helium, 2193
oxygen certified standard, 6185
Hematein, 6169
Hematoxylin, 6169
TS, Delafield's, 6228
Hematoxylin and eosin staining of sectioned tissue for microscopic examination ⟨1285.1⟩, 8374
Hemoglobin, bovine, 6169
Heparin
lock flush solution, 2194
sodium, 2195
sodium injection, 2200
Hepatitis B
immune globulin, 2201
1-Heptadecanol, 6169
Heptafluorobutyric acid, 6169
Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin, 6169
n-Heptane, 6169
chromatographic, 6152, 6169
Heptyl *p*-hydroxybenzoate, 6169
Hesperidin, 5084
Hexachlorophene, 2201
cleansing emulsion, 2202
liquid soap, 2202
Hexadecyl hexadecanoate, 6169
Hexadecyltrimethylammonium bromide, 6169
Hexadimethrine bromide, 6170
Hexamethyldisilazane, 6170
Hexamethyleneimine, 6170
Hexamethylenetetramine, 6170
n-Hexane, 6170
Hexane, solvent, 6170, 6189, 6207
chromatographic, 6152, 6170
Hexanes, 6170
Hexanitrodiphenylamine, 6162, 6170
Hexanophenone, 6170
Hexylamine, 6170
Hexylene glycol, 5814
Hexylresorcinol, 2203
lozenges, 2204
High-performance thin-layer chromatography procedure for identification of articles of botanical origin ⟨203⟩, 6602
Histamine
dihydrochloride, 6170
phosphate, 2205
phosphate injection, 2205
Histidine, 2206
L-Histidine hydrochloride monohydrate, 6170
Holy basil leaf, 5085
extract, powdered, 5089
powdered, 5087
Homatropine
hydrobromide, 2207
hydrobromide ophthalmic solution, 2208
methylbromide, 2208
methylbromide and hydrocodone bitartrate tablets, 2225
methylbromide tablets, 2210
Homosalate, 2210
Honey, purified, 5816
Horse chestnut, 4873
extract, powdered, 4876
powdered, 4875
Horseradish peroxidase conjugated to goat anti-mouse IgG, 6170
Human plasma ⟨1180⟩, 8008
Hyaluronidase
injection, 2211
for injection, 2211
Hydralazine hydrochloride, 2213
injection, 2214
oral solution, 2215
tablets, 2215
Hydrazine
dihydrochloride, 6170
hydrate, 85% in water, 6170
sulfate, 6170
Hydrindantin, 6170
Hydriodic acid, 6170
Hydrobromic acid, 6170
Hydrochloric acid, 5816, 6170
alcoholic, tenth-molar (0.1 M), 6242
buffer, 6145
diluted, 5817, 6159, 6170
half-normal (0.5 N), 6242
half-normal (0.5 N) in methanol, 6242
injection, 2217
normal (1 N), 6242
0.001 N TS, 6229
0.01 M TS, 6229
0.025 N TS, 6229
0.36 N TS, 6229
0.05 N TS, 6229
2 N TS, 6229
3 N TS, 6229
5 N TS, 6229
6 N TS, 6230
0.01 N VS, 6241
0.1 N VS, 6242
0.02 N VS, 6241
Hydrochloric acid
25 percent, 6171
4 N Hydrochloric acid
TS, 6229
0.08 N hydrochloric acid TS, 6230
0.125 N hydrochloric acid TS, 6230
Hydrochloride
fingolimod, 1891
nile blue, 6221
Hydrochlorothiazide, 2217
and amiloride hydrochloride tablets, 224
amlodipine, valsartan, tablets, 279
and bisoprolol fumarate tablets, 587
candesartan cilexetil, tablets, 719
capsules, 2219
and captopril tablets, 737
and enalapril maleate tablets, 1616
and fosinopril tablets, 2043
and irbesartan tablets, 2404
and lisinopril tablets, 2646
and losartan potassium tablets, 2690
and methyldopa tablets, 2878
and metoprolol tartrate tablets, 2928
and moexipril hydrochloride and tablets, 2996
and propranolol hydrochloride tablets, 3751
and quinapril tablets, 3803
and spironolactone oral suspension, 4113
and spironolactone tablets, 4113
tablets, 2222
and telmisartan tablets, 4246
and timolol maleate tablets, 4391
and triamterene capsules, 4496
and triamterene tablets, 4499
and valsartan tablets, 4580
Hydrocodone bitartrate, 2223
and acetaminophen tablets, 2224
and homatropine methylbromide tablets, 2225
tablets, 2224
Hydrocodone diol, 6171
Hydrocortisone, 2228
acetate, 2236
acetate and chloramphenicol for ophthalmic suspension, 932
acetate and colistin and neomycin sulfates otic suspension, 1150
acetate cream, 2237
acetate lotion, 2237
acetate, neomycin and polymyxin B sulfates, and bacitracin ointment, 3116
acetate, neomycin and polymyxin B sulfates, and bacitracin ophthalmic ointment, 3116
acetate, neomycin and polymyxin B sulfates, and bacitracin zinc ophthalmic ointment, 3120
acetate and neomycin and polymyxin B sulfates cream, 3125
acetate, neomycin and polymyxin B sulfates, and gramidicin cream, 3123
acetate and neomycin and polymyxin B sulfates ophthalmic suspension, 3125
acetate and neomycin sulfate cream, 3110
acetate and neomycin sulfate lotion, 3110

www.webofpharma.com

Hydrocortisone *(continued)*
acetate and neomycin sulfate ointment, 3110
acetate and neomycin sulfate ophthalmic suspension, 3110
acetate ointment, 2238
acetate and oxytetracycline hydrochloride ophthalmic suspension, 3363
acetate, penicillin G, neomycin, polymyxin B, and hydrocortisone sodium succinate topical suspension, 3430
acetate, penicillin G procaine, and neomycin and polymyxin B sulfates topical suspension, 3448
and acetic acid otic solution, 2235
and clioquinol cream, 1077
and clioquinol ointment, 1078
and neomycin and polymyxin B sulfates ophthalmic suspension, 3123
and neomycin and polymyxin B sulfates otic solution, 3124
and neomycin and polymyxin B sulfates otic suspension, 3124
and neomycin sulfate cream, 3109
and neomycin sulfate ointment, 3109
and neomycin sulfate otic suspension, 3109
and oxytetracycline hydrochloride ointment, 3364
and polymyxin B sulfate otic solution, 3592
butyrate, 2238
butyrate cream, 2239
cream, 2229
gel, 2230
hemisuccinate, 2240
lotion, 2232
neomycin and polymyxin B sulfates and bacitracin zinc ointment, 3118
neomycin and polymyxin B sulfates and bacitracin zinc ophthalmic ointment, 3119
ointment, 2232
rectal suspension, 2233
sodium phosphate, 2241
sodium phosphate injection, 2242
sodium succinate, 2243
sodium succinate for injection, 2243
sodium succinate, penicillin G, neomycin, polymyxin B, and hydrocortisone acetate topical suspension, 3430
tablets, 2234
valerate, 2245
valerate cream, 2245
valerate ointment, 2246
Hydroflumethiazide, 2247
tablets, 2247
Hydrofluoric acid, 6171
Hydrogen
peroxide, 10 percent, 6171
peroxide, 30 percent, 6171
peroxide, 30 percent, unstabilized, 6171
peroxide, 50 percent in water, 6171
peroxide concentrate, 2248
peroxide solution, 6171
peroxide topical solution, 2249
peroxide TS, 6230
sulfide, 6171
sulfide detector tube, 6171
sulfide TS, 6230
Hydrogenated lanolin, 5850
Hydrogenated polydextrose, 5936
Hydrogenated vegetable oil, 6099
Hydromorphone hydrochloride, 2249
injection, 2252
oral solution, 2252
tablets, 2254
Hydroquinone, 2255, 6171
cream, 2255
topical solution, 2255
Hydroxocobalamin, 2256
injection, 2257
Hydroxy naphthol blue, 6171
3′-Hydroxyacetophenone, 6171
4′-Hydroxyacetophenone, 6171
Hydroxyamphetamine hydrobromide, 2258
ophthalmic solution, 2258
Hydroxyanisole, butylated, 5651
p-Hydroxybenzoic acid, 6171
4-Hydroxybenzoic acid isopropyl ester, 6171
2-Hydroxybenzyl alcohol, 6171
4-Hydroxybutane-1-sulfonic acid, 6171
4-Hydroxy-2-butanone, 6171
Hydroxychloroquine sulfate, 2259
tablets, 2259
Hydroxyethyl cellulose, 5817
N-(2-Hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid), 6171
Hydroxylamine hydrochloride, 6171
TS, 6230
5-Hydroxymethylfurfural, 6172
10β-Hydroxynorandrostenedione, 6172
2′-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole trihydrochloride pentahydrate, 6172
4-(4-Hydroxyphenyl)-2-butanone, 6172
3-Hydroxyphenyldimethylethyl ammonium chloride, 6172
D-α-4-Hydroxyphenylglycine, 6172
4-Hydroxy-4-phenylpiperidine, 6171
Hydroxyprogesterone caproate, 2260
injection, 2261
Hydroxypropyl
betadex, 5818
cellulose, 5821
cellulose, low-substituted, 5822
cellulose ocular system, 2262
corn starch, 6045
pea starch, 6054
potato starch, 6060
Hydroxypropyl-β-cyclodextrin, 6172
Hydroxypropyl cellulose, 6172
8-Hydroxyquinoline, 6172
TS, 6230
Hydroxytoluene
butylated, 5652
butylated, reagent, 6146
Hydroxyurea, 2262
capsules, 2263
Hydroxyzine
hydrochloride, 2264
hydrochloride injection, 2265
hydrochloride oral solution, 2266
hydrochloride tablets, 2267
pamoate, 2269
pamoate capsules, 2271
Hymetellose, 5824
Hyoscyamine, 2273
hydrobromide, 2274
sulfate, 2275
sulfate elixir, 2276
sulfate injection, 2277
sulfate oral solution, 2277
sulfate tablets, 2278
tablets, 2273
Hypophosphorous acid, 5825
50 percent, 6172
Hypoxanthine, 6172
Hypromellose, 2279
acetate succinate, 5825
ophthalmic solution, 2281
phthalate, 5828

I

I 123
capsules, sodium iodide, 2365
injection, iobenguane, 2365
solution, sodium iodide, 2366
I 125
albumin injection, iodinated, 2367
injection, iothalamate sodium, 2367
I 131
albumin injection, iodinated, 2368
capsules, sodium iodide, 2368
solution, sodium iodide, 2368
Ibuprofen, 2281
and diphenhydramine citrate tablets, 1421
diphenhydramine hydrochloride capsules, 1431
and pseudoephedrine hydrochloride tablets, 2286
oral suspension, 2283
tablets, 2285
Ibutilide fumarate, 2287
Ichthammol, 2289
ointment, 2289
Idarubicin hydrochloride, 2290
injection, 2291
for injection, 2291
Identification
of articles of botanical origin ⟨563⟩, 6788
of articles of botanical origin by high-performance thin-layer chromatography procedure ⟨1064⟩, 7578
of fixed oils by thin-layer chromatography ⟨202⟩, 6601
organic nitrogenous bases ⟨181⟩, 6587
test, thin-layer chromatographic ⟨201⟩, 6600
tests—general ⟨191⟩, 6587
tests, spectrophotometric ⟨197⟩, 6593
tetracyclines ⟨193⟩, 6592
Idoxuridine, 2292
ophthalmic ointment, 2293
ophthalmic solution, 2293
Ifosfamide, 2294
for injection, 2296
IgG-coated red cells, 6172
Imidazole, 6172
Imidurea, 5829
Iminodiacetic acid, 6172
Imipenem, 2296
and cilastatin for injectable suspension, 2298
and cilastatin for injection, 2297
Imipramine pamoate, 2303
Imipramine hydrochloride, 2300
injection, 2301
tablets, 2301
Imipramine pamoate
capsules, 2304
Imiquimod, 2307
cream, 2309
Immunogenicity assays—design and validation of assays to detect anti-drug neutralizing antibody ⟨1106.1⟩, 7802
Immunogenicity assays—design and validation of immunoassays to detect anti-drug antibodies ⟨1106⟩, 7788

www.webofpharma.com

Immunological test methods—surface plasmon resonance ⟨1105⟩, 7773
Immunological test methods
enzyme-linked immunosorbent assay (ELISA) ⟨1103⟩, 7753
general considerations ⟨1102⟩, 7746
immunoblot analysis ⟨1104⟩, 7763
Impurities
ordinary ⟨466⟩, 6710
testing in medical gases ⟨413⟩, 6692
Impurities in drug substances and drug products ⟨1086⟩, 7678
Inamrinone, 2310
injection, 2311
Indapamide, 2313
tablets, 2314
Indene, 6172
Indicator and test papers, 6219
Indicators, 6220
indicator papers, 6219
reagents, and solutions, 6127
test papers, 6219
Indigo carmine, 6172
TS, 6230
Indigotindisulfonate sodium, 2315
injection, 2316
Indinavir sulfate, 2316
Indium In 111
capromab pendetide injection, 2318
chloride solution, 2318
oxyquinoline solution, 2320
pentetate injection, 2320
pentetreotide injection, 2321
Indocyanine green, 2322
for injection, 2322
Indole, 6172
Indole-3-carboxylic acid, 6172
Indomethacin, 2323
capsules, 2324
extended-release capsules, 2325
for injection, 2330
topical gel, 2330
oral suspension, 2332
sodium, 2334
suppositories, 2331
Indophenol-acetate TS, 6230
Inhalant
amyl nitrite, 332
propylhexedrine, 3754

Inhalation

Acetylcysteine and isoproterenol hydrochloride solution, 84
Cromolyn sodium powder, 1182
Cromolyn sodium solution, 1182
Epinephrine solution, 1648
Fluticasone propionate aerosol, 1988
Fluticasone propionate powder, 1993
Isoetharine solution, 2416
Isoproterenol solution, 2431
Isoproterenol sulfate aerosol, 2437
Isoproterenol sulfate solution, 2438
Levalbuterol solution, 2576
Racepinephrine solution, 3825
Ribavirin for solution, 3865
Salmeterol powder, 3973
Sodium chloride, solution, 4068
Sterile water for, 4652
Tobramycin solution, 4406

Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests ⟨601⟩, 6819
Inhalation and nasal drug products general information and product quality tests ⟨5⟩, 6427

Injection

Acepromazine maleate, 37
Acetazolamide for, 74
Acyclovir for, 90
Adenosine, 99
Alcohol, dehydrated, 117
Alcohol in dextrose, 117
Alfentanil, 123
Alprostadil, 156
Alteplase for, 160
Amifostine for, 217
Amikacin sulfate, 221
Aminocaproic acid, 236
Aminohippurate sodium, 237
Aminopentamide sulfate, 240
Aminophylline, 243
Amiodarone hydrochloride, 254
Ammonium chloride, 288
Ammonium molybdate, 291
Amobarbital sodium for, 292
Amphotericin B for, 315
Ampicillin for, 323
Ampicillin and sulbactam for, 328
Anileridine, 339
Aprotinin, 367
Arginine hydrochloride, 373
Articaine hydrochloride and epinephrine, 381
Ascorbic acid, 383
Atenolol, 406
Atracurium besylate, 426
Atropine sulfate, 430
Azaperone, 436
Azathioprine sodium for, 442
Azithromycin for, 451
Aztreonam, 462
Aztreonam for, 463
Bacitracin for, 466
Bacteriostatic sodium chloride, 4067
Bacteriostatic water for, 4652
Bendamustine hydrochloride, 495
Benztropine mesylate, 532
Benzylpenicilloyl polylysine, 536
Betamethasone sodium phosphate, 551
Bethanechol chloride, 562
Bleomycin for, 588
Bretylium tosylate, 590
Bretylium tosylate in dextrose, 591
Brompheniramine maleate, 602
Bumetanide, 607
Bupivacaine hydrochloride, 611
Bupivacaine hydrochloride in dextrose, 612
Bupivacaine hydrochloride and epinephrine, 612
Butorphanol tartrate, 656
Caffeine citrate, 662
Caffeine and sodium benzoate, 664
Calcitonin salmon, 674
Calcitriol, 677
Calcium chloride, 693
Calcium gluceptate, 697
Calcium gluconate, 700
Calcium levulinate, 705
Capreomycin for, 726
Carbenicillin for, 748
Carboplatin for, 769
Carboprost tromethamine, 772
Carmustine for, 784
Cefamandole nafate for, 819
Cefazolin, 822
Cefazolin for, 823
Cefepime for, 832
Cefmenoxime for, 839
Cefmetazole, 841
Cefmetazole for, 841
Cefonicid for, 842
Cefoperazone, 844
Cefoperazone for, 844
Ceforanide for, 846
Cefotaxime, 847
Cefotaxime for, 848
Cefotetan, 853
Cefotetan for, 854
Cefotiam for, 857
Cefoxitin, 859
Cefoxitin for, 860
Cefpiramide for, 862
Ceftazidime, 872
Ceftazidime for, 873
Ceftizoxime, 880
Ceftizoxime for, 881
Ceftriaxone, 881
Ceftriaxone for, 882
Cefuroxime, 886
Cefuroxime for, 886
Cephalothin, 901
Cephalothin for, 901
Cephapirin for, 903
Cephradine for, 908
Chloramphenicol, 928
Chloramphenicol sodium succinate for, 936
Chloroprocaine hydrochloride, 957
Chloroquine hydrochloride, 958
Chlorothiazide sodium for, 966
Chlorpheniramine maleate, 971
Chlorpromazine hydrochloride, 976
Chorionic gonadotropin for, 2149
Chromic chloride, 991
Cimetidine, 1006
Cimetidine in sodium chloride, 1007
Ciprofloxacin, 1012
Cisapride compounded, veterinary, 1024
Cisatracurium besylate, 1028
Cisplatin for, 1032
Cladribine, 1046
Clavulanic acid and ticarcillin, 4371
Clindamycin, 1062
Clindamycin for, 1063
Cloprostenol, 1113
Codeine phosphate, 1138
Colchicine, 1144
Colistimethate for, 1149
Corticotropin, 1170
Corticotropin for, 1172
Corticotropin, repository, 1174
Cr 51, sodium chromate, 992
Cupric chloride, 1188
Cupric sulfate, 1190
Cyanocobalamin, 1191
Cyclophosphamide for, 1204
Cyclosporine, 1214
Cysteine hydrochloride, 1222
Cytarabine for, 1224
Dacarbazine for, 1226
Dactinomycin for, 1228
Dantrolene sodium for, 1238
Daunorubicin hydrochloride for, 1242

www.webofpharma.com

Injection *(continued)*
Deferoxamine mesylate for, 1245
Dehydrated alcohol, 117
Deslanoside, 1259
Desmopressin acetate, 1266
Dexamethasone, 1286
Dexamethasone sodium phosphate, 1294
Dexamethasone sodium phosphate compounded, 1295
Dexmedetomidine, 1304
Dextran 40 in dextrose, 1311
Dextran 40 in sodium chloride, 1312
Dextran 70 in dextrose, 1315
Dextran 70 in sodium chloride, 1316
Dextrose, 1323
Dextrose and sodium chloride, 1323
Diatrizoate meglumine, 1325
Diatrizoate meglumine and diatrizoate sodium, 1326
Diatrizoate sodium, 1328
Diazepam, 1331
Diazoxide, 1334
Dibucaine hydrochloride, 1338
Dicyclomine hydrochloride, 1361
Digitoxin, 1381
Digoxin, 1384
Dihydroergotamine mesylate, 1389
Dihydrostreptomycin, 1391
Dimenhydrinate, 1409
Dimercaprol, 1412
Dinoprost tromethamine, 1416
Diphenhydramine hydrochloride, 1426
Dipyridamole, 1445
Dobutamine, 1468
Dobutamine for, 1469
Dobutamine in dextrose, 1470
Docetaxel, 1474
Dopamine hydrochloride, 1495
Dopamine hydrochloride and dextrose, 1495
Doxapram hydrochloride, 1503
Doxorubicin hydrochloride, 1514
Doxorubicin hydrochloride for, 1516
Doxycycline for, 1523
Droperidol, 1553
Dyphylline, 1573
Edetate calcium disodium, 1583
Edetate disodium, 1584
Edrophonium chloride, 1585
Electrolytes and dextrose type 1, multiple, 1598
Electrolytes and dextrose type 2, multiple, 1601
Electrolytes and dextrose type 3, multiple, 1605
Electrolytes type 1, multiple, 1593
Electrolytes type 2, multiple, 1595
Elements, trace, 1608
Enalaprilat, 1619
Enoxaparin sodium, 1626
Ephedrine sulfate, 1643
Epinephrine, 1648
Epirubicin hydrochloride, 1654
Ergonovine maleate, 1673
Ergotamine tartrate, 1677
Erythromycin, 1687
Erythromycin ethylsuccinate, 1698
Erythromycin lactobionate for, 1703
Estradiol cypionate, 1738
Estradiol valerate, 1741
Ethacrynate sodium for, 1759
Ethiodized oil, 1770
Etomidate, 1790
Etoposide, 1795
Exenatide, 1803
Famotidine, 1816
Fenoldopam mesylate, 1842
Fentanyl citrate, 1849
Fentanyl citrate and bupivacaine hydrochloride compounded injection, 1850
Fentanyl citrate compounded, 1848
Ferumoxides, 1866
Floxuridine for, 1901
Fluconazole, 1901
Fluconazole in dextrose, 1907
Fluconazole in sodium chloride, 1909
Fludarabine phosphate, 1919
Fludarabine phosphate for, 1921
Fludeoxyglucose F18, 1946
Flumazenil, 1926
Flunixin meglumine, 1932
Fluorescein, 1940
F 18, sodium fluoride, 1950
Fluorouracil, 1957
Fluphenazine decanoate, 1968
Fluphenazine enanthate, 1970
Fluphenazine hydrochloride, 1972
Folic acid, 2027
Fondaparinux sodium, 2033
Fosphenytoin sodium, 2047
Fructose, 2049
Fructose and sodium chloride, 2049
Furosemide, 2054
Gadodiamide, 2064
Gadopentetate dimeglumine, 2065
Gadoteridol, 2069
Gadoversetamide, 2073
Gallamine triethiodide, 2087
Gallium citrate Ga 67, 2088
Ganciclovir for, 2089
Gemcitabine for, 2094
Gentamicin, 2099
Glucagon for, 2122
Glycopyrrolate, 2139
Gold sodium thiomalate, 2143
Gonadorelin for, 2143
Gonadotropin, chorionic for, 2149
Granisetron hydrochloride, 2159
Guaifenesin compounded, veterinary, 2170
Guaifenesin for, 2171
Haloperidol, 2189
Heparin sodium, 2200
Histamine phosphate, 2205
Hyaluronidase, 2211
Hyaluronidase for, 2211
Hydralazine hydrochloride, 2214
Hydrochloric acid, 2217
Hydrocortisone sodium phosphate, 2242
Hydrocortisone sodium succinate for, 2243
Hydromorphone hydrochloride, 2252
Hydroxocobalamin, 2257
Hydroxyprogesterone caproate, 2261
Hydroxyzine hydrochloride, 2265
Hyoscyamine sulfate, 2277
I 123, iobenguane, 2365
I 125, iothalamate sodium, 2367
I 125, albumin, iodinated, 2367
I 131, albumin, iodinated, 2368
Idarubicin hydrochloride, 2291
Idarubicin hydrochloride for, 2291
Ifosfamide for, 2296
Imipenem and cilastatin for, 2297
Imipramine hydrochloride, 2301
Inamrinone, 2311
Indigotindisulfonate sodium, 2316
Indium In 111 capromab pendetide, 2318
Indium In 111 pentetate, 2320
Indium In 111 pentetreotide, 2321
Indocyanine green for, 2322
Indomethacin for, 2330
injection, 1000
Insulin, 2338
Insulin aspart, 2341
Insulin glargine, 2344
Insulin human, 2348
Insulin, human, and human insulin isophane suspension, 2349
Insulin lispro, 2355
Inulin in sodium chloride, 2361
Invert sugar, 4133
Iodipamide meglumine, 2370
Iodixanol, 2373
Iohexol, 2381
Iopamidol, 2383
Iopromide, 2386
Iothalamate meglumine, 2387
Iothalamate meglumine and iothalamate sodium, 2388
Ioversol, 2390
Ioxaglate meglumine and ioxaglate sodium, 2391
Ioxilan, 2394
Irinotecan hydrochloride, 2409
Iron dextran, 2411
Iron sorbitex, 2412
Iron sucrose, 2413
Isoniazid, 2425
Isoproterenol hydrochloride, 2433
Isoxsuprine hydrochloride, 2462
Ivermectin, 2472
Ivermectin and clorsulon, 2477
Kanamycin, 2501
Ketamine hydrochloride, 2506
Ketorolac tromethamine, 2514
Labetalol hydrochloride, 2518
Leucovorin calcium, 2568
leucovorin calcium for, 2569
Levetiracetam, 2601
Levocarnitine, 2599
Levorphanol tartrate, 2614
Lidocaine hydrochloride, 2625
Lidocaine hydrochloride and dextrose, 2628
Lidocaine hydrochloride and epinephrine, 2629
Lincomycin, 2632
Lorazepam, 2682
Magnesium sulfate, 2727
Magnesium sulfate in dextrose, 2727
Manganese chloride, 2731
Mannitol, 2736
Mannitol in sodium chloride, 2737
Mechlorethamine hydrochloride for, 2749
Meperidine hydrochloride, 2783
Mepivacaine hydrochloride, 2788
Mepivacaine hydrochloride and levonordefrin, 2789
Meropenem for, 2798
Metaraminol bitartrate, 2817
Methadone hydrochloride, 2837
Methocarbamol, 2851
Methohexital sodium for, 2853
Methotrexate, 2857
Methotrexate for, 2858
Methotrimeprazine, 2861
Methyldopate hydrochloride, 2880
Methylene blue, 2882
Methylene blue, veterinary, 2884
Methylergonovine maleate, 2886
Methylprednisolone sodium succinate for, 2903
Metoclopramide, 2909
Metoprolol tartrate, 2923
Metronidazole, 2936

www.webofpharma.com

Injection *(continued)*
Midazolam, 2953
Milrinone lactate, 2959
Minocycline for, 2963
Mitomycin for, 2986
Mitoxantrone, 2988
Morphine sulfate, 3027
Morphine sulfate compounded, 3028
Mycophenolate mofetil for, 3046
N 13, ammonia, 3183
Nafcillin, 3063
Nafcillin for, 3063
Nalorphine hydrochloride, 3067
Naloxone hydrochloride, 3068
Nandrolone decanoate, 3073
Neomycin for, 3102
Neostigmine methylsulfate, 3130
Niacin, 3140
Niacinamide, 3147
Nicardipine hydrochloride, 3149
Nitroglycerin, 3187
Norepinephrine bitartrate, 3198
Ondansetron, 3265
Orphenadrine citrate, 3284
Oxacillin, 3296
Oxacillin for, 3297
Oxaliplatin, 3301
Oxaliplatin for, 3303
Oxymorphone hydrochloride, 3353
Oxytetracycline, 3360
Oxytetracycline for, 3362
Oxytocin, 3367
Paclitaxel, 3370
Pamidronate disodium for, 3379
Pancuronium bromide, 3386
Papaverine hydrochloride, 3398
Paricalcitol, 3406
Particulate matter in injections ⟨788⟩, 7017
Pemetrexed, 3422
Penicillin G potassium, 3437
Penicillin G potassium for, 3437
Penicillin G sodium for, 3450
Pentazocine, 3465
Pentobarbital sodium, 3469
Phenobarbital sodium, 3500
Phentolamine mesylate for, 3509
Phenylbutazone, 3511
Phenylephrine hydrochloride, 3517
Phenytoin sodium, 3534
Physostigmine salicylate, 3535
Phytonadione injectable emulsion, 3538
Piperacillin for, 3565
Piperacillin and tazobactam for, 3567
Polymyxin B for, 3590
Potassium acetate, 3595
Potassium chloride concentrate for, 3604
Potassium chloride in dextrose, 3607
Potassium chloride in dextrose and sodium chloride, 3608
Potassium chloride in lactated ringer's and dextrose, 3610
Potassium chloride in sodium chloride, 3612
Potassium phosphates, 3629
Potassium phosphates compounded, 3629
Pralidoxime chloride for, 3637
Prednisolone sodium phosphate, 3667
Prednisolone sodium succinate for, 3668
Prilocaine and epinephrine, 3679
Prilocaine hydrochloride, 3678
Procainamide hydrochloride, 3690
Procaine hydrochloride, 3694
Procaine hydrochloride and epinephrine, 3694
Procaine and tetracaine hydrochlorides and levonordefrin, 3695
Prochlorperazine edisylate, 3699
Progesterone, 3705
Promazine hydrochloride, 3712
Promethazine hydrochloride, 3716
Propofol injectable emulsion, 3742
Propoxycaine and procaine hydrochlorides and levonordefrin, 3744
Propoxycaine and procaine hydrochlorides and norepinephrine bitartrate, 3745
Propranolol hydrochloride, 3749
Protamine sulfate, 3758
Pyridostigmine bromide, 3780
Pyridoxine hydrochloride, 3783
Quinidine gluconate, 3809
Ranitidine, 3845
Ranitidine in sodium chloride, 3848
Repository corticotropin, 1174
Riboflavin, 3869
Rifampin for, 3877
Ringer's, 3887
Ringer's and dextrose, 3889
Ringer's and dextrose, half-strength lactated, 3895
Ringer's and dextrose, lactated, 3893
Ringer's and dextrose, modified, lactated, 3898
Ringer's, lactated, 3891
Ritodrine hydrochloride, 3911
Ropivacaine hydrochloride, 3945
Rubidium chloride Rb 82, 3958
Sargramostim for, 3988
Scopolamine hydrobromide, 4012
Selenious acid, 4022
Sm 153 lexidronam, samarium, 3983
Sodium acetate, 4054
Sodium bicarbonate, 4060
Sodium bicarbonate compounded, 4061
Sodium bromide, veterinary, 4063
Sodium chloride, 4066
Sodium chloride, bacteriostatic, 4067
Sodium chromate Cr 51, 992
Sodium lactate, 4078
Sodium nitrite, 4082
Sodium nitroprusside for, 4084
Sodium phosphates, 4088
Sodium phosphates compounded, 4089
Sodium sulfate, 4096
Sodium thiosulfate, 4097
Somatropin for, 4099
Strontium chloride Sr 89, 4126
Streptomycin, 4125
Streptomycin for, 4125
Succinylcholine chloride, 4129
Sufentanil citrate, 4133
Sugar, invert, 4133
Sulfadiazine sodium, 4152
Sulfamethoxazole and trimethoprim, 4162
Sumatriptan, 4182
Technetium Tc 99m albumin aggregated, 4226
Technetium Tc 99m bicisate, 4227
Technetium Tc 99m disofenin, 4227
Technetium Tc 99m exametazime, 4228
Technetium Tc 99m mebrofenin, 4229
Technetium Tc 99m medronate, 4230
Technetium Tc 99m mertiatide, 4231
Technetium Tc 99m oxiseonate, 4232
Technetium Tc 99m pentetate, 4232
Technetium Tc 99m pertechnetate, sodium, 4233
Technetium Tc 99m pyrophosphate, 4234
Technetium Tc 99m (pyro- and trimeta-) phosphates, 4235
Technetium Tc 99m red blood cells, 4235
Technetium Tc 99m sestamibi, 4236
Technetium Tc 99m succimer, 4237
Technetium Tc 99m sulfur colloid, 4238
Technetium Tc 99m tetrofosmin, 4238
Temozolomide for injection, 4256
Teniposide, 4259
Terbutaline sulfate, 4272
Teriparatide, 4280
Testosterone cypionate, 4289
Testosterone enanthate, 4290
Testosterone propionate, 4291
Tetracaine hydrochloride, 4297
Tetracaine hydrochloride for, 4298
Tetracaine hydrochloride in dextrose, 4300
Tetracycline hydrochloride for, 4307
Thallous chloride Tl 201, 4318
Theophylline in dextrose, 4328
Thiamine hydrochloride, 4338
Thiopental sodium for, 4352
Thiotepa for, 4357
Thiothixene hydrochloride, 4360
Thiothixene hydrochloride for, 4361
Ticarcillin and clavulanic acid, 4371
Ticarcillin and clavulanic acid for, 4372
Ticarcillin for, 4370
Tigecycline for, 4381
Tiletamine and zolazepam for, 4383
Tilmicosin, 4385
Tobramycin, 4402
Tobramycin for, 4403
Tolazoline hydrochloride, 4418
Tolbutamide for, 4419
Tranexamic acid, 4463
Trifluoperazine hydrochloride, 4512
Triflupromazine hydrochloride, 4515
Trimethobenzamide hydrochloride, 4525
Tripelennamine hydrochloride, 4531
Tromethamine for, 4538
Tubocurarine chloride, 4548
Tylosin, 4550
Urea for, 4557
Valproate sodium, 4572
Vancomycin, 4585
Vancomycin hydrochloride for, 4588
Vasopressin, 4593
Verapamil hydrochloride, 4607
Verteporfin for, 4617
Vinblastine sulfate for, 4624
Vincristine sulfate, 4626
Vincristine sulfate for, 4628
Vinorelbine, 4631
Warfarin sodium for, 4648
Water for, bacteriostatic, 4652
Water for, sterile, 4653
Water for, 4651
Xylazine, 4659
Yohimbine, 4664
Yttrium Y 90 ibritumomab tiuxetan, 4665
Zidovudine, 4675
Zinc chloride, 4684
Zinc sulfate, 4692
Zolazepam and tiletamine for injection, 4383

Injections and implanted drug products (parenterals)—product quality tests ⟨1⟩, 6405
Inosine, 6172
Inositol, 5829, 6172
Insoluble matter in reagents, 6130
Insulin, 2335
aspart, 2339

www.webofpharma.com

Insulin *(continued)*
assays ⟨121⟩, 6547
glargine, 2342
glargine injection, 2344
human, 2346
human injection, 2348
human isophane suspension and human insulin injection, 2349
human suspension, isophane, 2352
injection, 2338
lispro, 2353
lispro injection, 2355
suspension, isophane, 2351
zinc suspension, 2356
zinc suspension, extended, 2357
zinc suspension, prompt, 2359
Insulin aspart
injection, 2341
Intestinal fluid, simulated, TS, 6230, 6234
Intramammary infusion
amoxicillin, 302
cloxacillin benzathine, 1126
Intrauterine contraceptive system
progesterone, 3705
Intrinsic viscosity table, 6349
Inulin, 2360
in sodium chloride injection, 2361
Invert sugar, 5832
In vitro
and in vivo evaluation of dosage forms ⟨1088⟩, 7684
biological reactivity tests ⟨87⟩, 6514
In vivo
biological reactivity tests ⟨88⟩, 6516
and in vitro evaluation of dosage forms ⟨1088⟩, 7684
Iobenguane
I 123 injection, 2365
sulfate, 6173
Iodic acid, 6173
Iodinated
I 125 albumin injection, 2367
I 131 albumin injection, 2368
Iodine, 2362, 6172
diluted TS, 6230
hundredth-normal (0.01 N), 6242
I 123 capsules, sodium iodide, 2365
I 123 injection, iobenguane, 2365
I 123 solution, sodium iodide, 2366
I 125 albumin injection, iodinated, 2367
I 125 injection, iothalamate sodium, 2367
I 131 albumin injection, iodinated, 2368
I 131 capsules, sodium iodide, 2368
I 131 solution, sodium iodide, 2368
monobromide, 6173
monochloride, 6173
monochloride TS, 6230
and potassium iodide TS 1, 6230
and potassium iodide TS 2, 6230
and potassium iodide TS 3, 6230
solution, strong, 2363
topical solution, 2362
tenth-normal (0.1 N), 6230, 6243
tincture, 2363
tincture, strong, 2364
TS, 6230
twentieth-normal (0.05 N), 6242
Iodipamide, 2369
meglumine injection, 2370
Iodixanol, 2370
injection, 2373
Iodobromide TS, 6230
Iodochloride TS, 6230
Iodoethane, 6173
Iodoform, 2376
Iodometric assay—antibiotics ⟨425⟩, 6696
p-Iodonitrotetrazolium violet, 6173
Iodoplatinate TS, 6230
Iodoquinol, 2377
tablets, 2378
Iohexol, 2378
injection, 2381
Ion chromatography ⟨1065⟩, 7588
Ion-exchange resin, 6173
Iopamidol, 2382
injection, 2383
Iopromide, 2384
injection, 2386
Iothalamate
meglumine injection, 2387
meglumine and iothalamate sodium injection, 2388
sodium I 125 injection, 2367
sodium and iothalamate meglumine injection, 2388
Iothalamic acid, 2389
Ioversol, 2390
injection, 2390
Ioxaglate
meglumine and ioxaglate sodium injection, 2391
sodium and ioxaglate meglumine injection, 2391
Ioxaglic acid, 2391
Ioxilan, 2392
injection, 2394
Ipecac, 2395
powdered, 2396
oral solution, 2397
Ipratropium bromide, 2398
Ipratropium bromide and albuterol sulfate inhalation solution, 2399
Irbesartan, 2402
and hydrochlorothiazide tablets, 2404
tablets, 2403
Irinotecan hydrochloride, 2406
injection, 2409
Iron
carbonyl, 2410
dextran injection, 2411
phenol TS, 6230
powder, 6173
salicylate TS, 6230
sorbitex injection, 2412
sucrose injection, 2413
wire, 6173
Iron ⟨241⟩, 6648
Isoamyl
alcohol, 6173
Isobutane, 5833
Isobutyl
acetate, 6173
alcohol, 5833, 6134, 6173
4-Isobutylacetophenone, 6173
Isobutyl chloroformate, 6173
N-Isobutylpiperidone, 6173
Isoetharine
hydrochloride, 2415
inhalation solution, 2416
mesylate, 2416
Isoflupredone acetate, 2418, 6173
injectable suspension, 2419
neomycin sulfate and tetracaine hydrochloride ointment, 3111
neomycin sulfate and tetracaine hydrochloride topical powder, 3112
Isoflurane, 2420
Isoleucine, 2422
L-Isoleucine, 6173
Isomalt, 5836
Isomaltotriose, 6173
Isometheptene mucate, 2423
dichloralphenazone, and acetaminophen capsules, 2423
2-Isoniazid, 2424, 6173
injection, 2425
and rifampin capsules, 3878
rifampin, pyrazinamide, and ethambutol hydrochloride tablets, 3881
rifampin and pyrazinamide tablets, 3879
oral solution, 2426
tablets, 2426
Isonicotinamide, 6173
Isonicotinic acid, 6173
hydrazide, 6173
Isooctane, 6174
Isopropamide iodide, 2427
tablets, 2428
Isopropyl
acetate, 6174
alcohol, 2428, 6134, 6174, 6194
alcohol, azeotropic, 2430
alcohol, dehydrated, 6134, 6174
alcohol, rubbing, 2430
ether, 6174
iodide, 6174
myristate, 5839, 6174
palmitate, 5840
salicylate, 6174
Isopropylamine, 6174
Isopropyl isostearate, 5838
Isopropyl stearate, 6174
Isoproterenol
hydrochloride, 2431
hydrochloride and acetylcysteine inhalation solution, 84
hydrochloride injection, 2433
hydrochloride tablets, 2434
inhalation solution, 2431
sulfate, 2436
sulfate inhalation aerosol, 2437
sulfate inhalation solution, 2438
Isorhamnetin, 6174
Isosorbide
concentrate, 2438
dinitrate extended-release capsules, 2441
dinitrate chewable tablets, 2444
dinitrate, diluted, 2440
dinitrate sublingual tablets, 2447
dinitrate extended-release tablets, 2445
mononitrate, diluted, 2448
mononitrate tablets, 2449
mononitrate extended-release tablets, 2451
oral solution, 2440
Isostearic acid, 6174
Isostearyl isostearate, 5840
Isotretinoin, 2457
capsules, 2458
Isovaleric acid, 6174
Isoxsuprine hydrochloride, 2462
injection, 2462
tablets, 2463
Isradipine, 2464
capsules, 2465
oral suspension, 2465
Itraconazole, 2466
capsules, 2468
Ivermectin, 2470
and clorsulon injection, 2477
injection, 2472
paste, 2473
and pyrantel pamoate tablets, 2478
topical solution, 2476
tablets, 2474

www.webofpharma.com

Ivermectin compounded
oral solution, veterinary, 2473
Ixabepilone, 2479

J

Japanese honeysuckle flower, 5091
dry extract, 5094
powder, 5097
Juniper tar, 2501

K

Kaempferol, 6174
Kanamycin
injection, 2501
sulfate, 2502
sulfate capsules, 2503
Kaolin, 2504
Kerosene, 6174
Ketamine hydrochloride, 2505
injection, 2506
Ketoconazole, 2506
oral suspension, 2507
tablets, 2508
Ketoprofen, 2509
capsules, 2510
extended-release capsules, 2511
Ketorolac tromethamine, 2513
injection, 2514
tablets, 2515
Krill oil
capsules, 5104
delayed-release capsules, 5107

L

L designations, 6174
Labeling ⟨7⟩, 6435
Labeling of inactive ingredients ⟨1091⟩, 7706
Labetalol hydrochloride, 2517
injection, 2518
oral suspension, 2519
tablets, 2519
alpha-Lactalbumin, 5841
Lactase, 2520
Lactic acid, 2520, 6174
Lactitol, 5845
Lactobacillus acidophilus
La-14, 5111
NCFM, 5113
Lactobacillus paracasei LPC-37, 5115
Lactobacillus rhamnosus HN001, 5117
Lactobionic acid, 5846
Lactose, 6174
anhydrous, 5847
beta, 6174
monohydrate, 5849
monohydrate, alpha, 6174
Lactulose
concentrate, 2521
solution, 2522
Lamivudine, 2523
oral solution, 2526
tablets, 2527
and abacavir tablets, 21
and zidovudine tablets, 2528
Lamotrigine, 2530
tablets, 2532
Lamotrigine
extended-release tablets, 2534
tablets for oral suspension, 2542
Lamotrigine compounded
oral suspension, 2545
Lanolin, 2545
alcohols, 5850
modified, 2548
Lansoprazole, 2551
delayed-release capsules, 2553
Lansoprazole compounded
oral suspension, 2556
Lanthanum
alizarin complexan mixture, 6175
chloride, 6175
nitrate hexahydrate, 6175
nitrate TS, 6230
oxide, 6175
Latanoprost, 2556
Lauric acid, 5852
Lauroyl polyoxylglycerides, 5852
Lauryl dimethyl amine oxide, 6175
Lead
acetate, 6175
acetate paper, 6175
acetate test paper, 6222
acetate TS, 6230
acetate TS, alcoholic, 6231
monoxide, 6175
nitrate, 6175
nitrate, hundredth-molar (0.01 M), 6243
nitrate stock solution TS, 6231
perchlorate, 6175
perchlorate, hundredth-molar (0.01 M), 6243
perchlorate, tenth-molar (0.1 M), 6243
solution, standard, 6236
subacetate TS, 6231
subacetate TS, diluted, 6228, 6231
tetraacetate, 6175
Lead ⟨251⟩, 6649
Leak rate ⟨604⟩, 6846
Lecithin, 5853, 6175
Leflunomide, 2558
tablets, 2560
Leflunomide compounded
oral suspension, 2560
Lemon
oil, 5856
tincture, 5857
Letrozole, 2562
tablets, 2563
Leucine, 2565
Leucovorin calcium, 2566
compounded oral suspension, 2570
injection, 2568
tablets, 2571
Leucovorin calcium for injection, 2569
Leuprolide acetate, 2572
Levalbuterol
inhalation solution, 2576
Levalbuterol hydrochloride, 2574
Levamisole hydrochloride, 2578
tablets, 2579
Levetiracetam, 2580
extended-release tablets, 2587
injection, 2582
oral solution, 2583
tablets, 2584
Levmetamfetamine, 2593
Levobunolol hydrochloride, 2594
ophthalmic solution, 2595
Levocabastine hydrochloride, 2596
Levocarnitine, 2597
injection, 2599
oral solution, 2600
tablets, 2601
Levocetirizine dihydrochloride
tablets, 2603
Levodopa, 2604
Levodopa
and carbidopa extended-release tablets, 753
and carbidopa orally disintegrating tablets, 759
and carbidopa tablets, 751
Levofloxacin, 2606
oral solution, 2608
tablets, 2609
Levonordefrin, 2611
and mepivacaine hydrochloride injection, 2789
and procaine and tetracaine hydrochlorides injection, 3695
and propoxycaine and procaine hydrochlorides injection, 3744
Levonorgestrel, 2612
and ethinyl estradiol tablets, 2612
Levorphanol tartrate, 2613
injection, 2614
tablets, 2614
Levothyroxine sodium, 2615
oral powder, 2618
tablets, 2618
L-alpha-glycerylphosphorylcholine, 5055
Licorice, 5119
extract, powdered, 5121
fluidextract, 5857
powdered, 5120
Lidocaine, 2620
topical aerosol, 2622
hydrochloride, 2623
hydrochloride and dextrose injection, 2628
hydrochloride and epinephrine injection, 2629
hydrochloride injection, 2625
hydrochloride jelly, 2625
hydrochloride oral topical solution, 2626
hydrochloride topical solution, 2627
neomycin and polymyxin B sulfates and bacitracin ointment, 3117
neomycin and polymyxin B sulfates and bacitracin zinc ointment, 3120
and neomycin and polymyxin B sulfates cream, 3126
ointment, 2622
and prilocaine cream, 2630
oral topical solution, 2623
Light diffraction measurement of particle size ⟨429⟩, 6697
Lime, 2632
Limestone
ground, 5121
Limit on number of large deviations when assessing content uniformity in large samples, 7744
(*R*)-(+)-Limonene, 6175
Linalool, 6175
Lincomycin
hydrochloride, 2633
hydrochloride capsules, 2634
hydrochloride soluble powder, 2634
injection, 2632

www.webofpharma.com

Lincomycin *(continued)*
oral solution, 2632
Lindane, 2634
cream, 2635
lotion, 2636
shampoo, 2636
Linezolid, 2637
Linoleic acid, 6175
Linoleic acids-free fatty acids
conjugated, 5123
Linolenic acid, 6175
Linoleoyl polyoxylglycerides, 5857
Liothyronine sodium, 2638
tablets, 2640
Liotrix tablets, 2640
Lipid injectable emulsion, 2641
Lipoic acid
alpha, 5124
capsules, alpha, 5125
tablets, alpha, 5126
α-Lipoic acid, 6175
Liquid petrolatum, 6175
Liquid-phase sterilization ⟨1229.6⟩, 8219
Lisinopril, 2642
and hydrochlorothiazide tablets, 2646
oral suspension, 2644
tablets, 2644
Lithium, 6175
carbonate, 2649
carbonate capsules, 2650
carbonate tablets, 2650
carbonate extended-release tablets, 2651
chloride, 6175
citrate, 2653
hydroxide, 2654, 6175
metaborate, 6175
methoxide, fiftieth-normal (0.02 N) in methanol, 6243
methoxide, tenth-normal (0.1 N) in chlorobenzene, 6243
methoxide, tenth-normal (0.1 N) in methanol, 6244
methoxide, tenth-normal (0.1 N) in toluene, 6244
nitrate, 6176
perchlorate, 6176
oral solution, 2648
sulfate, 6176
Lithocholic acid, 6176
Litmus, 6176, 6221
paper, blue, 6222
paper, red, 6222
TS, 6231
Litmus paper, neutral, 6222
Littorine hydrochloride (*R*)-(–)-, 6176
Locke-Ringer's
solution, 6231
TS, 6231
Locust bean gum, 6176
Lomustine, 2655
capsules, 2656
Loperamide hydrochloride, 2658
capsules, 2660
oral solution, 2661
tablets, 2661
Lopinavir, 2663
Lopinavir
and ritonavir oral solution, 2666
and ritonavir tablets, 2670
Loratadine, 2672
chewable tablets, 2676
oral solution, 2674
tablets, 2675
orally disintegrating tablets, 2678
Lorazepam, 2680
injection, 2682
oral concentrate, 2683
tablets, 2684
Losartan potassium, 2686
and hydrochlorothiazide tablets, 2690
tablets, 2687
Loss on drying ⟨731⟩, 6970
Loss on drying for reagents, 6131
Loss on ignition ⟨733⟩, 6970

Lotion

Amphotericin B, 316
Benzoyl peroxide, 527
Benzyl benzoate, 535
Betamethasone dipropionate, 548
Betamethasone valerate, 554
Clotrimazole, 1119
Flurandrenolide, 1976
Fluticasone propionate, 1998
Hydrocortisone, 2232
Hydrocortisone acetate, 2237
Lindane, 2636
Malathion, 2730
Methylbenzethonium chloride, 2873
Neomycin sulfate and flurandrenolide, 3108
Neomycin sulfate and hydrocortisone acetate, 3110
Nystatin, 3220
Padimate O, 3372
Triamcinolone acetonide, 4485

Lovastatin, 2694
Low molecular weight heparin molecular weight determinations ⟨209⟩, 6614
Loxapine
capsules, 2697
succinate, 2696
Lufenuron, 2697
Lumefantrine, 2699
Lutein, 5126
capsules, 5128
preparation, 5128
Lycopene, 5130
preparation, 5131
tomato extract containing, 5133
Lysine
acetate, 2700
hydrochloride, 2701
hydrochloride tablets, 5135
L-Lysine, 6176
Lysolecithin, 6176

M

Mafenide acetate, 2702
cream, 2703
for topical solution, 2704
Magaldrate, 2705
and simethicone chewable tablets, 2707
and simethicone oral suspension, 2706
Magnesia
alumina and calcium carbonate chewable tablets, 169
alumina, calcium carbonate, and simethicone chewable tablets, 170
alumina and calcium carbonate oral suspension, 168
alumina and simethicone chewable tablets, 174
alumina and simethicone oral suspension, 173
and alumina oral suspension, 166
and alumina tablets, 167
aspirin and alumina tablets, 397
aspirin, codeine phosphate, and alumina tablets, 404
calcium carbonate and simethicone chewable tablets, 689
and calcium carbonate chewable tablets, 688
milk of, 2708
mixture TS, 6231
tablets, 2709
Magnesium, 6176
acetate, 6176
aluminometasilicate, 5858
aluminosilicate, 5859
aluminum silicate, 5861
and calcium carbonates oral suspension, 691
and calcium carbonates tablets, 691
carbonate, 2709
carbonate and citric acid for oral solution, 2710
carbonate, citric acid, and potassium citrate for oral solution, 2711
carbonate and sodium bicarbonate for oral suspension, 2712
carbonate, alumina, and magnesium oxide tablets, 178
carbonate and alumina oral suspension, 176
carbonate and alumina tablets, 177
chloride, 2712, 6176
chloride, 0.01 M, 6244
citrate, 2713
citrate oral solution, 2714
citrate for oral solution, 2715
gluconate, 2716
gluconate tablets, 2717
hydroxide, 2718
hydroxide paste, 2719
nitrate, 6176
oxide, 2719, 6176
oxide, alumina, and magnesium carbonate tablets, 178
oxide, aspirin, and alumina tablets, 399
oxide capsules, 2721
oxide, chromatographic, 6152, 6176
oxide, citric acid, and sodium carbonate irrigation, 1044
oxide tablets, 2721
perchlorate, anhydrous, 6138, 6176
phosphate, 2722
salicylate, 2723
salicylate tablets, 2724
silicate, 5863
silicate, activated, 6133, 6176
silicate, chromatographic, 6176
stearate, 5864
sulfate, 2726, 6176
sulfate, anhydrous, 6138, 6176
sulfate in dextrose injection, 2727
sulfate injection, 2727
sulfate TS, 6231
trisilicate, 2728
trisilicate and alumina oral suspension, 179
trisilicate and alumina tablets, 180

www.webofpharma.com

Magnesium *(continued)*
trisilicate tablets, 2729
Magnesium carbonate, 6176
Malabar-nut-tree, leaf, 5136
powdered, 5137
extract, powdered, 5138
Malachite green
G, 6176
oxalate, 6221
TS, 6231
Malathion, 2729
lotion, 2730
Maleic acid, 5867, 6176
Malic acid, 5869
Mallory's stain, 6231
Malonic acid, 6176
Maltitol, 5869
solution, 5871
Maltodextrin, 5872
Maltol, 5875
Maltose, 5875
Maltotriose, 6176
Mandelic acid, 5876
Manganese, 6176
chloride, 2731
chloride injection, 2731
chloride for oral solution, 2732
dioxide, 6176
dioxide, activated, 6176, 6177
gluconate, 2732
sulfate, 2734
Manganese chloride tetrahydrate, 6177
Manganese sulfate, 6176
Mannitol, 2734
injection, 2736
in sodium chloride injection, 2737
D-Mannitol, 6177
Manufacturing practices for dietary supplements ⟨2750⟩, 8742
Maprotiline hydrochloride, 2737
tablets, 2739
Marbofloxacin compounded, veterinary
oral suspension, 2741
Marfey's reagent, 6177
Maritime pine, 5139
extract, 5141
Mass spectrometry ⟨736⟩, 6975
Mayer's reagent, 6231
Mazindol, 2742
Measurement of subvisible particulate matter in therapeutic protein injections ⟨1787⟩, 8562
Mebendazole, 2742
oral suspension, 2744
tablets, 2744
Mebrofenin, 2746
Mecamylamine hydrochloride, 2747
tablets, 2748
Mechlorethamine hydrochloride, 2749
for injection, 2749
Meclizine hydrochloride, 2750
tablets, 2751
Meclofenamate sodium, 2753
capsules, 2754
Medical air, 100
Medical devices—bacterial endotoxin and pyrogen tests ⟨161⟩, 6579
Medical gases assay ⟨415⟩, 6693
Medium-chain triglycerides, 6095, 6177
Medroxyprogesterone acetate, 2754
injectable suspension, 2755
tablets, 2756
Mefenamic acid, 2757
capsules, 2758
Mefloquine hydrochloride, 2760
tablets, 2761
Megestrol acetate, 2762
oral suspension, 2763
tablets, 2764
Meglumine, 2765
Melamine, 6177
Melatonin, 5143
tablets, 5144
Melengestrol acetate, 2766
Meloxicam, 2767
oral suspension, 2770
tablets, 2771
Melphalan, 2773
tablets, 2774
Melting range or temperature ⟨741⟩, 6980
Memantine hydrochloride, 2774
tablets, 2776
Members of the United States Pharmacopeial Convention, xviii
Menadiol sodium diphosphate, 2779
Menadione, 2780
Menaquinone-4, 5145
Menaquinone-7, 5146
capsules, 5147
extract, *Bacillus subtilis* subsp. *subtilis*, 5152
preparation, 5149
tablets, 5151
Menthol, 2781
and benzocaine topical aerosol, 518
lozenges, 2782
and tetracaine ointment, 4294
Meperidine hydrochloride, 2782
injection, 2783
oral solution, 2784
tablets, 2784
Mephobarbital, 2785
tablets, 2785
Mepivacaine hydrochloride, 2786
injection, 2788
and levonordefrin injection, 2789
Meprobamate, 2790
tablets, 2791
Meradimate, 2792
2-Mercaptoethanol, 6177
3-Mercaptopropionic acid, 6177
Mercaptopurine, 2793
tablets, 2794
Mercuric
acetate, 6177
acetate TS, 6231
ammonium thiocyanate TS, 6231
bromide, 6177
bromide test paper, 6222
bromide TS, alcoholic, 6226, 6231
chloride, 6177
chloride TS, 6231
iodide, red, 6177
iodide, TS, 6231
nitrate, 6177
nitrate, tenth-molar (0.1 M), 6244
nitrate TS, 6231
oxide, yellow, 6177, 6219
potassium iodide TS, 6231
potassium iodide TS, alkaline, 6226, 6231, 6232
sulfate, 6177
sulfate TS, 6228, 6231
thiocyanate, 6177
Mercurous nitrate
dihydrate, 6177
TS, 6231
Mercury, 6177
ammoniated, 2796
Mercury ⟨261⟩, 6650
Meropenem, 2796
for injection, 2798
Mesalamine, 2800
extended-release capsules, 2802
rectal suspension, 2803
delayed-release tablets, 2805
Mesityl oxide, 6177
Mesna, 2807
tablets, 2809
Mestranol, 2810
and ethynodiol diacetate tablets, 1781
and norethindrone tablets, 3203
Metacresol, 2811
Metanil
yellow, 6177
Metaphenylenediamine hydrochloride, 6177
TS, 6231
Metaphosphoric-acetic acid TS, 6231
Metaphosphoric acid, 6177
Metaproterenol sulfate, 2812
oral solution, 2813
tablets, 2814
Metaraminol bitartrate, 2815
injection, 2817
Metaxalone, 2817
tablets, 2819
Metformin hydrochloride, 2821
extended-release tablets, 2824
and glipizide tablets, 2116
and glyburide tablets, 2128
and pioglitazone tablets, 3558
tablets, 2822
Methacholine chloride, 2833
Methacrylic acid, 6177
and ethyl acrylate copolymer, 5878
and ethyl acrylate copolymer, partially-neutralized, 5881
and methyl methacrylate copolymer, 5880
Methacycline hydrochloride, 2835
oral suspension, 2835
Methadone hydrochloride, 2836
injection, 2837
oral concentrate, 2836
oral solution, 2838
tablets, 2838
tablets for oral suspension, 2839
Methamphetamine hydrochloride, 2840
tablets, 2840
Methanesulfonic acid, 6177
Methanol, 6134, 6138, 6177
aldehyde-free, 6177
anhydrous, 6177
deuterated, 6155
spectrophotometric, 6177
Methazolamide, 2841
tablets, 2842
Methenamine, 2843, 6170, 6177
hippurate, 2844
hippurate tablets, 2845
mandelate, 2845
mandelate tablets, 2846
tablets, 2843
Methimazole, 2847
tablets, 2848
Methionine, 2848
L-Methionine sulfoxide, 6177
Methocarbamol, 2850
injection, 2851
tablets, 2851
Methods for the determination of particulate matter in injections and ophthalmic solutions ⟨1788⟩, 8575
Methohexital, 2853
sodium for injection, 2853

www.webofpharma.com

Methotrexate, 2854
injection, 2857
for injection, 2858
tablets, 2860
Methotrimeprazine, 2860
injection, 2861
Methoxsalen, 2862
capsules, 2863
topical solution, 2865
5-Methoxy-1*H*-benzimidazole-2-thiol, 6178
Methoxyacetophenone *p*-, 6177
7-Methoxycoumarin, 6178
Methoxy determination ⟨431⟩, 6702
Methoxyethanol, 6178
2-Methoxyethanol, 6178
Methoxyflurane, 2866
5-Methoxy-2-methyl-3-indoleacetic acid, 6178
Methoxyphenylacetic acid, 6178
Methoxyphenylacetic TS, 6231
Methscopolamine bromide, 2866
tablets, 2868
Methsuximide, 2870
capsules, 2870
Methyclothiazide, 2871
tablets, 2872
Methyl
acetate, 6178
alcohol, 5882
4-aminobenzoate, 6178
arachidate, 6178
behenate, 6178
benzenesulfonate, 6178
caprate, 6178
caprylate, 6178
carbamate, 6178
chloroform, 6151, 6178, 6214
erucate, 6178
ethyl ketone, 6178
green, 6178
green–iodomercurate paper, 6222
heptadecanoate, 6178
iodide, 6179
isobutyl ketone, 5883, 6179
laurate, 6179
lignocerate, 6179
linoleate, 6179
linolenate, 6179
methacrylate, 6179
methacrylate and ethyl acrylate copolymer dispersion, 5760
myristate, 6179
oleate, 6179
orange, 6221
orange TS, 6231
palmitate, 6179
purple TS, 6231
red, 6179, 6221
red–methylene blue TS, 6231, 6232
red sodium, 6221
red TS, 6231
red TS 2, 6232
red TS, methanolic, 6232
salicylate, 5884
stearate, 6180
sulfoxide, 6180
violet TS, 6232
yellow, 6180, 6221
yellow–methylene blue TS, 6232
yellow paper, 6222
yellow TS, 6232
3-Methyl-2-benzothiazolinone hydrazone hydrochloride TS, 6232
Methylamine, 40 percent in water, 6180
Methylamine hydrochloride, 6180
p-Methylaminophenol sulfate, 6180
Methylbenzethonium chloride, 2873
lotion, 2873
4-Methylbenzophenone, 6180
Methylbenzothiazolone hydrazone hydrochloride, 6180
(*R*)-(+)-alpha-Methylbenzyl isocyanate, 6180
(*S*)-(−)-α-Methylbenzyl isocyanate, 6180
Methylcellulose, 2874
ophthalmic solution, 2876
oral solution, 2876
tablets, 2876
Methylcobalamin, 5153
tablets, 5154
Methyldopa, 2877
and hydrochlorothiazide tablets, 2878
tablets, 2878
Methyldopate hydrochloride, 2880
injection, 2880
Methyl cis-11-eicosenoate, 6178
Methylene
blue, 2881, 6180
blue injection, 2882
blue injection, veterinary, 2884
blue TS, 6232
chloride, 5885, 6157, 6180
5,5′-Methylenedisalicylic acid, 6180
Methylergonovine maleate, 2885
injection, 2886
tablets, 2887
3-*O*-Methylestrone, 6180
1-Methylimidazole, 6180
2-Methylimidazole, 6180
Methyl isobutyrate, 6179
Methyl 12-Ketostearate, 6179
Methyl methacrylate
and methacrylic acid copolymer, 5880
Methylnaltrexone bromide, 2888
2-Methyl-5-nitroimidazole, 6180
N-Methyl-*N*-nitroso-*p*-toluenesulfonamide, 6180
Methylparaben, 5886
sodium, 5887
4-Methylpentan-2-ol, 6180
2-Methylpentane, 6180
4-Methyl-2-pentanone, 6179, 6180
3-Methyl-2-pentanone, 6180
Methylphenidate hydrochloride, 2890
tablets, 2892
extended-release tablets, 2892
Methylprednisolone, 2898
acetate, 2899
acetate injectable suspension, 2901
acetate and neomycin sulfate cream, 3113
hemisuccinate, 2901
sodium succinate, 2902
sodium succinate for injection, 2903
tablets, 2899
2-Methyl-2-propyl-1,3-propanediol, 6180
Methyl *p*-toluenesulfonate, 6180
N-Methylpyrrolidine, 6181
Methylpyrrolidone, 5888
Methylsulfonylmethane, 5049
and glucosamine tablets, 5049
glucosamine, and chondroitin sulfate sodium tablets, 5051
tablets, 5156
Methyltestosterone, 2904
capsules, 2906
tablets, 2906
Methylthionine perchlorate TS, 6232
Methysergide maleate, 2907
Metoclopramide
hydrochloride, 2908
injection, 2909
oral solution, 2910
tablets, 2911
Metolazone, 2912
oral suspension, 2913
tablets, 2914
Metoprolol
fumarate, 2915
succinate, 2917
succinate extended-release tablets, 2918
tartrate, 2922
tartrate and hydrochlorothiazide tablets, 2928
tartrate injection, 2923
tartrate oral solution, 2925
tartrate oral suspension, 2926
tartrate tablets, 2926
Metrifonate, 2930
Metronidazole, 2931
benzoate, 2932
capsules, 2934
gel, 2935
injection, 2936
tablets, 2937
extended-release tablets, 2939
Metronidazole benzoate compounded oral suspension, 2933
Metyrapone, 2941
Metyrosine, 2942
capsules, 2942
Mexiletine hydrochloride, 2943
capsules, 2944
Mibolerone, 2944
oral solution, 2945
Miconazole, 2946
compounded ophthalmic solution, 2946
nitrate, 2947
nitrate cream, 2949
nitrate topical powder, 2949
nitrate vaginal suppositories, 2951
Microbial characterization, identification, and strain typing ⟨1113⟩, 7823
Microbial enumeration tests—nutritional and dietary supplements ⟨2021⟩, 8687
Microbiological attributes of nonsterile nutritional and dietary supplements ⟨2023⟩, 8698
Microbiological best laboratory practices ⟨1117⟩, 7845
Microbiological control and monitoring of aseptic processing environments ⟨1116⟩, 7833
Microbiological examination of nonsterile products: acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use ⟨1111⟩, 7819
Microbiological examination of nonsterile products: microbial enumeration tests ⟨61⟩, 6457
Microbiological examination of nonsterile products: tests for specified microorganisms ⟨62⟩, 6463
Microbiological examination of nonsterile products tests for Burkholderia Cepacia complex ⟨60⟩, 6454
Microbiological procedures for absence of specified microorganisms—nutritional and dietary supplements ⟨2022⟩, 8692
Microscopy, optical ⟨776⟩, 6998
Midazolam, 2952
injection, 2953
Mid-infrared spectroscopy ⟨854⟩, 7151
Mid-infrared spectroscopy—theory and practice ⟨1854⟩, 8650

www.webofpharma.com

Midodrine hydrochloride, 2954
tablets, 2955
Milbemycin oxime, 2957
Milk thistle, 5157
capsules, 5162
extract, powdered, 5160
powdered, 5159
tablets, 5163
Millon's reagent, 6232
Milrinone, 2958
Milrinone lactate
injection, 2959
Mineral
acid, 6181
oil, 2961
oil emulsion, 2962
oil, light, 5889
oil, rectal, 2962
oil, topical light, 2962
Minerals
with calcium and vitamin D tablets, 4845
capsules, 5164
oil- and water-soluble vitamins with, capsules, 5435
oil- and water-soluble vitamins with, oral solution, 5462
oil- and water-soluble vitamins with, tablets, 5476
tablets, 5172
water-soluble vitamins with, capsules, 5523
water-soluble vitamins with, oral solution, 5543
water-soluble vitamins with, tablets, 5552
Minimum fill ⟨755⟩, 6982
Minocycline
hydrochloride, 2964
hydrochloride capsules, 2965
periodontal system, 2970
hydrochloride oral suspension, 2966
hydrochloride tablets, 2966
hydrochloride extended-release tablets, 2967
for injection, 2963
Minoxidil, 2972
topical solution, 2975
tablets, 2974
Mirtazapine, 2976
tablets, 2978
orally disintegrating tablets, 2980
Mirtazapine compounded, veterinary
oral suspension, 2977
Misoprostol, 2981
dispersion, 2983
Mission
and preface, v
statement, v
Mitomycin, 2984
for injection, 2986
Mitotane, 2986
tablets, 2987
Mitoxantrone
hydrochloride, 2987
injection, 2988
Modafinil, 2990
tablets, 2991
Moexipril hydrochloride, 2992
Moexipril hydrochloride
and hydrochlorothiazide tablets, 2996
tablets, 2994
Moist heat sterilization of aqueous liquids ⟨1229.2⟩, 8202
Molindone hydrochloride, 2999
tablets, 3000
Molybdenum, 6181
Molybdic acid, 6181
Molybdo-phosphotungstate TS, 6232
Mometasone furoate, 3001
cream, 3002
ointment, 3003
topical solution, 3005
Monensin, 3006
granulated, 3007
premix, 3008
sodium, 3009
Monitoring devices—time, temperature, and humidity ⟨1118⟩, 7850
Monitoring of bioburden ⟨1229.3⟩, 8206
Monobasic
potassium phosphate, 5979, 6181
sodium phosphate, 4088, 6181
0.1 M Monobasic sodium phosphate TS, 6232
0.05 M Monobasic sodium phosphate TS, 6232
Monobenzone, 3009
Monochloroacetic acid, 6181
Mono- and di-glycerides, 5890
Monoethanolamine, 5891, 6181
Monoglyceride citrate, 5891
Monograph and reference material donors 2014 recognition, xxv
Monosaccharide Analysis, 6616
Monosodium glutamate, 5892, 6181
Monothioglycerol, 5892
Montelukast
sodium oral granules, 3012
sodium tablets, 3015
sodium chewable tablets, 3018
Montelukast sodium, 3010
Montelukast sodium hydrate, 6181
Morantel tartrate, 3021
Moricizine hydrochloride, 3021
Morin, 6181
Morphine sulfate, 3023
extended-release capsules, 3024
injection, 3027
suppositories, 3028
Morphine sulfate
compounded injection, 3028
Morpholine, 6181
4-Morpholine propane sulfonic acid, 6181
Moxidectin, 3029
Moxifloxacin
hydrochloride, 3032
ophthalmic solution, 3034
tablets, 3036
Mucosal drug products—performance tests ⟨1004⟩, 7227
Mucosal drug products—product quality tests ⟨4⟩, 6423
Mupirocin, 3038
calcium, 3038
cream, 3040
ointment, 3041
nasal ointment, 3042
Mycophenolate
sodium, 3052
Mycophenolate mofetil, 3043
capsules, 3044
for injection, 3046
for oral suspension, 3047
tablets, 3049
Mycophenolic acid
delayed-release tablets, 3054
Mycoplasma tests ⟨63⟩, 6472
Myristic acid, 5893
Myristyl alcohol, 5895
Myristyltrimethylammonium bromide, 6181
Myrrh, 3056
topical solution, 3057

N

N 13 injection, ammonia, 3183
Nabumetone, 3058
tablets, 3059
Nadolol, 3059
and bendroflumethiazide tablets, 3062
tablets, 3061
Nafcillin
injection, 3063
for injection, 3063
sodium, 3064
Naftifine hydrochloride, 3064
cream, 3065
gel, 3066
Nalorphine hydrochloride, 3066
injection, 3067
Naloxone
hydrochloride, 3067
hydrochloride injection, 3068
and pentazocine tablets, 3463
Naltrexone hydrochloride, 3069
tablets, 3071
Nandrolone
decanoate, 3072
decanoate injection, 3073
Naphazoline hydrochloride, 3074
nasal solution, 3075
ophthalmic solution, 3076
and pheniramine maleate ophthalmic solution, 3076
Naphthalene, 6181
1,3-Naphthalenediol, 6181
2,7-Naphthalenediol, 6159, 6181
2-Naphthalenesulfonic acid, 6181
Naphthol
dipotassium disulfonate, 6181
disodium disulfonate, 6181
1-Naphthol, 6134, 6181
reagent, 6232
TS, 6232
2-Naphthol, 6142, 6181
TS, 6227, 6232
p-Naphtholbenzein, 6182, 6221
TS, 6232
β-Naphthoquinone-4-sodium sulfonate, 6182
Naphthoresorcinol, 6182
1-Naphthylamine, 6182
1-Naphthylamine hydrochloride, 6182
2-Naphthyl chloroformate, 6182
N-(1-Naphthyl)ethylenediamine
dihydrochloride, 6182
TS, 6232
Naproxen, 3077
sodium, 3082
sodium tablets, 3082
oral suspension, 3079
tablets, 3079
delayed-release tablets, 3081
Naproxen compounded
oral suspension, 3078
Naproxen sodium and pseudoephedrine hydrochloride extended-release tablets, 3084
Narasin
granular, 3088
premix, 3090
Naratriptan
hydrochloride, 3093
hydrochloride oral suspension, 3094
tablets, 3091

www.webofpharma.com

Nasal solution

Calcitonin salmon, 675
Cromolyn sodium, 1184
Ephedrine sulfate, 1644
Epinephrine, 1649
Flunisolide, 1929
Naphazoline hydrochloride, 3075
Oxymetazoline hydrochloride, 3349
Phenylephrine hydrochloride, 3518
Tetrahydrozoline hydrochloride, 4315
Xylometazoline hydrochloride, 4661

Nasal spray

Butorphanol tartrate, 657
Desmopressin acetate, 1267
Fluticasone propionate, 2000
Natamycin, 3095
  ophthalmic suspension, 3096
Nateglinide, 3096
  tablets, 3098
Near-infrared spectroscopy ⟨856⟩, 7161
Nefazodone hydrochloride, 3099
  tablets, 3100
Neohesperidin dihydrochalcone, 5896
Neomycin
  boluses, 3102
  and colistin sulfates and hydrocortisone acetate otic suspension, 1150
  for injection, 3102
  penicillin G, polymyxin B, hydrocortisone acetate, and hydrocortisone sodium succinate topical suspension, 3430
  and polymyxin B sulfates, bacitracin, and hydrocortisone acetate ointment, 3116
  and polymyxin B sulfates, bacitracin, and hydrocortisone acetate ophthalmic ointment, 3116
  and polymyxin B sulfates, bacitracin, and lidocaine ointment, 3117
  and polymyxin B sulfates and bacitracin ointment, 3115
  and polymyxin B sulfates and bacitracin ophthalmic ointment, 3115
  and polymyxin B sulfates, bacitracin zinc, and hydrocortisone ointment, 3118
  and polymyxin B sulfates, bacitracin zinc, and hydrocortisone ophthalmic ointment, 3119
  and polymyxin B sulfates, bacitracin zinc, and hydrocortisone acetate ophthalmic ointment, 3120
  and polymyxin B sulfates, bacitracin zinc, and lidocaine ointment, 3120
  and polymyxin B sulfates and bacitracin zinc ointment, 3118
  and polymyxin B sulfates and bacitracin zinc ophthalmic ointment, 3118
  and polymyxin B sulfates cream, 3114
  and polymyxin B sulfates and dexamethasone ophthalmic ointment, 3121
  and polymyxin B sulfates and dexamethasone ophthalmic suspension, 3122
  and polymyxin B sulfates and gramicidin cream, 3122
  and polymyxin B sulfates, gramicidin, and hydrocortisone acetate cream, 3123
  and polymyxin B sulfates and gramicidin ophthalmic solution, 3123
  and polymyxin B sulfates and hydrocortisone ophthalmic suspension, 3123
  and polymyxin B sulfates and hydrocortisone otic solution, 3124
  and polymyxin B sulfates and hydrocortisone otic suspension, 3124
  and polymyxin B sulfates and hydrocortisone acetate cream, 3125
  and polymyxin B sulfates and hydrocortisone acetate ophthalmic suspension, 3125
  and polymyxin B sulfates and lidocaine cream, 3126
  and polymyxin B sulfates ophthalmic ointment, 3114
  and polymyxin B sulfates ophthalmic solution, 3115
  and polymyxin B sulfates, penicillin G procaine, and hydrocortisone acetate topical suspension, 3448
  and polymyxin B sulfates and pramoxine hydrochloride cream, 3126
  and polymyxin B sulfates and prednisolone acetate ophthalmic suspension, 3127
  and polymyxin B sulfates solution for irrigation, 3114
  sulfate, 3102
  sulfate and bacitracin ointment, 3104
  sulfate and bacitracin zinc ointment, 3104
  sulfate cream, 3103
  sulfate and dexamethasone sodium phosphate cream, 3104
  sulfate and dexamethasone sodium phosphate ophthalmic ointment, 3105
  sulfate and dexamethasone sodium phosphate ophthalmic solution, 3106
  sulfate and fluocinolone acetonide cream, 3107
  sulfate and fluorometholone ointment, 3107
  sulfate and flurandrenolide cream, 3107
  sulfate and flurandrenolide lotion, 3108
  sulfate and flurandrenolide ointment, 3108
  sulfate and gramicidin ointment, 3108
  sulfate and hydrocortisone cream, 3109
  sulfate and hydrocortisone ointment, 3109
  sulfate and hydrocortisone otic suspension, 3109
  sulfate and hydrocortisone acetate cream, 3110
  sulfate and hydrocortisone acetate lotion, 3110
  sulfate and hydrocortisone acetate ointment, 3110
  sulfate and hydrocortisone acetate ophthalmic suspension, 3110
  sulfate, isoflupredone acetate, and tetracaine hydrochloride ointment, 3111
  sulfate, isoflupredone acetate, and tetracaine hydrochloride topical powder, 3112
  sulfate and methylprednisolone acetate cream, 3113
  sulfate, nystatin, gramicidin, and triamcinolone acetonide cream, 3222
  sulfate, nystatin, gramicidin, and triamcinolone acetonide ointment, 3222
  sulfate, nystatin, thiostrepton, and triamcinolone acetonide cream, 3223
  sulfate, nystatin, thiostrepton, and triamcinolone acetonide ointment, 3223
  sulfate ointment, 3103
  sulfate ophthalmic ointment, 3103
  sulfate and prednisolone acetate ophthalmic suspension, 3128
  sulfate oral solution, 3103
  sulfate tablets, 3104
  sulfate and triamcinolone acetonide cream, 3129
Neostigmine
  bromide, 3129
  bromide tablets, 3129
  methylsulfate, 3130
  methylsulfate injection, 3130
Neotame, 5898
Nephelometry, turbidimetry, and visual comparison ⟨855⟩, 7155
Nessler's reagent, 6232
Neutralized
  alcohol, 6182
  phthalate buffer, 6145
Neutral red, 6221
  TS, 6232
Nevirapine, 3131
Nevirapine
  extended release tablets, 3135
  oral suspension, 3132
  tablets, 3134
New sterilization methods ⟨1229.12⟩, 8233
Niacin, 3138
  extended-release tablets, 3141
  injection, 3140
  or niacinamide assay ⟨441⟩, 6704
  tablets, 3140
Niacinamide, 3146
  injection, 3147
  or niacin assay ⟨441⟩, 6704
  tablets, 3147
Nicardipine hydrochloride, 3148
  injection, 3149
Nickel-aluminum catalyst, 6182
Nickel, 6182
  standard solution TS, 6232
  sulfate, 6182
  (II) sulfate heptahydrate, 6182
Nickel nitrate hexahydrate, 6182
β-Nicotinamide adenine dinucleotide, 6182
Nicotinamide adenine dinucleotide phosphate-adenosine-5′-triphosphate mixture, 6182
Nicotine, 3152
  polacrilex, 3156
  polacrilex gum, 3159
  transdermal system, 3153
Nicotinic acid, 6182
Nifedipine, 3160
  capsules, 3161
  extended-release tablets, 3163
Nile blue hydrochloride, 6221
Nilutamide, 3170
Nimodipine, 3171
Ninhydrin, 6182
  TS, 6232
Nitrate
  mercurous, dihydrate, 6177
  mercurous, TS, 6231
  ophthalmic solution, silver, 4041
  in reagents, 6131
  silver, 4041, 6200
  silver, TS, 6234
  tenth-normal (0.1 N), silver, 6234, 6247
  toughened silver, 4042
Nitric
  acid, 5900, 6182
  acid, diluted, 6159, 6182
  acid, fuming, 6168, 6182
  acid, lead-free, 6182

www.webofpharma.com

Nitric *(continued)*
oxide–nitrogen dioxide detector tube, 6182
Nitric acid
0.01 N TS, 6232
0.2 N TS, 6232
1 N TS, 6232
2 N TS, 6232
ultratrace, 6182
Nitrilotriacetic acid, 6182
Nitrite titration ⟨451⟩, 6709
4′-Nitroacetophenone, 6182
o-Nitroaniline, 6183
p-Nitroaniline, 6183
TS, 6232
Nitrobenzene, 6183
p-Nitrobenzenediazonium tetrafluoroborate, 6183
4-Nitrobenzoic acid, 6183
p-Nitrobenzyl bromide, 6183
4-(*p*-Nitrobenzyl) pyridine, 6183
Nitrofurantoin, 3172
capsules, 3174
oral suspension, 3179
tablets, 3180
Nitrofurazone, 3181
ointment, 3182
topical solution, 3183
Nitrogen, 5900
97 percent, 5901
certified standard, 6183
compounds in reagents, 6131
determination ⟨461⟩, 6709
N 13 injection, ammonia, 3183
Nitroglycerin
diluted, 3186
injection, 3187
ointment, 3187
sublingual tablets, 3188
Nitromersol, 3189
topical solution, 3190
Nitromethane, 6183
5-Nitro-1,10-phenanthroline, 6182
Nitrophenanthroline TS, 6232
1-Nitroso-2-naphthol, 6183
Nitroso R salt, 6183
Nitrous
oxide, 3190
oxide certified standard, 6183
Nizatidine, 3191
capsules, 3192
Nomenclature ⟨1121⟩, 7862
Nonadecane, 6183
Nonanoic acid, 6183
Nonionic wetting agent, 6183, 6184
Nonoxynol 9, 3193, 6184
1-Nonyl alcohol, 6184
n-Nonylamine, 6184
Nonylphenol polyoxyethylene ether, 6184
Nonylphenoxypoly(ethyleneoxy)ethanol, 6184
Norelgestromin, 3196
Norepinephrine bitartrate, 3197
injection, 3198
and propoxycaine and procaine hydrochlorides injection, 3745
Norethindrone, 3198
acetate, 3204
acetate and estradiol tablets, 1733
acetate and ethinyl estradiol tablets, 3208
acetate tablets, 3205
and ethinyl estradiol tablets, 3201
and mestranol tablets, 3203
tablets, 3199
Norgestimate, 3209
and ethinyl estradiol tablets, 3211
Norgestrel, 3212
and ethinyl estradiol tablets, 3214
tablets, 3213
Normal
butyl acetate, 6145
butyl alcohol, 6145
butylamine, 6184
Northern schisandra fruit, 5261
dry extract, 5263
powder, 5265
Nortriptyline hydrochloride, 3214
capsules, 3216
oral solution, 3216
Noscapine, 3217
Novobiocin
sodium, 3218
sodium intramammary infusion, 3218
sodium and penicillin G procaine intramammary infusion, 3448
sodium, tetracycline hydrochloride, and prednisolone tablets, 4312
sodium and tetracycline hydrochloride tablets, 4312
Nuclear magnetic resonance spectroscopy ⟨761⟩, 6984
Nuclear magnetic resonance spectroscopy identity testing of bacterial polysaccharides used in vaccine manufacture ⟨198⟩, 6596
Nucleic acid-based techniques
amplification ⟨1127⟩, 7879
approaches for detecting trace nucleic acids (residual DNA testing) ⟨1130⟩, 7897
extraction, detection, and sequencing ⟨1126⟩, 7870
general ⟨1125⟩, 7865
genotyping ⟨1129⟩, 7893
microarray ⟨1128⟩, 7887
Nystatin, 3218
cream, 3219
lotion, 3220
lozenges, 3220
neomycin sulfate, gramicidin, and triamcinolone acetonide cream, 3222
neomycin sulfate, gramicidin, and triamcinolone acetonide ointment, 3222
neomycin sulfate, thiostrepton, and triamcinolone acetonide cream, 3223
neomycin sulfate, thiostrepton, and triamcinolone acetonide ointment, 3223
ointment, 3220
topical powder, 3220
oral suspension, 3221
for oral suspension, 3221
tablets, 3221
and tetracycline hydrochloride capsules, 4313
and triamcinolone acetonide cream, 3224
and triamcinolone acetonide ointment, 3225
vaginal inserts, 3222
vaginal suppositories, 3220

O

n-Octadecane, 6184
Octadecyl silane, 6184
Octane-*n*, 6184
Octanesulfonic acid sodium salt, 6204
Octanesulfonic acid sodium salt monohydrate, 6184
1-Octanol, 6184
Octanophenone, 6184
Octinoxate, 3225
Octisalate, 3226
Octocrylene, 3227
Octoxynol 9, 5901, 6184
Octreotide acetate, 3228
Octylamine, 6184
Octyldodecanol, 5904
(*p-tert*-Octylphenoxy)nonaethoxyethanol, 6163, 6184
(*p-tert*-Octylphenoxy)polyethoxyethanol, 6184
Octyl sulfate, sodium salt, 6184
Officers (2015–2020), ix
Ofloxacin, 3230
ophthalmic solution, 3231
tablets, 3232

Oil

Almond, 5609
Anise, 5624
Borage seed, 4830
Borage seed, capsules, 4831
Canola, 5664
Caraway, 5667
Cardamom, 5688
Castor, 802
Castor, aromatic, 807
Castor, capsules, 805
Castor, emulsion, 806
Castor, hydrogenated, 5691
Cedar, 6149
Chia seed, 4877
Clove, 5718
Coconut, 5719
Coconut, hydrogenated, 5720
Cod liver, 1135
Cod liver, capsules, 4910
Coriander, 5723
Corn, 5723
Cottonseed, 5733
Cottonseed, hydrogenated, 5734
Crypthecodinium cohnii, 4922
Crypthecodinium cohnii, capsules, 4925
Ethiodized injection, 1770
Evening primrose, 4980
Evening primrose, capsules, 4980
Fats and fixed oils ⟨401⟩, 6676
Fennel, 5776
Flax seed, 4998
Flax seed, capsules, 4999
Krill, capsules, 5104
Krill delayed-release capsules, 5107
Lemon, 5856
Mineral, 2961
Mineral emulsion, 2962
Mineral, light, 5889
Mineral, rectal, 2962
Mineral, topical light, 2962
Olive, 5910
Orange, 5914
Palm, 5916
Palm, hydrogenated, 5916
Palm kernel, 5917
Peanut, 5921
Peppermint, 5923
Polyoxyl 35 castor, 5956
Polyoxyl 40 hydrogenated castor, 5958
Fully hydrogenated rapeseed, 5998

www.webofpharma.com

Oil *(continued)*
Superglycerinated fully hydrogenated rapeseed, 5999
Rose, 6001
Safflower, 3968
Schizochytrium, 5266
Schizochytrium, capsules, 5269
Sesame, 6003
Soybean, 4105
Soybean, hydrogenated, 6040
Star anise, 6043
Sunflower, 6085
Vegetable, hydrogenated, 6099
Vitamins capsules, oil- and water-soluble, 5389
Vitamins capsules, oil-soluble, 5346
Vitamins with minerals capsules, oil- and water-soluble, 5435
Vitamins with minerals oral solution, oil- and water-soluble, 5462
Vitamins with minerals tablets, oil- and water-soluble, 5476
Vitamins oral solution, oil- and water-soluble, 5409
Vitamins tablets, oil- and water-soluble, 5419
Vitamins tablets, oil-soluble, 5356

Oil-soluble vitamins
capsules, 5346
tablets, 5356
Oil- and water-soluble vitamins
capsules, 5389
with minerals capsules, 5435
with minerals oral solution, 5462
with minerals tablets, 5476
oral solution, 5409
tablets, 5419

Ointment

Acyclovir, 91
Alclometasone dipropionate, 112
Amcinonide, 215
Amphotericin B, 316
Anthralin, 344
Atropine sulfate ophthalmic, 431
Bacitracin ophthalmic, 467
Bacitracin zinc, 470
Bacitracin zinc and polymyxin B sulfate, 471
Bacitracin zinc and polymyxin B sulfate ophthalmic, 471
Benzocaine, 509
Benzocaine, butamben, and tetracaine hydrochloride, 516
Benzoic and salicylic acids, 521
Betamethasone dipropionate, 548
Betamethasone valerate, 556
Bland lubricating ophthalmic, 3273
Calcipotriene, 668
Chloramphenicol and polymyxin B sulfate ophthalmic, 933
Chloramphenicol ophthalmic, 929
Chlortetracycline hydrochloride, 980
Chlortetracycline hydrochloride ophthalmic, 987
Ciprofloxacin ophthalmic, 1014
Clioquinol, 1076
Clioquinol and hydrocortisone, 1078
Clobetasol propionate, 1081
Coal tar, 1133
Desoximetasone, 1275
Dibucaine, 1337
Diflorasone diacetate, 1375
Erythromycin, 1688
Erythromycin ophthalmic, 1689
Fluocinolone acetonide, 1935
Fluocinonide, 1938
Flurandrenolide, 1976
Fluticasone propionate, 2003
Gentamicin and prednisolone acetate ophthalmic, 2106
Gentamicin sulfate, 2101
Gentamicin sulfate and betamethasone valerate, 2103
Gentamicin sulfate ophthalmic, 2102
Halcinonide, 2185
Hydrocortisone, 2232
Hydrocortisone acetate, 2238
Hydrocortisone valerate, 2246
Hydrophilic, 3233
Ichthammol, 2289
Idoxuridine ophthalmic, 2293
Lidocaine, 2622
Mometasone furoate, 3003
Mupirocin, 3041
Mupirocin nasal, 3042
Neomycin and polymyxin B sulfates and bacitracin, 3115
Neomycin and polymyxin B sulfates, bacitracin, and hydrocortisone acetate, 3116
Neomycin and polymyxin B sulfates, bacitracin, and hydrocortisone acetate ophthalmic, 3116
Neomycin and polymyxin B sulfates, bacitracin, and lidocaine, 3117
Neomycin and polymyxin B sulfates and bacitracin ophthalmic, 3115
Neomycin and polymyxin B sulfates and bacitracin zinc, 3118
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone, 3118
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone acetate ophthalmic, 3120
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone ophthalmic, 3119
Neomycin and polymyxin B sulfates, bacitracin zinc, and lidocaine, 3120
Neomycin and polymyxin B sulfates and bacitracin zinc ophthalmic, 3118
Neomycin and polymyxin B sulfates and dexamethasone ophthalmic, 3121
Neomycin and polymyxin B sulfates ophthalmic, 3114
Neomycin sulfate, 3103
Neomycin sulfate and bacitracin, 3104
Neomycin sulfate and bacitracin zinc, 3104
Neomycin sulfate and dexamethasone sodium phosphate ophthalmic, 3105
Neomycin sulfate and fluorometholone, 3107
Neomycin sulfate and flurandrenolide, 3108
Neomycin sulfate and gramicidin, 3108
Neomycin sulfate and hydrocortisone, 3109
Neomycin sulfate and hydrocortisone acetate, 3110
Neomycin sulfate, isoflupredone acetate, and tetracaine hydrochloride, 3111
Neomycin sulfate ophthalmic, 3103
Nitrofurazone, 3182
Nitroglycerin, 3187
Nystatin, 3220
Nystatin, neomycin sulfate, gramicidin, and triamcinolone acetonide, 3222
Nystatin, neomycin sulfate, thiostrepton, and triamcinolone acetonide, 3223
Nystatin and triamcinolone acetonide, 3225
Oxytetracycline hydrochloride and hydrocortisone, 3364
Oxytetracycline hydrochloride and polymyxin B sulfate, 3364
Oxytetracycline hydrochloride and polymyxin B sulfate ophthalmic, 3365
Polyethylene glycol, 5941
Povidone–iodine, 3635
Prednicarbate, 3658
Resorcinol ointment, compound, 3861
Rose water, 3946
Sodium chloride ophthalmic, 4068
Sulfacetamide sodium ophthalmic, 4142
Sulfacetamide sodium and prednisolone acetate ophthalmic, 4144
Sulfur, 4176
Tetracaine, 4294
Tetracaine and menthol, 4294
Tetracycline hydrochloride, 4307
Tetracycline hydrochloride ophthalmic, 4308
Tobramycin and dexamethasone ophthalmic, 4409
Tobramycin ophthalmic, 4405
Triamcinolone acetonide, 4485
Undecylenic acid, compound, 4554
White, 3233
Yellow, 3233
Zinc oxide, 4689

Olanzapine, 3234
and fluoxetine capsules, 3237
tablets, 3235
Olanzapine orally disintegrating tablets, 3240
Olefin detector tube, 6184
Oleic acid, 5906
Oleoresin, capsicum, 730
Oleovitamin A and D, 3242
capsules, 3242
Oleoyl polyoxylglycerides, 5908
Oleyl
alcohol, 5909
oleate, 5910
Oligo-deoxythymidine, 6184
Oligosaccharide analysis ⟨212⟩, 6623
Olive leaf, 5181
dry extract, 5182
powder, 5184
Olive oil, 5910
Olmesartan medoxomil, 3242
and amlodipine tablets, 272
tablets, 3244
Olopatadine hydrochloride ophthalmic solution, 3249
Omega-3
acids triglycerides, 5185
ethyl esters capsules, 3253
ethyl esters, 3250
Omega-3 free fatty acids, 5188
Omeprazole, 3256
delayed-release capsules, 3258
magnesium, 3260

www.webofpharma.com

Ondansetron, 3262
compounded topical gel, 3272
hydrochloride, 3263
hydrochloride oral suspension, 3268
injection, 3265
oral solution, 3266
tablets, 3268
orally disintegrating tablets, 3271

Ophthalmic ointment

Atropine sulfate, 431
Bacitracin, 467
Bacitracin zinc and polymyxin B sulfate, 471
Bland lubricating, 3273
Chloramphenicol, 929
Chloramphenicol and polymyxin B sulfate, 933
Chlortetracycline hydrochloride, 987
Ciprofloxacin, 1014
Erythromycin, 1689
Gentamicin and prednisolone acetate, 2106
Gentamicin sulfate, 2102
Idoxuridine, 2293
Neomycin and polymyxin B sulfates, 3114
Neomycin and polymyxin B sulfates and bacitracin, 3115
Neomycin and polymyxin B sulfates, bacitracin, and hydrocortisone acetate, 3116
Neomycin and polymyxin B sulfates and bacitracin zinc, 3118
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone, 3119
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone acetate, 3120
Neomycin and polymyxin B sulfates and dexamethasone, 3121
Neomycin sulfate, 3103
Neomycin sulfate and dexamethasone sodium phosphate, 3105
Oxytetracycline hydrochloride and polymyxin B sulfate, 3365
Sodium chloride, 4068
Sulfacetamide sodium, 4142
Sulfacetamide sodium and prednisolone acetate, 4144
Tetracycline hydrochloride, 4308
Tobramycin, 4405
Tobramycin and dexamethasone, 4409

Ophthalmic products—performance tests ⟨1771⟩, 8549
Ophthalmic products—quality tests ⟨771⟩, 6993

Ophthalmic solution

Acetylcholine chloride for, 81
Apraclonidine, 359
Atropine sulfate, 432
Azelastine hydrochloride, 445
Benoxinate hydrochloride, 499
Betaxolol, 558
Carbachol, 739
Carteolol hydrochloride, 789
Chloramphenicol, 929
Chloramphenicol for, 930
Chymotrypsin for, 994
Ciprofloxacin, 1015
Cromolyn sodium, 1185
Cyclopentolate hydrochloride, 1201
Cyclosporine compounded, veterinary, 1217
Demecarium bromide, 1247
Dexamethasone sodium phosphate, 1296
Dipivefrin hydrochloride, 1442
Dorzolamide hydrochloride, 1497
Dorzolamide hydrochloride and timolol maleate, 1499
Echothiophate iodide for, 1579
Emedastine, 1610
Epinephrine, 1649
Epinephrine bitartrate, 1651
Epinephrine bitartrate for, 1652
Epinephryl borate, 1652
Fluorescein sodium and benoxinate hydrochloride, 1944
Fluorescein sodium and proparacaine hydrochloride, 1945
Flurbiprofen sodium, 1982
Gentamicin sulfate, 2102
Gentamicin sulfate and betamethasone acetate, 2102
Glycerin, 2133
Homatropine hydrobromide, 2208
Hydroxyamphetamine hydrobromide, 2258
Hypromellose, 2281
Idoxuridine, 2293
Levobunolol hydrochloride, 2595
Methylcellulose, 2876
Miconazole compounded, 2946
Moxifloxacin, 3034
Naphazoline hydrochloride, 3076
Naphazoline hydrochloride and pheniramine maleate, 3076
Neomycin and polymyxin B sulfates, 3115
Neomycin and polymyxin B sulfates and gramicidin, 3123
Neomycin sulfate and dexamethasone sodium phosphate, 3106
Ofloxacin, 3231
Olopatadine hydrochloride, 3249
Oxymetazoline hydrochloride, 3350
Phenylephrine hydrochloride, 3519
Physostigmine salicylate, 3536
Pilocarpine hydrochloride, 3543
Pilocarpine nitrate, 3545
Polymyxin B sulfate and trimethoprim, 3592
Prednisolone sodium phosphate, 3668
Proparacaine hydrochloride, 3739
Scopolamine hydrobromide, 4013
Silver nitrate, 4041
Sodium chloride, 4068
Sulfacetamide sodium, 4143
Tetracaine hydrochloride, 4299
Tetrahydrozoline hydrochloride, 4315
Timolol maleate, 4389
Tobramycin, 4408
Travoprost, 4471
Tropicamide, 4541
Voriconazole compounded, veterinary, 4645
Zinc sulfate, 4693

Ophthalmic suspension

Brinzolamide, 594
Chloramphenicol and hydrocortisone acetate for, 932
Dexamethasone, 1287
Fluorometholone, 1952
Gentamicin and prednisolone acetate, 2107
Natamycin, 3096
Neomycin and polymyxin B sulfates and dexamethasone, 3122
Neomycin and polymyxin B sulfates and hydrocortisone, 3123
Neomycin and polymyxin B sulfates and hydrocortisone acetate, 3125
Neomycin and polymyxin B sulfates and prednisolone acetate, 3127
Neomycin sulfate and hydrocortisone acetate, 3110
Neomycin sulfate and prednisolone acetate, 3128
Oxytetracycline hydrochloride and hydrocortisone acetate, 3363
Prednisolone acetate, 3663
Rimexolone, 3887
Sulfacetamide sodium and prednisolone acetate, 4145
Tetracycline hydrochloride, 4310
Tobramycin and dexamethasone, 4411
Tobramycin and fluorometholone acetate, 4412

Opium, 3273
powdered, 3274
tincture, 3274
Optical
microscopy ⟨776⟩, 6998
rotation ⟨781⟩, 7001
Oral drug products—product quality tests ⟨2⟩, 6410
Orally inhaled and nasal drug products ⟨1664.1⟩, 8467

Oral powder

Containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 54
Levothyroxine sodium, 2618
Sodium bicarbonate, 4061

Oral solution

Abacavir, 19
Acacia syrup, 5597
Acetaminophen, 41
Containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 56
Acetaminophen and codeine phosphate, 54

www.webofpharma.com

Oral solution *(continued)*
Acetaminophen, dextromethorphan hydrobromide, doxylamine succinate, and pseudoephedrine hydrochloride, 67
Acetaminophen for effervescent, 42
Amantadine hydrochloride, 212
Aminocaproic acid, 236
Aminophylline, 245
Amprolium, 331
Aromatic elixir, 5624
Ascorbic acid, 384
Ascorbic acid compounded, 385
Aspirin effervescent tablets for, 395
Atenolol, 407
Beclomethasone dipropionate compounded, 484
Benzaldehyde elixir, compound, 5634
Betamethasone, 542
Bethanechol chloride, 563
Bromodiphenhydramine hydrochloride, 599
Bromodiphenhydramine hydrochloride and codeine phosphate, 600
Brompheniramine maleate, 602
Brompheniramine maleate and pseudoephedrine sulfate, 603
Butabarbital sodium, 642
Caffeine citrate, 663
Calcium glubionate syrup, 695
Captopril, 734
C 13 for, urea, 767
Cetirizine hydrochloride, 912
Cherry syrup, 5709
Chloral hydrate, 924
Chloramphenicol, 930
Chlorpheniramine maleate, 971
Chlorpheniramine maleate and pseudoephedrine hydrochloride, 973
Chlorpromazine hydrochloride syrup, 976
Chocolate syrup, 5716
Citalopram, 1034
Clindamycin hydrochloride, 1066
clindamycin hydrochloride compounded, 1066
Clindamycin palmitate hydrochloride for, 1068
Cloxacillin sodium for, 1130
Codeine phosphate, 1138
Codeine sulfate, 1141
Cyclosporine, 1216
Cyproheptadine hydrochloride, 1219
Dexamethasone, 1288
Dexamethasone elixir, 1286
Dexbrompheniramine maleate and pseudoephedrine sulfate, 1298
Dexchlorpheniramine maleate, 1301
Dextromethorphan hydrobromide, 1321
Diclofenac potassium for, 1344
Dicyclomine hydrochloride, 1361
Didanosine for, 1366
Digoxin, 1385
Dihydrotachysterol, 1392
Diltiazem hydrochloride, 1405
Dimenhydrinate, 1410
Diphenhydramine hydrochloride, 1429
Diphenoxylate hydrochloride and atropine sulfate, 1438
Docusate sodium syrup, 1481
Dolasetron mesylate, 1485
Doxepin hydrochloride, 1510
Doxylamine succinate, 1546
Dyphylline, 1573
Dyphylline and guaifenesin, 1575
Entecavir, 1636
Ephedrine sulfate, 1644
Ergocalciferol, 1667
Ergoloid mesylates, 1671
Escitalopram, 1706
Ethosuximide, 1776
Ferric ammonium citrate for, 290
Ferrous gluconate, 1860
Ferrous sulfate, 1863
Ferrous sulfate syrup, 1863
Fluoxetine, 1961
Fluphenazine hydrochloride, 1973
Fluphenazine hydrochloride elixir, 1971
Folic acid, compounded, 2027
Furosemide, 2055
Galantamine, 2079
Glycerin, 2134
Guaifenesin, 2171
Guaifenesin and codeine phosphate, 2172
Haloperidol, 2189
Hydralazine hydrochloride, 2215
Hydromorphone hydrochloride, 2252
Hydroxyzine hydrochloride, 2266
Hyoscyamine sulfate, 2277
Hyoscyamine sulfate elixir, 2276
Ipecac, 2397
Isoniazid, 2426
Isosorbide, 2440
Ivermectin compounded, veterinary, 2473
Lamivudine, 2526
Leucovorin calcium compounded, 2570
Levetiracetam, 2583
Levocarnitine, 2600
Levofloxacin, 2608
Lincomycin, 2632
Lithium, 2648
Loperamide hydrochloride, 2661
Lopinavir and ritonavir, 2666
Loratadine, 2674
Magnesium carbonate, citric acid, and potassium citrate for, 2711
Magnesium carbonate and citric acid for, 2710
Manganese chloride for, 2732
Magnesium citrate, 2714
Magnesium citrate for, 2715
Meperidine hydrochloride, 2784
Metaproterenol sulfate, 2813
Methadone hydrochloride, 2838
Methylcellulose, 2876
Metoclopramide, 2910
Metoprolol tartrate, 2925
Mibolerone, 2945
Neomycin sulfate, 3103
Nortriptyline hydrochloride, 3216
Ondansetron, 3266
Orange syrup, 5915
Oxybutynin chloride, 3328
Oxycodone hydrochloride, 3337
Paromomycin, 3409
Penicillin G potassium for, 3439
Penicillin V potassium for, 3456
Phenobarbital, 3498
Piperazine citrate syrup, 3575
Polyethylene glycol 3350 and electrolytes for, 3587
Potassium bicarbonate effervescent tablets for, 3596
Potassium bicarbonate and potassium chloride for effervescent, 3597
Potassium bicarbonate and potassium chloride effervescent tablets for, 3597
Potassium bicarbonate, potassium chloride, and potassium citrate effervescent tablets for, 3609
Potassium bromide, veterinary, 3600
Potassium chloride, 3604
Potassium chloride for, 3605
Potassium citrate and citric acid, 3617
Potassium gluconate, 3619
Potassium gluconate and potassium chloride, 3621
Potassium gluconate and potassium chloride for, 3621
Potassium gluconate and potassium citrate, 3622
Potassium gluconate, potassium citrate, and ammonium chloride, 3622
Potassium iodide, 3624
Potassium and sodium bicarbonates and citric acid effervescent tablets for, 3598
Prednisolone, 3661
Prednisolone sodium phosphate compounded, 3667
Prednisone, 3671
Prochlorperazine, 3697
Promazine hydrochloride, 3713
Promazine hydrochloride syrup, 3713
Promethazine and phenylephrine hydrochloride, 3721
Promethazine and phenylephrine hydrochloride and codeine phosphate, 3724
Promethazine hydrochloride, 3717
Pseudoephedrine hydrochloride, 3762
Pseudoephedrine hydrochloride, carbinoxamine maleate, and dextromethorphan hydrobromide, 3767
Pyridostigmine bromide, 3780
Ranitidine, 3846
Risperidone, 3905
Ritonavir, 3917
Saccharin sodium, 3967
Senna, 4026
Sertraline hydrochloride, 4032
Sodium benzoate compounded, 4056
Sodium bromide, veterinary, 4063
Sodium citrate and citric acid, 4069
Sodium fluoride, 4072
Sodium phosphates, 4090
Stavudine for, 4122
Sulfaquinoxaline, 4169
Syrup, 6087
Terpin hydrate, 4284
Terpin hydrate and codeine, 4284
Theophylline, 4325
Theophylline and guaifenesin, 4332
Theophylline sodium glycinate, 4334
Thiamine hydrochloride, 4338
Thiamine mononitrate, 4341
Thioridazine hydrochloride, 4354
Thiothixene hydrochloride, 4361
Tolu balsam syrup, 6090
Triamcinolone diacetate, 4491
Tricitrates, 4504
Trifluoperazine, 4511
Trihexyphenidyl hydrochloride, 4519
Trikates, 4521
Trimeprazine, 4522
Triprolidine hydrochloride, 4533
Triprolidine and pseudoephedrine hydrochlorides, 4534
Valproic acid, 4574
Vancomycin hydrochloride for, 4590
Vancomycin hydrochloride compounded, 4589
Vehicle for, 5913
Vehicle for, sugar free, 5913
Verapamil hydrochloride, 4609
Vigabatrin for, 4619
Vitamins with minerals, oil-soluble, 5372

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Oral solution *(continued)*
Vitamins with minerals, oil- and water-soluble, 5462
Vitamins with minerals, water-soluble, 5543
Vitamins, oil-soluble, 5353
Vitamins, oil- and water-soluble, 5409
Zidovudine, 4676
Zinc acetate, 4682
Zinc sulfate, 4693

Oral suspension

Acetaminophen, 42
Acetaminophen and codeine phosphate, 56
Acetazolamide, 76
Acyclovir, 92
Albendazole, 102
Allopurinol, 133
Alprazolam, 145
Alumina and magnesia, 166
Alumina, magnesia, and calcium carbonate, 168
Alumina, magnesia, and simethicone, 173
Alumina and magnesium carbonate, 176
Alumina and magnesium trisilicate, 179
Amiodarone hydrochloride, 257
Amlodipine, 264
Amoxicillin, 303
Amoxicillin and clavulanate potassium for, 308
Amoxicillin for, 303
Amoxicillin tablets for, 306
Ampicillin for, 324
Ampicillin and probenecid for, 326
Atenolol compounded, 408
Atenolol compounded, veterinary, 409
Atovaquone, 423
Azathioprine, 440
Azithromycin for, 454
Baclofen, 473
Benazepril hydrochloride compounded, veterinary, 490
Bethanechol chloride, 564
Biotin compounded, 571
Bismuth subsalicylate, 582
Calcium carbonate, 686
Calcium and magnesium carbonates, 691
Captopril, 735
Carbamazepine, 742
Cefaclor for, 810
Cefadroxil for, 816
Cefdinir for, 830
Cefixime for, 837
Cefpodoxime proxetil for, 864
Cefprozil for, 869
Cefuroxime axetil for, 888
Cellulose sodium phosphate for, 896
Cephalexin for, 898
Cephalexin tablets for, 899
Cephradine for, 909
Chloramphenicol palmitate, 934
Chloroquine phosphate, 960
Chlorothiazide, 963
Chlorothiazide compounded, 963
Cholestyramine for, 990
Ciprofloxacin for, 1019
Cisapride compounded, veterinary, 1025
Clarithromycin for, 1049
Clavulanate potassium and amoxicillin for, 308
Clomipramine compounded, veterinary, 1091
Clonazepam, 1097
Clonidine hydrochloride compounded, 1103
Clopidogrel compounded, 1111
Colestipol hydrochloride for, 1147
Colistin sulfate for, 1150
Cyclophosphamide compounded, 1208
Dapsone, 1240
Diazoxide, 1335
Didanosine tablets for, 1367
Diltiazem hydrochloride, 1406
Dipyridamole, 1445
Dolasetron mesylate, 1485
Doxycycline for, 1525
Doxycycline calcium, 1529
Doxycycline compounded, veterinary, 1530
Enalapril maleate, 1613
Enalapril maleate compounded, veterinary, 1613
Enrofloxacin compounded, veterinary, 1630
Erythromycin estolate, 1693
Erythromycin estolate for, 1694
Erythromycin estolate and sulfisoxazole acetyl, 1695
Erythromycin ethylsuccinate, 1698
Erythromycin ethylsuccinate for, 1699
Erythromycin ethylsuccinate and sulfisoxazole acetyl for, 1701
Ethambutol hydrochloride compounded, 1763
Famciclovir compounded, 1812
Famotidine for, 1818
Felbamate, 1822
Ferumoxsil, 1868
Flecainide acetate, 1897
Fluconazole for, 1912
Flucytosine, 1917
Furazolidone, 2052
Ganciclovir, 2090
Granisetron hydrochloride, 2161
Griseofulvin, 2166
Ibuprofen, 2283
Indomethacin, 2332
Isradipine, 2465
Ketoconazole, 2507
Labetalol hydrochloride, 2519
Lamotrigine compounded, 2545
Lamotrigine tablets, 2542
Lansoprazole compounded, 2556
Leflunomide compounded, 2560
Lisinopril, 2644
Magaldrate and simethicone, 2706
Magnesium carbonate and sodium bicarbonate for, 2712
Marbofloxacin compounded, veterinary, 2741
Mebendazole, 2744
Megestrol acetate, 2763
Meloxicam, 2770
Methacycline hydrochloride, 2835
Methadone hydrochloride tablets for, 2839
Metolazone, 2913
Metoprolol tartrate, 2926
Metronidazole benzoate compounded, 2933
Minocycline hydrochloride, 2966
Mirtazapine compounded, veterinary, 2977
Mycophenolate mofetil for, 3047
Naproxen, 3079
Naproxen compounded, 3078
Naratriptan hydrochloride, 3094
Nevirapine, 3132
Nitrofurantoin, 3179
Nystatin, 3221
Nystatin for, 3221
Ondansetron hydrochloride, 3268
Oxcarbazepine, 3317
Oxfendazole, 3322
Penicillin G benzathine, 3433
Penicillin V for, 3452
Penicillin V benzathine, 3454
Pentoxifylline, 3470
Pergolide, veterinary, 3478
Phenobarbital, 3498
Phenoxybenzamine hydrochloride compounded, 3504
Phenytoin, 3526
Phytonadione compounded, 3538
Piroxicam compounded, 3579
Prazosin hydrochloride compounded, 3655
Prednisolone compounded, veterinary, 3664
Primidone, 3684
Propylthiouracil, 3755
Psyllium hydrophilic mucilloid for, 3771
Pyrantel pamoate, 3774
Pyrazinamide, 3776
Pyrimethamine, 3787
Pyrvinium pamoate, 3789
Quinidine sulfate, 3814
Rifabutin, 3874
Rifampin, 3877
Sildenafil citrate, 4040
Simethicone, 4045
Sodium phenylbutyrate, 4086
Sotalol hydrochloride, 4104
Spironolactone, 4111
Spironolactone and hydrochlorothiazide, 4113
Spironolactone compounded, 4111
Sulfadimethoxine, 4153
Sulfamethizole, 4158
Sulfamethoxazole, 4161
Sulfamethoxazole and trimethoprim, 4163
Sulfisoxazole acetyl, 4175
Sumatriptan succinate, 4188
Tacrolimus, 4202
Tadalafil compounded, 4207
Temozolomide, 4257
Terbinafine, 4267
Terbutaline, 4273
Tetracycline, 4302
Tetracycline hydrochloride, 4310
Theophylline, 4327
Thiabendazole, 4335
Tiagabine hydrochloride, 4367
Topiramate compounded, 4436
Tramadol hydrochloride, 4443
Tramadol hydrochloride and acetaminophen, 4452
Tramadol hydrochloride compounded, veterinary, 4443
Trifluprazine, 4514
Trisulfapyrimidines, 4536
Ursodiol, 4559
Valacyclovir, 4561
Vehicle for, 5913
Verapamil hydrochloride, 4609
Zonisamide compounded, 4721

Orange
G, 6184
oil, 5914
peel tincture, sweet, 5915

www.webofpharma.com

Orange *(continued)*
spirit, compound, 5914
syrup, 5915
Orbifloxacin, 3275
tablets, 3276
Orcinol, 6185
Ordinary impurities ⟨466⟩, 6710
Organic
nitrogenous bases—identification ⟨181⟩, 6587
nitrogenous bases, salts of ⟨501⟩, 6741
Orlistat, 3277
capsules, 3280
Orphenadrine citrate, 3281
aspirin and caffeine tablets, 3288
injection, 3284
extended-release tablets, 3285
Orthophenanthroline, 6185
TS, 6232
Oseltamivir phosphate, 3291
capsules, 3293
Osmium tetroxide, 6185
Osmolality and osmolarity ⟨785⟩, 7008
Otic solution
acetic acid, 78
antipyrine and benzocaine, 352
antipyrine, benzocaine, and phenylephrine hydrochloride, 354
benzocaine, 510
chloramphenicol, 931
gentamicin sulfate and betamethasone valerate, 2104
hydrocortisone and acetic acid, 2235
neomycin and polymyxin B sulfates and hydrocortisone, 3124
polymyxin B sulfate and hydrocortisone, 3592
Otic suspension
Ciprofloxacin and dexamethasone, 1021
Oxacillin
injection, 3296
for injection, 3297
sodium, 3294
Oxalic acid, 6185
tenth-normal (0.1 N), 6244
TS, 6232
Oxaliplatin, 3297
injection, 3301
for injection, 3303
Oxandrolone, 3306
tablets, 3308
Oxaprozin, 3310
tablets, 3311
Oxazepam, 3312
capsules, 3314
Oxcarbazepine, 3315
oral suspension, 3317
tablets, 3319
Oxfendazole, 3322
oral suspension, 3322
Oxidized cellulose, 893
regenerated, 894
Oxprenolol hydrochloride, 3323
extended-release tablets, 3324
Oxtriphylline, 3324
extended-release tablets, 3325
Oxybenzone, 3326
and dioxybenzone cream, 1418
Oxybutynin chloride, 3326
oral solution, 3328
tablets, 3328
tablets, extended-release, 3329
Oxycodone
and acetaminophen capsules, 3341
and acetaminophen tablets, 3343
and aspirin tablets, 3344
terephthalate, 3345
Oxycodone hydrochloride, 3335
oral solution, 3337
tablets, 3338
extended-release tablets, 3339
3,3′-Oxydipropionitrile, 6185
Oxygen, 3347
21 percent certified standard, 6185
93 percent, 3347
93 percent certified standard, 6185
certified standard, 6185
flask combustion ⟨471⟩, 6735
helium certified standard, 6185
Oxymetazoline hydrochloride, 3348
nasal solution, 3349
ophthalmic solution, 3350
Oxymetholone, 3350
tablets, 3351
Oxymorphone hydrochloride, 3352
injection, 3353
tablets, 3354
extended-release tablets, 3356
Oxyquinoline sulfate, 5915
Oxytetracycline, 3359
for injection, 3362
hydrochloride, 3361
hydrochloride capsules, 3362
hydrochloride and hydrocortisone acetate ophthalmic suspension, 3363
hydrochloride and hydrocortisone ointment, 3364
hydrochloride and polymyxin B sulfate ointment, 3364
hydrochloride and polymyxin B sulfate ophthalmic ointment, 3365
hydrochloride and polymyxin B sulfate topical powder, 3365
hydrochloride and polymyxin B sulfate vaginal inserts, 3366
hydrochloride soluble powder, 3363
injection, 3360
tablets, 3361
Oxytocin, 3366
injection, 3367

P

Package integrity and test method selection ⟨1207.1⟩, 8086
Package integrity leak test technologies ⟨1207.2⟩, 8097
Package seal quality test technologies ⟨1207.3⟩, 8111
Packaging and repackaging—single unit containers ⟨1136⟩, 7919
Packaging and storage requirements ⟨659⟩, 6876
Paclitaxel, 3367
injection, 3370
Padimate O, 3371
lotion, 3372
Paired ion chromatography reagent, 6185
Paliperidone, 3373
Palladium
catalyst, 6185
chloride, 6185
chloride TS, buffered, 6232
Palladous chloride, 6185
Pallida
echinacea, 4944
extract, powdered echinacea, 4948
powdered echinacea, 4946
Palm
oil, 5916
oil, hydrogenated, 5916
kernel oil, 5917
Palmitic acid, 5918
Palonosetron
hydrochloride, 3374
Pamabrom, 3376
Pamidronate disodium, 3377
for injection, 3379
Pancreatic digest of casein, 6185, 6216
Pancreatin, 3379, 6185
tablets, 3381
Pancreatin ⟨1025⟩, 7272
Pancrelipase, 3382
capsules, 3384
delayed-release capsules, 3384
tablets, 3385
Pancuronium bromide, 3385
injection, 3386
Panthenol, 3388
Pantoprazole sodium, 3388
delayed-release tablets, 3391
Papaic digest of soybean meal, 6185
Papain, 3396
tablets for topical solution, 3396
Papaverine hydrochloride, 3397
injection, 3398
tablets, 3398
Paper
lead acetate, 6175
Para-aminobenzoic acid, 6135, 6185
Parachlorophenol, 3399
camphorated, 3399
Paraffin, 5920
synthetic, 5921
Paraformaldehyde, 6186
Paraldehyde, 3400
Paregoric, 3401
Paricalcitol, 3402
capsules, 3404
injection, 3406
Paromomycin
oral solution, 3409
sulfate, 3408
sulfate capsules, 3408
Paroxetine
hydrochloride, 3409
tablets, 3412
extended-release tablets, 3413
Partially-neutralized methacrylic acid and ethyl acrylate copolymer, 5881
Particle size distribution estimation by analytical sieving ⟨786⟩, 7010
Particulate matter in injections ⟨788⟩, 7017
Particulate matter in ophthalmic solutions ⟨789⟩, 7020
Peanut oil, 5921
Pea starch, 6053
Pectate lyase, 6186
Pectin, 3418
Pemetrexed
disodium, 3420
for injection, 3422
Penbutolol sulfate, 3424
tablets, 3425
Penicillamine, 3426
capsules, 3427
tablets, 3429
Penicillin
G benzathine, 3432
G benzathine injectable suspension, 3433

www.webofpharma.com

Penicillin *(continued)*
G benzathine and penicillin G procaine injectable suspension, 3434
G benzathine oral suspension, 3433
G benzathine tablets, 3434
G, neomycin, polymyxin B, hydrocortisone acetate, and hydrocortisone sodium succinate topical suspension, 3430
G potassium, 3436
G potassium injection, 3437
G potassium for injection, 3437
G potassium for oral solution, 3439
G potassium tablets, 3439
G procaine, 3441
G procaine, dihydrostreptomycin sulfate, chlorpheniramine maleate, and dexamethasone injectable suspension, 3445
G procaine and dihydrostreptomycin sulfate injectable suspension, 3444
G procaine and dihydrostreptomycin sulfate intramammary infusion, 3444
G procaine, dihydrostreptomycin sulfate, and prednisolone injectable suspension, 3447
G procaine injectable suspension, 3443
G procaine for injectable suspension, 3443
G procaine intramammary infusion, 3442
G procaine, neomycin and polymyxin B sulfates, and hydrocortisone acetate topical suspension, 3448
G procaine and novobiocin sodium intramammary infusion, 3448
G procaine and penicillin G benzathine injectable suspension, 3434
G sodium, 3449
G sodium for injection, 3450
V, 3451
V benzathine, 3453
V benzathine oral suspension, 3454
V potassium, 3454
V potassium for oral solution, 3456
V potassium tablets, 3456
V for oral suspension, 3452
V tablets, 3453
Penicillinase, 6186
Pentadecane, 6186
1-Pentadecanol, 6186
Pentafluoropropionic acid, 6186
Pentamidine isethionate, 3457
Pentane, 6186
1-Pentanesulfonic acid sodium salt, 6186
2-Pentanone, 6186
Pentazocine, 3458
and acetaminophen tablets, 3460
and aspirin tablets, 3461
hydrochloride, 3459
injection, 3465
and naloxone tablets, 3463
Pentetic acid, 3466
Pentobarbital, 3467
sodium, 3468
sodium injection, 3469
Pentoxifylline, 3469
oral suspension, 3470
extended-release tablets, 3471
People, ix
Peppermint, 5922
oil, 5923
spirit, 3474
water, 5923
Pepsin, 6186
purified, 6188
Peptic digest of animal tissue, 6188
Peptone, dried, 6163, 6188
Perchloric acid, 6189
tenth-normal (0.1 N) in dioxane, 6244
tenth-normal (0.1 N) in glacial acetic acid, 6244
TS, 6233
Perflubron, 3474
Perflutren protein-type A microspheres injectable suspension, 3475
Pergolide
mesylate, 3477
oral suspension veterinary, 3478
tablets, 3478
Perindopril
erbumine, 3480
erbumine tablets, 3483
Periodic acid, 6189
Periodontal system
minocycline, 2970
Peroxidase, 6189
Perphenazine, 3485
and amitriptyline hydrochloride tablets, 3487
tablets, 3486
Pertussis
immune globulin, 3488
Petrolatum, 3488
hydrophilic, 3489
white, 3489
Petroleum benzin, 6189
Petroleum ether, 6189
pH ⟨791⟩, 7022
Pharmaceutical calculations in pharmacy practice ⟨1160⟩, 7953
Pharmaceutical compounding
nonsterile preparations ⟨795⟩, 7025
sterile preparations ⟨797⟩, 7037
Pharmaceutical dosage forms ⟨1151⟩, 7929
Phellandrene
(R)-(–)-alpha, 6189
Phenacetin, 6189
1,10-Phenanthroline, 6185, 6189
o-Phenanthroline monohydrochloride monohydrate, 6189
Phenazopyridine hydrochloride, 3490
tablets, 3491
Phendimetrazine tartrate, 3492
capsules, 3493
tablets, 3494
Phenelzine sulfate, 3495
tablets, 3496
Pheniramine maleate, 3497
and naphazoline hydrochloride ophthalmic solution, 3076
Phenobarbital, 3497
sodium, 3499
sodium injection, 3500
sodium for injection, 3500
oral solution, 3498
oral suspension, 3498
tablets, 3499
theophylline and ephedrine hydrochloride tablets, 4330
Phenol, 3501, 6189
alcohol TS, 6225
topical gel, camphorated, 3501
iron, TS, 6230
liquefied, 3502
red, 6189, 6221
red, sodium, 6189
red TS, 6233
red TS, pH 4.7, 6233
camphorated, topical solution, 3502
TS, 6233
Phenolated
calamine topical suspension, 665
Phenoldisulfonic acid TS, 6233
Phenolphthalein, 6221
paper, 6222
Phenolphthalein TS, 6233
Phenolsulfonphthalein, 5924, 6189
Phenoxybenzamine hydrochloride, 3503, 6189
capsules, 3503
Phenoxybenzamine hydrochloride compounded
oral suspension, 3504
3-Phenoxybenzoic acid, 6189
2-Phenoxyethanol, 6189
Phenoxyethanol, 5925
Phentermine hydrochloride, 3505
capsules, 3506
tablets, 3507
Phentolamine mesylate, 3508
for injection, 3509
Phenyl
ether, 6189
isocyanate, 6189
2-Phenylacetamide, 6189
Phenylacetic acid, 6189
Phenylalanine, 3509, 6189
dl-Phenylalanine, 6189
Phenylbutazone, 3510
boluses, 3511
injection, 3511
tablets, 3512
p-Phenylenediamine
dihydrochloride, 6189
hydrochloride, 6189
o-Phenylenediamine dihydrochloride, 6189
Phenylephrine
bitartrate, 3513
diphenhydramine, hydrochloride tablets, 1433
hydrochloride, 3515
hydrochloride, antipyrine, and benzocaine otic solution, 354
hydrochloride and promethazine and codeine phosphate oral solution, 3724
hydrochloride and promethazine oral solution, 3721
hydrochloride injection, 3517
hydrochloride nasal jelly, 3518
hydrochloride nasal solution, 3518
hydrochloride ophthalmic solution, 3519
hydrochloride tablets, 3520
Phenylethyl alcohol, 3522
Phenylglycine, 6190
Phenylhydrazine, 6190
acetate TS, 6233
hydrochloride, 6190
sulfuric acid TS, 6233
Phenylmercuric
acetate, 5926
nitrate, 5926
Phenylmethylsulfonyl fluoride, 6190
3-Phenylphenol, 6190
Phenylpropanolamine hydrochloride, 3522
Phenyltoloxamine citrate, 3523
Phenytoin, 3524
chewable tablets, 3528
sodium, 3529
sodium capsules, extended, 3531
sodium injection, 3534
oral suspension, 3526
Phenytoin compounded topical gel, 3525
pH indicator paper, short-range, 6222
Phloroglucinol, 6190
TS, 6233

www.webofpharma.com

Phloxine B, 6190
Phosphatase enzyme, alkaline, 6134, 6190
Phosphate
  acidulated, and sodium fluoride topical solution, 4074
  buffer, 6145
  diethylamine, 6158
  in reagents, 6131
Phosphatic enzyme, 6190
  TS, 6233
Phosphomolybdic acid, 6190
  TS, 6233
Phosphoric acid, 5927, 6190
  diluted, 5927
  and sodium fluoride gel, 4075
  0.01% TS, 6233
  0.05 M TS, 6233
  0.06 M TS, 6233
  10 TS, 6233
  1 N TS, 6233
  20% TS, 6233
  0.01 M TS, 6233
18 N Phosphoric acid
  TS, 6233
1 M Phosphoric acid
  TS, 6233
0.75 M Phosphoric acid TS, 6233
0.02 M Phosphoric acid TS, 6233
1.5 M Phosphoric acid TS, 6233
Phosphorous acid, 6190
Phosphorus
  pentoxide, 6190
  red, 6190, 6197
Phosphotungstic acid, 6190
  TS, 6233
o-Phthalaldehyde, 6190
Phthalazine, 6191
Phthalic
  acid, 6191
  anhydride, 6191
Phthalimide, 6191
Phyllanthus amarus, 5191
  powdered, 5193
Physical environments that promote safe medication use ⟨1066⟩, 7590
Physicochemical analytical procedures for insulins ⟨121.1⟩, 6550
Physicochemical integrators and indicators for sterilization ⟨1229.9⟩, 8227
Physostigmine
  salicylate, 3535
  salicylate injection, 3535
  salicylate ophthalmic solution, 3536
Phytonadione, 3537
  injectable emulsion, 3538
  tablets, 3539
Phytonadione compounded oral suspension, 3538
2-Picoline, 6191
Picrate TS, alkaline, 6226, 6233
Picric acid, 6191, 6215
  TS, 6233
Picrolonic acid, 6191
Pilocarpine, 3539
  hydrochloride, 3542
  hydrochloride ophthalmic solution, 3543
  hydrochloride tablets, 3543
  nitrate, 3545
  nitrate ophthalmic solution, 3545
  ocular system, 3541
Pimobendan, 3546
Pimozide, 3547
  tablets, 3548
Pindolol, 3549
  tablets, 3550
Pinene
  (+)-alpha, 6191
  beta, 6191
Pioglitazone
  and glimepiride tablets, 3554
  hydrochloride, 3551
  and metformin hydrochloride tablets, 3558
  tablets, 3553
Pipemidic acid, 6191
Piperacillin, 3562
  for injection, 3565
  sodium, 3564
  and tazobactam for injection, 3567
Piperazine, 3573, 6191
  adipate, 3573
  citrate, 3574
  citrate syrup, 3575
  citrate tablets, 3575
  dihydrochloride, 3575
  phosphate, 3576
Piperidine, 6191
Piroxicam, 3577
  capsules, 3578
  cream, 3579
Piroxicam compounded oral suspension, 3579
Plantago seed, 3580
Plant Stanol Esters, 5194
Plasma protein fraction, 3581
Plasma spectrochemistry ⟨730⟩, 6967
Plasma spectrochemistry—theory and practice ⟨1730⟩, 8484
Plastic materials of construction ⟨661.1⟩, 6893
Plastic packaging systems and their materials of construction ⟨661⟩, 6887
Plastic packaging systems for pharmaceutical use ⟨661.2⟩, 6912
Platinic
  chloride, 6191
  chloride TS, 6233
Platinum
  cobalt TS, 6233
Podophyllum, 3581
  resin, 3582
  resin topical solution, 3582
Polacrilin potassium, 5928
Polarography ⟨801⟩, 7089
Policies, USP, xxviii
Poloxalene, 3582
Poloxamer, 5929
Poly(dimethylsiloxane-co-methylphenylsiloxane), 6191
Polycarbophil, 3583
  calcium, 713
Polydecene
  hydrogenated, 5932
Polydextrose, 5933
  hydrogenated, 5936
Polydimethylsiloxane, viscosity 0.65 centistokes, 6191
Polyethylene
  glycol, 5939
  glycol 200, 6191
  glycol 600, 6191
  glycol 20,000, 6191
  glycol 3350 and electrolytes for oral solution, 3587
  glycol monomethyl ether, 5942
  glycol ointment, 5941
  oxide, 5944
Polyethylene glycol 3350, 3584
Polyethylene glycol 400, 6191
Polyethylene glycol 4000, 6191
Polyethylene glycol standards with molecular weights of 1000, 2000, 3000, 4000, and 6000 daltons (g/mol), 6192
Polyglyceryl
  3 diisostearate, 5948
  dioleate, 5946
Polyisobutylene, 5949
Polymyxin B
  for injection, 3590
  and neomycin sulfates, bacitracin, and hydrocortisone acetate ointment, 3116
  and neomycin sulfates, bacitracin, and hydrocortisone acetate ophthalmic ointment, 3116
  and neomycin sulfates, bacitracin, and lidocaine ointment, 3117
  and neomycin sulfates and bacitracin ointment, 3115
  and neomycin sulfates and bacitracin ophthalmic ointment, 3115
  and neomycin sulfates, bacitracin zinc, and hydrocortisone acetate ophthalmic ointment, 3120
  and neomycin sulfates, bacitracin zinc, and hydrocortisone ointment, 3118
  and neomycin sulfates, bacitracin zinc, and hydrocortisone ophthalmic ointment, 3119
  and neomycin sulfates, bacitracin zinc, and lidocaine ointment, 3120
  and neomycin sulfates and bacitracin zinc ointment, 3118
  and neomycin sulfates and bacitracin zinc ophthalmic ointment, 3118
  and neomycin sulfates cream, 3114
  and neomycin sulfates and dexamethasone ophthalmic ointment, 3121
  and neomycin sulfates and dexamethasone ophthalmic suspension, 3122
  and neomycin sulfates and gramidicin cream, 3122
  and neomycin sulfates, gramidicin, and hydrocortisone acetate cream, 3123
  and neomycin sulfates and gramidicin ophthalmic solution, 3123
  and neomycin sulfates and hydrocortisone acetate cream, 3125
  and neomycin sulfates and hydrocortisone acetate ophthalmic suspension, 3125
  and neomycin sulfates and hydrocortisone ophthalmic suspension, 3123
  and neomycin sulfates and hydrocortisone otic solution, 3124
  and neomycin sulfates and hydrocortisone otic suspension, 3124
  and neomycin sulfates and lidocaine cream, 3126
  and neomycin sulfates ophthalmic ointment, 3114
  and neomycin sulfates ophthalmic solution, 3115
  and neomycin sulfates, penicillin G procaine, and hydrocortisone acetate topical suspension, 3448
  and neomycin sulfates and pramoxine hydrochloride cream, 3126
  and neomycin sulfates and prednisolone acetate ophthalmic suspension, 3127
  and neomycin sulfates solution for irrigation, 3114
  penicillin G, neomycin, hydrocortisone acetate, and hydrocortisone sodium succinate topical suspension, 3430
  sulfate, 3588
  sulfate and bacitracin topical aerosol, 468

www.webofpharma.com

Polymyxin B *(continued)*
sulfate and bacitracin zinc topical aerosol, 3591
sulfate and bacitracin zinc ointment, 471
sulfate and bacitracin zinc ophthalmic ointment, 471
sulfate and bacitracin zinc topical powder, 3591
sulfate and chloramphenicol ophthalmic ointment, 933
sulfate and hydrocortisone otic solution, 3592
sulfate and oxytetracycline hydrochloride ointment, 3364
sulfate and oxytetracycline hydrochloride ophthalmic ointment, 3365
sulfate and oxytetracycline hydrochloride topical powder, 3365
sulfate and oxytetracycline hydrochloride vaginal inserts, 3366
sulfate and trimethoprim ophthalmic solution, 3592
Polyoxyethylene 10 lauryl ether, 6192
Polyoxyethylene (20) sorbitan monolaurate, 6192
Polyoxyethylene (23) lauryl ether, 6192
Polyoxyl
10 oleyl ether, 5950
15 hydroxystearate, 5951
20 cetostearyl ether, 5955
35 castor oil, 5956
40 hydrogenated castor oil, 5958
lauryl ether, 5960
oleate, 5961
stearate, 5961
stearyl ether, 5962
Polypeptone, 6192
Polypropylene glycol 11 stearyl ether, 5963
Polysaccharide molecular weight standards, 6192
Polysorbate
20, 5965
40, 5966
60, 5966
80, 5967
Polysorbate 20, 6192
Polysorbate 80, 6192
Polystyrene
cation-exchange resin, 6192
Polytef, 6192
Polyvinyl
acetate, 5969
acetate dispersion, 5972
acetate phthalate, 5974
alcohol, 3593, 6192
alcohol and ethylene glycol graft copolymer, 5769
Porosimetry by mercury intrusion ⟨267⟩, 6653
Porosity by nitrogen adsorption–desorption ⟨268⟩, 6655
Positron emission tomography drugs for compounding, investigational, and research uses ⟨823⟩, 7100
Positron emission tomography drugs—information ⟨1823⟩ , 8617
Potash, sulfurated, 3594
Potassium
acetate, 3595, 6192
acetate injection, 3595
acetate TS, 6233
alginate, 5975
alum, 166, 6192
arsenate monobasic, 6192
arsenite, tenth-normal (0.1 N), 6245
benzoate, 5976
bicarbonate, 3596, 6192
bicarbonate effervescent tablets for oral solution, 3596
bicarbonate and potassium chloride for effervescent oral solution, 3597
bicarbonate and potassium chloride effervescent tablets for oral solution, 3597
bicarbonate, potassium chloride, and potassium citrate effervescent tablets for oral solution, 3609
biphosphate, 6192
biphthalate, 6192
bismuth iodide TS, 6233
bisulfate, 6192
bitartrate, 3599
bromate, 6192
bromate, tenth-normal (0.1 N), 6245
bromide, 3600, 6192
bromide-bromate, tenth-normal (0.1 N), 6245
bromide oral solution, veterinary, 3600
carbonate, 3601, 6193
carbonate, anhydrous, 6138, 6193
carbonate TS, 6233
chlorate, 6193
chloride, 3601, 6193
chloride extended-release capsules, 3602
chloride in dextrose injection, 3607
chloride in dextrose and sodium chloride injection, 3608
chloride for injection concentrate, 3604
chloride in lactated ringer's and dextrose injection, 3610
chloride, potassium bicarbonate, and potassium citrate effervescent tablets for oral solution, 3609
chloride and potassium bicarbonate for effervescent oral solution, 3597
chloride and potassium bicarbonate effervescent tablets for oral solution, 3597
chloride and potassium gluconate oral solution, 3621
chloride and potassium gluconate for oral solution, 3621
chloride in sodium chloride injection, 3612
chloride oral solution, 3604
chloride for oral solution, 3605
chloride extended-release tablets, 3605
chloroplatinate, 6193
chromate, 6193
chromate TS, 6234
citrate, 3613
citrate and citric acid oral solution, 3617
citrate, magnesium carbonate, and citric acid for oral solution, 2711
citrate, potassium chloride, and potassium bicarbonate effervescent tablets for oral solution, 3609
citrate, potassium gluconate, and ammonium chloride oral solution, 3622
citrate and potassium gluconate oral solution, 3622
citrate tablets, 5196
citrate extended-release tablets, 3614
cyanide, 6193
dichromate, 6193
dichromate, tenth-normal (0.1 N), 6245
dichromate TS, 6234
ferricyanide, 6193
ferricyanide TS, 6234
ferricyanide, twentieth-molar (0.05 M), 6245
ferrocyanide, 6193
ferrocyanide TS, 6234
gluconate, 3618
gluconate and potassium chloride oral solution, 3621
gluconate and potassium chloride for oral solution, 3621
gluconate, potassium citrate, and ammonium chloride oral solution, 3622
gluconate and potassium citrate oral solution, 3622
gluconate oral solution, 3619
gluconate tablets, 3620
guaiacolsulfonate, 3623
hyaluronate, 6193
hydrogen sulfate, 6193
hydroxide, 5977, 6193
hydroxide, alcoholic, half-normal (0.5 N), 6234, 6246
hydroxide, alcoholic, tenth-molar (0.1 M), 6246
hydroxide, methanolic, tenth-normal (0.1 N), 6246
hydroxide, normal (1 N), 6246
hydroxide TS, 6234
hydroxide 2 N TS, 6234
hydroxide TS, alcoholic, 6234
hydroxide TS 2, alcoholic, 6234
iodate, 6193
iodate, twentieth-molar (0.05 M), 6246
iodide, 3624, 6193
iodide and iodine TS 1, 6230
iodide and iodine TS 2, 6230
iodide and iodine TS 3, 6230
iodide oral solution, 3624
iodide and starch TS, 6234
iodide tablets, 3624
iodide delayed-release tablets, 3625
iodide TS, 6234
iodide 20% TS, 6234
iodoplatinate TS, 6234
metabisulfite, 5978, 6193
metaphosphate, 5978
nitrate, 3625, 6193
nitrate solution, 3626
nitrite, 6193
perchlorate, 3627, 6193
periodate, 6193
permanganate, 3627, 6193
permanganate, tenth-normal (0.1 N), 6234, 6246
permanganate TS, 6234
persulfate, 6193
phosphate, dibasic, 3628, 6156, 6193
phosphate, dibasic, trihydrate, 6193
phosphate, monobasic, 5979, 6181, 6192, 6193
phosphate, tribasic, 6194
phosphates injection, 3629
pyroantimonate, 6194
pyroantimonate TS, 6234
pyrophosphate, 6194
pyrosulfate, 6194
and sodium bicarbonates and citric acid effervescent tablets for oral solution, 3598
sodium tartrate, 3630, 6194
sorbate, 5980
sulfate, 6194
sulfate TS, 6234
tellurite, 6194
thiocyanate, 6194
thiocyanate, tenth-normal (0.1 N), 6247
thiocyanate TS, 6234
0.025 N Potassium dichromate VS, 6245

www.webofpharma.com

Potassium hydroxide
1.8 N TS, 6234
45% TS, 6234
10 M TS, 6234
0.1 N VS, 6246
Potassium phosphate
0.02 M TS, 6234
0.2 M TS, 6234
Potassium phosphates
compounded injection, 3629
Potato starch, 6058, 6194
Povidone, 3631
Povidone–iodine, 3634
topical aerosol, 3635
cleansing solution, 3635
ointment, 3635
topical solution, 3636

Powder

Absorbable dusting, 1569
Ampicillin soluble, 323
Amprolium soluble, 330
Astragalus root, 4785
Bacitracin methylene disalicylate soluble, 467
Bacitracin zinc soluble, 470
Banaba leaf, 4803
Chinese skullcap root, 4885
Chlortetracycline and sulfamethazine bisulfates soluble, 980
Chlortetracycline hydrochloride soluble, 987
Cinnamomum cassia twig, 4904
Compound clioquinol topical, 1076
Coix seed, 4917
Cromolyn sodium inhalation, 1182
Echinacea species, 4966
Eleuthero root and rhizome, capsules, 4976
Fenugreek seed, 4984
Fluticasone propionate and salmeterol, inhalation, 2010
Fluticasone propionate inhalation, 1993
Ganoderma lucidum fruiting body, 5008
Iron, 6173
Japanese honeysuckle flower, 5097
Levothyroxine sodium oral, 2618
Lincomycin hydrochloride soluble, 2634
Miconazole nitrate topical, 2949
Neomycin sulfate, isoflupredone acetate, and tetracaine hydrochloride topical, 3112
Northern schisandra fruit, 5265
Nystatin topical, 3220
Olive leaf, 5184
Oral, containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 54
Oxytetracycline hydrochloride and polymyxin B sulfate topical, 3365
Oxytetracycline hydrochloride soluble, 3363
Polymyxin B sulfate and bacitracin zinc topical, 3591
Rhodiola crenulata root and rhizome, 5216
St. John's wort flowering top, 5238
Salix species bark, 5249
Salmeterol inhalation, 3973
Sodium bicarbonate oral, 4061
Soy isoflavones, powdered extract, 5274
Sulfadimethoxine soluble, 4153
Tangerine peel, 5296
Tetracycline hydrochloride soluble, 4309
Tienchi ginseng root and rhizome, 5301
Tolnaftate topical, 4426
Valerian root, capsules, 5333
Vitamins with minerals oral, 5338
Zinc oxide, 4690

Powdered

American ginseng, 4752
American ginseng extract, 4753
andrographis, 4760
andrographis extract, 4762
ashwagandha root, 4771
ashwagandha root extract, 4768
Asian ginseng, 4775
Asian ginseng extract, 4776
bilberry extract, 4813
black cohosh, 4817
black cohosh extract, 4820
black pepper, 4827
black pepper extract, 4829
cat's claw, 4854
cat's claw extract, 4855
cellulose, 5700
Chinese salvia, 4880
digitalis, 1379
Echinacea angustifolia, 4938
Echinacea angustifolia extract, 4941
Echinacea pallida, 4946
Echinacea pallida extract, 4948
Echinacea purpurea, 4955
Echinacea purpurea extract, 4958
eleuthero, 4975
eleuthero extract, 4970
fenugreek seed, extract, 4987
feverfew, 4990
garlic, 5020
garlic extract, 5022
ginger, 5028
ginkgo extract, 5038
goldenseal, 5063
goldenseal extract, 5064
green tea extract, decaffeinated, 5067
gymnema, 5075
hawthorn leaf with flower, 5082
holy basil leaf, 5087
holy basil leaf extract, 5089
horse chestnut, 4875
horse chestnut extract, 4876
ipecac, 2396
licorice, 5120
licorice extract, 5121
malabar-nut-tree, leaf, 5137
milk thistle, 5159
milk thistle extract, 5160
opium, 3274
Phyllanthus amarus, 5193
rauwolfia serpentina, 3850
Rhodiola rosea, 5220
Rhodiola rosea extract, 5221
rosemary, 5232
saw palmetto, 5254
stinging nettle, 5288
stinging nettle extract, 5290
turmeric, 5316
turmeric extract, 5317
valerian, 5326
valerian extract, 5327
zinc chloride, anhydrous, 6219

Powder fineness ⟨811⟩, 7093
Powder flow ⟨1174⟩, 7993
Pralidoxime
chloride, 3636
chloride for injection, 3637
Pramipexole dihydrochloride, 3639
Pramoxine
hydrochloride, 3640
hydrochloride cream, 3641
hydrochloride jelly, 3642
hydrochloride and neomycin and polymyxin B sulfates cream, 3126
Prasugrel hydrochloride, 3642
Pravastatin sodium, 3645
tablets, 3647
Praziquantel, 3649
tablets, 3650
Prazosin hydrochloride, 3651
capsules, 3653
Prazosin hydrochloride compounded oral suspension, 3655
Prednicarbate, 3655
cream, 3656
ointment, 3658
Prednisolone, 3659
acetate, 3662
acetate and gentamicin ophthalmic ointment, 2106
acetate and gentamicin ophthalmic suspension, 2107
acetate injectable suspension, 3663
acetate and neomycin and polymyxin B sulfates ophthalmic suspension, 3127
acetate and neomycin sulfate ophthalmic suspension, 3128
acetate ophthalmic suspension, 3663
acetate and sulfacetamide sodium ophthalmic ointment, 4144
acetate and sulfacetamide sodium ophthalmic suspension, 4145
cream, 3660
hemisuccinate, 3665
penicillin G procaine, and dihydrostreptomycin sulfate injectable suspension, 3447
sodium phosphate, 3665
sodium phosphate injection, 3667
sodium phosphate ophthalmic solution, 3668
sodium succinate for injection, 3668
oral solution, 3661
tablets, 3661
tebutate, 3669
tebutate injectable suspension, 3670
tetracycline hydrochloride and novobiocin sodium tablets, 4312
Prednisolone compounded oral suspension, veterinary, 3664
Prednisolone sodium phosphate compounded
oral solution, 3667
Prednisone, 3670
injectable suspension, 3672
oral solution, 3671
tablets, 3672
Preface
and mission, v
Pregabalin, 3674
Pregnenolone acetate, 6194
Prekallikrein activator ⟨165⟩, 6583
Preparation of biological specimens for histologic and immunohistochemical analysis ⟨1285⟩, 8370

www.webofpharma.com

Prescription balances and volumetric apparatus ⟨1176⟩, 7997
Prescription container labeling ⟨17⟩, 6445
Prilocaine, 3675
and epinephrine injection, 3679
hydrochloride, 3676
hydrochloride injection, 3678
and lidocaine cream, 2630
Primaquine phosphate, 3680
tablets, 3681
Primidone, 3683
oral suspension, 3684
tablets, 3685
Prion inactivation ⟨1229.16⟩, 8239
Probenecid, 3686
and ampicillin for oral suspension, 326
and colchicine tablets, 3687
tablets, 3687
Procainamide hydrochloride, 3688
capsules, 3689
injection, 3690
tablets, 3690
extended-release tablets, 3691
Procaine
hydrochloride, 3693
hydrochloride and epinephrine injection, 3694
hydrochloride injection, 3694
and propoxycaine hydrochlorides and levonordefrin injection, 3744
and propoxycaine hydrochlorides and norepinephrine bitartrate injection, 3745
and tetracaine hydrochlorides and levonordefrin injection, 3695
Procarbazine hydrochloride, 3696
capsules, 3696
Prochlorperazine, 3697
edisylate, 3699
edisylate injection, 3699
maleate, 3700
maleate tablets, 3701
oral solution, 3697
suppositories, 3698
Procyclidine hydrochloride, 3703
tablets, 3704
Products for nebulization—characterization tests ⟨1601⟩, 8407
Progesterone, 3704
injectable suspension, 3706
injection, 3705
intrauterine contraceptive system, 3705
vaginal suppositories, 3707
Proguanil hydrochloride, 3708
Proline, 3711
Promazine hydrochloride, 3712
injection, 3712
oral solution, 3713
syrup, 3713
tablets, 3714
Promethazine
and phenylephrine hydrochloride and codeine phosphate oral solution, 3724
and phenylephrine hydrochloride oral solution, 3721
Promethazine hydrochloride, 3714
injection, 3716
oral solution, 3717
suppositories, 3718
tablets, 3719
Propafenone hydrochloride, 3727
extended-release capsules, 3729
tablets, 3734
Propane, 5982
Propanediol, 5983
Propantheline bromide, 3736
tablets, 3737
Proparacaine hydrochloride, 3738
and fluorescein sodium ophthalmic solution, 1945
ophthalmic solution, 3739
Propellants ⟨602⟩, 6844
Propionaldehyde, 6194
Propionic
acid, 5985
anhydride, 6194
Propionic acid, 6194
Propiophenone, 6194
Propofol, 3739
injectable emulsion, 3742
Propoxycaine
hydrochloride, 3743
and procaine hydrochlorides and levonordefrin injection, 3744
and procaine hydrochlorides and norepinephrine bitartrate injection, 3745
Propranolol hydrochloride, 3746
extended-release capsules, 3747
and hydrochlorothiazide tablets, 3751
injection, 3749
tablets, 3750
iso-Propyl alcohol, 6194
n-Propyl alcohol, 6134, 6194
Propyl gallate, 5985
Propylamine hydrochloride, 6194
Propylene
carbonate, 5986
glycol, 3753
glycol alginate, 5986
glycol dicaprylate/dicaprate, 5988
glycol dilaurate, 5989
glycol monocaprylate, 5990
glycol monolaurate, 5991
glycol monostearate, 5993
Propylene glycol, 6194
Propylene Glycol Diacetate, 5987
Propylhexedrine, 3754
inhalant, 3754
Propyliodone, 3754
Propylparaben, 5994, 6194
sodium, 5995
Propylthiouracil, 3755
oral suspension, 3755
tablets, 3756
Protamine sulfate, 3757
injection, 3758
Protein
molecular weight standard, 6194
standard solution (8 g/dL), 6194
Protein A quality attributes ⟨130⟩, 6570
Protein determination procedures ⟨507⟩, 6744
Protocatechuic acid, 6194
Protriptyline hydrochloride, 3759
tablets, 3760
Pseudoephedrine
chlorpheniramine, dextromethorphan (salts of), and acetaminophen, capsules containing at least three of the following, 52
chlorpheniramine, dextromethorphan (salts of), and acetaminophen, oral powder containing at least three of the following, 54
chlorpheniramine, dextromethorphan (salts of), and acetaminophen, oral solution containing at least three of the following, 56
chlorpheniramine, dextromethorphan (salts of) and acetaminophen, tablets containing at least three of the following, 58
and diphenhydramine capsules, 1435
hydrochloride, 3761
hydrochloride, acetaminophen, dextromethorphan hydrobromide, and doxylamine succinate oral solution, 67
hydrochloride, acetaminophen, and diphenhydramine hydrochloride tablets, 70
hydrochloride and acetaminophen tablets, 72
hydrochloride extended-release capsules, 3762
hydrochloride, carbinoxamine maleate, and dextromethorphan hydrobromide oral solution, 3767
hydrochloride and chlorpheniramine maleate extended-release capsules, 972
hydrochloride and chlorpheniramine maleate oral solution, 973
hydrochloride and guaifenesin capsules, 2174
hydrochloride, guaifenesin, and dextromethorphan hydrobromide capsules, 2175
hydrochloride and ibuprofen tablets, 2286
hydrochloride oral solution, 3762
hydrochloride tablets, 3763
hydrochloride extended-release tablets, 3764
hydrochloride and cetrizine hydrochloride extended-release tablets, 917
hydrochloride and fexofenadine hydrochloride extended-release tablets, 1876
sulfate, 3768
sulfate and brompheniramine maleate oral solution, 603
sulfate and dexbrompheniramine maleate oral solution, 1298
and triprolidine hydrochlorides oral solution, 4534
and triprolidine hydrochlorides tablets, 4535
Psyllium
hemicellulose, 3768
husk, 3770
hydrophilic mucilloid for oral suspension, 3771
Pullulan, 5996
Pullulanase, 6194
5,800, 23,700, and 100,000 molecular weight (MW) pullulan standards, 6181
Pumice, 3772, 6195
Pure steam, 4654
Purine, 6196
Purpurea
extract, powdered *Echinacea*, 4958
powdered *Echinacea*, 4955
root, *Echinacea*, 4953
Putrescine dihydrochloride, 6196
Pygeum, 5198
capsules, 5200
extract, 5198
Pyrantel pamoate, 3772
and ivermectin tablets, 2478
oral suspension, 3774
Pyrantel tartrate, 3775
Pyrazinamide, 3776
rifampin, isoniazid, and ethambutol hydrochloride tablets, 3881
rifampin and isoniazid tablets, 3879

www.webofpharma.com

Pyrazinamide *(continued)*
oral suspension, 3776
tablets, 3777
Pyrazole, 6196
Pyrene, 6196
Pyrethrum extract, 3778
4-(2-Pyridylazo)resorcinol, 6196
Pyridine, 6196
dried, 6196
Pyridine, anhydrous, 6196
Pyridine–pyrazolone TS, 6234
Pyridostigmine bromide, 3778
injection, 3780
oral solution, 3780
tablets, 3781
Pyridoxal
hydrochloride, 6196
5-phosphate, 6196
Pyridoxamine dihydrochloride, 6196
Pyridoxine hydrochloride, 3782
injection, 3783
tablets, 3784
1-(2-Pyridylazo)-2-naphthol, 6196
3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′, 5″-disulfonic acid, disodium salt, 6196
Pyrilamine maleate, 3785
tablets, 3786
Pyrimethamine, 3786
and sulfadoxine tablets, 4155
oral suspension, 3787
tablets, 3787
Pyrogallol, 6196
TS, alkaline, 6234
Pyrogen test ⟨151⟩, 6577
Pyroxylin, 3788
Pyrrole, 6196
Pyruvic acid, 6196
Pyrvinium pamoate, 3789
oral suspension, 3789
tablets, 3790

Q

Quality assurance in pharmaceutical compounding ⟨1163⟩, 7979
Quality attributes of tablets labeled as having a functional score ⟨705⟩, 6944
Quality of biotechnological products
analysis of the expression construct in cells used for production of r-DNA derived protein products ⟨1048⟩, 7452
stability testing of biotechnological/biological products ⟨1049⟩, 7454
Quazepam, 3790
tablets, 3791
Quercetin, 5202
Quetiapine
tablets, 3792
extended-release tablets, 3795
Quetiapine fumarate, 3800
Quinaldine red, 6221
TS, 6234
Quinapril
hydrochloride, 3802
and hydrochlorothiazide tablets, 3803
tablets, 3806
Quinhydrone, 6197
Quinidine gluconate, 3807
injection, 3809
extended-release tablets, 3810
Quinidine sulfate, 3812
capsules, 3813
oral suspension, 3814
tablets, 3814
extended-release tablets, 3816
Quinine sulfate, 3818
capsules, 3819
tablets, 3821
Quinone, 6197
TS, 6234

R

Rabeprazole
sodium, 3822
Rabies
immune globulin, 3824
Racemethionine, 5997
Racemic
calcium pantothenate, 708
Racepinephrine, 3824
hydrochloride, 3826
inhalation solution, 3825
Ractopamine hydrochloride
suspension, 3826
Radiation sterilization ⟨1229.10⟩, 8228
Radioactivity ⟨821⟩, 7094
Radioactivity—theory and practice ⟨1821⟩, 8604

Radiopharmaceuticals

C 13, urea, 766
C 13, urea for oral solution, 767
C 14, urea capsules, 768
Cr 51, sodium chromate injection, 992
F 18, fludeoxyglucose injection, 1946
F 18, sodium fluoride injection, 1950
Ga 67 injection, gallium citrate, 2088
Indium In 111 capromab pendetide injection, 2318
Indium In 111 chloride solution, 2318
Indium In 111 oxyquinoline solution, 2320
Indium In 111 pentetate injection, 2320
Indium In 111 pentetreotide injection, 2321
I 123, iobenguane injection, 2365
I 123, sodium iodide capsules, 2365
I 123, sodium iodide solution, 2366
I 125, iodinated albumin injection, 2367
I 125, iothalamate sodium injection, 2367
I 131, iodinated albumin injection, 2368
I 131, sodium iodide capsules, 2368
I 131, sodium iodide solution, 2368
N 13, ammonia injection, 3183
Rubidium chloride Rb 82 injection, 3958
Samarium Sm 153 lexidronam injection, 3983
Sr 89 injection, strontium chloride, 4126
Technetium Tc 99m albumin aggregated injection, 4226
Technetium Tc 99m bicisate injection, 4227
Technetium Tc 99m disofenin injection, 4227
Technetium Tc 99m exametazime injection, 4228
Technetium Tc 99m mebrofenin injection, 4229
Technetium Tc 99m medronate injection, 4230
Technetium Tc 99m mertiatide injection, 4231
Technetium Tc 99m oxidronate injection, 4232
Technetium Tc 99m pentetate injection, 4232
Technetium Tc 99m pertechnetate injection, sodium, 4233
Technetium Tc 99m pyrophosphate injection, 4234
Technetium Tc 99m (pyro- and trimeta-) phosphates injection, 4235
Technetium Tc 99m red blood cells injection, 4235
Technetium Tc 99m sestamibi injection, 4236
Technetium Tc 99m succimer injection, 4237
Technetium Tc 99m sulfur colloid injection, 4238
Technetium Tc 99m tetrofosmin injection, 4238
Thallous chloride Tl 201 injection, 4318
Xenon Xe 133, 4656
Yttrium Y 90 ibritumomab tiuxetan injection, 4665

Radiopharmaceuticals—preparation, compounding, dispensing, and repackaging ⟨825⟩, 7109
Raloxifene hydrochloride, 3828
tablets, 3830
Raltegravir
chewable tablets, 3835
tablets, 3834
Raltegravir potassium, 3832
Raman spectroscopy ⟨1120⟩, 7856
Ramipril, 3837
capsules, 3839
tablets, 3841
Ranitidine
hydrochloride, 3843
injection, 3845
in sodium chloride injection, 3848
oral solution, 3846
tablets, 3847
Rapeseed oil
fully hydrogenated, 5998
superglycerinated fully hydrogenated, 5999
Rapid sterility testing of short life products a risk based approach ⟨1071⟩, 7602
Rat tail collagen, 6153
Rauwolfia serpentina, 3849
powdered, 3850
tablets, 3851
Rayon, 6197
purified, 3851
Rb 82
injection, rubidium chloride, 3958
Readily carbonizable substances test ⟨271⟩, 6660
Reagents, 6128
arsenic in, 6128
boiling or distilling range for, 6128
chloride in, 6129
flame photometry for, 6129
general tests for, 6128
heavy metals in, 6130
indicators and solutions, 6127
insoluble matter in, 6130

www.webofpharma.com

Reagents *(continued)*
loss on drying for, 6131
nitrate in, 6131
nitrogen compounds in, 6131
phosphate in, 6131
residue on ignition in, 6131
sulfate in, 6131
Rectal solution
aminophylline, 246
sodium phosphates, 4090
Red
80, direct, 6197
phosphorus, 6197
Red-cell lysing agent, 6197
Red clover
aerial parts isoflavone aglycones dry extract, 5206
Reference standards
USP ⟨11⟩, 6442
Reference tables, 6259
Alcoholometric, 6346
Atomic weights, 6344
Container specifications for capsules and tablets, 6259
Description and relative solubility of USP and NF articles, 6273
Intrinsic viscosity table, 6349
Relative atomic masses and half-lives of selected radionuclides, 6345
Solubilities, 6334
Refractive index ⟨831⟩, 7137
Rehydration salts, oral, 3851
Relative atomic masses and half-lives of selected radionuclides, 6345
Repaglinide, 3854
tablets, 3856
Resazurin (sodium), 6197
Reserpine, 3857
and chlorothiazide tablets, 3859
tablets, 3858
Residual DNA testing ⟨509⟩, 6748
Residual host cell protein measurement in biopharmaceuticals ⟨1132⟩, 7900
Residual solvents ⟨467⟩, 6712
Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures, 8404
Residue on ignition ⟨281⟩, 6660
Residue on ignition in reagents, 6131

Resin

Anion-exchange, 50- to 100-mesh, styrene-divinylbenzene, 6138, 6208
Anion-exchange, chloromethylated polystyrene-divinylbenzene, 6138, 6151
Anion-exchange, strong, lightly cross-linked, in the chloride form, 6138
Anion-exchange, styrene-divinylbenzene, 6138
Capsicum oleoresin, 730
Carboxylate (sodium form) cation-exchange (50- to 100-mesh), 6148
Cation-exchange, 6149
Cation-exchange, carboxylate (sodium form) 50- to 100-mesh, 6148, 6149
Cation-exchange, polystyrene, 6149, 6192
Cation-exchange, styrene-divinylbenzene, 6149
Cation-exchange, styrene-divinylbenzene, strongly acidic, 6149, 6208
Cation-exchange, sulfonic acid, 6149, 6209
Chloromethylated polystyrene-divinylbenzene anion-exchange, 6151
Cholestyramine, 989
Ion-exchange, 6173
Podophyllum, 3582
Podophyllum topical solution, 3582
Polystyrene cation-exchange, 6192
Styrene-divinylbenzene anion-exchange, 50- to 100-mesh, 6208
Styrene-divinylbenzene cation-exchange, strongly acidic, 6208
Sulfonic acid cation-exchange, 6209

Resorcinol, 3861, 6197
monoacetate, 3862
ointment, compound, 3861
and sulfur topical suspension, 3862
TS, 6234
6*Z*-retinoic acid, 6197
Retinyl palmitate, 6197
Reverse transcriptase, 6197
Rheometry ⟨1911⟩, 8679
Rhodamine 6G, 6197
Rhodamine B, 6197
Rhodiola crenulata
root and rhizome dry extract, 5215
root and rhizome powder, 5216
Rhodiola crenulata
root and rhizome, 5213
Rhodiola rosea, 5218
capsules, 5224
extract, 5221
powdered, 5220
tablets, 5227
tincture, 5223
Ribavirin, 3863
capsules, 3863
for inhalation solution, 3865
tablets, 3866
Riboflavin, 3869
assay ⟨481⟩, 6735
injection, 3869
5′-phosphate sodium, 3870
tablets, 3870
Ribonuclease inhibitor, 6197
Ribose, 5229
Rifabutin, 3872
capsules, 3873
oral suspension, 3874
Rifampin, 3875
capsules, 3876
for injection, 3877
and isoniazid capsules, 3878
isoniazid, pyrazinamide, and ethambutol hydrochloride tablets, 3881
isoniazid, and pyrazinamide tablets, 3879
oral suspension, 3877
Riluzole, 3882
tablets, 3883
Rimantadine hydrochloride, 3884
tablets, 3885
Rimexolone, 3886
ophthalmic suspension, 3887
Ringer's
and dextrose injection, 3889
and dextrose injection, half-strength lactated, 3895
and dextrose injection, lactated, 3893
and dextrose injection, modified lactated, 3898
injection, 3887
injection, lactated, 3891
irrigation, 3900
lactated, and dextrose injection, potassium chloride in, 3610
Risedronate sodium, 3900
tablets, 3902
Risperidone, 3904
oral solution, 3905
tablets, 3907
orally disintegrating tablets, 3908
Ritodrine hydrochloride, 3910
injection, 3911
tablets, 3911
Ritonavir, 3912
capsules, 3914
and lopinavir oral solution, 2666
and lopinavir tablets, 2670
oral solution, 3917
tablets, 3920
Rivastigmine, 3922
Rivastigmine tartrate, 3924
capsules, 3925
Rizatriptan benzoate, 3927
tablets, 3928
orally disintegrating tablets, 3930
Rocuronium bromide, 3932
Root and rhizome
dry extract capsules and eleuthero, 4972
dry extract and *Rhodiola crenulata*, 5215
dry extract tablets and eleuthero root, 4973
powder capsules and eleuthero, 4976
powder and *Rhodiola crenulata*, 5216
and *Rhodiola crenulata*, 5213
Ropinirole
tablets, 3934
extended-release tablets, 3936
Ropinirole hydrochloride, 3940
Ropivacaine hydrochloride, 3943
injection, 3945
Rose
bengal sodium, 6197
oil, 6001
water ointment, 3946
water, stronger, 6001
Rosiglitazone maleate, 3946
Rosuvastatin
tablets, 3950
Rosuvastatin calcium, 3948
Rotigotine, 3953
Rotigotine transdermal system, 3955
Roxarsone, 3957
Rubidium chloride Rb 82 injection, 3958
Rufinamide, 3959
tablets, 3960
Rules and procedures, xxviii
Ruthenium red, 6198
TS, 6234
Rutin, 5235

S

Sabinene, 6198
Saccharin, 6001
calcium, 3963
sodium, 3965
sodium oral solution, 3967
sodium tablets, 3968
Saccharose, 6198
Safflower oil, 3968
Safranin O, 6198
St. John's wort flowering top, 5237
dry extract capsules, 5242

www.webofpharma.com

St. John's wort flowering top *(continued)*
extract, dry, 5240
powder, 5238
dry extract tablets, 5244
Salicylaldazine, 6198
Salicylaldehyde, 6198
Salicylamide, 3969
Salicylic acid, 3970, 6198
and benzoic acids ointment, 521
collodion, 3972
gel, 3972
plaster, 3973
topical foam, 3972
and zinc paste, 4690
Saline TS, 6202, 6234
pyrogen-free, 6234
Salix species bark, 5245
dry extract, 5247
powder, 5249
Salmeterol
fluticasone propionate, inhalation aerosol, 2005
fluticasone propionate, inhalation powder, 2010
inhalation powder, 3973
Salmeterol xinafoate, 3978
Salsalate, 3980
capsules, 3981
tablets, 3982
Salt
octanesulfonic acid sodium, 6204
Salts of organic nitrogenous bases ⟨501⟩, 6741
Samarium Sm 153 lexidronam injection, 3983
Sand
standard 20- to 30-mesh, 6198, 6207
washed, 6198, 6218
Saquinavir mesylate, 3983
capsules, 3984
Sargramostim, 3985
for injection, 3988
Sawdust, purified, 6198
Saw palmetto, 5251
capsules, 5259
extract, 5256
powdered, 5254
Scaffold
bovine dermis, 3990
human dermis, 3998
porcine bladder, 4001
silk fibroin, 4006
Scaffold human amniotic membrane allograft, 3993
Scandium oxide, 6198
Scanning electron microscopy ⟨1181⟩, 8025
Schizochytrium oil, 5266
capsules, 5269
Schweitzer's reagent, 6234
Scopolamine hydrobromide, 4010
injection, 4012
ophthalmic solution, 4013
tablets, 4013
Screening for undeclared drugs and drug analogues ⟨2251⟩, 8725
S designations, 6198
Secobarbital, 4013
sodium, 4014
sodium capsules, 4015
Secondary butyl alcohol, 6198
Selamectin, 4016
Selegiline hydrochloride, 4017
capsules, 4018
tablets, 4020
Selegiline hydrochloride compounded topical gel, 4021
Selenious acid, 4022, 6198
injection, 4022
Selenium, 6199
sulfide, 4023
sulfide topical suspension, 4023
Selenium ⟨291⟩, 6661
Selenomethionine, 5272, 6199
Semisolid drug products—performance tests ⟨1724⟩, 8473
Senna
fluidextract, 4025
leaf, 4023
pods, 4025
oral solution, 4026
Sennosides, 4026
tablets, 4028
Sensitization testing ⟨1184⟩, 8035
Serine, 4029
Sertraline
hydrochloride, 4030
hydrochloride oral solution, 4032
hydrochloride tablets, 4033
Sesame oil, 6003
Sevoflurane, 4036
Shear cell methodology for powder flow testing ⟨1063⟩, 7566
Shellac, 6005
Significant change guide for bulk pharmaceutical excipients ⟨1195⟩, 8049
Sildenafil
tablets, 4038
Sildenafil citrate, 4039
oral suspension, 4040
Silica
calcined diatomaceous, 6199
chromatographic, silanized, flux-calcined, acid-washed, 6199
colloidal, hydrophobic, 6007
dental-type, 6006
gel, 6199
gel, binder-free, 6152, 6199
gel, chromatographic, 6152, 6199
gel-impregnated glass microfiber sheet, 6199
gel mixture, chromatographic, 6152, 6199
gel mixture, chromatographic, with chemically bound amino groups, 6199
gel mixture, dimethylsilanized, chromatographic, 6199
gel mixture, octadecylsilanized chromatographic, 6199
gel mixture, octylsilanized, chromatographic, 6199
gel, octadecylsilanized chromatographic, 6199
gel, porous, 6199
microspheres, 6199
Silica gel, pre-coated plates, with fluorescence indicator F254, 6199
Siliceous earth
chromatographic, 6152, 6199
chromatographic, silanized, 6152, 6200
purified, 6008
Silicic
acid, 6200
acid—impregnated glass microfilament sheets with fluorescent indicator, 6200
Silicon
carbide, 6200
dioxide, 6009
dioxide colloidal, 6009
Silicone
75 percent phenyl, methyl, 6200
Silicotungstic acid, *n*-hydrate, 6200
Silicified
microcrystalline cellulose, 5699
Silver
diethyldithiocarbamate, 6200
diethyldithiocarbamate TS, 6234
nitrate, 4041, 6200
nitrate ophthalmic solution, 4041
nitrate, tenth-normal (0.1 N), 6234, 6247
nitrate, toughened, 4042
nitrate TS, 6234
oxide, 6200
Silver–ammonia–nitrate TS, 6234
Silver–ammonium nitrate TS, 6234
Silver nitrate
0.002 N VS, 6247
0.05 N VS, 6247
Silver sulfate, 6200
Simethicone, 4042
alumina, magnesia, and calcium carbonate chewable tablets, 170
alumina and magnesia oral suspension, 173
alumina and magnesia chewable tablets, 174
calcium carbonate and magnesia chewable tablets, 689
capsules, 4043
emulsion, 4044
and magaldrate chewable tablets, 2707
and magaldrate oral suspension, 2706
oral suspension, 4045
tablets, 4045
Simulated gastric fluid TS, 6234
Simulated intestinal fluid TS, 6234
Simvastatin, 4046
tablets, 4047
Single-steroid assay ⟨511⟩, 6750
Sipuleucel-T, 4049
Sitagliptin
phosphate, 4052
tablets, 4051
β-Sitosterol, 6200
Six-month implementation guideline, ii
Sm 153 lexidronam injection, samarium, 3983
Soda lime, 6010, 6201
Sodium phosphate
tribasic, anhydrous, 6205
Sodium, 6201
acetate, 4053, 6201
acetate, anhydrous, 6138, 6201
acetate injection, 4054
acetate solution, 4055
acetate TS, 6234
alendronate, tablets, 120
alginate, 6011
alizarinsulfonate, 6201
alizarinsulfonate TS, 6235
aminoacetate TS, 6235
ammonium phosphate, 6201
arsenate, 6201
arsenite, 6201
arsenite, twentieth-molar (0.05 M), 6247
ascorbate, 4055
azide, 6201
benzoate, 6011
benzoate and caffeine injection, 664
bicarbonate, 4056, 6201
bicarbonate injection, 4060
bicarbonate and magnesium carbonate for oral suspension, 2712
bicarbonate oral powder, 4061
bicarbonate tablets, 4062
biphenyl, 6201

www.webofpharma.com

Sodium *(continued)*
biphosphate, 6202
bisulfite, 6202
bisulfite TS, 6235
bitartrate, 6202
bitartrate TS, 6235
borate, 6012, 6202
borohydride, 6202
bromide, 4062, 6202
bromide injection, veterinary, 4063
bromide oral solution, veterinary, 4063
butyrate, 4064
caprylate, 6013
carbonate, 6014, 6202
carbonate, anhydrous, 6138, 6202
carbonate, citric acid, and magnesium oxide irrigation, 1044
carbonate, monohydrate, 6202
carbonate TS, 6235
carboxymethylcellulose, 773
carboxymethylcellulose, and microcrystalline cellulose, 5698
carboxymethylcellulose, paste, 774
carboxymethylcellulose, tablets, 774
carboxymethylcellulose, 12, 5684
cefazolin, 824
cefmetazole, 842
cefoperazone, 845
cefotaxime, 850
cetostearyl sulfate, 6014
chloride, 4064, 6202
chloride and dextrose injection, 1323
chloride and fructose injection, 2049
chloride inhalation solution, 4068
chloride injection, 4066
chloride injection, bacteriostatic, 4067
chloride injection, dextran 40 in, 1312
chloride injection, dextran 70 in, 1316
chloride injection, mannitol in, 2737
chloride injection, potassium chloride in, 3612
chloride injection, potassium chloride in dextrose injection and, 3608
chloride injection, ranitidine in, 3848
chloride irrigation, 4067
chloride ophthalmic ointment, 4068
chloride ophthalmic solution, 4068
chloride solution, isotonic, 6202
chloride tablets, 4068
chloride tablets for solution, 4069
chloride TS, alkaline, 6235
cholate hydrate, 6202
chromate, 6202
chromate, Cr 51 injection, 992
chromotropate, 6202
cilastatin, 1001
citrate, 4069
citrate and citric acid oral solution, 4069
citrate dihydrate, 6203
citrate TS, 6235
citrate TS, alkaline, 6235
cobaltinitrite, 6203
cobaltinitrite TS, 6235
cyanide, 6203
dalteparin, 1230
1-decanesulfonate, 6203
dehydroacetate, 6016
desoxycholate, 6203
dichromate, 6203
diethyldithiocarbamate, 6203
2,2-dimethyl-2-silapentane-5-sulfonate, 6203
dithionite, 6203
dodecyl sulfate, 6203, 6204
ethylparaben, 5773
ferrocyanide, 6203
fluconazole, chloride injection, 1909
fluorescein, 6203
fluoride, 4071, 6203
fluoride and acidulated phosphate topical solution, 4074
fluoride F18 injection, 1950
fluoride and phosphoric acid gel, 4075
fluoride oral solution, 4072
fluoride tablets, 4073
fluoride TS, 6235
formaldehyde sulfoxylate, 6017
gluconate, 4076
glycocholate, 6203
1-heptanesulfonate, 6203
1-heptanesulfonate, monohydrate, 6203
1-hexanesulfonate, 6203
1-hexanesulfonate, monohydrate, 6203
hydrogen sulfate, 6203
hydrosulfite, 6203
hydrosulfite TS, alkaline, 6226, 6235
hydroxide, 6018, 6204
hydroxide, alcoholic, tenth-normal (0.1 N), 6248
hydroxide, normal (1 N), 6248
hydroxide TS, 6235
hydroxide TS 2, 6235
hydroxide TS 3, 6235
hypobromite TS, 6235
hypochlorite solution, 4076, 6235
hypochlorite topical solution, 4077
hypochlorite TS, 6235
iodate, 6204
iodide, 4077
iodide I 123 capsules, 2365
iodide I 123 solution, 2366
iodide I 131 capsules, 2368
iodide I 131 solution, 2368
iodohydroxyquinolinesulfonate TS, 6235
lactate injection, 4078
lactate solution, 4078
lauryl sulfate, 6019, 6204
low-substituted carboxymethylcellulose, 5682
metabisulfite, 6020, 6204
metaperiodate, 6204
methoxide, 6204
methoxide, half-normal (0.5 N) in methanol, 6248
methoxide, tenth-normal (0.1 N) in toluene, 6248
molybdate, 6204
monofluorophosphate, 4079
montelukast, oral granules, 3012
montelukast, tablets, 3015
montelukast, chewable tablets, 3018
mycophenolate, 3052
nitrate, 6204
nitrite, 4080, 6204
nitrite injection, 4082
nitrite, tenth-molar (0.1 M), 6249
nitroferricyanide, 6204
nitroferricyanide TS, 6235
nitroprusside, 4083
nitroprusside for injection, 4084
1-octanesulfonate, 6204
oxalate, 6204
(tri) pentacyanoamino ferrate, 6204
1-pentanesulfonate, 6186, 6205
1-pentanesulfonate, anhydrous, 6205
perchlorate, 6205
peroxide, 6205
pertechnetate Tc 99m injection, 4233
phenylbutyrate, 4085
phenylbutyrate oral suspension, 4086
phosphate, dibasic, 4087, 6205
phosphate, dibasic, anhydrous, 6138, 6205
phosphate, dibasic, dihydrate, 6205
phosphate, dibasic, dodecahydrate, 6205
phosphate, dibasic, heptahydrate, 6205
phosphate, dibasic, TS, 6235
phosphate, monobasic, 4088, 6181, 6205
phosphate, monobasic, anhydrous, 6205
phosphate, monobasic, dihydrate, 6205
phosphates injection, 4088
phosphates oral solution, 4090
phosphates rectal solution, 4090
phosphate, tribasic, 6021, 6205
phosphite pentahydrate, 6205
phosphotungstate TS, 6235
picosulfate, 4090
polystyrene sulfonate, 4092
polystyrene sulfonate suspension, 4092
and potassium bicarbonates and citric acid effervescent tablets for oral solution, 3598
propionate, 6022
pyrophosphate, 6205
pyruvate, 6205
rabeprazole, 3822
salicylate, 4093, 6205
salicylate tablets, 4094
selenite, 6206
starch glycolate, 6022
stearate, 6024
stearyl fumarate, 6025
sulfate, 4095, 6206
sulfate, anhydrous, 6138, 6206
sulfate decahydrate, 6206
sulfate injection, 4096
sulfide, 4096, 6206
sulfide topical gel, 4096
sulfide TS, 6235
sulfite, 6027, 6206
sulfite, anhydrous, 6138, 6206
p-sulfophenylazochromotropate, 6206
tartrate, 6028, 6206
tartrate TS, 6235
tetraphenylborate, 6206
tetraphenylboron, 6206
tetraphenylboron, fiftieth-molar (0.02 M), 6249
tetraphenylboron TS, 6236
thioglycolate, 6206
thioglycolate TS, 6236
thiosulfate, 4097, 6207
thiosulfate injection, 4097
thiosulfate, tenth-normal (0.1 N), 6236, 6249
thiosulfate TS, 6236
L-thyroxine, 6207
3-(trimethylsilyl)-1-propane sulfonate, 6203, 6207
tungstate, 6207
Sodium benzoate compounded oral solution, 4056
Sodium bicarbonate compounded injection, 4061
Sodium butyrate, 6202
Sodium chloride
TS, 6235
0.5 M TS, 6235
0.1 N Sodium chloride TS, 6235
Sodium 1-dodecanesulfonate, 6203
Sodium ferrous citrate, 5273
Sodium hydroxide
0.0025 N TS, 6235
0.2 N, TS, 6235
0.02 N TS, 6235

www.webofpharma.com

Sodium hydroxide *(continued)*
10 N TS, 6235
2.5 N TS, 6235
2 N TS, 6235
5 N, TS, 6235
0.025 N VS, 6247
0.1 N VS, 6248
0.01 N VS, 6247
0.5 N VS, 6247
50 percent sodium hydroxide TS, 6235
Sodium oleate, 6204
Sodium phenylbutyrate, 4085
Sodium phosphate, 6205
Sodium phosphates compounded injection, 4089
Sodium propionate, 6205
Sodium succinate, 6026
Sodium taurocholate, 6206
Sodium taurodeoxycholate, 6206
Sodium thiosulfate
0.002 N VS, 6249
0.01 M VS, 6249
Solubilities, 6334
Soluble starch, 6207

Solution

Acetaminophen and codeine phosphate oral, 54
Acetaminophen, dextromethorphan hydrobromide, doxylamine succinate, and pseudoephedrine hydrochloride oral, 67
Acetaminophen for effervescent oral, 42
Acetaminophen oral, 41
Acetic acid otic, 78
Acetylcholine chloride for ophthalmic, 81
Acetylcysteine, 82
Acidulated phosphate and sodium fluoride topical, 4074
Aluminum acetate topical, 181
Aluminum chlorohydrate, 183
Aluminum dichlorohydrate, 186
Aluminum sesquichlorohydrate, 191
Aluminum subacetate topical, 193
Aluminum sulfate and calcium acetate for topical, 194
Aluminum sulfate and calcium acetate tablets for topical, 195
Aluminum zirconium octachlorohydrate, 196
Aluminum zirconium octachlorohydrex gly, 198
Aluminum zirconium pentachlorohydrate, 199
Aluminum zirconium pentachlorohydrex gly, 201
Aluminum zirconium tetrachlorohydrate, 203
Aluminum zirconium tetrachlorohydrex gly, 204
Aluminum zirconium trichlorohydrate, 206
Aluminum zirconium trichlorohydrex gly, 207
Amantadine hydrochloride oral, 212
Aminocaproic acid oral, 236
Aminophylline oral, 245
Aminophylline rectal, 246
Ammonia, diluted, 6136
Ammonia, strong, 5616
Amprolium oral, 331
Anticoagulant citrate dextrose, 345
Anticoagulant citrate phosphate dextrose, 347
Anticoagulant citrate phosphate dextrose adenine, 348
Anticoagulant heparin, 2194
Anticoagulant sodium citrate, 350
Antipyrine and benzocaine otic, 352
Antipyrine, benzocaine, and phenylephrine hydrochloride otic, 354
Apraclonidine ophthalmic, 359
Aromatic elixir, 5624
Ascorbic acid oral, 384
Aspirin effervescent tablets for oral, 395
Atenolol oral, 407
Atropine sulfate ophthalmic, 432
Benoxinate hydrochloride ophthalmic, 499
Benzaldehyde elixir, compound, 5634
Benzalkonium chloride, 5636
Benzethonium chloride topical, 501
Benzocaine, butamben, and tetracaine hydrochloride topical, 517
Benzocaine otic, 510
Benzocaine topical, 511
Betamethasone oral, 542
Betaxolol ophthalmic, 558
Bethanechol chloride oral, 563
Bromodiphenhydramine hydrochloride and codeine phosphate oral, 600
Bromodiphenhydramine hydrochloride oral, 599
Brompheniramine maleate and pseudoephedrine sulfate oral, 603
Brompheniramine maleate oral, 602
Buprenorphine compounded buccal, veterinary, 615
Butabarbital sodium oral, 642
Caffeine citrate oral, 663
Calcitonin salmon nasal, 675
Calcium hydroxide topical, 702
Captopril oral, 734
Carbachol intraocular, 739
Carbachol ophthalmic, 739
Carbamide peroxide topical, 747
Carbol-fuchsin topical, 765
C 13 for oral, urea, 767
Carteolol hydrochloride ophthalmic, 789
Cetylpyridinium chloride topical, 923
Chloral hydrate oral, 924
Chloramphenicol for ophthalmic, 930
Chloramphenicol ophthalmic, 929
Chloramphenicol oral, 930
Chloramphenicol otic, 931
Chlorhexidine gluconate, 948
Chlorpheniramine maleate and pseudoephedrine hydrochloride oral, 973
Chlorpheniramine maleate oral, 971
Cholecalciferol, 987
Chymotrypsin for ophthalmic, 994
Ciprofloxacin ophthalmic, 1015
Clindamycin hydrochloride oral, 1066
Clindamycin palmitate hydrochloride for oral, 1068
Clindamycin phosphate topical, 1072
Clobetasol propionate topical, 1082
Clotrimazole topical, 1122
Cloxacillin sodium for oral, 1130
Coal tar topical, 1133
Cocaine hydrochloride tablets for topical, 1134
Codeine sulfate oral, 1141
Cromolyn sodium ophthalmic, 1185
Cupriethylenediamine hydroxide, 1.0 M, 6154
Cyclopentolate hydrochloride ophthalmic, 1201
Cyclosporine oral, 1216
Cyproheptadine hydrochloride oral, 1219
Demecarium bromide ophthalmic, 1247
Dexamethasone elixir, 1286
Dexamethasone oral, 1288
Dexamethasone sodium phosphate ophthalmic, 1296
Dexbrompheniramine maleate and pseudoephedrine sulfate oral, 1298
Dexchlorpheniramine maleate oral, 1301
Dextromethorphan hydrobromide oral, 1321
Diatrizoate meglumine and diatrizoate sodium, 1327
Diatrizoate sodium, 1329
Dichlorophenol–indophenol, standard, 6240
Dicyclomine hydrochloride oral, 1361
Diethyltoluamide topical, 1373
Digoxin oral, 1385
Dihydrotachysterol oral, 1392
Diltiazem hydrochloride oral, 1405
Dimenhydrinate oral, 1410
Dimethyl sulfoxide topical, 1414
Diphenhydramine hydrochloride oral, 1429
Diphenoxylate hydrochloride and atropine sulfate oral, 1438
Dipivefrin hydrochloride ophthalmic, 1442
Docusate sodium, 1480
Dolasetron mesylate oral, 1485
Dorzolamide hydrochloride and timolol maleate ophthalmic, 1499
Doxepin hydrochloride oral, 1510
Doxylamine succinate oral, 1546
Dyclonine hydrochloride topical, 1571
Dyphylline and guaifenesin oral, 1575
Dyphylline oral, 1573
Ecamsule, 1576
Echothiophate iodide for ophthalmic, 1579
Emedastine ophthalmic, 1610
Ephedrine sulfate oral, 1644
Epinephrine bitartrate for ophthalmic, 1652
Epinephrine bitartrate ophthalmic, 1651
Epinephrine ophthalmic, 1649
Epinephryl borate ophthalmic, 1652
Ergocalciferol oral, 1667
Ergoloid mesylates oral, 1671
Erythromycin topical, 1690
Escitalopram oral, 1706
Ethosuximide oral, 1776
Fehling's, 6229
Ferric ammonium citrate for oral, 290
Ferric subsulfate, 1852
Ferrous gluconate oral, 1860
Ferrous sulfate oral, 1863
Fluocinolone acetonide topical, 1936
Fluocinonide topical, 1939
(+)-1-(9-Fluorenyl) ethyl chloroformate, 6166
Fluorescein sodium and benoxinate hydrochloride ophthalmic, 1944
Fluorescein sodium and proparacaine hydrochloride ophthalmic, 1945
Fluorouracil topical, 1957
Fluoxetine oral, 1961
Fluphenazine hydrochloride elixir, 1971
Fluphenazine hydrochloride oral, 1973
Flurbiprofen sodium ophthalmic, 1982
Formaldehyde, 2035, 6167, 6229
Furosemide oral, 2055
Gentamicin sulfate and betamethasone acetate ophthalmic, 2102

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Solution *(continued)*
Gentamicin sulfate and betamethasone valerate otic, 2104
Gentamicin topical, 2105
Gentamicin sulfate ophthalmic, 2102
Gentian violet topical, 2109
Glutaral disinfectant, 5789
Glycerin ophthalmic, 2133
Glycerin oral, 2134
Guaifenesin and codeine phosphate oral, 2172
Guaifenesin oral, 2171
Halazone tablets for, 2182
Halcinonide topical, 2186
Haloperidol oral, 2189
Heparin lock flush, 2194
Homatropine hydrobromide ophthalmic, 2208
Hydralazine hydrochloride oral, 2215
Hydrocortisone and acetic acid otic, 2235
Hydrogen peroxide, 6171
Hydrogen peroxide topical, 2249
Hydroquinone topical, 2255
Hydroxyamphetamine hydrobromide ophthalmic, 2258
Hydroxyzine hydrochloride oral, 2266
Hyoscyamine sulfate elixir, 2276
Hyoscyamine sulfate oral, 2277
Hypromellose ophthalmic, 2281
Idoxuridine ophthalmic, 2293
Indium In 111 chloride, 2318
Indium In 111 oxyquinoline, 2320
Iodine, strong, 2363
Sodium iodide I 123, 2366
Sodium iodide I 131, 2368
Iodine topical, 2362
Ipecac oral, 2397
Ipratropium bromide and albuterol sulfate inhalation, 2399
Isoniazid oral, 2426
Isosorbide oral, 2440
Ivermectin topical, 2476
Lactulose, 2522
Lead, standard, 6236
Levalbuterol inhalation, 2576
Levobunolol hydrochloride ophthalmic, 2595
Levocarnitine oral, 2600
Levofloxacin oral, 2608
Lidocaine hydrochloride topical, 2627
Lincomycin oral, 2632
Lithium oral, 2648
Locke-Ringer's, 6231
Loperamide hydrochloride oral, 2661
Loratadine oral, 2674
Mafenide acetate for topical, 2704
Magnesium carbonate and citric acid for oral, 2710
Magnesium carbonate, citric acid, and potassium citrate for oral, 2711
Manganese chloride for oral, 2732
Magnesium citrate for oral, 2715
Magnesium citrate oral, 2714
Maltitol, 5871
Meperidine hydrochloride oral, 2784
Metaproterenol sulfate oral, 2813
Methadone hydrochloride oral, 2838
Methoxsalen topical, 2865
Methylcellulose ophthalmic, 2876
Methylcellulose oral, 2876
Metoclopramide oral, 2910
Metoprolol tartrate oral, 2925
Mibolerone oral, 2945
Minoxidil topical, 2975
Mometasone furoate topical, 3005
Moxifloxacin ophthalmic, 3034
Myrrh topical, 3057
Naphazoline hydrochloride ophthalmic, 3076
Naphazoline hydrochloride and pheniramine maleate ophthalmic, 3076
Neomycin and polymyxin B sulfates and gramicidin ophthalmic, 3123
Neomycin and polymyxin B sulfates and hydrocortisone otic, 3124
Neomycin and polymyxin B sulfates for irrigation, 3114
Neomycin and polymyxin B sulfates ophthalmic, 3115
Neomycin sulfate and dexamethasone sodium phosphate ophthalmic, 3106
Neomycin sulfate oral, 3103
Nickel standard TS, 6232
Nitrofurazone topical, 3183
Nitromersol topical, 3190
Nortriptyline hydrochloride oral, 3216
Ofloxacin ophthalmic, 3231
Olopatadine hydrochloride ophthalmic, 3249
Ondansetron, oral, 3266
Oral, containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 56
Oxybutynin chloride oral, 3328
Oxycodone hydrochloride oral, 3337
Oxymetazoline hydrochloride ophthalmic, 3350
Papain tablets for topical, 3396
Paromomycin oral, 3409
Penicillin G potassium for oral, 3439
Penicillin V potassium for oral, 3456
Phenobarbital oral, 3498
Phenol, topical, camphorated, 3502
Phenylephrine hydrochloride ophthalmic, 3519
Physostigmine salicylate ophthalmic, 3536
Pilocarpine hydrochloride ophthalmic, 3543
Pilocarpine nitrate ophthalmic, 3545
Podophyllum resin topical, 3582
Polyethylene glycol 3350 and electrolytes for oral, 3587
Polymyxin B sulfate and hydrocortisone otic, 3592
Polymyxin B sulfate and trimethoprim ophthalmic, 3592
Potassium bicarbonate effervescent tablets for oral, 3596
Potassium bicarbonate and potassium chloride for effervescent oral, 3597
Potassium bicarbonate and potassium chloride effervescent tablets for oral, 3597
Potassium bicarbonate, potassium chloride, and potassium citrate effervescent tablets for oral, 3609
Potassium bromide oral, veterinary, 3600
Potassium chloride for oral, 3605
Potassium chloride oral, 3604
Potassium citrate and citric acid oral, 3617
Potassium gluconate and potassium chloride for oral, 3621
Potassium gluconate and potassium chloride oral, 3621
Potassium gluconate, potassium citrate, and ammonium chloride oral, 3622
Potassium gluconate and potassium citrate oral, 3622
Potassium gluconate oral, 3619
Potassium iodide oral, 3624
Potassium nitrate, 3626
Potassium and sodium bicarbonates and citric acid effervescent tablets for oral, 3598
Povidone–iodine cleansing, 3635
Povidone–iodine topical, 3636
Prednisolone oral, 3661
Prednisolone sodium phosphate compounded oral, 3667
Prednisolone sodium phosphate ophthalmic, 3668
Prednisone oral, 3671
Prochlorperazine oral, 3697
Promazine hydrochloride oral, 3713
Promethazine and phenylephrine hydrochloride and codeine phosphate oral, 3724
Promethazine and phenylephrine hydrochloride oral, 3721
Promethazine hydrochloride oral, 3717
Proparacaine hydrochloride ophthalmic, 3739
Protein standard (8 g/dL), 6194
Pseudoephedrine hydrochloride, carbinoxamine maleate, and dextromethorphan hydrobromide oral, 3767
Pseudoephedrine hydrochloride oral, 3762
Pyridostigmine bromide oral, 3780
Ranitidine oral, 3846
Risperidone oral, 3905
Saccharin sodium oral, 3967
Scopolamine hydrobromide ophthalmic, 4013
Senna oral, 4026
Silver nitrate ophthalmic, 4041
Sodium acetate, 4055
Sodium bromide oral, veterinary, 4063
Sodium chloride, isotonic, 6202
Sodium chloride ophthalmic, 4068
Sodium chloride tablets for, 4069
Sodium citrate and citric acid oral, 4069
Sodium fluoride and acidulated phosphate topical, 4074
Sodium fluoride oral, 4072
Sodium hypochlorite, 4076, 6235, 6235
Sodium hypochlorite topical, 4077
Sodium lactate, 4078
Sodium phosphates oral, 4090
Sodium phosphates rectal, 4090
Sorbitol, 4101
Sorbitol noncrystallizing, 6036
Sorbitol sorbitan, 6038
Stavudine for oral, 4122
Sulfacetamide sodium ophthalmic, 4143
Sulfaquinoxaline oral, 4169
Terpin hydrate and codeine oral, 4284
Terpin hydrate oral, 4284
Tetracaine hydrochloride ophthalmic, 4299
Tetracaine hydrochloride topical, 4299
Tetracycline hydrochloride for topical, 4309
Tetrahydrozoline hydrochloride ophthalmic, 4315
Tetramethylammonium hydroxide, in methanol, 6211
Theophylline and guaifenesin oral, 4332
Theophylline oral, 4325
Theophylline sodium glycinate oral, 4334
Thiamine hydrochloride oral, 4338
Thiamine mononitrate oral, 4341
Thimerosal topical, 4347
Thioridazine hydrochloride oral, 4354
Thiothixene hydrochloride oral, 4361

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Solution *(continued)*
Timolol maleate ophthalmic, 4389
Tobramycin ophthalmic, 4408
Tolnaftate topical, 4427
Travoprost ophthalmic, 4471
Tretinoin topical, 4479
Triamcinolone diacetate oral, 4491
Tricitrates oral, 4504
Trifluoperazine oral, 4511
Trihexyphenidyl hydrochloride oral, 4519
Trikates oral, 4521
Trimeprazine oral, 4522
Triprolidine hydrochloride oral, 4533
Triprolidine and pseudoephedrine hydrochlorides oral, 4534
Tropicamide ophthalmic, 4541
Valproic acid oral, 4574
Valrubicin intravesical, 4577
Vancomycin hydrochloride for oral, 4590
Vehicle for oral, 5913
Vehicle for oral, sugar free, 5913
Verapamil hydrochloride oral, 4609
Vitamins with minerals, water-soluble oral, 5543
Vitamins with minerals, oil- and water-soluble oral, 5462
Vitamins, oil- and water-soluble oral, 5409
Xanthan gum, 6104
Zidovudine oral, 4676
Zinc sulfate ophthalmic, 4693
Zinc sulfate oral, 4693

Solutions,
reagents, and indicators, 6127
Solvent hexane, 6207
Somatropin, 4098
for injection, 4099
Somatropin bioidentity tests ⟨126⟩, 6558
Sorbic acid, 6029
Sorbitan
monolaurate, 6030
monooleate, 6031
monopalmitate, 6032
monostearate, 6033
sesquioleate, 6033
sorbitol, solution, 6038
trioleate, 6034
Sorbitol, 6035, 6207
solution, 4101
solution noncrystallizing, 6036
sorbitan solution, 6038
Sotalol hydrochloride, 4102
oral suspension, 4104
tablets, 4105
Soybean oil, 4105
hydrogenated, 6040
Soy isoflavones
capsules, 5276
powdered extract, 5274
tablets, 5278
Spacers and valved holding chambers used with inhalation aerosols—characterization tests ⟨1602⟩ , 8411
Specific gravity ⟨841⟩, 7137
Specific surface area ⟨846⟩, 7139
Spectinomycin
hydrochloride, 4108
for injectable suspension, 4108
Spectrophotometric identification tests ⟨197⟩, 6593
Spironolactone, 4109
and hydrochlorothiazide oral suspension, 4113
and hydrochlorothiazide tablets, 4113
tablets, 4112
Spironolactone compounded
oral suspension, 4111
Spironolactone compounded, veterinary
oral suspension, 4111
Spirulina, 5279
tablets, 5283
Squalane, 6041
Sr 89 injection, strontium chloride, 4126
Stability considerations in dispensing practice ⟨1191⟩, 8044
Stachyose hydrate, 6207
Standard sand, 20- to 30-mesh, 6207
Stannous
chloride, 6041, 6207
chloride acid, stronger, TS, 6225, 6236
chloride acid TS, 6225, 6236
fluoride, 4114
fluoride gel, 4115
Stanozolol, 4117
tablets, 4118
Star anise
oil, 6043
Starch
corn, 6044
corn, pregelatinized hydroxypropyl, 6047
hydrolysate, hydrogenated, 6049
hydroxypropyl corn, 6045
iodate paper, 6222
iodide-free TS, 6236
iodide paper, 6222
iodide paste TS, 6236
modified, 6052
pea, 6053
pea, pregelatinized hydroxypropyl, 6056
potassium iodide TS, 6236
potassium iodide and, TS, 6234
potato, 6058, 6194, 6207
potato, pregelatinized hydroxypropyl, 6061
pregelatinized, 6063
pregelatinized modified, 6064
sodium, glycolate, 6022
soluble, 6207
soluble, purified, 6207
tapioca, 6066
topical, 4119
TS, 6236
wheat, 6066
Stavudine, 4119
capsules, 4120
for oral solution, 4122
Steam, pure, 4654
Steam sterilization by direct contact ⟨1229.1⟩, 8199
Stearic acid, 6068, 6207
purified, 6070
Stearoyl polyoxylglycerides, 6071
Stearyl alcohol, 6072, 6207

Sterile

Erythromycin ethylsuccinate, 1698
Erythromycin gluceptate, 1702
Erythromycin lactobionate, 1703
Pharmaceutical compounding—sterile preparations ⟨797⟩, 7037
Sterile product packaging—integrity evaluation ⟨1207⟩, 8079
Sterility assurance ⟨1211⟩, 8129
Sterility testing—validation of isolator systems ⟨1208⟩, 8113
Water, purified, 4654
Water for inhalation, 4652
Water for injection, 4653
Water for irrigation, 4653

Sterile product packaging—integrity evaluation ⟨1207⟩, 8079
Sterility
testing—validation of isolator systems ⟨1208⟩, 8113
tests ⟨71⟩, 6481
Sterility assurance ⟨1211⟩, 8129
Sterilization cycle development, 8235
Sterilization filtration of gases, 8238
Sterilization-in-place ⟨1229.13⟩, 8233
Sterilization of compendial articles ⟨1229⟩, 8194
Sterilizing filtration of liquids ⟨1229.4⟩, 8209
Stinging nettle, 5286
extract, powdered, 5290
powdered, 5288
Storax, 4123
Streptomycin
injection, 4125
for injection, 4125
sulfate, 4124
Stronger
ammonia water, 6207
cupric acetate TS, 6236
Strontium
acetate, 6207
chloride Sr 89 injection, 4126
hydroxide, 6208
Strychnine sulfate, 6208
Styrene-divinylbenzene
anion-exchange resin, 50- to 100-mesh, 6208
cation-exchange resin, strongly acidic, 6208
copolymer beads, 6208
Subvisible particulate matter in therapeutic protein injections ⟨787⟩, 7014
Succinic acid, 6074, 6208
Succinylcholine chloride, 4127
injection, 4129
Sucralfate, 4130
tablets, 4131
Sucralose, 6074
Sucrose, 6076
octaacetate, 6077
palmitate, 6078
stearate, 6079
Sudan
III, 6208
III TS, 6236
IV, 6208
IV TS, 6236
Sufentanil citrate, 4132
injection, 4133
Sugar
compressible, 6081
confectioner's, 6082
free suspension structured vehicle, 6087
injection, invert, 4133
spheres, 6083
Sulbactam
and ampicillin for injection, 328
sodium, 4134
Sulconazole nitrate, 4135
Sulfa
vaginal cream, triple, 4137
vaginal inserts, triple, 4137
Sulfabenzamide, 4138

www.webofpharma.com

Sulfacetamide, 4139
sodium, 4140
sodium ophthalmic ointment, 4142
sodium ophthalmic solution, 4143
sodium and prednisolone acetate ophthalmic ointment, 4144
sodium and prednisolone acetate ophthalmic suspension, 4145
sodium topical suspension, 4143
Sulfachlorpyridazine, 4146
Sulfadiazine, 4147
cream, silver, 4150
silver, 4149
sodium, 4151
sodium injection, 4152
tablets, 4148
Sulfadimethoxine, 4152
sodium, 4154
soluble powder, 4153
oral suspension, 4153
tablets, 4153
Sulfadoxine, 4154
and pyrimethamine tablets, 4155
Sulfaguanidine, 6209
Sulfamerazine, 6209
Sulfamethazine, 4156
and chlortetracycline bisulfates soluble powder, 980
granulated, 4156
Sulfamethizole, 4157
oral suspension, 4158
tablets, 4158
Sulfamethoxazole, 4159
oral suspension, 4161
tablets, 4161
and trimethoprim injection, 4162
and trimethoprim oral suspension, 4163
and trimethoprim tablets, 4166
Sulfamic acid, 6209
Sulfan blue, 6209
Sulfanilamide, 6209
Sulfanilic
acid, 6209
acid, diazotized TS, 6236
acid TS, 6236
1-naphthylamine TS, 6236
α-naphthylamine TS, 6236
Sulfapyridine, 4167
tablets, 4167
Sulfaquinoxaline, 4168
oral solution, 4169
Sulfasalazine, 4169
tablets, 4170
delayed-release tablets, 4170
Sulfatase enzyme preparation, 6209
Sulfate
acid, ferrous, TS, 6225, 6229
and chloride ⟨221⟩, 6636
ferrous, TS, 6229
magnesium, TS, 6231
mercuric, TS, 6228, 6231
potassium, 6194
potassium, TS, 6234
in reagents, 6131
strychnine, 6208
Sulfathiazole, 4171
sodium, 6209
Sulfinpyrazone, 4172
capsules, 4172
tablets, 4173
Sulfiram, 6209
Sulfisoxazole, 4174
acetyl, 4175
acetyl and erythromycin estolate oral suspension, 1695
acetyl and erythromycin ethylsuccinate for oral suspension, 1701
acetyl oral suspension, 4175
tablets, 4175
Sulfomolybdic acid TS, 6236
Sulfonic acid cation-exchange resin, 6209
2-(4-Sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid, trisodium salt, 6220, 6221
Sulfosalicylic acid, 6209
Sulfur, 6209
dioxide, 6084
dioxide detector tube, 6209
ointment, 4176
precipitated, 4176
and resorcinol topical suspension, 3862
sublimed, 4176
Sulfur dioxide ⟨525⟩, 6751
Sulfuric acid, 6084, 6209
diluted, 6159, 6209
fluorometric, 6209
fuming, 6168, 6209
half-normal (0.5 N) in alcohol, 6250
nitrogen free, 6209
normal (1 N), 6250
phenylhydrazine, TS, 6233
TS, 6236
0.02 N TS, 6236
0.2 N TS, 6236
0.5 N TS, 6236
1 M TS, 6236
2 N TS, 6236
6 N TS, 6236
7 N TS, 6236
0.01 N VS, 6250
0.02 N Sulfuric acid VS, 6250
10 N Sulfuric acid TS, 6236
Sulfuric acid–formaldehyde TS, 6236
0.05 N Sulfuric acid VS, 6250
Sulfurous acid, 6209
Sulindac, 4177
tablets, 4178
Sulisobenzone, 4180
Sumatriptan, 4180
nasal spray, 4183
injection, 4182
succinate, 4187
succinate oral suspension, 4188
tablets, 4185
Sunflower oil, 6085, 6209
Supplemental information for articles of botanical origin ⟨2030⟩, 8702

Suppositories

Acetaminophen, 44
Aminophylline, 247
Aspirin, 391
Bisacodyl, 573
Chlorpromazine, 974
Ergotamine tartrate and caffeine, 1680
Glycerin, 2134
Indomethacin, 2331
Miconazole nitrate vaginal, 2951
Morphine sulfate, 3028
Nystatin vaginal, 3220
Prochlorperazine, 3698
Progesterone vaginal, 3707
Promethazine hydrochloride, 3718
Thiethylperazine maleate, 4342

Suspension

Acetaminophen and codeine phosphate oral, 56
Acetaminophen oral, 42
Acetazolamide oral, 76
Acyclovir oral, 92
Albendazole oral, 102
Allopurinol oral, 133
Alprazolam oral, 145
Alumina, magnesia, and calcium carbonate oral, 168
Alumina and magnesia oral, 166
Alumina, magnesia, and simethicone oral, 173
Alumina and magnesium carbonate oral, 176
Alumina and magnesium trisilicate oral, 179
Amoxicillin and clavulanate potassium for oral, 308
Amoxicillin for oral, 303
Amoxicillin for injectable, 302
Amoxicillin oral, 303
Amoxicillin tablets for oral, 306
Ampicillin for injectable, 324
Ampicillin for oral, 324
Ampicillin and probenecid for oral, 326
Atenolol compounded oral, 408
Atenolol compounded oral, veterinary, 409
Atovaquone oral, 423
Aurothioglucose injectable, 434
Azathioprine oral, 440
Azithromycin for oral, 454
Baclofen oral, 473
Barium sulfate, 481
Barium sulfate for, 482
Benazepril hydrochloride compounded oral, veterinary, 490
Betamethasone sodium phosphate and betamethasone acetate injectable, 551
Bethanechol chloride oral, 564
Bisacodyl rectal, 574
Bismuth subsalicylate oral, 582
Brinzolamide ophthalmic, 594
Calamine topical, 665
Calamine topical, phenolated, 665
Calcium carbonate oral, 686
Calcium and magnesium carbonates oral, 691
Captopril oral, 735
Carbamazepine oral, 742
Cefaclor for oral, 810
Cefadroxil for oral, 816
Cefdinir for oral, 830
Cefixime for oral, 837
Cefpodoxime proxetil for oral, 864
Cefprozil for oral, 869
Cefuroxime axetil for oral, 888
Cellulose sodium phosphate for oral, 896
Cephalexin for oral, 898
Cephradine for oral, 909
Chloramphenicol and hydrocortisone acetate for ophthalmic, 932
Chloramphenicol palmitate oral, 934
Chlorothiazide oral, 963
Cholestyramine for oral, 990
Ciclopirox olamine topical, 998
Ciprofloxacin and dexamethasone otic, 1021
Clarithromycin for oral, 1049
Clavulanate potassium and amoxicillin for oral, 308

www.webofpharma.com

Suspension *(continued)*
Clindamycin phosphate topical, 1072
Clonazepam oral, 1097
Clopidogrel compounded oral, 1111
Colestipol hydrochloride for oral, 1147
Colistin and neomycin sulfates and hydrocortisone acetate otic, 1150
Colistin sulfate for oral, 1150
Desoxycorticosterone pivalate injectable, 1279
Dexamethasone acetate injectable, 1291
Dexamethasone ophthalmic, 1287
Diazoxide oral, 1335
Didanosine tablets for oral, 1367
Diltiazem hydrochloride oral, 1406
Dipyridamole oral, 1445
Dolasetron mesylate oral, 1485
Doxycycline calcium oral, 1529
Doxycycline compounded oral, veterinary, 1530
Doxycycline for oral, 1525
Enalapril maleate compounded oral, veterinary, 1613
Erythromycin estolate for oral, 1694
Erythromycin estolate oral, 1693
Erythromycin estolate and sulfisoxazole acetyl oral, 1695
Erythromycin ethylsuccinate for oral, 1699
Erythromycin ethylsuccinate oral, 1698
Erythromycin ethylsuccinate and sulfisoxazole acetyl for oral, 1701
Estrone injectable, 1752
Famotidine for oral, 1818
Ferumoxsil oral, 1868
Flucytosine oral, 1917
Fluorometholone ophthalmic, 1952
Furazolidone oral, 2052
Ganciclovir oral, 2090
Gentamicin and prednisolone acetate ophthalmic, 2107
Griseofulvin oral, 2166
Hydrocortisone rectal, 2233
Ibuprofen oral, 2283
Imipenem and cilastatin for injectable, 2298
Indomethacin oral, 2332
Isophane insulin human, 2352
Human insulin isophane and human insulin injection, 2349
Isophane insulin, 2351
Insulin zinc, 2356
Insulin zinc, extended, 2357
Insulin zinc, prompt, 2359
Isoflupredone acetate injectable, 2419
Ketoconazole oral, 2507
Labetalol hydrochloride oral, 2519
Lamotrigine compounded oral, 2545
Lansoprazole compounded oral, 2556
Magaldrate and simethicone oral, 2706
Magnesium carbonate and sodium bicarbonate for oral, 2712
Mebendazole oral, 2744
Medroxyprogesterone acetate injectable, 2755
Megestrol acetate oral, 2763
Meloxicam oral, 2770
Mesalamine rectal, 2803
Methacycline hydrochloride oral, 2835
Methadone hydrochloride tablets for oral, 2839
Methylprednisolone acetate injectable, 2901
Metolazone oral, 2913
Metoprolol tartrate oral, 2926
Metronidazole benzoate compounded oral, 2933
Minocycline hydrochloride oral, 2966
Naproxen oral, 3079
Natamycin ophthalmic, 3096
Neomycin and polymyxin B sulfates and dexamethasone ophthalmic, 3122
Neomycin and polymyxin B sulfates and hydrocortisone otic, 3124
Neomycin and polymyxin B sulfates and hydrocortisone acetate ophthalmic, 3125
Neomycin and polymyxin B sulfates and hydrocortisone ophthalmic, 3123
Neomycin and polymyxin B sulfates and prednisolone acetate ophthalmic, 3127
Neomycin sulfate and hydrocortisone otic, 3109
Neomycin sulfate and hydrocortisone acetate ophthalmic, 3110
Neomycin sulfate and prednisolone acetate ophthalmic, 3128
Nevirapine oral, 3132
Nitrofurantoin oral, 3179
Nystatin for oral, 3221
Nystatin oral, 3221
Ondansetron hydrochloride oral, 3268
Oxfendazole oral, 3322
Oxytetracycline hydrochloride and hydrocortisone acetate ophthalmic, 3363
Penicillin G benzathine injectable, 3433
Penicillin G benzathine and penicillin G procaine injectable, 3434
Penicillin G benzathine oral, 3433
Penicillin G, neomycin, polymyxin B, hydrocortisone acetate, and hydrocortisone sodium succinate topical, 3430
Penicillin G procaine, dihydrostreptomycin sulfate, chlorpheniramine maleate, and dexamethasone injectable, 3445
Penicillin G procaine and dihydrostreptomycin sulfate injectable, 3444
Penicillin G procaine, dihydrostreptomycin sulfate, and prednisolone injectable, 3447
Penicillin G procaine, neomycin and polymyxin B sulfates, and hydrocortisone acetate topical, 3448
Penicillin G procaine injectable, 3443
Penicillin G procaine for injectable, 3443
Penicillin V benzathine oral, 3454
Penicillin V for oral, 3452
Perflutren protein-type A microspheres injectable, 3475
Pergolide, oral, veterinary, 3478
Phenoxybenzamine hydrochloride compounded oral, 3504
Phenytoin oral, 3526
Piroxicam compounded oral, 3579
Prednisolone acetate injectable, 3663
Prednisolone acetate ophthalmic, 3663
Prednisolone compounded oral, veterinary, 3664
Prednisone injectable, 3672
Prednisolone tebutate injectable, 3670
Primidone oral, 3684
Progesterone injectable, 3706
Psyllium hydrophilic mucilloid for oral, 3771
Pyrantel pamoate oral, 3774
Pyrvinium pamoate oral, 3789
Quinidine sulfate oral, 3814
Ractopamine hydrochloride, 3826
Resorcinol and sulfur topical, 3862
Rifampin oral, 3877
Rimexolone ophthalmic, 3887
Selenium sulfide topical, 4023
Simethicone oral, 4045
Sodium polystyrene sulfonate, 4092
Spectinomycin for injectable, 4108
Spironolactone compounded oral, 4111
Structured vehicle, 6086
Structured vehicle, sugar-free, 6087
Sulfacetamide sodium and prednisolone acetate ophthalmic, 4145
Sulfacetamide sodium topical, 4143
Sulfadimethoxine oral, 4153
Sulfamethizole oral, 4158
Sulfamethoxazole oral, 4161
Sulfamethoxazole and trimethoprim oral, 4163
Sulfisoxazole acetyl oral, 4175
Sumatriptan succinate oral, 4188
Temozolomide oral, 4257
Testosterone injectable, 4287
Tetracycline hydrochloride ophthalmic, 4310
Tetracycline hydrochloride oral, 4310
Tetracycline oral, 4302
Thiabendazole oral, 4335
Tobramycin and dexamethasone ophthalmic, 4411
Tobramycin and fluorometholone acetate ophthalmic, 4412
Topiramate compounded oral, 4436
Triamcinolone acetonide injectable, 4490
Triamcinolone diacetate injectable, 4491
Triamcinolone hexacetonide injectable, 4493
Trifluprazine oral, 4514
Trisulfapyrimidines oral, 4536
Vehicle for oral, 5913
Verapamil hydrochloride oral, 4609
Zinc sulfide topical, 4695

Suspension structured vehicle, 6086
sugar-free, 6087
Suture
absorbable surgical, 4189
nonabsorbable surgical, 4191
Sutures
diameter ⟨861⟩, 7175
needle attachment ⟨871⟩, 7178

Syrup

Acacia, 5597
Calcium glubionate, 695
Cherry, 5709
Chlorpromazine hydrochloride, 976
Chocolate, 5716
Corn, 5724
Corn, solids, 5731
High fructose corn, 5727
Docusate sodium, 1481
Ferrous sulfate, 1863
Orange, 5915
Piperazine citrate, 3575
Promazine hydrochloride, 3713
Syrup, 6087
Tolu balsam, 6090

www.webofpharma.com

T

Tablet breaking force ⟨1217⟩, 8138
Tablet compression characterization, 7556
Tablet friability ⟨1216⟩, 8137

Tablets

Abacavir, 6066
Abiraterone acetate, 26
Acarbose, 31
Acepromazine maleate, 38
Acetaminophen, 45
Containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 58
Acetaminophen and aspirin, 48
Acetaminophen, aspirin, and caffeine, 49
Acetaminophen and caffeine, 51
Acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide, 60
Acetaminophen and codeine phosphate, 66
Acetaminophen and diphenhydramine citrate, 69
Acetaminophen, diphenhydramine hydrochloride, and pseudoephedrine hydrochloride, 70
Acetaminophen extended-release, 47
Acetaminophen and hydrocodone bitartrate, 2224
Acetaminophen and pseudoephedrine hydrochloride, 72
Acetazolamide, 76
Acetohydroxamic acid, 79
Acyclovir, 92
Albendazole, 103
Albuterol, 105
Albuterol extended-release, 106
Alendronate sodium, 120
Alfuzosin hydrochloride extended-release, 125
Allopurinol, 134
Almotriptan, 138
Alosetron, 142
Alprazolam, 146
Alprazolam extended-release, 148
Alprazolam orally disintegrating, 152
Alumina and magnesia, 167
Alumina, magnesia, and calcium carbonate chewable, 169
Alumina, magnesia, calcium carbonate, and simethicone chewable, 170
Alumina, magnesia, and simethicone chewable, 174
Alumina and magnesium carbonate, 177
Alumina, magnesium carbonate, and magnesium oxide, 178
Alumina and magnesium trisilicate, 180
Aluminum hydroxide gel, dried, 190
Aluminum sulfate and calcium acetate for topical solution, 195
Amiloride hydrochloride, 223
Amiloride hydrochloride and hydrochlorothiazide, 224
Aminocaproic acid, 237
Aminopentamide sulfate, 240
Aminophylline, 248
Aminophylline delayed-release, 250
Amiodarone hydrochloride, 257
Amitriptyline hydrochloride, 263
amlodipine and atorvastatin, 265
Amlodipine and olmesartan medoxomil, 272
Amlodipine and valsartan, 275
Amlodipine besylate, 286
Amlodipine, valsartan and hydrochlorothiazide, 279
Ammonium chloride delayed-release, 288
Amodiaquine hydrochloride, 294
Amoxapine, 297
Amoxicillin, 304
Amoxicillin and clavulanic acid extended-release, 310
Amoxicillin and clavulanate potassium, 308
Amphetamine sulfate, 313
Ampicillin, 325
Anastrozole, 337
Anileridine hydrochloride, 341
Apomorphine hydrochloride, 358
Arginine, 4764
Aripiprazole, 375
Aripiprazole orally disintegrating, 377
Ascorbic acid, 385
Aspirin, 392
Aspirin, alumina, and magnesia, 397
Aspirin, alumina, and magnesium oxide, 399
Aspirin, buffered, 393
Aspirin and codeine phosphate, 403
Aspirin, codeine phosphate, alumina, and magnesia, 404
Aspirin delayed-release, 394
Aspirin effervescent, for oral solution, 395
Aspirin extended-release, 396
Atenolol, 407
Atenolol and chlorthalidone, 409
Atorvastatin calcium, 418
Azatadine maleate, 438
Azathioprine, 441
Azithromycin, 457
Baclofen, 473
Barium sulfate, 483
Belladonna extract, 487
Benazepril hydrochloride, 491
Bendroflumethiazide, 497
benzphetamine hydrochloride, 530
Benztropine mesylate, 533
Betaxolol, 559
Bethanechol chloride, 564
Bicalutamide, 567
Biotin, 571
Bisacodyl delayed-release, 574
Bismuth subsalicylate, 583
Bisoprolol fumarate, 586
Bisoprolol fumarate and hydrochlorothiazide, 587
Black cohosh, 4823
Bromocriptine mesylate, 597
Brompheniramine maleate, 603
Bumetanide, 608
Buprenorphine and naloxone sublingual, 616
Bupropion hydrochloride, 620
Bupropion hydrochloride extended-release, 621
Buspirone hydrochloride, 637
Busulfan, 640
Butabarbital sodium, 643
Butalbital, acetaminophen, and caffeine, 646
Butalbital and aspirin, 646
Butalbital, aspirin, and caffeine, 649
Cabergoline, 659
Calcium acetate, 682
Calcium carbonate, 687
Calcium carbonate, magnesia, and simethicone chewable, 689
Calcium citrate, 4835
Calcium gluconate, 701
Calcium L-5-methyltetrahydrofolate, 4842
Calcium lactate, 703
Calcium and magnesium carbonates, 691
Calcium pantothenate, 707
Calcium phosphate, dibasic, 712
Calcium with vitamin D, 4843
Calcium and vitamin D with minerals, 4845
Candesartan cilexetil, 717
Candesartan cilexetil and hydrochlorothiazide, 719
Capecitabine, 723
Captopril, 735
Captopril and hydrochlorothiazide, 737
Carbamazepine, 743
Carbamazepine extended-release, 746
Carbenicillin indanyl sodium, 749
Carbidopa and levodopa, 751
Levodopa and carbidopa extended-release, 753
Carbidopa and levodopa orally disintegrating, 759
Carbinoxamine maleate, 763
Calcium carbonate and magnesia chewable, 688
Carboxymethylcellulose sodium, 774
Carisoprodol, 777
Carisoprodol, aspirin, and codeine phosphate, 780
Carisoprodol and aspirin, 778
Carprofen, 786
Carteolol hydrochloride, 790
Carvedilol, 793
Cascara, 800
Cat's claw, 4858
Cefaclor extended-release, 811
Cefadroxil, 817
Cefixime, 838
Cefpodoxime proxetil, 864
Cefprozil, 870
Cefuroxime axetil, 889
Cephalexin, 899
Cephalexin, for oral suspension, 899
Cephradine, 909
Cetirizine hydrochloride, 913
Cetirizine hydrochloride orally disintegrating, 915
Cetrizine hydrochloride and pseudoephedrine hydrochloride extended-release, 917
Chlorambucil, 926
Chloramphenicol, 932
Chlordiazepoxide, 937
Chlordiazepoxide and amitriptyline hydrochloride, 938
Chloroquine phosphate, 961
Chlorothiazide, 964
Chlorpheniramine maleate, 971
Chlorpromazine hydrochloride, 977
Chlorpropamide, 978
Chlortetracycline hydrochloride, 987
Chlorthalidone, 983
Chlorzoxazone, 984
Cholecalciferol, 988
Chondroitin sulfate sodium, 4897
Chromium picolinate, 4902
Cilostazol, 1004
Cimetidine, 1007
Ciprofloxacin, 1015
Ciprofloxacin extended-release, 1017

www.webofpharma.com

Tablets *(continued)*
Citalopram, 1035
Clarithromycin, 1049
Clarithromycin extended-release, 1051
Clemastine fumarate, 1059
Clomiphene citrate, 1090
Clonazepam, 1098
Clonazepam orally disintegrating, 1099
Clonidine hydrochloride, 1103
Clonidine hydrochloride and chlorthalidone, 1104
Clopidogrel, 1111
Clorazepate dipotassium, 1115
Clover, red, 5212
Clozapine, 1132
Cocaine hydrochloride, for topical solution, 1134
Codeine phosphate, 1139
Codeine sulfate, 1142
Colchicine, 1145
Colestipol hydrochloride, 1147
Cortisone acetate, 1176
Curcuminoids, 4930
Cyanocobalamin, 1192
Cyclizine hydrochloride, 1195
Cyclobenzaprine hydrochloride, 1199
Cyclophosphamide, 1209
Cyproheptadine hydrochloride, 1220
Dapsone, 1241
Demeclocycline hydrochloride, 1249
Desipramine hydrochloride, 1256
Desloratadine, 1261
Desloratadine orally disintegrating, 1263
Desogestrel and ethinyl estradiol, 1270
Dexamethasone, 1289
Dexchlorpheniramine maleate, 1301
Dextroamphetamine sulfate, 1318
Diazepam, 1332
Dichlorphenamide, 1340
Diclofenac potassium, 1345
Diclofenac sodium and misoprostol delayed-release, 1353
Diclofenac sodium delayed-release, 1348
Diclofenac sodium extended-release, 1350
Dicyclomine hydrochloride, 1362
Didanosine, for oral suspension, 1367
Diethylcarbamazine citrate, 1369
Diethylpropion hydrochloride, 1371
Diflunisal, 1376
Digitalis, 1380
Digitoxin, 1382
Digoxin, 1385
Dihydrotachysterol, 1393
Dihydroxyaluminum sodium carbonate chewable, 1396
Diltiazem hydrochloride, 1406
Dimenhydrinate, 1410
Diphenhydramine citrate and ibuprofen, 1421
Diphenhydramine and phenylephrine hydrochloride, 1433
Diphenoxylate hydrochloride and atropine sulfate, 1439
Dipyridamole, 1446
Dirithromycin delayed-release, 1448
Disulfiram, 1452
Divalproex sodium delayed-release, 1457
Divalproex sodium extended-release, 1458
Docusate sodium, 1481
Donepezil hydrochloride, 1488
Donepezil hydrochloride orally disintegrating, 1492
Doxazosin, 1506
Doxycycline, 1527
Doxycycline hyclate, 1537
Doxycycline hyclate delayed-release, 1539
Doxylamine succinate, 1546
Dronedarone, 1551
Drospirenone and ethinyl estradiol, 1556
Dydrogesterone, 1572
Dyphylline, 1574
Dyphylline and guaifenesin, 1575
Echinacea Species dry extract tablets, 4963
Efavirenz, 1591
Eleuthero root and rhizome dry extract, 4973
Enalapril maleate, 1614
Enalapril maleate and hydrochlorothiazide, 1616
Entacapone, 1632
Entecavir, 1637
Ergocalciferol, 1668
Ergoloid mesylates, 1671
Ergonovine maleate, 1674
Ergotamine tartrate, 1678
Ergotamine tartrate and caffeine, 1681
Ergotamine tartrate sublingual, 1679
Erythromycin, 1691
Erythromycin delayed-release, 1691
Erythromycin estolate, 1694
Erythromycin ethylsuccinate, 1699
Erythromycin stearate, 1706
Escitalopram, 1708
Estazolam, 1723
Estradiol, 1732
Estradiol and norethindrone acetate, 1733
Estrogens, conjugated, 1746
Estrogens, esterified, 1750
Estropipate, 1754
Eszopiclone, 1757
Ethacrynic acid, 1761
Ethambutol hydrochloride, 1764
Ethinyl estradiol, 1768
Ethionamide, 1771
Ethotoin, 1778
Ethynodiol diacetate and ethinyl estradiol, 1781
Ethynodiol diacetate and mestranol, 1781
Etidronate disodium, 1784
Etodolac, 1787
Etodolac extended-release, 1788
Ezetimibe, 1806
famciclovir, 1812
Famotidine, 1819
Felbamate, 1824
Felodipine extended-release, 1827
Fenofibrate, 1837
Fenoprofen calcium, 1845
Ferrous fumarate, 1855
Ferrous fumarate and docusate sodium extended-release, 1856
Ferrous gluconate, 1861
Ferrous sulfate, 1864
Fexofenadine hydrochloride, 1872
Fexofenadine hydrochloride and pseudoephedrine hydrochloride extended-release, 1876
Finasteride, 1890
Flavoxate hydrochloride, 1894
Flecainide acetate, 1898
Fluconazole, 1914
Fludrocortisone acetate, 1923
Fluoxetine, 1962
Fluoxymesterone, 1966
Flurbiprofen, 1980
Fluvoxamine maleate, 2023
Folic acid, 2028
Fosinopril sodium, 2042
Fosinopril sodium and hydrochlorothiazide, 2043
Furazolidone, 2053
Furosemide, 2056
Gabapentin, 2060
Galantamine, 2081
Garlic delayed-release, 5024
Gemfibrozil, 2098
Ginkgo, 5041
Ginseng, American, 4757
Ginseng, Asian, 4778
Glimepiride, 2111
Glipizide, 2115
Glipizide and metformin hydrochloride, 2116
Glucosamine, 5046
Glucosamine and chondroitin sodium sulfate, 5043
Glucosamine, chondroitin sulfate sodium, and methylsulfonylmethane, 5051
Glucosamine and methylsulfonylmethane, 5049
Glyburide, 2126
Glyburide and metformin hydrochloride, 2128
Glycopyrrolate, 2140
Granisetron hydrochloride, 2161
Griseofulvin, 2166
Griseofulvin, ultramicrosize, 2168
Guaifenesin, 2172
Guanabenz acetate, 2177
Guanethidine monosulfate, 2179
Guanfacine, 2180
Guggul, 5072
Halazone for solution, 2182
Haloperidol, 2190
Homatropine methylbromide, 2210
Hydralazine hydrochloride, 2215
Hydrochlorothiazide, 2222
Hydrochlorothiazide and amiloride hydrochloride, 224
Hydrocodone bitartrate, 2224
Hydrocodone bitartrate and acetaminophen, 2224
Hydrocodone bitartrate and homatropine methylbromide, 2225
Hydrocortisone, 2234
Hydroflumethiazide, 2247
Hydromorphone hydrochloride, 2254
Hydroxychloroquine sulfate, 2259
Hydroxyzine hydrochloride, 2267
Hyoscyamine, 2273
Hyoscyamine sulfate, 2278
Ibuprofen, 2285
Ibuprofen and pseudoephedrine hydrochloride, 2286
Imipramine hydrochloride, 2301
Indapamide, 2314
Iodoquinol, 2378
Irbesartan, 2403
Irbesartan and hydrochlorothiazide, 2404
Isoniazid, 2426
Isopropamide iodide, 2428
Isoproterenol hydrochloride, 2434
Isosorbide dinitrate chewable, 2444
Isosorbide dinitrate extended-release, 2445
Isosorbide dinitrate sublingual, 2447
Isosorbide mononitrate, 2449
Isosorbide mononitrate extended-release, 2451
Isoxsuprine hydrochloride, 2463
Ivermectin, 2474
Ivermectin and pyrantel pamoate, 2478
Ketoconazole, 2508
Ketorolac tromethamine, 2515
Labetalol hydrochloride, 2519
Lamivudine, 2527

www.webofpharma.com

Tablets *(continued)*
Lamivudine and zidovudine, 2528
Lamotrigine, 2532
Lamotrigine extended-release, 2534
Lamotrigine, for oral suspension, 2542
Leflunomide, 2560
Letrozole, 2563
Leucovorin calcium, 2571
Levamisole hydrochloride, 2579
Levetiracetam, 2584
Levetiracetam extended-release, 2587
Levocarnitine, 2601
Levocetirizine dihydrochloride, 2603
Levofloxacin, 2609
Levonorgestrel and ethinyl estradiol, 2612
Levorphanol tartrate, 2614
Levothyroxine sodium, 2618
Liothyronine sodium, 2640
Liotrix, 2640
Lipoic acid, alpha, 5126
Lisinopril, 2644
Lisinopril and hydrochlorothiazide, 2646
Lithium carbonate, 2650
Lithium carbonate extended-release, 2651
Loperamide hydrochloride, 2661
Lopinavir and ritonavir, 2670
Loratadine, 2675
Loratadine chewable, 2676
Loratadine orally disintegrating, 2678
Lorazepam, 2684
Losartan potassium, 2687
Losartan potassium and hydrochlorothiazide, 2690
Lysine hydrochloride, 5135
Magaldrate and simethicone chewable, 2707
Magnesia, 2709
Magnesium gluconate, 2717
Magnesium oxide, 2721
Magnesium salicylate, 2724
Magnesium trisilicate, 2729
Maprotiline hydrochloride, 2739
Mebendazole, 2744
Mecamylamine hydrochloride, 2748
Meclizine hydrochloride, 2751
Medroxyprogesterone acetate, 2756
Mefloquine hydrochloride, 2761
Megestrol acetate, 2764
Melatonin, 5144
Meloxicam, 2771
Melphalan, 2774
Memantine hydrochloride, 2776
Menaquinone-7, 5151
Meperidine hydrochloride, 2784
Mephobarbital, 2785
Meprobamate, 2791
Mercaptopurine, 2794
Mesalamine delayed-release, 2805
Mesna, 2809
Metaproterenol sulfate, 2814
Metaxalone, 2819
Metformin hydrochloride, 2822
Metformin hydrochloride extended-release, 2824
Methadone hydrochloride, 2838
Methamphetamine hydrochloride, 2840
Methazolamide, 2842
Methenamine, 2843
Methenamine hippurate, 2845
Methenamine mandelate, 2846
Methimazole, 2848
Methocarbamol, 2851
Methotrexate, 2860
Methscopolamine bromide, 2868
Methyclothiazide, 2872
Methylcellulose, 2876
Methylcobalamin, 5154
Methyldopa, 2878
Methyldopa and hydrochlorothiazide, 2878
Methylergonovine maleate, 2887
Methylphenidate hydrochloride, 2892
Methylphenidate hydrochloride extended-release, 2892
Methylprednisolone, 2899
Methylsulfonylmethane, 5156
Methyltestosterone, 2906
Metoclopramide, 2911
Metolazone, 2914
Metoprolol succinate extended-release, 2918
Metoprolol tartrate, 2926
Metoprolol tartrate and hydrochlorothiazide, 2928
Metronidazole, 2937
Metronidazole extended-release, 2939
Midodrine hydrochloride, 2955
Milk thistle, 5163
Minerals, 5172
Minocycline hydrochloride, 2966
Minocycline hydrochloride extended-release, 2967
Minoxidil, 2974
Mirtazapine, 2978
Mirtazapine orally disintegrating, 2980
Mitotane, 2987
Modafinil, 2991
Memantine hydrochloride, 2994
Moexipril hydrochloride and hydrochlorothiazide, 2996
Molindone hydrochloride, 3000
Montelukast sodium, 3015
Montelukast sodium chewable, 3018
Moxifloxacin, 3036
Mycophenolate mofetil, 3049
Mycophenolic acid delayed-release, 3054
Nabumetone, 3059
Nadolol, 3061
Nadolol and bendroflumethiazide, 3062
Naltrexone hydrochloride, 3071
Naproxen, 3079
Naproxen delayed-release, 3081
Naproxen sodium, 3082
Naproxen sodium and pseudoephedrine hydrochloride extended-release, 3084
Naratriptan, 3091
Nateglinide, 3098
Nefazodone hydrochloride, 3100
Neomycin sulfate, 3104
Neostigmine bromide, 3129
Nevirapine, 3134
Niacin, 3140
Niacinamide, 3147
Niacin extended-release, 3141
Nifedipine extended-release, 3163
Nitrofurantoin, 3180
Nitroglycerin sublingual, 3188
Norethindrone, 3199
Norethindrone acetate, 3205
Norethindrone acetate and ethinyl estradiol, 3208
Norethindrone and ethinyl estradiol, 3201
Norethindrone and mestranol, 3203
Norgestimate and ethinyl estradiol, 3211
Norgestrel, 3213
Norgestrel and ethinyl estradiol, 3214
Nystatin, 3221
Ofloxacin, 3232
Olanzapine, 3235
Olanzapine orally disintegrating, 3240
Olmesartan medoxomil, 3244
Ondansetron, 3268
Ondansetron orally disintegrating, 3271
Orbifloxacin, 3276
Orphenadrine citrate, aspirin, and caffeine, 3288
Orphenadrine citrate extended-release, 3285
Oxandrolone, 3308
Oxaprozin, 3311
Oxcarbazepine, 3319
Oxprenolol hydrochloride extended-release, 3324
Oxtriphylline extended-release, 3325
Oxybutynin chloride, 3328
Oxybutynin chloride extended-release, 3329
Oxycodone and acetaminophen, 3343
Oxycodone and aspirin, 3344
Oxycodone hydrochloride, 3338
Oxycodone hydrochloride extended-release, 3339
Oxymetholone, 3351
Oxymorphone hydrochloride, 3354
Oxymorphone hydrochloride extended-release, 3356
Oxytetracycline, 3361
Pancreatin, 3381
Pancrelipase, 3385
Pantoprazole sodium delayed-release, 3391
Papain, for topical solution, 3396
Papaverine hydrochloride, 3398
Paroxetine, 3412
Paroxetine extended-release, 3413
Penbutolol sulfate, 3425
Penicillamine, 3429
Penicillin G benzathine, 3434
Penicillin G potassium, 3439
Penicillin V, 3453
Penicillin V potassium, 3456
Pentazocine and acetaminophen, 3460
Pentazocine and aspirin, 3461
Pentazocine and naloxone, 3463
Pentoxifylline extended-release, 3471
Pergolide, 3478
Perindopril erbumine, 3483
Perphenazine, 3486
Perphenazine and amitriptyline hydrochloride, 3487
Phenazopyridine hydrochloride, 3491
Phendimetrazine tartrate, 3494
Phenelzine sulfate, 3496
Phenobarbital, 3499
Phentermine hydrochloride, 3507
Phenylbutazone, 3512
Phenylephrine hydrochloride, 3520
Phenytoin chewable, 3528
Phytonadione, 3539
Pilocarpine hydrochloride, 3543
Pimozide, 3548
Pindolol, 3550
Pioglitazone, 3553
Pioglitazone and glimepiride, 3554
Pioglitazone and metformin hydrochloride, 3558
Piperazine citrate, 3575
Potassium and sodium bicarbonates and citric acid effervescent, for oral solution, 3598
Potassium bicarbonate effervescent, for oral solution, 3596
Potassium bicarbonate and potassium chloride effervescent, for oral solution, 3597
Potassium chloride extended-release, 3605

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Tablets *(continued)*
Potassium chloride, potassium bicarbonate, and potassium citrate effervescent, for oral solution, 3609
Potassium citrate, 5196
Potassium citrate extended-release, 3614
Potassium gluconate, 3620
Potassium iodide, 3624
Potassium iodide delayed-release, 3625
Pravastatin sodium, 3647
Praziquantel, 3650
Prednisolone, 3661
Prednisone, 3672
Primaquine phosphate, 3681
Primidone, 3685
Probenecid, 3687
Probenecid and colchicine, 3687
Procainamide hydrochloride, 3690
Procainamide hydrochloride extended-release, 3691
Prochlorperazine maleate, 3701
Procyclidine hydrochloride, 3704
Promazine hydrochloride, 3714
Promethazine hydrochloride, 3719
Propafenone hydrochloride, 3734
Propantheline bromide, 3737
Propranolol hydrochloride, 3750
Propranolol hydrochloride and hydrochlorothiazide, 3751
Propylthiouracil, 3756
Protriptyline hydrochloride, 3760
Pseudoephedrine hydrochloride, 3763
Pseudoephedrine hydrochloride extended-release, 3764
Pyrazinamide, 3777
Pyridostigmine bromide, 3781
Pyridoxine hydrochloride, 3784
Pyrilamine maleate, 3786
Pyrimethamine, 3787
Pyrvinium pamoate, 3790
Quazepam, 3791
Quetiapine, 3792
Quetiapine, extended-release, 3795
Quinapril, 3806
Quinapril and hydrochlorothiazide, 3803
Quinidine gluconate extended-release, 3810
Quinidine sulfate, 3814
Quinidine sulfate extended-release, 3816
Quinine sulfate, 3821
Raloxifene hydrochloride, 3830
Raltegravir, 3834
Raltegravir chewable, 3835
Ramipril, 3841
Ranitidine, 3847
Rauwolfia serpentina, 3851
Repaglinide, 3856
Reserpine, 3858
Reserpine and chlorothiazide, 3859
Rhodiola rosea, 5227
Ribavirin, 3866
Riboflavin, 3870
Rifampin, isoniazid, and pyrazinamide, 3879
Rifampin, isoniazid, pyrazinamide, and ethambutol hydrochloride, 3881
Riluzole, 3883
Rimantadine hydrochloride, 3885
Risedronate sodium, 3902
Risperidone, 3907
Risperidone orally disintegrating, 3908
Ritodrine hydrochloride, 3911
Ritonavir, 3920
Rizatriptan benzoate, 3928
Rizatriptan benzoate orally disintegrating, 3930
Ropinirole, 3934
Ropinirole extended-release, 3936
Rosuvastatin, 3950
Rufinamide, 3960
Saccharin sodium, 3968
St. John's wort flowering top dry extract, 5244
Salsalate, 3982
Scopolamine hydrobromide, 4013
Selegiline hydrochloride, 4020
Sennosides, 4028
Sertraline hydrochloride, 4033
Sildenafil, 4038
Simethicone, 4045
Simvastatin, 4047
Sitagliptin, 4051
Sodium bicarbonate, 4062
Sodium chloride, 4068
Sodium chloride, for solution, 4069
Sodium fluoride, 4073
Sodium salicylate, 4094
Sotalol hydrochloride, 4105
Soy isoflavones, 5278
Spironolactone, 4112
Spironolactone and hydrochlorothiazide, 4113
Spirulina, 5283
Stanozolol, 4118
Sucralfate, 4131
Sulfadiazine, 4148
Sulfadimethoxine, 4153
Sulfadoxine and pyrimethamine, 4155
Sulfamethizole, 4158
Sulfamethoxazole, 4161
Sulfamethoxazole and trimethoprim, 4166
Sulfapyridine, 4167
Sulfasalazine, 4170
Sulfasalazine delayed-release, 4170
Sulfinpyrazone, 4173
Sulfisoxazole, 4175
Sulindac, 4178
Sumatriptan, 4185
Tadalafil, 4205
Tamoxifen citrate, 4210
Telmisartan, 4240
Telmisartan and amlodipine, 4242
Telmisartan and hydrochlorothiazide, 4246
Terazosin, 4263
Terbinafine, 4268
Terbutaline sulfate, 4273
Testolactone, 4286
Tetracycline hydrochloride, 4311
Tetracycline hydrochloride and novobiocin sodium, 4312
Tetracycline hydrochloride, novobiocin sodium, and prednisolone, 4312
Theophylline, 4328
Theophylline, ephedrine hydrochloride, and phenobarbital, 4330
Theophylline sodium glycinate, 4334
Thiabendazole chewable, 4336
Thiamine hydrochloride, 4339
Thiethylperazine maleate, 4343
Thioguanine, 4350
Thioridazine hydrochloride, 4355
Thyroid, 4364
Ticlopidine hydrochloride, 4378
Tienchi ginseng root and rhizome dry extract, 5311
Tienchi ginseng root and rhizome powder, 5306
Timolol maleate, 4389
Timolol maleate and hydrochlorothiazide, 4391
Tizanidine, 4399
Tolazamide, 4417
Tolbutamide, 4419
Tolcapone, 4421
Tolmetin sodium, 4424
Topiramate, 4434
Torsemide, 4439
Tramadol hydrochloride, 4444
Tramadol hydrochloride and acetaminophen, 4450
Tramadol hydrochloride extended-release, 4446
Trandolapril, 4454
Trandolapril and verapamil hydrochloride extended-release, 4456
Tranexamic acid, 4464
Tranylcypromine, 4466
Trazodone hydrochloride, 4474
Triamcinolone, 4482
Triamterene and hydrochlorothiazide, 4499
Triazolam, 4502
Trifluoperazine hydrochloride, 4512
Triflupromazine hydrochloride, 4515
Trihexyphenidyl hydrochloride, 4519
Trimeprazine tartrate, 4523
Trimethoprim, 4526
Trioxsalen, 4529
Tripelennamine hydrochloride, 4531
Triprolidine hydrochloride, 4534
Triprolidine and pseudoephedrine hydrochlorides, 4535
Trisulfapyrimidines, 4537
Trospium chloride, 4543
Ubidecarenone, 5321
Ursodiol, 4559
Valacyclovir, 4562
Valerian, 5329
Valganciclovir, 4566
Valsartan, 4579
Valsartan and hydrochlorothiazide, 4580
Venlafaxine, 4595
Verapamil hydrochloride, 4610
Verapamil hydrochloride extended-release, 4611
Vigabatrin, 4621
Vinpocetine, 5337
Vitamin A, 4635
Vitamins with minerals, oil-soluble, 5378
Vitamins with minerals, oil- and water-soluble, 5476
Vitamins with minerals, water-soluble, 5552
Vitamins, oil-soluble, 5356
Vitamins, oil- and water-soluble, 5419
Vitamins, water-soluble, 5512
Warfarin sodium, 4650
Zalcitabine, 4667
Zidovudine, 4678
Zinc citrate, 5575
Zinc gluconate, 4686
Zinc sulfate, 4694
Zolmitriptan, 4706
Zolmitriptan orally disintegrating, 4709
Zolpidem tartrate, 4712
Zolpidem tartrate extended-release, 4714

Tacrolimus, 4193
capsules, 4196
oral suspension, 4202
Tadalafil, 4203
tablets, 4205

www.webofpharma.com

Tadalafil compounded
oral suspension, 4207
Tagatose, 6087
Talc, 4207
Tamoxifen citrate, 4209
tablets, 4210
Tamsulosin hydrochloride, 4211
capsules, 4213
Tangerine peel, 5292
dry extract, 5294
powder , 5296
Tannic acid, 4223, 6209
TS, 6236
Tape, adhesive, 4223
Tapioca starch, 6066
Tartaric acid, 6088, 6209
TS, 6237
Taurine, 4223
Tazobactam, 4224
and piperacillin for injection, 3567
Tc 99m
albumin aggregated injection, technetium, 4226
bicisate injection, technetium, 4227
disofenin injection, technetium, 4227
exametazime injection, technetium, 4228
mebrofenin injection, technetium, 4229
medronate injection, technetium, 4230
mertiatide injection, technetium, 4231
oxidronate injection, technetium, 4232
pentetate injection, technetium, 4232
pertechnetate injection, sodium, 4233
(pyro- and trimeta-) phosphates injection, technetium, 4235
pyrophosphate injection, technetium, 4234
red blood cells injection, technetium, 4235
sestamibi injection, technetium, 4236
succimer injection, technetium, 4237
sulfur colloid injection, technetium, 4238
tetrofosmin injection, technetium, 4238
T-dodecyl mercaptan ethoxylate, 6163
Technetium
Tc 99m albumin aggregated injection, 4226
Tc 99m bicisate injection, 4227
Tc 99m disofenin injection, 4227
Tc 99m exametazime injection, 4228
Tc 99m mebrofenin injection, 4229
Tc 99m medronate injection, 4230
Tc 99m mertiatide injection, 4231
Tc 99m oxidronate injection, 4232
Tc 99m pentetate injection, 4232
Tc 99m pertechnetate injection, sodium, 4233
Tc 99m pyrophosphate injection, 4234
Tc 99m (pyro- and trimeta-) phosphates injection, 4235
Tc 99m red blood cells injection, 4235
Tc 99m sestamibi injection, 4236
Tc 99m succimer injection, 4237
Tc 99m sulfur colloid injection, 4238
Tc 99m tetrofosmin injection, 4238
Telmisartan, 4239
and hydrochlorothiazide tablets, 4246
tablets, 4240
Telmisartan and amlodipine
tablets, 4242
Temazepam, 4250
capsules, 4251
Temozolomide, 4252
capsules, 4253
for injection, 4256
oral suspension, 4257
Temperature
congealing ⟨651⟩, 6874
Teniposide, 4258
injection, 4259
Tensile strength ⟨881⟩, 7179
Terazosin
capsules, 4261
hydrochloride, 4264
tablets, 4263
Terbinafine
hydrochloride, 4269
oral suspension, 4267
tablets, 4268
Terbutaline
sulfate, 4271
sulfate injection, 4272
sulfate tablets, 4273
oral suspension, 4273
Terconazole, 4274
Teriparatide, 4275
injection, 4280
Terminally sterilized pharmaceutical products—parametric release ⟨1222⟩, 8142
Terpin hydrate, 4283
and codeine oral solution, 4284
oral solution, 4284
tert-Butyl hydroperoxide solution, 6209
Tertiary butyl alcohol, 6134, 6209
Test for 1,6-anhydro derivative for enoxaparin sodium ⟨207⟩, 6605
Testolactone, 4285
tablets, 4286
Testosterone, 4287
benzoate, 6209
cypionate, 4288
cypionate injection, 4289
enanthate, 4289
enanthate injection, 4290
injectable suspension, 4287
propionate, 4290
propionate injection, 4291
topical solution, 4291
Test papers
and indicator, 6219
indicators and indicator, 6220
Test solutions, 6225
Tetanus
immune globulin, 4292
2′,4′,5′,7′-Tetrabromofluorescein, 6209
Tetrabromophenolphthalein ethyl ester, 6209
TS, 6237
Tetrabutylammonium
bromide, 6209
hydrogen sulfate, 6209
hydrogen sulfate ion pairing reagent, 6209
hydroxide, 1.0 M in methanol, 6210
hydroxide, 0.4 M aqueous, 6210
hydroxide 30-hydrate, 6210
hydroxide in methanol/isopropyl alcohol (0.1 N), 6251
hydroxide, tenth-normal (0.1 N), 6250
iodide, 6210
phosphate, 6210
Tetrabutylammonium hydrogen sulfate
0.02 M TS, 6237
Tetrabutylammonium hydroxide
25% TS, 6237
Tetrabutylammonium hydroxide, 40 percent in water, 6210
Tetracaine, 4292
hydrochloride, 4295
hydrochloride, benzocaine, and butamben topical aerosol, 513
hydrochloride, benzocaine, and butamben gel, 514
hydrochloride, benzocaine, and butamben ointment, 516
hydrochloride, benzocaine, and butamben topical solution, 517
hydrochloride cream, 4296
hydrochloride in dextrose injection, 4300
hydrochloride injection, 4297
hydrochloride for injection, 4298
hydrochloride, neomycin sulfate, and isoflupredone acetate ointment, 3111
hydrochloride, neomycin sulfate, and isoflupredone acetate topical powder, 3112
hydrochloride ophthalmic solution, 4299
hydrochloride topical solution, 4299
and menthol ointment, 4294
ointment, 4294
and procaine hydrochlorides and levonordefrin injection, 3695
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled, 6210
2,3,7,8-Tetrachlorodibenzofuran, ^{13}C-labeled, 6210
1,1,2,2-Tetrachloroethane, 6210
Tetracosane, 6210
Tetracycline, 4301
boluses, 4302
hydrochloride, 4303
hydrochloride capsules, 4305
hydrochloride for injection, 4307
hydrochloride, novobiocin sodium, and prednisolone tablets, 4312
hydrochloride and novobiocin sodium tablets, 4312
hydrochloride and nystatin capsules, 4313
hydrochloride ointment, 4307
hydrochloride ophthalmic ointment, 4308
hydrochloride ophthalmic suspension, 4310
hydrochloride soluble powder, 4309
hydrochloride for topical solution, 4309
hydrochloride oral suspension, 4310
hydrochloride tablets, 4311
oral suspension, 4302
Tetradecane, 6210
Tetradecylammonium bromide, 6210
Tetraethylammonium chloride, 6210
Tetraethylammonium perchlorate, 6210
Tetraethylene glycol, 6210
Tetraethylenepentamine, 6210
Tetraheptylammonium bromide, 6210
Tetrahexylammonium hydrogen sulfate, 6210
Tetrahydrofuran, 6211
peroxide-free, 6211
stabilizer-free, 6211
Tetrahydro-2-furancarboxylic acid, 6211
N-(2-Tetrahydrofuroyl)piperazine, 6211
1,2,3,4-Tetrahydronaphthalene, 6211
Tetrahydrozoline hydrochloride, 4314
nasal solution, 4315
ophthalmic solution, 4315
Tetramethylammonium
bromide, 6211
bromide, tenth-molar (0.1 M), 6251
chloride, 6211
chloride, tenth-molar (0.1 M), 6251
hydroxide, 6211
hydroxide, pentahydrate, 6211
hydroxide solution in methanol, 6211
hydroxide TS, 6237
nitrate, 6211
Tetramethylbenzidine, 6211
1,1,3,3-Tetramethylbutylamine, 6211
4,4′-Tetramethyldiaminodiphenylmethane, 6211
Tetramethylethylenediamine, 6211
Tetramethylsilane, 6211

www.webofpharma.com

Tetrapropylammonium
chloride, 6211
Tetrasodium ethylenediaminetetraacetate, 6212
Thalidomide, 4316
capsules, 4318
Thallous chloride, 6212
Tl 201 injection, 4318
Theobromine, 6212
Theophylline, 4319
capsules, 4321
extended-release capsules, 4322
in dextrose injection, 4328
ephedrine hydrochloride, and phenobarbital tablets, 4330
and guaifenesin capsules, 4331
and guaifenesin oral solution, 4332
sodium glycinate, 4333
sodium glycinate oral solution, 4334
sodium glycinate tablets, 4334
oral solution, 4325
oral suspension, 4327
tablets, 4328
Theory and practice of electrical conductivity measurements of solutions ⟨1644⟩, 8423
Thermal analysis ⟨891⟩, 7180
Thiabendazole, 4335
chewable tablets, 4336
oral suspension, 4335
Thiamine
hydrochloride, 4337
hydrochloride injection, 4338
hydrochloride oral solution, 4338
hydrochloride tablets, 4339
mononitrate, 4340
mononitrate oral solution, 4341
Thiamine assay ⟨531⟩, 6756
Thiazole yellow, 6212
paper, 6222
Thiethylperazine maleate, 4342
suppositories, 4342
tablets, 4343
Thimerosal, 4344
topical aerosol, 4346
topical solution, 4347
tincture, 4348
Thin-layer chromatographic identification test ⟨201⟩, 6600
Thioacetamide, 6212
TS, 6237
Thioacetamide-glycerin base TS, 6237
2-Thiobarbituric acid, 6212
2,2′-Thiodiethanol, 6212
Thioglycolic acid, 6212
Thioguanine, 4349
tablets, 4350
Thionine acetate, 6212
Thiopental sodium, 4351
for injection, 4352
Thioridazine, 4353
hydrochloride, 4353
hydrochloride oral solution, 4354
hydrochloride tablets, 4355
Thiostrepton, 4355
nystatin, neomycin sulfate, and triamcinolone acetonide cream, 3223
nystatin, neomycin sulfate, and triamcinolone acetonide ointment, 3223
Thiotepa, 4356
for injection, 4357
Thiothixene, 4358
capsules, 4359
hydrochloride, 4360
hydrochloride injection, 4360
hydrochloride for injection, 4361
hydrochloride oral solution, 4361
Thiourea, 6212
Thorium nitrate, 6212
TS, 6237
Threonine, 4362
Thrombin human, 6212
Thromboplastin, 6212
Thymidine, 6212
Thymol, 6088, 6212
blue, 6221
blue TS, 6237
Thymolphthalein, 6221
TS, 6237
Thyroglobulin, 6213
Thyroid, 4362
tablets, 4364
Tiagabine hydrochloride, 4365
oral suspension, 4367
Tiamulin, 4368
fumarate, 4369
Ticarcillin
and clavulanic acid injection, 4371
and clavulanic acid for injection, 4372
disodium, 4373
for injection, 4370
monosodium, 4375
Ticlopidine hydrochloride, 4376
tablets, 4378
Tienchi ginseng root and rhizome, 5299
dry extract capsules, 5309
powder capsules, 5304
dry extract, 5307
powder, 5301
dry extract tablets, 5311
powder tablets, 5306
Tigecycline, 4379
for injection, 4381
Tiletamine
hydrochloride, 4382
and zolazepam for injection, 4383
Tilmicosin, 4384
injection, 4385
Timolol
maleate, 4387
maleate and hydrochlorothiazide tablets, 4391
maleate ophthalmic solution, 4389
maleate tablets, 4389
Timolol maleate
and dorzolamide hydrochloride ophthalmic solution, 1499
Tin, 6213

Tincture

Belladonna, 488
Benzethonium chloride, 501
Benzoin, compound, 522
Capsicum, 732
Cardamom, compound, 5689
Ginger, 5030
Green soap, 2164
Iodine, 2363
Iodine, strong, 2364
Lemon, 5857
Opium, 3274
Orange peel, sweet, 5915
Rhodiola rosea, 5223
Thimerosal, 4348
Tolu balsam, 6090
Valerian, 5330
Vanilla, 6098

Tinidazole, 4392
Tioconazole, 4393
Tissue human amnion chorion membrane dehydrated, 4395
Titanium
dioxide, 4396
tetrachloride, 6213
trichloride, 6213
trichloride–sulfuric acid TS, 6237
trichloride, tenth-normal (0.1 N), 6251
trichloride TS, 6237
Titration, nitrite ⟨451⟩, 6709
Titrimetry ⟨541⟩, 6764
Tizanidine
hydrochloride, 4397
tablets, 4399
Tl 201
injection, thallous chloride, 4318
Tobramycin, 4401
and dexamethasone ophthalmic ointment, 4409
and dexamethasone ophthalmic suspension, 4411
and fluorometholone acetate ophthalmic suspension, 4412
inhalation solution, 4406
injection, 4402
for injection, 4403
ophthalmic ointment, 4405
ophthalmic solution, 4408
sulfate, 4414
Tocopherols excipient, 6089
Tolazamide, 4415
tablets, 4417
Tolazoline hydrochloride, 4417
injection, 4418
Tolbutamide, 4418
for injection, 4419
tablets, 4419
Tolcapone, 4420
tablets, 4421
o-Tolidine, 6213
Tolmetin sodium, 4422
capsules, 4423
tablets, 4424
Tolnaftate, 4425
topical aerosol, 4425
cream, 4426
gel, 4426
topical powder, 4426
topical solution, 4427
Tolterodine tartrate, 4427
Tolualdehyde, 6213
p-Tolualdehyde, 6213
Tolu balsam, 4429
syrup, 6090
tincture, 6090
Toluene, 6213
p-Toluenesulfonic acid, 6213
TS, 6237
p-Toluenesulfonyl-L-arginine methyl ester hydrochloride, 6213
p-Toluic acid, 6213
Toluidine
blue, 6213
blue O, 6213
o-Toluidine, 6213
p-Toluidine, 6213
Tomato extract containing lycopene, 5133
Topical aerosols ⟨603⟩, 6845

www.webofpharma.com

Topical and transdermal drug products—product quality tests ⟨3⟩, 6415

Topical solution

Aluminum acetate, 181
Aluminum subacetate, 193
Aluminum sulfate and calcium acetate for, 194
Aluminum sulfate and calcium acetate tablets for, 195
Benzethonium chloride, 501
Benzocaine, 511
Benzocaine, butamben, and tetracaine hydrochloride, 517
Calcium hydroxide, 702
Carbamide peroxide, 747
Carbol-fuchsin, 765
Cetylpyridinium chloride, 923
Chlorhexidine acetate, 947
Chlorhexidine gluconate, 951
Ciclopirox, 996
Clindamycin phosphate, 1072
Clobetasol propionate, 1082
Clotrimazole, 1122
Coal tar, 1133
Cocaine hydrochloride tablets for, 1134
Diclofenac sodium , 1352
Diethyltoluamide, 1373
Dimethyl sulfoxide, 1414
Dyclonine hydrochloride, 1571
Erythromycin, 1690
Fluocinolone acetonide, 1936
Fluocinonide, 1939
Fluorouracil, 1957
Gentamicin sulfate and betamethasone valerate, 2105
Gentian violet, 2109
Halcinonide, 2186
Hydrogen peroxide, 2249
Hydroquinone, 2255
Iodine, 2362
Ivermectin, 2476
Lidocaine hydrochloride, 2627
Mafenide acetate for, 2704
Methoxsalen, 2865
Minoxidil, 2975
Mometasone furoate, 3005
Myrrh, 3057
Nitrofurazone, 3183
Nitromersol, 3190
Papain tablets for, 3396
Phenol, camphorated, 3502
Podophyllum resin, 3582
Povidone–iodine, 3636
Sodium fluoride and acidulated phosphate, 4074
Sodium hypochlorite, 4077
Testosterone, 4291
Tetracaine hydrochloride, 4299
Tetracycline hydrochloride for, 4309
Thimerosal, 4347
Tolnaftate, 4427
Tretinoin, 4479

Topical suspension

Calamine, 665
Calamine, phenolated, 665
Ciclopirox olamine, 998
Clindamycin phosphate, 1072
Penicillin G, neomycin, polymyxin B, hydrocortisone acetate, and hydrocortisone sodium succinate, 3430
Penicillin G procaine, neomycin and polymyxin B sulfates, and hydrocortisone acetate, 3448
Resorcinol and sulfur, 3862
Selenium sulfide, 4023
Sulfacetamide sodium, 4143
Zinc sulfide, 4695

Topiramate, 4429
  capsules, 4432
  tablets, 4434
Topiramate compounded
  oral suspension, 4436
Torsemide, 4437
  tablets, 4439
Tosylchloramide sodium, 6213
Total organic carbon ⟨643⟩, 6867
Tragacanth, 6091
Tramadol hydrochloride, 4441
  and acetaminophen oral suspension, 4452
  and acetaminophen tablets, 4450
  oral suspension, 4443
  tablets, 4444
  extended-release tablets, 4446
Tramadol hydrochloride compounded, veterinary
  oral suspension, 4443
Trandolapril, 4453
  tablets, 4454
Trandolapril and verapamil hydrochloride extended-release tablets, 4456
Tranexamic acid, 4462
  injection, 4463
Transdermal system
  clonidine, 1105
  nicotine, 3153
Transfer of analytical procedures ⟨1224⟩, 8164
Tranylcypromine
  sulfate, 4468
  tablets, 4466
Travoprost, 4469
  ophthalmic solution, 4471
Trazodone hydrochloride, 4472
  tablets, 4474
Trehalose, 6091
Trenbolone acetate, 4476
Tretinoin, 4478
  cream, 4478
  gel, 4479
  topical solution, 4479
Triacetin, 4480, 6213
n-Triacontane, 6213
Triamcinolone, 4481
  acetonide, 4482
  acetonide cream, 4484
  acetonide dental paste, 4485
  acetonide injectable suspension, 4490
  acetonide topical aerosol, 4483
  acetonide lotion, 4485
  acetonide and neomycin sulfate cream, 3129
  acetonide and nystatin cream, 3224
  acetonide, nystatin, neomycin sulfate, and gramicidin cream, 3222
  acetonide, nystatin, neomycin sulfate, and gramicidin ointment, 3222
  acetonide, nystatin, neomycin sulfate and thiostrepton cream, 3223
  acetonide, nystatin, neomycin sulfate, and thiostrepton ointment, 3223
  acetonide and nystatin ointment, 3225
  acetonide ointment, 4485
  acetonide nasal spray, 4485
  diacetate, 4490
  diacetate injectable suspension, 4491
  diacetate oral solution, 4491
  hexacetonide, 4492
  hexacetonide injectable suspension, 4493
  tablets, 4482
2,4,6-Triamino-5-nitrosopyrimidine, 6213
Triamterene, 4494
  capsules, 4495
  and hydrochlorothiazide capsules, 4496
  and hydrochlorothiazide tablets, 4499
Triazolam, 4501, 6213
  tablets, 4502
Tribasic calcium phosphate, 5657
Tribasic sodium phosphate, 6021
Tributyl
  citrate, 6093
  phosphate, 6213
Tributylethylammonium hydroxide, 6214
Tributyrin, 6214
Trichlormethiazide, 4502
Trichloroacetic acid, 6214
Trichloroethane, 6214
Trichloroethanol2,2,2-, 6214
Trichlorofluoromethane, 6214
Trichloromonofluoromethane, 6093
Trichlorotrifluoroethane, 6214
Tricitrates oral solution, 4504
Triclocarban, 4505
Triclosan, 4507
Trientine hydrochloride, 4509
  capsules, 4510
Triethanolamine, 6214
Triethylamine, 6214
  hydrochloride, 6214
  phosphate, 6214
Triethylammonium Acetate 1M, 6214
Triethyl citrate, 6094
Triethylenediamine, 6214
Triethylene glycol, 6214
Trifluoperazine
  hydrochloride, 4511
  hydrochloride injection, 4512
  hydrochloride tablets, 4512
  oral solution, 4511
Trifluoroacetic
  acid, 6214
  anhydride, 6214
Trifluoroacetic acid ethyl ester, 6214
Trifluoroacetic acid (TFA) in peptides ⟨503.1⟩, 6743
0.1% Trifluoroacetic acid TS, 6237
2,2,2-Trifluoroethanol, 6215
2,2,2-Trifluoroethyldifluoromethyl ether, 6215
(*m*-Trifluoromethylphenyl) trimethylammonium hydroxide in methanol, 6215
5-(Trifluoromethyl)uracil, 6215
α,α,α-Trifluoro-*p*-cresol, 6215
Trifluorovinyl chloride polymer, 6215
Triflupromazine, 4513
  hydrochloride, 4514
  hydrochloride injection, 4515
  hydrochloride tablets, 4515
  oral suspension, 4514
Trifluridine, 4516
Triglycerides medium-chain, 6095

www.webofpharma.com

Trihexyphenidyl hydrochloride, 4517
extended-release capsules, 4518
oral solution, 4519
tablets, 4519
Trikates oral solution, 4521
Triketohydrindene hydrate
TS, 6232, 6237
Trimeprazine
oral solution, 4522
tartrate, 4521
tartrate tablets, 4523
Trimethobenzamide hydrochloride, 4524
capsules, 4524
injection, 4525
Trimethoprim, 4525
and polymyxin B sulfate ophthalmic solution, 3592
and sulfamethoxazole injection, 4162
and sulfamethoxazole oral suspension, 4163
and sulfamethoxazole tablets, 4166
sulfate, 4527
tablets, 4526
Trimethylacethydrazide ammonium chloride, 6168, 6215
Trimethylamine hydrochloride, 6215
Trimethylchlorosilane, 6215
2,2,4-Trimethylpentane, 6174, 6215
2,4,6-Trimethylpyridine, 6215
N-(Trimethylsilyl)-imidazole, 6215
Trimethyltin bromide, 6215
Trimipramine maleate, 4527
2,4,6-Trinitrobenzenesulfonic acid, 6215
Trinitrophenol, 6215
TS, 6233, 6237
Trioctylphosphine oxide, 6215
Trioxsalen, 4529
tablets, 4529
Tripelennamine hydrochloride, 4530
injection, 4531
tablets, 4531
1,3,5-Triphenylbenzene, 6215
Triphenylene, 6215
Triphenylmethane, 6215
Triphenylmethanol, 6216
Triphenylphosphine oxide, 6216
Triphenyltetrazolium
chloride, 6216
chloride TS, 6237
Triprolidine
hydrochloride, 4532
hydrochloride oral solution, 4533
hydrochloride tablets, 4534
and pseudoephedrine hydrochlorides oral solution, 4534
and pseudoephedrine hydrochlorides tablets, 4535
Tris(2-aminoethyl)amine, 6216
Tris(hydroxymethyl)aminomethane, 6216
acetate, 6216
hydrochloride, 6216
N-Tris(hydroxymethyl)methylglycine, 6216
Trisulfapyrimidines
oral suspension, 4536
tablets, 4537
Tritirachium album proteinase K, 6216
Trolamine, 6097
salicylate, 4537
Tromethamine, 4538, 6216
carboprost, 771
carboprost, injection, 772
for injection, 4538
Tropaeolin OO, 6216
Tropic acid, 6216
Tropicamide, 4539
ophthalmic solution, 4541
Tropine, 6216
Trospium chloride, 4541
tablets, 4543
Trypan blue, 6216
Trypsin, crystallized, 4545
Tryptamine hydrochloride, 6216
Tryptone, 6216
Tryptophan, 4546
5-Hydroxy-L-, 5313
L-Tryptophane, 6216
Tuberculin purified protein derivative *(Tuberculin PPD)*, 6216
Tubocurarine chloride, 4547, 6217
injection, 4548
Tungstic acid, 6217
Turmeric, 5314
powdered, 5316
extract, powdered, 5317
Turmeric paper, 6222
Tylosin, 4548
granulated, 4549
injection, 4550
tartrate, 4550
Tyloxapol, 4551
Tyrosine, 4553
L-Tyrosine disodium, 6217
Tyrosol, 6217
Tyrothricin, 4554

U

Ubidecarenone, 5319
capsules, 5320
tablets, 5321
Ubiquinol, 5322
capsules, 5323
Ultraviolet-visible spectroscopy ⟨857⟩, 7166
Ultraviolet-visible spectroscopy—theory and practice ⟨1857⟩, 8670
Undecylenic acid, 4554
ointment, compound, 4554
Uniformity of dosage units ⟨905⟩, 7183
Uracil, 6217
Uranyl acetate, 6217
cobalt, TS, 6227
zinc, TS, 6237
Urea, 4555, 6217
C 13, 766
C 13 for oral solution, 767
C 14 capsules, 768
for injection, 4557
Urea compounded irrigation, 4556
Urethane, 6217
Uridine, 6217
Ursodiol, 4557
capsules, 4558
oral suspension, 4559
tablets, 4559
USP and NF excipients listed by category, 5584
USP policies, xxviii
USP reference standards ⟨11⟩, 6442

V

Vaccines for human use
bacterial vaccines ⟨1238⟩, 8338
general considerations ⟨1235⟩, 8291
polysaccharide and glycoconjugate vaccines ⟨1234⟩, 8276
Vaccinia immune globulin, 4561
Valacyclovir
oral suspension, 4561
tablets, 4562
Valacyclovir hydrochloride, 4563
Valerian, 5324
extract, powdered, 5327
powdered, 5326
tablets, 5329
tincture, 5330
Valerian root
powder capsules, 5333
Valerian root dry
extract capsules, 5332
Valeric acid, 6217
Valerophenone, 6217
Valganciclovir
hydrochloride, 4568
tablets, 4566
Validation
of alternative microbiological methods ⟨1223⟩, 8144
of compendial procedures ⟨1225⟩, 8166
of microbial recovery from pharmacopeial articles ⟨1227⟩, 8172
Validation of alternative methods to antibiotic microbial assays ⟨1223.1⟩, 8157
Valine, 4571
Valproate sodium
injection, 4572
Valproic acid, 4572
capsules, 4573
oral solution, 4574
Valrubicin, 4575
intravesical solution, 4577
Valsartan, 4577
tablets, 4579
amlodipine, and hydrochlorothiazide tablets, 279
and amlodipine tablets, 275
and hydrochlorothiazide tablets, 4580
Vanadium pentoxide, 6217
Vanadyl sulfate, 6217
Vancomycin, 4583
hydrochloride, 4586
hydrochloride capsules, 4588
hydrochloride for injection, 4588
hydrochloride for oral solution, 4590
injection, 4585
Vancomycin hydrochloride compounded oral solution, 4589
Vanilla, 6097
tincture, 6098
Vanillin, 6098
Vapor phase sterilization ⟨1229.11⟩, 8231
Varicella-zoster immune globulin, 4592
Vasopressin, 4592
injection, 4593
Vecuronium bromide, 4593
Vegetable oil, hydrogenated, 6099
Vehicle
for oral solution, 5913
for oral solution, sugar free, 5913
for oral suspension, 5913
suspension structured, 6086
suspension structured, sugar-free, 6087

www.webofpharma.com

Venlafaxine
hydrochloride, 4597
hydrochloride extended-release capsules, 4598
tablets, 4595
Verapamil hydrochloride, 4604
extended-release capsules, 4605
injection, 4607
oral solution, 4609
oral suspension, 4609
tablets, 4610
extended-release tablets, 4611
Vardenafil
hydrochloride, 4590
Verification of compendial procedures ⟨1226⟩, 8171
Verteporfin, 4615
for injection, 4617

Veterinary

Atenolol compounded oral suspension, 409
Benazepril hydrochloride compounded oral suspension, 490
Buprenorphine compounded buccal solution, 615
Doxycycline compounded oral suspension, veterinary, 1530
Enalapril maleate compounded oral suspension, 1613
Methylene blue injection, 2884
Pergolide oral suspension, 3478
Potassium bromide oral solution, 3600
Prednisolone compounded oral suspension, 3664
Sodium bromide injection, 4063
Sodium bromide oral solution, 4063
Spironolactone compounded oral suspension, 4111
Tramadol hydrochloride compounded oral suspension, 4443
Voriconazole compounded ophthalmic solution, 4645

Vibrational circular dichroism spectroscopy ⟨782⟩, 7002
Vibrational circular dichroism spectroscopytheory and practice ⟨1782⟩, 8549
Vigabatrin, 4618
for oral solution, 4619
tablets, 4621
Vinblastine sulfate, 4623
for injection, 4624
Vincristine sulfate, 4625
injection, 4626
for injection, 4628
Vinorelbine
injection, 4631
tartrate, 4630
Vinpocetine, 5335
capsules, 5336
tablets, 5337
Vinyl acetate, 6217
2-Vinylpyridine, 6218
Vinylpyrrolidinone, 6218
Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin ⟨1050⟩, 7459
Virology test methods ⟨1237⟩, 8319
Virus testing of human plasma for further manufacture ⟨1240⟩, 8350
Viscosity—capillary methods ⟨911⟩, 7187
Viscosity—pressure driven methods ⟨914⟩, 7195
Viscosity—rolling ball method ⟨913⟩, 7193
Viscosity—rotational methods ⟨912⟩, 7189
Visible particulates in injections ⟨790⟩, 7021
Visual Comparison ⟨630⟩, 6865
Visual inspection of injections, 8587
Vitamin
A, 4632
A assay ⟨571⟩, 6801
A capsules, 4633
A oral liquid preparation, 4634
A tablets, 4635
B_{12} activity assay ⟨171⟩, 6584
C assay ⟨580⟩, 6805
C and zinc lozenges, 5576
D assay ⟨581⟩, 6808
D and calcium with minerals tablets, 4845
D with calcium tablets, 4843
E, 4637
E assay ⟨551⟩, 6767
E capsules, 4639
E polyethylene glycol succinate, 6099
E preparation, 4641
Vitamins
capsules, oil-soluble, 5346
capsules, oil- and water-soluble, 5389
capsules, water-soluble, 5499
with minerals capsules, oil- and water-soluble, 5435
with minerals capsules, water-soluble, 5523
with minerals oral solution, oil- and water-soluble, 5462
with minerals oral solution, water-soluble, 5543
with minerals tablets, oil- and water-soluble, 5476
with minerals tablets, water-soluble, 5552
with minerals capsules, oil-soluble, 5361
with minerals oral solution, oil-soluble, 5372
with minerals tablets, oil-soluble, 5378
oral solution, oil-soluble, 5353
oral solution, oil- and water-soluble, 5409
tablets, oil-soluble, 5356
tablets, oil- and water-soluble, 5419
tablets, water-soluble, 5512
Vitamins with minerals oral powder, 5338
Volumetric
apparatus ⟨31⟩, 6448
solutions, 6237
Voriconazole, 4643
Voriconazole compounded, veterinary ophthalmic solution, 4645

W

Warfarin sodium, 4646
for injection, 4648
tablets, 4650
Washed sand, 6218

Water

ammonia, stronger, 6207, 6207, 6226, 6226
ammonia, 25 percent, 6136
ammonia-free, 6218
carbon dioxide-free, 6218
cetyltrimethylammonium chloride, 25 percent in, 6150
conductivity ⟨645⟩, 6871
deaerated, 6218
determination ⟨921⟩, 7196
deuterated, 6155
D-Gluconic acid, 50 percent in, 6168
for hemodialysis, 4651
for hemodialysis applications ⟨1230⟩, 8240
hydrazine hydrate, 85% in, 6170
for inhalation, sterile, 4652
for injection, 4651
for injection, bacteriostatic, 4652
for injection, sterile, 4653
for irrigation, sterile, 4653
methylamine, 40 percent in, 6180
organic-free, 6218
particle-free, 6218
peppermint, 5923
for pharmaceutical purposes ⟨1231⟩, 8242
pure steam, 4654
purified, 4653
purified, sterile, 4654
rose, ointment, 3946
rose, stronger, 6001
solid interactions in pharmaceutical systems ⟨1241⟩, 8359
soluble vitamins capsules, 5499
soluble vitamins with minerals capsules, 5523
soluble vitamins with minerals oral solution, 5543
soluble vitamins with minerals tablets, 5552
soluble vitamins tablets, 5512
Stronger ammonia, 6207
vapor detector tube, 6218
vitamins capsules, and oil-soluble, 5389
vitamins with minerals capsules, and oil-soluble, 5435
vitamins with minerals oral solution, and oil-soluble, 5462
vitamins with minerals tablets, and oil-soluble, 5476
vitamins oral solution, and oil-soluble, 5409
vitamins tablets, and oil-soluble, 5419

Wax
carnauba, 6101
emulsifying, 6101
microcrystalline, 6101
white, 6102
yellow, 6103
Weighing on an analytical balance ⟨1251⟩, 8363
Weight variation of dietary supplements ⟨2091⟩, 8718
Wheat
bran, 4654
starch, 6066
Witch hazel, 4656
Wound matrix small intestinal submucosa, 4002
Wright's stain, 6218

www.webofpharma.com

Written prescription drug information—guidelines ⟨1265⟩, 8368

X

Xanthan gum, 6103
solution, 6104
Xanthine, 6218
Xanthydrol, 6218
Xenon Xe 133, 4656
X-ray fluorescence spectrometry ⟨735⟩, 6971
X-ray fluorescence spectrometry—theory and practice ⟨1735⟩, 8490
Xylazine, 4656
hydrochloride, 4658
injection, 4659
Xylene, 6218
m-Xylene, 6218
o-Xylene, 6218
p-Xylene, 6218
Xylene cyanole FF, 6219
Xylenol orange, 6221
TS, 6237
Xylitol, 6105
Xylometazoline hydrochloride, 4659, 6219
nasal solution, 4661
Xylose, 4662, 6219

Y

Yeast extract, 6219
Yellow mercuric oxide, 6219
Yohimbine
hydrochloride, 4663
injection, 4664
Yttrium Y 90 ibritumomab tiuxetan injection, 4665

Z

Zalcitabine, 4666
tablets, 4667
Zaleplon, 4668
capsules, 4669
Zanamivir, 4671
meso-Zeaxanthin, 5570
preparation, 5572
Zein, 6106
Zidovudine, 4673
capsules, 4674
injection, 4675
and lamivudine tablets, 2528
oral solution, 4676
tablets, 4678
Zileuton, 4679
Zinc, 6219
acetate, 4681, 6219
acetate oral solution, 4682
activated, 6219
amalgam, 6219
carbonate, 4682
chloride, 4683
chloride, anhydrous, powdered, 6219
chloride injection, 4684
citrate, 5574
citrate tablets, 5575
determination ⟨591⟩, 6817
gluconate, 4684
gluconate tablets, 4686
oxide, 4687
oxide neutral, 4688
oxide ointment, 4689
oxide paste, 4690
oxide and salicylic acid paste, 4690
stearate, 4691
sulfate, 4692
sulfate heptahydrate, 6219
sulfate injection, 4692
sulfate ophthalmic solution, 4693
sulfate oral solution, 4693
sulfate tablets, 4694
sulfate, twentieth-molar (0.05 M), 6252
sulfide topical suspension, 4695
undecylenate, 4695
uranyl acetate TS, 6237
and vitamin C lozenges, 5576
Zinc oxide
powder, 4690
Zinc sulfate
0.02 M VS, 6252
0.1 M VS, 6252
Ziprasidone
capsules, 4695
Ziprasidone hydrochloride, 4699
Zirconyl
nitrate, 6219
Zolazepam
hydrochloride, 4701
and tiletamine for injection, 4383
Zolmitriptan, 4702
nasal spray, 4704
tablets, 4706
orally disintegrating tablets, 4709
Zolpidem tartrate, 4711
tablets, 4712
extended-release tablets, 4714
Zonisamide, 4718
capsules, 4719
Zonisamide compounded
oral suspension, 4721

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